Handbook of Neurochemistry and Molecular Neurobiology

Schizophrenia

Abel Lajtha (Ed.)

Handbook of Neurochemistry and Molecular Neurobiology

Schizophrenia

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With 62 Figures and 46 Tables

Springer

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Library of Congress Control Number: 2006922553

ISBN: 978-0-387-30365-9

Additionally, the whole set will be available upon completion under ISBN: 978-0-387-35443-9
The electronic version of the whole set will be available under ISBN: 978-0-387-30426-7
The print and electronic bundle of the whole set will be available under ISBN: 978-0-387-35478-1

springer.com

Printed on acid-free paper SPIN: 11531043 2109 – 5 4 3 2 1 0

Preface

The field of Neuroscience has, in the past decades, undergone an explosive growth and developed very impressive approaches and very exciting and important findings on how the mechanisms in the nervous system operate. In spite of these really significant advances, our efforts to understand, cure, and prevent pathological changes have not been so successful. Mental disease to many means life-long incapacity, which affects more people in its various forms and for a longer time than any other disease, and represents a major financial burden on society, but its elimination remains unfulfilled. We still do not know whether schizophrenia or any other mental disease is a possible manifestation of separate pathologies and if so, what the biological similarities and differences between our diagnostic classes are. Of course even temporary help that diminishes the manifestations of the illness, and diminishes the associated suffering of the specific hospital populations, is of great importance and represents a crucial contribution. Clearly mental illness affects not only the patients; it is a burden on families and on the whole society; it diminishes contributions to society, the productive life and the happiness of the patients suffering from it.

In spite of this, there is a dramatic decrease in hospital population and in incapacitated patients, showing that the field is advancing, and that old dogmas have been replaced by new ones, and we have new hope. The field is constantly advancing: we possess better drugs, better understanding, and more knowledge. Certainly, knowledge of the basic mechanisms that ultimately modulate behavior and its changes, determine pathology, the structure of receptors, of the cells, the interaction of the components, connections, plasticity, measuring physiological processes, is constantly advancing. With such increasing knowledge comes our recognition of the complexities of neural processes – especially the ones that govern behavior, and the ones that are responsible for malfunction. The complexity requires a multifaceted research approach; it therefore works best if a number of subdivisions collaborate. The use of structural, imaging, genetic, electrophysiological, pharmacological, and biological expertise, looking at interaction of specific proteins and of structural elements is needed. The knowledge of susceptibility and much more is now essential for a better understanding of these complex mechanisms, which, however, seem more and more within reach. Clearly a better understanding will represent a major accomplishment for mankind, with immense medical, cultural, human and economic importance.

This volume also shows that it is impossible to predict from where the next advances in schizophrenia research will come. For example, in the early 1980s, I led a study looking at the effects of a series of amino acids and amino acid derivatives on the behavioral effects of phencyclidine. Of the amino acids we tested, only glycine and the glycine derivative glycyldodecylamide were found to have a significant behavioral effect. At the time, NMDA receptors had not yet been described, nor the inhibitory effects of phencyclidine on NMDA function. Certainly, the glycine site of the NMDA complex had not been described. It would have been impossible to predict that about a decade later these findings would contribute to the development of new classes of medication, such as glycine transport inhibitors, that are currently in clinical trials for schizophrenia, or that much of the work underlying this development would be conducted at my own institution. Hopefully, the clues that will lead to further advances in the understanding and treatment of schizophrenia can be found in this volume.

The multifaceted approach needed for further research development is clearly accomplished in the present volume thanks to its Editors – multidisciplinary researchers themselves. Each chapter in itself – even though it represents an approach to specific aspects of schizophrenia distinctly different from the others – still reflects this need for a complex approach. Each deals with molecular mechanisms, but of different genes, expressing different proteins; each deals with structures, which derive larger structures such as the

cortex, to smaller structures such as specific receptors, from the relevance of models to the relevance of family studies. They show the wealth of available approaches and the exciting results of this work. These fine reviews should help the field by pointing out not only what has been found and established, but also what else needs to be found. It shows the successes of collaborative studies and the possibilities of such collaborative studies in the future. Mental disease is not only a major burden, it also represents as shown in these chapters, a major possibility to approach and understand behavior, its factors and mechanisms. By pointing out current knowledge, it also indicates the promise of further understanding and improvement of available therapies for patients. A great deal has been accomplished – a great deal is yet to be accomplished. This volume shows us not only the accomplishments of the present, but also a hope for our future.

Abel Lajtha

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Section 1

Neurochemical Systems

1.1 Cortical Dopamine in Schizophrenia

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia*, DOI 10.1007/978-0-387-30410-6_1.1,

Abstract: This chapter is a review of the evidence implicating a dysfunction of dopamine transmission in the prefrontal cortex in schizophrenia, underlying negative symptoms and cognitive deficits. The evidence derives essentially from brain imaging studies, post-mortem studies and clinical pharmacological studies. The imaging studies have suggested alterations in the main mediator of dopamine transmission in the cortex, the D1 receptors. Furthermore, studies have suggested a link between NMDA deficits and D1 alterations in the cortex in schizophrenia, suggesting that therapeutic interventions at these targets may have beneficial effects on cognition and negative symptoms.

List of Abbreviations: CKU, chronic ketamine users; COMT, catechol-*O*-methyltransferase; DA, dopamine; DAT, DA transporters; DLPFC, dorsolateral prefrontal cortex; EC, entorhinal cortex; MPFC, medial prefrontal cortex; NET, norepinephrine transporters; PFC, prefrontal cortex; SN, substantia nigra; VTA, ventral tegmental area; TH, tyrosine hydroxylase; WCST, Wisconsin Cart Sort Task; WM, working memory

1 Introduction

Converging lines of evidence have shown that hyperstimulation of subcortical D_2 receptors contributes to the positive symptoms of schizophrenia: hallucinations, delusions, and thought disorder. On the other hand, negative symptoms (flattening of affect, apathy, poverty of speech, anhedonia, and social withdrawal) and cognitive impairment (deficits in attention, working memory (WM), and executive functions), which are resistant to D_2 receptor antagonism, have a more complex pathophysiology with multiple components of which cortical dopamine deficit may be a major contributor.

The hypothesis of a cortical dopaminergic deficit underlying negative and cognitive symptoms (Weinberger, 1987; Davis et al., 1991) derives from clinical and preclinical observations. Functional brain imaging studies engaging prefrontal cortex (PFC) function have shown alterations in patients with schizophrenia (for reviews see Knable and Weinberger, 1997). Studies have shown an important role for the dorsolateral prefrontal cortex (DLPFC) in the execution of WM tasks (Jonides et al., 1993; Petrides et al., 1993; Cohen et al., 1994; McCarthy et al., 1994; Courtney et al., 1996; Braver et al., 1997; Klingberg et al., 1997; Carlson et al., 1998; D'Esposito et al., 1998a; D'Esposito et al., 1998b; Callicott et al., 1999; Konishi et al., 1999; Jansma et al., 2000; Zarahn et al., 2000) and alterations in DLPFC activation during completion of WM tasks in schizophrenia (Stevens et al., 1998; Honey et al., 1999; Manoach et al., 1999; Callicott et al., 2000; Manoach et al., 2000; Barch et al., 2001; Menon et al., 2001; Perlstein et al., 2001), suggesting that pathology of the DLPFC or its connectivity is implicated in the WM deficits in schizophrenia. Preclinical studies showed that prefrontal DA transmission at D_1 receptors (the main DA receptor in the neocortex) is a critical modulator of PFC performance (for review see Goldman-Rakic et al., 2000).

Together, these studies implicate altered dopaminergic transmission at the D_1 receptor in the pathophysiology of cognitive and negative symptoms in schizophrenia. These deficits are part of a larger picture of prefrontal alterations that involve other transmitters and neuronal ensembles. Prefrontal cortical pathology in schizophrenia is multifactorial as evidenced by the neuropathological studies showing decreases in soma size, dendritic length, and spine density of pyramidal neurons, and alterations in various indices of GABAergic transmission as well as fewer thalamic projections to the DLPFC. These may all be interconnected, synergistic, and contribute to the clinical symptoms observed. This review will focus only on the current evidence for prefrontal hypodopaminergia in schizophrenia.

We will first review the dopaminergic system in the cortex from the anatomical and functional point of view, followed by a review of the evidence for a cortical deficit with special emphasis on the imaging data of cortical D_1 receptors as a probe of dopaminergic function in schizophrenia and in related conditions used to model the cortical dopamine deficit.

2 Overview of Prefrontal DA

Dopaminergic projections are classically divided in nigrostriatal, mesolimbic, and mesocortical systems. The nigrostriatal system projects from the substantia nigra (SN) to the dorsal striatum, and has been

classically involved in movement initiation, habituation, and sensorimotor integration. The mesolimbic system projects from the ventral tegmental area (VTA) and medial SN to limbic structures such as ventral striatum, septum, hippocampus, and amygdala. The mesocortical system projects primarily from the VTA and, in primates, from the dorsal tier of the SN to cortical regions, mostly orbitofrontal, medial prefrontal, and cingulate cortices, but also to the DLPFC, temporal, and parietal cortices (Haber and Fudge, 1997). The mesolimbic and mesocortical systems are involved in regulation of motivation, attention, and reward (Mogenson et al., 1980).

DA receptors include a D_1-like family (D_1 and D_5 receptors), and a D_2-like family (D_2, D_3, and D_4 receptors), which differ in their distributions on neurons (Missale et al., 1998) and their regional localization in the human brain (for reviews see Seeman, 1992; Joyce and Meador Woodruff, 1997). D_1 receptors show a widespread neocortical distribution, and are also present in high concentration in striatum. D_5 receptors are mostly detected in the hippocampus and entorhinal cortex. D_2 receptors are concentrated in the striatum, with low concentration in medial temporal structures (hippocampus, entorhinal cortex, amygdala) and thalamus. The concentration of D_2 receptors in the PFC is extremely low. D_3 receptors are present in the striatum, where their concentration is particularly high in the ventral striatum. D_4 receptors are present in the PFC and hippocampus, but not detected in the striatum.

In the PFC, D1 receptors display a bilaminar distribution with highest concentration in layers I, II, IIIa, V, and Vi and lower concentration in IIIb and IV (Dawson et al., 1984; Lidow et al., 1990). D_1 receptors are localized on pyramidal cells (dendritic spines and shafts); D_1, D_2, and D_4 receptors are localized on GABA interneurons (Smiley et al., 1994; Mrzljak et al., 1996).

The effects of dopamine on cortical activity are very complex and vary as a function of the intensity and duration of the stimulation as well as the receptor, membrane potential state, and history of the neurons; for a recent review, see Seamans and Yang (2004). DA receptors modulate pyramidal cell excitability, both directly and indirectly via modulation of GABAergic interneurons. Stimulation of DA receptors located on GABAergic interneurons is generally viewed as promoting a GABA-mediated inhibition of pyramidal cells (Grobin and Deutch, 1998; Del Arco and Mora, 2000; Seamans et al., 2001b; Gorelova et al., 2002). There is also evidence that presynaptic D_1 receptors on glutamatergic axon terminals decrease presynaptic glutamate release (Gao et al., 2001; Urban et al., 2002).

The postsynaptic effects of D_1 receptor stimulation on prefrontal pyramidal neurons are more complex. D_1 stimulation is neither "excitatory" nor "inhibitory," but depends on the functional status of the neuron (Yang et al., 1999). Evidence in cortical and striatal spiny projection neurons indicates that stimulation of D_1 receptors stabilizes inactivation during periods of irregular glutamate input, but enhances spike firing and plasticity in response to a high level of glutamatergic stimulation. When excitatory inputs to a neuron are not active and the neuron is hyperpolarized, D_1 receptors may decrease glutamate-mediated responses, in part by reducing sodium and N-type calcium currents (Fienberg et al., 1998). However, when glutamatergic inputs are synchronously active, i.e., both AMPA and NMDA receptors are stimulated at multiple synapses, D_1 receptor stimulation facilitates NMDA-mediated excitatory synaptic responses and increases evoked spike activity (Cepeda et al., 1992; Seamans et al., 2001a). These "activity-dependent" actions of DA increase the "gain" of cortical output signals and promote plasticity during the processing of task-relevant information, while suppressing task-irrelevant neural activity. Such mechanisms are likely to underlie a DA-mediated enhancement of cognitive "signal-to-noise" in the cortex (Seamans et al., 2001b; Gorelova et al., 2002).

3 Prefrontal DA and Cognition

A large body of evidence indicates that prefrontal DA activity is involved in cognitive processes subserved by the DLPFC and its connections, such as tasks involving working memory (WM). In 1979, Brozowski et al. reported in a seminal paper that selective DA depletion in the DLPFC in monkeys markedly impaired spatial WM performance (Brozowski et al., 1979), which was restored by treatment with DA agonists. These observations prompted a large number of pharmacological studies focusing primarily on D_1 receptors, showing that local intracerebral and systemic injections of selective D_1 receptor antagonists impaired WM

performance (Sawaguchi and Goldman-Rakic, 1991; Arnsten et al., 1994; Seamans et al., 1998). Conversely, stimulation of the D_1 receptor with low doses of selective D_1 agonists improved WM (Arnsten et al., 1994). The beneficial effects of D_1 receptor agonists on WM were mostly observed in animals with impaired prefrontal DA function. In MPTP-treated monkeys, D_1, but not D_2 agonists, improved accuracy of cognitive performance (Schneider et al., 1994). In aged monkeys, low doses of D_1 agonists improved WM performance (Cai and Arnsten, 1997). In monkeys treated with haloperidol (a drug that downregulates D_1 receptors in the PFC), WM impairment is also reversed by D_1 agonists (Castner et al., 2000). On the other hand, high doses of DA agonists impaired WM performance, an effect reversed by pretreatment with D_1 antagonists (Arnsten and Goldman-Rakic, 1990; Zahrt et al., 1997b).

Put together, these observations suggest that both insufficient and excessive D_1 receptor stimulation can be detrimental to WM performance (Arnsten, 1997). These observations are consistent with the reinforcing properties of D_1 receptor stimulation. Deficient stimulation of D_1 receptors on pyramidal cells might impair sustained NMDA-mediated response of pyramidal cells required for WM processing, while excessive stimulation of D_1 receptors might lead to a GABAergic overdrive (Goldman-Rakic et al., 2000; Gorelova et al., 2002).

In humans, the importance of DA for cognitive function is supported by cognitive impairment presented by patients with Parkinson's disease (Bowen et al., 1975; Stern and Langston, 1985). The concept that an optimal level of DA activity is required for WM tasks is also supported by studies of DA agonists in healthy volunteers (see for example Mattay et al., 2000; Mehta et al., 2000), showing that stimulant-induced improvement in performance is only observed in subjects with low baseline performance level (and presumably low DA tone). In contrast, in subjects with high baseline performance (and presumably optimal DA tone), pharmacologically induced excess of DA tone results in deterioration of performance. The inverted U-shape suggested by these studies is reminiscent of the inverted U-shape of DA agonist effects on WM performance in preclinical studies reviewed earlier. In addition, functional imaging studies suggest that optimal DA tone is associated with optimal activation "efficiency" (less activation required for a given performance level). Improved efficiency within optimal DA tone is also consistent with the view that DA improves the signal-to-noise in prefrontal circuitry. Recent studies of the functional consequences of a polymorphism of COMT support the involvement of cortical DA in WM tasks. In healthy subjects, the presence of the valine allele of the catechol-*O*-methyltransferase (*COMT*) gene, an allele associated with increased *COMT* activity and DA catabolism, is associated with lower performance and altered DLPFC activation during completion of the Wisconsin Cart Sort Task (WCST), a test of executive function involving WM (Egan et al., 2001). COMT knockout mice have higher prefrontal dopamine levels and enhanced memory performance (Gogos et al., 1998). The genetically determined level of COMT activity may be most influential in the PFC compared to other brain regions because DA transporters (DAT) are expressed in low levels in this region (Sesack et al., 1998). As a consequence, inactivation of released synaptic dopamine by COMT may play a more important role in PFC than in striatum, where DA transmission is dominated by DAT activity. While evidence for an overall contribution of the val allele to the susceptibility to develop schizophrenia is inconclusive (Daniels et al., 1996; Li et al., 1996; Karayiorgou et al., 1998; Ohmori et al., 1998; Wei and Hemmings, 1999; de Chaldee et al., 2001; Egan et al., 2001; Herken and Erdal, 2001), its presence might further compromise prefrontal DA function in these patients.

4 Prefrontal DA Function and Schizophrenia

We will review here the evidence for a deficit in prefrontal DA function in schizophrenia.

4.1 Clinical Studies

Clinical studies have suggested a relationship between low cerebrospinal fluid homovanillic acid, a measure reflecting DA activity in the PFC, and poor performance at tasks involving WM in schizophrenia (Kahn et al., 1994; Weinberger et al., 1998). DA agonists have beneficial effects on the pattern of prefrontal activation measured with PET during these tasks (Daniel et al., 1991; Dolan et al., 1995).

4.2 Postmortem Studies

4.2.1 D_1 Receptors

In the PFC, one study reported no change (Laruelle et al., 1990), and one reported a nonsignificant increase (Knable et al., 1996). Postmortem studies of D_1 transmission associated proteins have shown an upregulation of calcyon in PFC, a finding which has been replicated across different studies at this point (Lidow et al., 2001; Koh et al., 2003; Bai et al., 2004).

4.2.2 Tyrosine Hydroxylase Immunolabeling

A recent and interesting postmortem finding regarding DA parameters in patients with schizophrenia is the observation of decreased tyrosine hydroxylase (TH)-labeled axons in layers III and VI of the entorhinal cortex (EC) and in layer VI of the PFC, a finding suggesting that schizophrenia might be associated with a deficit in DA transmission in the EC and PFC (Akil et al., 1999, 2000). This finding was clearly unrelated to premortem neuroleptic exposure. Benes et al. (1997) observed no significant changes in TH-positive varicosities in the DLPFC. In the anterior cingulate region (layer II), these authors observed a significant shift in the distribution of TH varicosities from large neurons to small neurons.

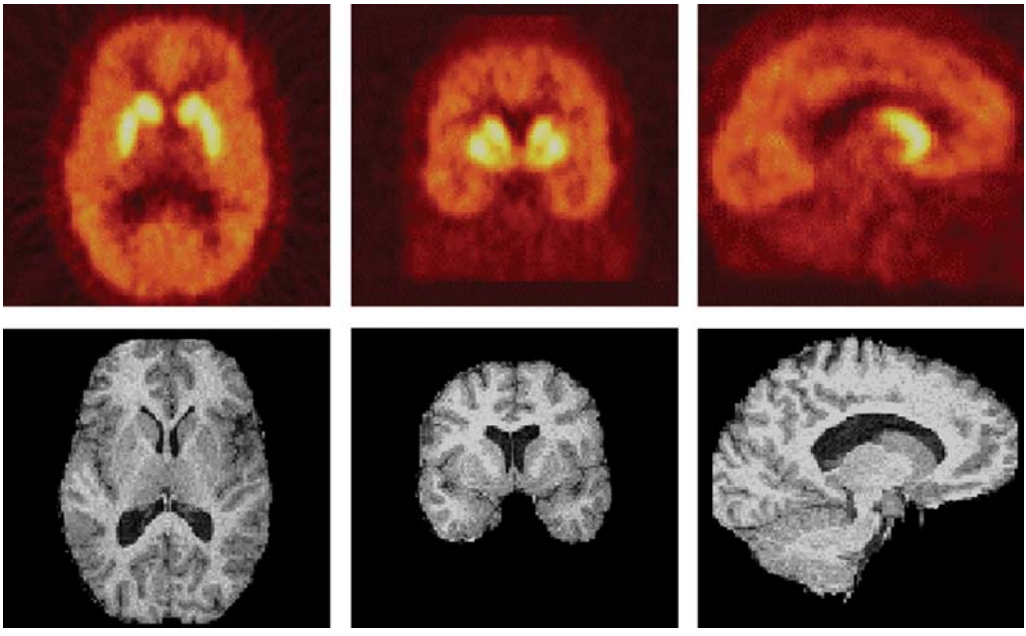
4.3 Imaging Studies

4.3.1 Extrastriatal D_1 Receptors

The main parameter of extrastriatal DA transmission that is currently quantifiable using noninvasive in vivo studies is D_1 receptor availability (*Figure 1.1-1*). Three PET studies of prefrontal D_1 receptor availability in patients with schizophrenia have recently been published. Two studies were performed with the D_1 radiotracer [^{11}C]SCH 23390. The first reported decreased [^{11}C]SCH 23390 binding potential in the PFC (Okubo et al., 1997), and the other reported no change (Karlsson et al., 2002). One study was performed with [^{11}C]NNC 112 (Abi-Dargham et al., 2002), and reported increased [^{11}C]NNC 112 binding potential in the DLPFC, and no change in other regions of the PFC such as the medial prefrontal cortex (MPFC) or the orbitofrontal cortex. In patients with schizophrenia, increased [^{11}C]NNC 112 binding in the DLPFC was predictive of poor performance on a working memory task (Abi-Dargham et al., 2002). Many potential factors, including patient heterogeneity and differences in the boundaries of the sampled regions, might account for these discrepancies. However, severity of deficits at tasks involving working memory were reported to be associated with both decreased PFC [^{11}C]SCH 23390 binding potential in one study (Okubo et al., 1997) and increased PFC [^{11}C]NNC 112 binding potential in our study (Abi-Dargham et al., 2002), suggesting that both alterations might reflect a common underlying deficit (*Figure 1.1-2*).

Because of the prevalent view that schizophrenia is associated with a deficit in prefrontal DA activity, the impact of acute and subchronic DA depletion on the in vivo binding of [^{11}C]SCH 23390 and [^{11}C]NNC 112 is highly relevant to the interpretation of these data. We investigated the effect of DA depletion on the in vivo binding of both tracers in rats (Guo et al., 2001). Acute DA depletion does not affect the in vivo binding of [^{11}C]NNC 112, but results in decreased in vivo binding of [^{3}H]SCH 23390, a paradoxical response that might be related to DA-depletion-induced translocation of D_1 receptors from the cytoplasmic to cell surface compartment (Dumartin et al., 2000; Laruelle, 2001; Scott et al., 2002). [^{11}C]NNC 112 in vivo binding was upregulated following chronic DA depletion, supporting the biological plausibility of the hypothesis that, in schizophrenia, upregulated [^{11}C]NNC 112 BP is secondary to sustained DA depletion (*Figure 1.1-3*). However, no change or a decrease in [^{3}H]SCH 23390 binding was observed after chronic depletion, demonstrating that the in vivo binding of both radiotracers is differentially regulated by chronic DA depletion, and potentially accounting for the discrepant results observed in schizophrenia.

Figure 1.1-1
[^{11}C]NNC 112 in a human volunteer. *Upper Row*. PET: Transaxial, coronal, and sagittal views of a 20-min frame obtained 40 min following the injection of 16 mCi of [^{11}C]NNC 112 in a 22-year old male. *Lower Row*. MRI: SPGR acquisitions in the corresponding planes. Activity concentrated in caudate, putamen, neocortex, and hippocampus. Background activity is measured in the cerebellum. Thalamic activity is very low. This distribution corresponds to the anatomical distribution of the dopamine D_1receptors



Interestingly, a recent study showed an effect of genetic loading on cortical D_1 levels measured with [^{11}C]SCH 23390, with higher values associated with more loading; this study also showed lower cortical D_1 in medicated patients (Hirvonen et al., 2006). A downregulation of D_1 receptors by D_2 antagonists had been previously shown in cortex of nonhuman primates (Lidow et al., 1997). This suggests that the discrepancies in findings with the two tracers may be related to differences in the patients' populations rather than differences in the tracers' in vivo behavior.

Studies with both radiotracers in the same patients are required to clarify this issue. In addition, more selective tracers are needed for future investigations, as we recently observed that 25% of the cortical binding of both tracers in monkeys is due to the 5HT2A receptor (Ekelund et al., 2006). Similar results were obtained in humans for [^{11}C]NNC 112 (Slifstein et al., 2007). No investigations in humans have been performed for [^{11}C]SCH23390. This finding flags the need for better tracers going forward with investigations of cortical dopaminergic transmission in schizophrenia.

4.3.2 Extrastriatal D_2 Receptors

Assessing extrastriatal cortical D_2 receptors may be an alternative way to probe cortical dopaminergic function. The recent availability of high-affinity D_2 radiotracers made the study of D_2 receptors in low-density regions possible. However, the current tracers that are available allow for the quantification of extrastriatal D_2 receptors in only the following regions: Substantia nigra, thalamus, and temporal cortex. Unfortunately, the signal in the PFC remains below reliable quantification, despite the higher affinity of the tracers. Using these tracers, a first study found decreases in temporal cortex in both hemispheres in a very

Figure 1.1-2

***Left*: Mean ± SD of DLPFC [^{11}C]NNC 112 BP (ml/g) in patients with schizophrenia (SCH) compared to controls (CTR). The mean ± SD of control subjects is displayed in white bars, and the mean ± SD of the SCH group is displayed in gray bars. Subjects with SCH displayed significantly higher levels of D_1 receptors in the DLPFC. *Right*: Relationship between upregulation of D_1 receptors in the DLPFC of untreated patients with schizophrenia and performance at WM task (three back AHR, lower values are poorer performances, $r^2 = 0.28$, $p = 0.006$)**

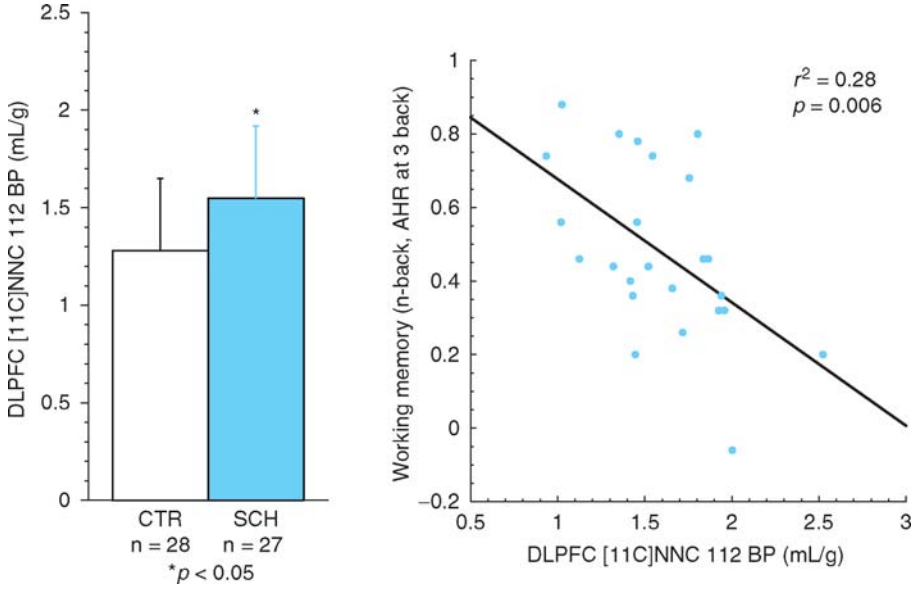
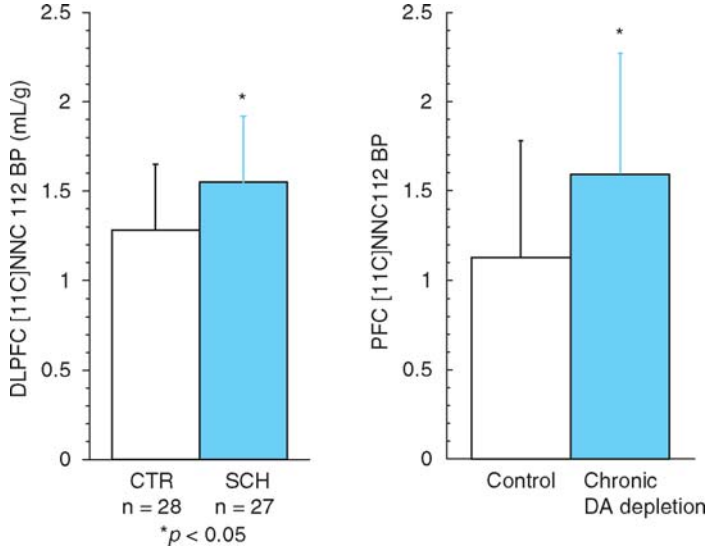


Figure 1.1-3

Mean ± SD of DLPFC [^{11}C]NNC 112 BP (ml/g), *Left*: patients with schizophrenia (SCH) compared to controls. *Right*: normal (white bars) versus dopamine-depleted rats (gray bars)



small group of patients compared to controls (seven patients and seven controls) (Tuppurainen et al., 2003). Another, similarly small study found low thalamic binding that was later confirmed in a larger sample of drug-naïve patients (Talvik et al., 2003, 2006). Suhara et al. (2002) found decreased D_2receptors in the anterior cingulate cortex and thalamic subregions in patients with schizophrenia, while Glenthoj et al. (2006) found a significant correlation between frontal D_2 receptor and positive symptoms in male schizophrenic patients (Glenthoj et al., 2006). In conclusion, the thalamic decreases seem to be a consistent observation so far; however, more research is needed to find tools to examine directly D_2 transmission in the PFC.

5 Prefrontal Cortical Dopamine in a Human Model of NMDA Dysfunction

Dysregulation of DA function in schizophrenia might be a consequence of alterations in NMDA transmission. Preclinical studies suggest that chronic hypofunction of NMDA receptors is associated with dysregulated prefrontal DA function (for review, see Jentsch et al., 1997; Jentsch and Roth, 1999). Furthermore, in nonhuman primates, chronic, intermittent exposure to the NMDA antagonist MK801 resulted in decreased performance at prefrontal tasks, decreased extracellular levels of DA in the DLPFC, and increase in [^{11}C] NNC 112 BP in the DLPFC (Kakiuchi et al., 2001; Tsukada et al., 2005). Together, these preclinical data suggest that NMDA dysfunction might lead to decreased prefrontal DA activity and increased D_1 receptor availability, all three dysregulations contributing to WM deficits. The description by Sesack et al. of direct glutamatergic tracks from the PFC stimulating midbrain DA neurons projecting back to the cortex provides an anatomical framework for this hypothesis (Carr and Sesack, 2000).

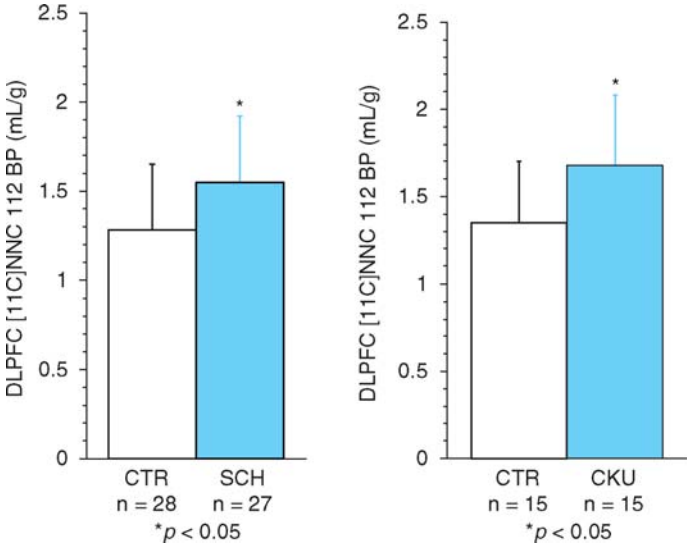
We explored this hypothesis by measuring [^{11}C]NNC 112 BP in human volunteers who abuse NMDA antagonists. Ketamine ("Vitamin K") and phencyclidine ("angel dust") are NMDA antagonists with abuse potential, and are found illegally on the streets. Exclusive ketamine or PCP use was confirmed by hair analysis. PET scans were acquired and were analyzed using the same methods used for the study in patients with schizophrenia (Abi-Dargham et al., 2002). DLPFC D_1 receptor availability was significantly upregulated in chronic ketamine users (CKU) compared to controls (Narendran et al., 2005). No significant differences were noted in other cortical, limbic, or striatal regions. In the CKU group, DLPFC [^{11}C]NNC 112 BP upregulation correlated with the number of vials (200–300 mg) of ketamine used per week ($r^2 = 0.48$, $p = 0.005$). The second interesting similarity between patients with schizophrenia and CKU subjects is that the DLPFC was the only region where a significant increase in [^{11}C]NNC 112 was detected (*Figure 1.1-4*). This observation suggests that the DLPFC DA system is more vulnerable to disruption due to intermittent, but repeated, states of NMDA hypofunction. These data suggest that the repeated use of ketamine for recreational purposes affects prefrontal dopaminergic transmission—a system critically involved in working memory and executive function, and support the concept of NMDA dysfunction leading to dopaminergic alterations observed in schizophrenia.

6 Significance and Therapeutic Implications

The current status of the prefrontal hypodopaminergia hypothesis in schizophrenia is characterized by a wealth of preclinical and clinical evidence incriminating altered prefrontal DA transmission at D_1 receptors as a putative factor in cognitive impairment presented by these patients, and mostly indirect evidence documenting alterations in brains of patients with schizophrenia. This situation is ultimately due to the lack of an appropriate method to measure prefrontal DA function in vivo with noninvasive techniques. Imaging studies of the cortical D_1 receptor as a probe of dopaminergic function are indirect ways of assessing DA in cortex. In addition, these studies have yielded inconsistent results across different tracers. However, when one examines results from one tracer, such as those with [^{11}C]NNC112, there is a consistent pattern of upregulation, modest in magnitude but statistically significant throughout conditions, showing increased cortical D_1 in schizophrenia, in a DA depletion rat model and in the human ketamine user's model of

Figure 1.1-4

Mean ± SD of DLPFC [^{11}C]NNC 112 BP (ml/g), *Left*: patients with schizophrenia (SCH) compared to controls (CTR). *Right*: healthy controls (white bars) versus chronic ketamine users (CKU) (gray bars)



NMDA dysfunction. Although, the functional meaning of this upregulation is subject to speculation, it has potential therapeutic implications that we will discuss here.

The first and most likely interpretation, supported by the rat model of DA depletion, is that D_1 increases represent a compensatory upregulation in response to a chronic deficiency in DA and a chronic "hypostimulation" of D_1 receptors. This explanation is congruent with all the clinical data reviewed earlier. An added validation to this hypothesis is the observation that a deficit in prefrontal DA innervation in schizophrenia could contribute to the disinhibition of subcortical DA observed in these patients as DA activity in PFC exerts a negative feedback on VTA activity. It has been shown that selective lesions of DA neurons in the PFC are associated with increased striatal DA release in rats (Pycock et al., 1980; Deutch 1990; Bubser and Koch, 1994; Thompson and Moss, 1995) and primates (Roberts et al., 1994), while augmentation of DA or GABA activity in the PFC inhibits striatal DA release (Kolachana et al., 1995; Karreman and Moghaddam, 1996; for review see Tzschentke, 2001). The reciprocal interaction has recently been shown in a mouse model of striatal D_2overexpression, showing resulting long-lasting negative effects on cortical cognitive performance (Kellendonk et al., 2006).

The hypothesis of D_1 hypostimulation can be tested with the use of D_1 agonists in conjunction with antipsychotic treatment as a cognitive enhancement strategy. This strategy has been difficult to implement because of the lack of D_1 agonists available to administer to humans. Recently, an investigational drug, DAR100, with poor bioavalability, has been tested in patients with schizophrenia in subcutaneous administration. Initial studies showed safety and absence of negative or side effects. Further testing is currently underway to show efficacy against cognitive impairment. Alternatively, drugs aiming at increasing cortical dopaminergic transmission by inhibition of COMT or norepinephrine transporters (NET), two main mechanisms by which DA is removed in the cortex, can be examined.

Because the evidence for cortical dopamine dysfunction in schizophrenia is indirect, the data can be interpreted in different ways. For example, the increase in DLPFC D_1 receptors could be seen as a primary phenomenon, and the alteration in WM performance in these patients could result from increased postsynaptic response to DA released in the DLPFC during task performance (Watanabe et al., 1997). According to this alternate hypothesis, WM impairment observed in these patients results from *excessive D_1 receptor transmission* (Arnsten et al., 1994; Murphy et al., 1996a, b; Cai and Arnsten, 1997;

Zahrt et al., 1997a). A third scenario, that combines elements of the first and second interpretations, can also be entertained. A persistent decrease in prefrontal DA activity might induce upregulation of D_1 receptors. This upregulation may be associated with increased receptor sensitivity, creating conditions in which the increase in DA release associated with stress or cognitive demands results in "overstimulation" of these upregulated D_1 receptors. In other terms, the inverted U-shaped relationship between D_1 receptor stimulation and WM performance (Goldman-Rakic et al., 2000) is narrower in patients with schizophrenia. In this model, the effects of atypical antipsychotic drugs might result from a subtle balance between D_1 receptors' downregulation (Lidow and Goldman-Rakic, 1994; Lidow et al., 1997) and sustained increase in DA release in the PFC (Pehek and Yamamoto, 1994; Yamamoto and Cooperman, 1994; Rollema et al., 1997; Melis et al., 1999; Youngren et al., 1999; Gessa et al., 2000; Ichikawa et al., 2001; Westerink et al., 2001).

7 Conclusion

While imaging studies have conclusively shown that schizophrenia is associated with hyperactivity of subcortical transmission at D_2 receptors (Laurelle et al., 1996; Breier et al., 1997; Abi-Dargham et al., 1998) the evidence supporting a deficit in DA transmission in the PFC in schizophrenia underlying cognitive and negative symptoms remains largely indirect. A better characterization of cortical dopamine transmission is needed. This will derive from use of more direct ways of measuring cortical dopaminergic transmission, by developing tracers that are agonists for the D_1 receptor or sensitive to acute fluctuations of dopamine tone in the cortex.

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1.2 Cholinergic Mechanisms in Schizophrenia

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Abstract: Substantial evidence exists for the involvement of both muscarinic and nicotinic cholinergic neurotransmission in the pathophysiology of schizophrenia. Molecular, genetic, postmortem, and pharmacological findings are included in this evidence. The role of both types of receptors is not unexpected, given their diverse functions in the brain. The common observation of heavy smoking in schizophrenia has directed attention to the nicotinic receptors, particularly the α7-nicotinic receptor, which requires high levels of nicotine for activation. Cholinesterase inhibitors, which raise acetylcholine levels have had limited therapeutic success in schizophrenia. Nicotine has some positive electrophysiological and cognitive effects in schizophrenia, but the profound tachyphylaxis that occurs with this substance limits the effectiveness of this agent. In contrast, promising findings with both muscarinic and selective nicotinic agonists are beginning to appear.

List of Abbreviations: DMXB-A, 3-(2,4-dimethoxybenzylidene)-anabaseine; mAChR, muscarinic acetylcholine receptor; nAChR, nicotinic acetylcholine receptor; NDMC, *N*-desmethylclozapine; PPI, prepulse inhibition; QNB, quinuclidinyl benzilate

1 Introduction

Since the discovery of dopamine as a neurotransmitter (Carlsson, 1959), schizophrenia has been associated with changes in the dopaminergic system. This association was supported by early findings of an antagonistic effect of antipsychotics on dopamine receptors (van Rossum, 1988; Creese et al., 1976; Seeman et al., 1976). In addition, the administration of dopamine-enhancing substances (e.g., amphetamine) frequently results in psychotic symptoms (Angrist and Gershon, 1970). These findings led to the dopamine hypothesis of schizophrenia suggesting that a hyperdopaminergic state causes the positive symptoms of schizophrenia (Carlsson, 1988; Abi-Dargham, 2004). Initially a global dopamine excess was postulated in schizophrenia. More recent formulations of the dopamine hypothesis describe a dopamine excess in the mesolimbic dopaminergic projections as well as a dopamine deficiency in mesocortical dopaminergic projections (e.g., dopaminergic projections to the prefrontal cortex) (see Abi-Dargham, 2004; Goldman-Rakic et al., 2004). In addition, an abnormal reactivity of midbrain dopamine neurons (Kapur, 2003), a hyperexcitability of dopamine D_2 receptors (Seeman et al., 2006) as well as an alteration in the function of extrastriatal (Takahashi et al., 2006) and extrasynaptic dopamine receptors (Carlsson and Carlsson, 2006) are thought to contribute to alterations of the dopamine system in schizophrenia.

Despite these modifications, the dopamine hypothesis cannot entirely explain the variety of symptoms associated with schizophrenia. Therefore, research in schizophrenia has also focused on the role of other neurotransmitter systems including glutamate (see Javitt and Zukin, 1991; Olney and Farber, 1995; Goff and Coyle, 2001; Konradi and Heckers, 2003), GABA (Benes and Berretta, 2001), serotonin (Meltzer, 1989), and acetylcholine (ACh) (Freedman et al., 2000; Olincy et al., 2006; Raedler et al., 2007) in schizophrenia. In this review, we focus on the role of the cholinergic system in the pathophysiology and treatment of schizophrenia.

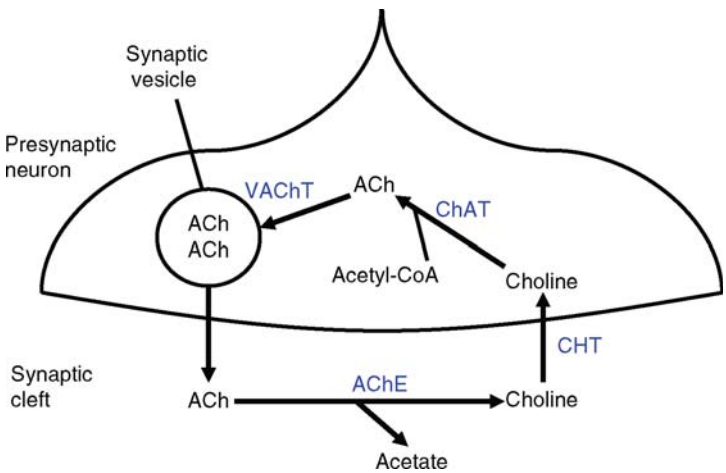
2 Acetylcholine

For almost one century, acetylcholine has been recognized as a neurotransmitter both in the central nervous system and the peripheral nervous system. In the peripheral nervous system, acetylcholine has been identified as the neurotransmitter of autonomic ganglia and the neuromuscular junction. Acetylcholine is involved in different peripheral functions such as heart rate, blood flow, gastrointestinal tract motility, and sweat production and smooth muscle activity. In the CNS, cholinergic neurotransmission plays a crucial role in a variety of CNS functions including sensory perception, motor function, cognitive processing, memory, arousal, attention, sleep, nociception, motivation, reward, mood, and psychosis.

2.1 Synthesis of Acetylcholine

Acetylcholine is synthesized in neurons from acetyl-CoA and choline in a reaction catalyzed by the enzyme choline acetyltransferase. This enzyme is almost exclusively located in high concentrations in cholinergic neurons. Glucose and citrate serve as a source for acetyl-CoA, while choline is transported into the brain from the blood stream. Choline is also recycled from the synaptic cleft through the presynaptic choline transporter (Sarter and Parikh, 2005). Despite these two mechanisms, the availability of choline seems to be the rate-limiting step of acetylcholine synthesis (Bazalakova and Blakely, 2006). After synthesis acetylcholine is stored in synaptic vesicles, from where it is released into the synaptic cleft following the activation of the neuron. Following its release into the synaptic cleft, acetylcholine either binds to pre- and postsynaptic receptors (see later) or is inactivated in the synaptic cleft through hydrolysis by the enzyme cholinesterase. Once acetylcholine is hydrolyzed, choline is transported back into the presynaptic neuron through a choline transporter and is recycled into the synthesis of acetylcholine (Smythies, 2005). Different substances (e.g., organophosphates, physostigmine, acetylcholinesterase inhibitors) inhibit the enzymatic inactivation of acetylcholine in the synaptic cleft and thus increase the concentration of acetylcholine. Acetylcholine synthesis and release are shown in *Figure 1.2-1*.

Figure 1.2-1
Acethylcholine synthesis and release in the presynaptic cholinergic terminal. Ach, acetylcholine; ChAT, choline acetyltransferase; VAChT, vesicular acetylcholine transporter; AChE, acetylcholineesterase; CHT, choline transporter



2.2 Cholinergic Projections in the CNS

Both cholinergic interneurons and cholinergic projection neurons can be found in the CNS. Cholinergic interneurons are mainly located in the striatum and nucleus accumbens. Most cholinergic projection neurons originate from the basal forebrain (projections to the cerebral cortex and hippocampus) and the brainstem (projections to mid-brain and brainstem) (Mesulam, 2004; Smythies, 2005).

3 Cholinergic Receptors

Different toxins with agonist and antagonist effects on the cholinergic system (e.g., muscarine, atropine, nicotine, and d-tubocurarine) helped to differentiate the muscarinic system and the nicotinic system as two

distinct families of the cholinergic system (Dale, 1914). Muscarinic and nicotinic acetylcholine receptors (nAChR) differ with regards to their function as well as their receptor structure. Nicotinic cholinergic receptors are ligand-gated ion channel receptors, whereas muscarinic acetylcholine receptors (mAChRs) are G-protein-coupled receptors.

3.1 Nicotinic Acetylcholine Receptors

Human nAChRs are pentamers of homomeric or heteromeric combinations of nine α (α_2–α_{10}) subunits and three β (β_2–β_4) subunits. These subunits combine in various sequences, to form a ligand-gated ion channel (Picciotto et al., 2000). The different combinations of these subunits have different distribution in the brain as well as different pharmacological properties (Gotti et al., 2006). The α_7 and the $\alpha_4\beta_2$ nAChRs predominate among the large number of AChRs present in the adult CNS with noticeable changes in distribution during brain development (Tribollet et al., 2004). In addition to their synaptic location, many nAChRs have an extrasynaptic location on somatodendritic locations or presynaptic terminals (Jones and Wonnacott, 2004). nAChRs are also expressed in nonneuronal tissues (Gotti and Clementi, 2004).

3.2 Muscarinic Acetylcholine Receptors

mAChRs are part of the superfamily of G-protein-coupled receptors and consist of seven transmembrane spanning domains (Wess, 1993). As such, mAChRs either activate or inhibit message transduction systems, thus having an effect on the intracellular second messengers (e.g., cAMP or IP_3).

mAChRs can be found on cholinergic and noncholinergic cells, both as auto- and heteroreceptors. Five different mAChRs (M_1–M_5) are known, that are encoded by five different genes. All five subtypes are found in the human CNS, albeit in regionally varying concentrations (Levey et al., 1991). The M_1, M_2, and M_4 subtypes are the predominant mAChR subtypes in the CNS (Volpicelli and Levey, 2004). M_1 mAChRs can be found in high concentration in cortex and striatum and in low concentration in thalamus and cerebellum. M_4 mAChRs are mainly expressed in the striatum, whereas M_2 mAChRs are most evident in the cerebellum and thalamus (Piggott et al., 2002). The M_5-subtype is the least abundant in the CNS. However, the M_5-subtype still might be relevant to schizophrenia as it is located in the brain-stem and midbrain, where it has an effect on dopamine release (Miller and Blaha, 2005).

4 The Role of mAChRs in Schizophrenia

Based on clinical observations, in 1989, Tandon and Greden proposed the concept of an alteration of the muscarinic cholinergic system in schizophrenia (Tandon and Greden, 1989). Several years later, Yeomans suggested that schizophrenia involves an overactivation of cholinergic neurons in the pedunculopontine and the laterodorsal tegmental nucleus, resulting in an overactivation of dopaminergic neurons (Yeomans, 1995). Recent neuropsychopharmacological, neuropathological, and brain-imaging studies have added to the concept that the muscarinic cholinergic system plays a crucial role in the pathology and treatment of schizophrenia and will be reviewed in detail.

4.1 Postmortem Studies

Postmortem studies have investigated both the number and distribution of cholinergic neurons and muscarinic receptors in schizophrenia, as summarized in *Table 1.2-1*. Few studies have looked at the distribution of cholinergic neurons in schizophrenia. A decade ago, Miller suggested that neuroleptic administration results in a degeneration of striatal cholinergic neurons, predisposing to tardive dyskinesia as well as refractory schizophrenia (Miller and Chouinard, 1993). Moreover, reduced numbers of cholinergic

Table 1.2-1
Postmortem expression of muscarinic acetylcholine receptors in schizophrenia

References	Muscarinic receptor subtype	Brain area	Result
Mancama et al. (2003)	M_1	Frontal cortex	M_1 mRNA decreased
Crook et al. (2001)	M_1, M_4	Prefrontal cortex	Significant decrease of M1 and M4 receptors in schizophrenia with and without pretreatment with anticholinergics
Scarr (2005)	M_2, M_3	Dorsolateral prefrontal cortex	No change in schizophrenia
Dean et al. (2002)	M_1, M_4	Dorsolateral prefrontal cortex	Significant decrease of M1 receptors in schizophrenia
Zavitsanou et al. (2005)	M_2, M_4	Anterior cingulate cortex	No changes in schizophrenia, depression, and bipolar disorder
Zavitsanou et al. (2004)	M_1, M_4	Anterior cingulate cortex	Significant decrease of M_1 and M_4 receptors in schizophrenia but not in bipolar disorder or depression
Newell et al. (2007)	M_1, M_2, M_4	Posterior cingulate cortex	Significant decrease of M_1 and M_4 receptors
Deng and Huang (2005)	M_1, M_2, M_4	Superior temporal gyrus	Significant decrease of M_1 and M_4 receptors; trend reduction in M2 and M4 receptors
Scarr et al. (2007)	M_1, M_4	Hippocampus	Significant decrease of M_1 and M_4; M_4 mRNA but not M_1 mRNA levels decreased
Crook et al. (2000)	M_1, M_4	Hippocampus	Significant decrease in schizophrenia
Dean et al. (2000)	M_1 and M_2 mRNA	Caudate, putamen	No differences in mRNA
Crook et al. (1999)	M_2, M_4	Caudate, putamen	Significant decrease in schizophrenia
Dean et al. (1996)	M_1	Caudate, putamen	Significant decrease in schizophrenia

interneurons have been found in the ventral striatum in schizophrenia (Holt et al., 1999; Holt et al., 2005). Studies of the distribution of mesopontine cholinergic neurons have yielded conflicting results, finding both increased numbers as well as decreased numbers of cholinergic neurons (Karson et al., 1993; Garcia-Rill et al., 1995; German et al., 1999).

Using ^{3}H-QNB as a marker for all five subtypes of the mAChRs, an early study reported a significant reduction in the level of mAChR-binding in the frontal cortex of subjects with schizophrenia compared with healthy controls (Bennett et al., 1979). This result was not replicated in two later studies, which reported an increased number of mAChRs in frontal cortex in medicated subjects with schizophrenia (Watanabe et al, 1983; Toru et al., 1988).

Newer studies have used more selective ligands (e.g., [^{3}H]pirenzepine as a marker of M_1 and M_4 mAChRs and [^{3}H]AF-DX 384 as a marker of M_2 and M_4 mAChRs) to assess the distribution of subtypes of the mAChR in schizophrenia (for more details see Raedler et al., 2007). Decreased levels of M_1 and M_4 mAChRs were found in schizophrenia in caudate and putamen (Dean et al., 1996; Crook et al., 1999), hippocampus (Crook et al., 2000; Scarr et al., 2007), anterior cingulate cortex (Zavitsanou et al., 2004), posterior cingulate cortex (Newell et al., 2007), and the prefrontal cortex (Crook et al., 2001; Dean et al., 2002), but not in the parietal cortex (Dean et al., 2002). Compared with healthy controls, M_2 and M_4 mAChR levels in anterior cingulate cortex (Zavitsanou et al., 2005) and superior temporal gyrus (Deng and Huang, 2005) showed no significant changes in schizophrenia.

Looking at the mRNA levels, the decrease in M_1/M_4 receptor levels is associated with decreased levels of M_4-, but not M_1-receptor mRNA in the hippocampus (Scarr et al., 2007). At the same time M_1-receptor mRNA levels were significantly decreased in the superior prefrontal gyrus (Mancama et al., 2003) and dorsolateral prefrontal cortex (Dean et al., 2000) in subjects with schizophrenia.

With regards to the effects of antipsychotic medications on muscarinic and nAChR in the brain, only olanzapine resulted in a temporary increase of muscarinic binding sites in a long-term study of different typical and atypical antipsychotics (Terry et al., 2006). This result is in accordance with in vitro (Bymaster et al., 1996; Schotte et al., 1996) and in vivo (Raedler et al., 2000) studies showing that olanzapine has considerable affinity to mAChRs.

In summary, these postmortem studies found a subtype-specific and region-specific decrease in mAChR density in schizophrenia. This decrease in mAChR density in schizophrenia may be disease-specific as subjects with bipolar disorder or major depression did not show similar changes (Zavitsanou et al., 2004). In addition to the small sample sizes, potential confounders should be taken into consideration (e.g., smoking, adaptive and residual effects of antipsychotic and anticholinergic medication) when assessing the results of the neuropathological studies. Despite these limitations, several neuropathological studies have repeatedly found region-specific, subtype-specific, and potentially disease-specific decreases in mAChR density in schizophrenia suggestive of an alteration of the muscarinic acetylcholine system in schizophrenia.

4.2 Animal Models

Further support for a potential role of the muscarinic acetylcholine system in schizophrenia comes from animal models. Neonatal hippocampal lesions in rats have been used as an animal model of schizophrenia (Lipska, 2004). In postpubertal rats, neonatal ventral hippocampal lesions are associated with increased levels of M_1/M_4- and M_2/M_4 mAChR binding in striatum, nucleus accumbens, and dorsal hippocampus as well as signs of increased mAChR responsiveness (Laplante et al., 2005). In addition, these lesioned animals show signs of a reorganization of the prefrontal cortical cholinergic system with increased acetylcholine release in response to dopaminergic stimulation (Laplante et al., 2004a) as well as to stressful stimuli (Laplante et al., 2004b). Thus, this animal model of schizophrenia results in enhanced muscarinic reactiveness, a finding similar to increased mAChR hyperreactivity suggested in humans with schizophrenia (Tandon and Greden, 1989; Tandon et al., 1991).

4.3 Brain Imaging Studies

Only few studies have used modern imaging techniques to further clarify the role of mAChRs in schizophrenia. [I-123]IQNB (quinuclidinyl benzilate) is a SPECT ligand that binds with very high affinity to all five subtypes of the mAChR. IQNB has been used to assess mAChRs in unmedicated subjects with schizophrenia. Compared with matched healthy controls, the mAChR availability was significantly decreased in cortex and basal ganglia in schizophrenia (Raedler et al., 2003b). This result has not yet been replicated. However, a recent PET study has challenged the specificity of this finding. Using the selective M_2 receptor ligand [18F]FP-TZTP, a decrease in M_2 receptor binding was found in bipolar disorder, but not in major depression (Cannon et al., 2006).

SPECT imaging has also been used to study the in vivo effects of antipsychotics on mAChRs. Using SPECT imaging, decreased mAChR availability was shown in schizophrenic subjects under treatment with the antipsychotics clozapine (Raedler et al., 2003a) and olanzapine (Raedler et al., 2000; Lavalaye et al., 2001) with a stronger reduction of mAChR availability under treatment with clozapine (Raedler et al., 2007).

5 Pharmacological Studies

Different pharmacological agents with an effect on the mAChR system have been used in the treatment of schizophrenia. These include agents that affect the concentration of intrasynaptic acetylcholine as well as agonists and antagonists of mAChRs.

5.1 Cholinesterase Inhibitors

Cholinesterase inhibitors are a group of pharmaceutical agents with known effects on the cholinergic system. Cholinesterase inhibitors (e.g., donepezil, rivastigmine, and galantamine) increase the synaptic levels of acetylcholine through an inhibition of the enzyme cholinesterase. Cholinesterase inhibitors are frequently used with moderate efficacy to improve cognitive functioning in dementia.

Cholinesterase inhibitors have been evaluated in schizophrenia as adjunctive treatments to ameliorate cognitive deficits as well as negative symptoms. *Table 1.2-2* summarizes studies on the adjunctive use of cholinesterase inhibitors for the treatment of schizophrenia. Overall, the effects of cholinesterase inhibitors on cognitive function in schizophrenia are modest (Ferreri et al., 2006). Beyond cognition, cholinesterase inhibitors also have modest effects on tardive dyskinesia (Tammenmaa et al., 2004).

Due to its dual mechanism of action that combines cholinesterase inhibition with allosteric modulation of nicotinic receptors, adjunctive treatment with galantamine may be more beneficial than other cholinesterase inhibitors in schizophrenia. In rats, galantamine increases dopamine levels in the prefrontal cortex (Schilstrom et al, 2007), which may contribute to a better control of cognition and affect. Only few studies have so far used galantamine as an adjunctive in schizophrenia. In a small trial in 16 schizophrenic subjects treated with risperidone, a significant improvement in memory and attention was observed after the addition of galantamine (Schubert et al., 2006). In a small case-series, the addition of galantamine to clozapine improved attention and psychomotor speed (Bora et al., 2005). Large-scale studies seem warranted to assess the potential benefits of adjunctive use of galantamine in schizophrenia.

5.2 Muscarinic Antagonists

Anticholinergics have been used for centuries as hallucinogens (Muller, 1998), particularly nightshade alkaloids (e.g., atropine, hyoscyamine, and scopolamine). Therapeutically, mAChR antagonists (anticholinergics) are an established treatment to alleviate motor side effects caused by antipsychotics. mAChR antagonists have been associated with impaired cognition in subjects with schizophrenia (Minzenberg et al., 2004) as well as in healthy controls (Ellis et al., 2006). In several studies, treatment with anticholinergics resulted in a worsening of positive symptoms coupled with a moderate improvement of negative symptoms of schizophrenia (Johnstone et al., 1983; Tandon et al., 1991; Tandon et al., 1992). Subjects with schizophrenia frequently report an activating effect of higher doses of anticholinergics, which occasionally results in an abuse of these medications (Zemishlany et al., 1996). In addition, the antimuscarinic drug scopolamine has recently shown direct antidepressant efficacy (Furey and Drevets, 2006).

5.3 Muscarinic Agonists

Betel nut chewing is a common practice in some Asian and Pacific cultures. In subjects with schizophrenia the chewing of betel nut has been preliminarily associated with fewer positive and negative symptoms (Sullivan et al., 2000). Constituents of betel nut have cholinomimetic properties as well as possibly also inhibitory effects on cholinesterase (Gilani et al., 2004). Arecoline, a major component of betel nut, is a known muscarinic and nicotinic agonist (Tripathi, 1983).

Xanomeline, a synthetic arecoline derivative, is an M_1/M_4 mAChR agonist that has been evaluated for potential use in schizophrenia (Mirza et al., 2003). Recent data suggest that xanomeline is also an antagonist at the M_5 receptor (Grant and El-Fakahany, 2005). In clinical studies, xanomeline resulted in cognitive improvement in Alzheimer Disease (Veroff et al., 1998). Looking at behavioral effects, xanomeline showed a dose-dependent efficacy against psychotic symptoms (agitation, delusions and hallucinations) in Alzheimer disease (Bodick et al., 1997). In a small pilot study in schizophrenia, monotherapy with xanomeline resulted in an improvement in positive symptoms as well as in cognitive function (Shekhar et al., 2001).

The results of the few clinical studies with xanomeline are consistent with animal studies that showed an antipsychotic-like effect of xanomeline in rodents and monkeys (Shannon et al., 1999; Shannon et al., 2000;

Table 1.2-2
Effects of cholinesterase inhibitors in schizophrenia

References	Subjects	Design	Duration	Cholinesterase Inhibitor	Antipsychotic medication	Result
Lee et al. (2007)	24	Double blind placebo-controlled	3 months	Donepezil 5 mg	Haloperidol 5–30 mg	Impovement in verbal recognition and visual recall memory
Risch et al. (2006)	13	Double blind crossover	12 weeks	Donepezil 5–10 mg	Atypical antipsychotics	No effect on mood
Erickson (2005)	15	Double blind	18 weeks	Donepezil	Standard antipsychotics	Modest improvement in psychiatric symptoms and verbal learning
Freudenreich (2005)	36	Double blind placebo-controlled	8 weeks	Donepezil 5–10 mg	Standard antipsychotics	No improvement in cognitive or psychopathological measures
Stryjer (2004)	8	Double blind crossover	18 weeks	Donepezil 5–10 mg	Clozapine	No overall change in PANSS three patients improved during donepezil
Tugal (2004)	12	Double blind placebo-controlled	12 weeks	Donezepil 5 mg	High potency typical P	No changes in PANSS or cognitive measures
Stryjer (2003)	6	Single blind	4 weeks	Donepezil 5 mg	Standard antipsychotic medication	Improvement in MMSE, CGI and PANSS
Buchanan (2003)	15	Open label	6 weeks	Donepezil	Olanzapine	Improvement in memory and processing speed
Friedman (2002)	36	Double blind placebo-controlled	12 weeks	Donepezil 5–10 mg	Risperidone	No significant improvement
Tandon (1999)	12	Open label	8 weeks	Donepezil 10 mg	Risperidone/ olanzapine	Improvement in positive symptoms
Aasen (2005)	20	Double blind	12 weeks	Rivastigmine	Standard antipsychotics	Nonsignificant improvement in sustained attention
Lenzi (2003)	16	Open	12 months	Rivastigmine 6 mg bid	Not specified	Improvement in quality of life and cognition
Sharma (2006)	21	Double blind placebo-controlled	24 weeks	Rivastigmine	not specified	No significant improvement
Bora et al. (2005)	5	Case-series	8 weeks	Galantamine 16 mg	Clozapine	Improvement in sustained attention, selective attention, psychomotor speed
Schubert et al. (2006)	16	Double blind placebo controlled	8 weeks	Galantamine	Risperidone	Improvement in memory and attention

Stanhope et al., 2001; Andersen et al., 2003). Like atypical antipsychotics, xanomeline has also been shown to increase extracellular concentrations of dopamine in prefrontal cortex (Perry et al., 2001).

PTAC and BuTAC are muscarinic agents that have been evaluated in animal models. PTAC and BuTAC are agonists at muscarinic M_2 and M_4 receptors and antagonists at M_1, M_3, and M_5 receptors. Both substances have antipsychotic-like properties with no affinity to dopamine D_2-receptors (Bymaster et al., 1998; Rasmussen et al., 2001). BuTAC may have favorable effects on learning and memory (Rasmussen et al., 2001). Prepulse inhibition (PPI) has been used as a model of sensorimotor gating. PPI is frequently impaired in schizophrenia (Kumari et al., 2000) and PPI has been used in animal-models of schizophrenia. Xanomeline and BuTAC have similar effects on PPI as haloperidol and olanzapine (Jones et al., 2005). However, neither PTAC nor BuTAC have been evaluated in humans so far.

5.4 Muscarinic Effects of Antipsychotics

Several antipsychotics have antimuscarinic properties in vitro and cause anticholinergic-like side effects (urinary hesitancy, dry mouth, constipation) in clinical practice. Among the second-generation antipsychotics, clozapine and olanzapine have strong in vitro binding to mAChRs (Bymaster et al., 1996; Schotte et al., 1996), while quetiapine shows moderate binding to mAChRs (Chew et al., 2006). In rodents, prolonged treatment with second generation antipsychotics has been associated with changes in key components of the cholinergic system, including choline acetyltransferase, as well as vesicular acetylcholine receptor, α7-nicotinic and M_2 mAChRs (Terry and Mahadik, 2007).

Both clozapine and olanzapine bind to mAChRs in vivo (Raedler, 2007a). Based on in vitro studies as well as its side-effect profile (e.g., severe constipation), clozapine has traditionally been regarded as a muscarinic antagonist. However, the concept of a pure muscarinic antagonism of clozapine has been challenged by the clinical observation that higher doses of clozapine frequently result in hypersalivation that can be treated successfully with anticholinergics such as pirenzepine (Schneider et al., 2004; Praharaj et al., 2006). In cell cultures, clozapine has shown to be an agonist at muscarinic M_4-receptors (Zorn et al., 1994; Zeng et al., 1997; Michal et al., 1999) as well as a partial agonist at M_1, M_2, and M_3 receptors (Michal et al., 1999; Olianas et al., 1999). Other studies, however, failed to show an agonist effect of clozapine on mAChRs (Olianas et al., 1997; Bymaster et al., 1999).

Recently, *N*-desmethylclozapine (NDMC = norclozapine), the major active metabolite of clozapine, has received increasing attention as it combines unique pharmacological properties on the mAChR system as well as other receptor systems. The *N*-demethylation of clozapine is predominantly regulated through CYP1A2 and CYP3A4 (Olesen and Linnet, 2001). In clinical practice, the NDMC/clozapine ratio shows high variability both between subjects as well as within subjects (Raedler, in press). NDMC has been shown to be a potent partial agonist at cloned human M_1 receptors (Weiner et al., 2004). This makes NDMC the only antipsychotic compound with M_1 agonist activity (Davies et al., 2005). NDMC is also a partial agonist at dopamine D_2 and D_3 receptors (Burstein et al., 2005). NDMC, but not clozapine, leads to an increased release of dopamine and acetylcholine in the prefrontal cortex and the hippocampus (Li et al., 2005). NDMC potentiates NMDA receptor activity in the hippocampus (Sur et al., 2003) and acts as a selective δ-opioid receptor agonist (Onali and Olianas, 2007). In preclinical binding studies, NDMC shows strong binding to 5-HT_2 receptors in rat brain, but only minimal dopamine D_2 occupancy (Natesan et al., 2007). NDMC (ACP-104) is currently being evaluated as a potential new pharmacological agent for the treatment of schizophrenia. Initial clinical studies with this compound have shown encouraging results.

5.5 Muscarinic Receptors as Therapeutic Targets

In summary, different muscarinic agents show promise as potential new treatments of schizophrenia. Potential treatment targets for these agents include both positive and negative symptoms as well as cognitive symptoms. However, more studies are necessary before the utility of a muscarinic approach to the treatment of schizophrenia can be assessed.

6 Interactions between the Muscarinic Cholinergic System and the Dopaminergic System

Interactions between the cholinergic system and the dopaminergic system are complex, bidirectional and occur at different levels in the brain (Sarter et al., 2005; Jones et al., 2005). In addition, interactions between the muscarinic acetylcholine system and the dopaminergic system occur directly as well as via other neurotransmitters. Cholinergic neurons have synaptic contact with dopaminergic neurons in the substantia nigra (Bolam et al., 1991). Functionally active mAChRs are located on dopaminergic neurons in the midbrain (De Klippel et al., 1993; Gronier and Rasmussen, 1998). These mAChRs on dopaminergic neurons in the substantia nigra and VTA are predominantly of the M_5-subtype (Vilaro et al., 1990; Weiner et al, 1990) with coexpression of D_2 receptor mRNA and M_5 receptor mRNA in the substantia nigra (Weiner et al., 1990). In addition, stimulation of M_1 receptors also results in an activation of dopaminergic neurons in the midbrain (Gronier and Rasmussen, 1999). Stimulation of mAChRs on dopaminergic neurons in the VTA results in an increase of dopamine release (Gronier et al., 2000). Similarly, the firing rate of the dopaminergic system increases when muscarinic agonists are applied to midbrain dopaminergic neurons (Gronier and Rasmussen, 1998; Gronier et al., 2000).

The effects of a muscarinic stimulus on the dopaminergic system depend on both the mAChRs involved as well as the brain regions involved. In the striatum, activation of mAChRs can result in both an increase in dopamine release (Raiteri et al., 1984; De Klippel et al., 1993) as well as a decrease in dopamine release (De Klippel et al., 1993). At the same time, the functional effect of the application of muscarinic agonists on dopaminergic neurons is influenced by the temporal pattern of activation (Fiorillo and Williams, 2000).

The interactions between the muscarinic acetylcholine system and the dopaminergic system are bidirectional and dopamine also has a modulatory effect on the release of acetylcholine (Di Chiara et al., 1994). Dopamine D_2 receptors (Alcantara et al., 2003) and dopamine D_5 receptors (Berlanga et al., 2005) are localized on cholinergic neurons and interneurons in the striatum, nucleus accumbens, forebrain, and diencephalon.

As pointed out earlier, muscarinic agents may be beneficial for the treatment of schizophrenia with regards to improving psychotic symptoms as well as improving cognitive symptoms. Given the close interactions between the muscarinic cholinergic system and the dopaminergic system both a direct dopamine-independent effect of muscarinic agents as well as a therapeutic effect that is mediated through a modulation of the dopaminergic system seem possible.

7 Nicotinic Receptors and the Pathophysiology of Schizophrenia

Convergent neurobiological and genetic evidence identifies involvement of the α7-nicotinic receptor in schizophrenia. An elementary deficit in psychosis is inability to filter or gate responses to sensory stimuli (Venables, 1967). Diminished inhibition of the P50 component of the cerebral evoked response to repeated auditory stimuli is a physiological demonstration of one brain mechanism that underlies abnormal sensory gating (Freedman et al., 1997). For paired auditory stimuli 500 ms apart, normal subjects inhibit the response to the second stimulus compared with the first; persons with schizophrenia generally have less inhibition. Their deficit is correlated with impairment in sustained attention (Cullum et al., 1993). In animal models, cholinergic stimulation of postsynaptic α7-nicotinic receptors on inhibitory interneurons is essential for this inhibition (Frazier et al., 1998). Positive effects of nicotine on human P50 inhibition are further support for a hypothesis of failure of normal nicotinic cholinergic neurotransmission as a pathophysiological mechanism in schizophrenia (Adler et al., 1993). Genetic studies, utilizing a linkage strategy independent of this neurobiological hypothesis, showed that the P50 gating deficit, like several other indicators of diminished attentional capability (Harris et al., 1996) is heritable in the families of schizophrenic probands. The maximal linkage is at the chromosome 15q14 locus of CHRNA7 (Freedman et al., 1997). Polymorphisms in the gene and its nearby partial duplication are associated with diminished P50 inhibition and cognitive function (Leonard et al., 2002; Houy et al., 2004; Dempster et al., 2006).

Several subsequent studies have found evidence for linkage of schizophrenia to this site, as well as for linkage of psychotic mania (Table 1.2-3). Polymorphisms in CHRNA7, especially in the promoter region, are likely to decrease gene expression; decreased expression of the receptor and its protein has been observed in schizophrenics' postmortem brain tissue (Table 1.2-4).

There is also emerging evidence for the influence of other nicotinic receptors in schizophrenia. The *CHRNA2* gene is located near a linkage peak for schizophrenia at chromosome 8p22, and high affinity nicotinic receptors, mostly composed of *CHRNA4* and *CHRNB2* subunits are depleted in postmortem brain (Breese et al., 2000; Blaveri et al., 2001). Mecamylamine, a nonspecific ion channel blocking agent, blocks nicotine's effects at these high affinity nicotinic receptors more readily than at α7-receptors. It appears to block many of the effects of nicotine on cognition in schizophrenia (Sacco et al., 2005).

Table 1.2-3
Genetic findings in schizophrenia at chromosome 15q14 and *CHRNA7*

Population	Phenotype	Finding	References
Linkage at chromosome 15q14			
Caucasians	P50 inhibition	LOD = 5.3	Freedman et al. (1997)
African Americans	Schizophrenia	NPL = 2.3	Kaufmann et al. (1998)
Bantu Africans	Schizophrenia	NPL = 1.8	Riley et al. (2000)
Mixed U.S.	Schizophrenia	LOD = 3.9	Freedman et al. (2001)
Caucasians	Schizophrenia	LOD = 1.7	Tsuang et al. (2001)
Caucasians	Schizophrenia	MLS = 2.0	Gejman et al. (2001)
Azorean	Schizophrenia	ETDT $P = 0.0006$	Xu et al. (2001)
Han Chinese	Schizophrenia	NPL = 3.3	Liu et al. (2001)
Caucasians	Schizophrenia	FBAT Z = 2.6	DeLuca et al. (2004a)
Association at CHRNA7			
Caucasians	Schizophrenia	$P = 0.0001$	Stassen et al. (2000)
Mixed U.S.	Schizophrenia	$P = 0.04$	Leonard et al. (2002)
Caucasians	P50 inhibition	$P = 0.02$	Raux et al. (2002)
Caucasians	Schizophrenia	$P = 0.0002$	Houy et al. (2004)
Caucasians	Memory in SZ	$P = 0.006$	Dempster et al. (2006)
Caucasians	Smoking in SZ	$P = 0.015$	DeLuca et al. (2004b)

Note: This is a summary of positive findings, to indicate that linkage and association in this area has been found in multiple studies. There are also negative and equivocal reports at this site, as is true for all genetic findings in schizophrenia. Association has been found to SNPs in the CHRNA7 promoter, a dinucleotide repeat in intron 2, and a polymorphism in a nearby duplicated portion of CHRNA7

Table 1.2-4
Expression of the α7-nicotinic receptor in schizophrenia

Brain region	Method	Finding	References
Hippocampus	^{125}I-αbungarotoxoin	Decreased 45%	Freedman et al. (1995)
Thalamic reticular nucleus	^{125}I-αbungarotoxoin	Decreased 25%	Court et al. (1999)
Frontal cortex	Immunoreactivity	Decreased 40%	Guan et al. (1999)
Cingulate cortex	^{125}I-αbungarotoxoin	Decreased 54%	Marutle et al. (2001)
Dorsolateral prefrontal cortex	Immunoreactivity	Decreased 28%	Martin-Ruiz et al. (2003)

7.1 Response to Nicotine in Schizophrenia

Animal models of decreased α7-nicotinic receptor expression exhibit diminished sensory inhibition and cognitive dysfunction (Stevens et al., 1996). Pharmacological stimulation of their α7-receptors by nicotine normalizes some of their functional impairments, presumably by increasing activation of their diminished receptors beyond that provided by their endogenous acetylcholine. This activation normalizes several aspects of sensory dysfunction in schizophrenia, including the increased hemodynamic activity that has been observed in the schizophrenia during elementary sensorimotor tasks such as smooth pursuit eye tracking (Tregellas et al., 2005).

Nicotine is indeed heavily abused by schizophrenics. Many smoke over two packs of cigarettes per day, and per cigarette, they extract 50% more nicotine than other smokers (Olincy et al., 1997). Higher nicotine levels are consistent with activity at α7-receptors, which are less sensitive to nicotine than α4β2-nicotinic receptors found on presynaptic terminals of many different neuronal types. Nicotine has neurocognitive effects, particularly on attention, but clinical use is severely limited by tachyphylaxis (*Table 1.2-5*). Although nicotine itself has no useful therapeutic effect because of this tachyphylaxis and its numerous toxic effects, its actions prompt investigation of less toxic nicotinic agonists.

Table 1.2-5
Effects of nicotine on neurocognition in schizophrenia

Cognitive function	References
Spatial/attention	Levin et al. (1996)
Spatial organization	Smith (2002)
Visuospatial working memory	George et al. (2002)
Sustained attention	Depatie et al. (2002)
Delayed recognition	Myers et al. (2004)
Sustained attention	Harris et al. (2004)
Working memory/selective attention	Jacobsen et al. (2004)

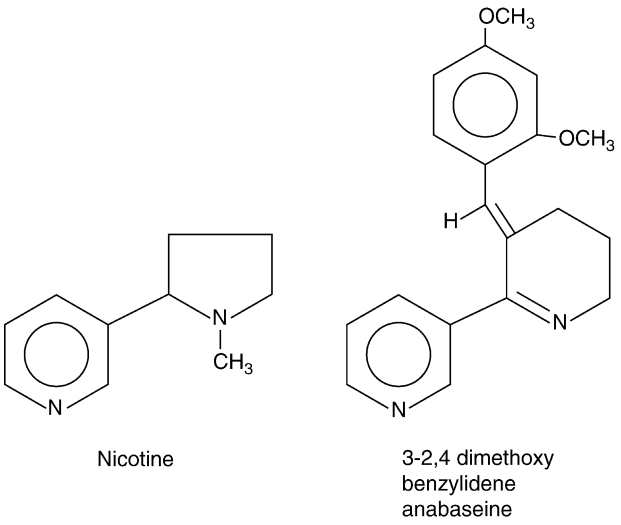
7.2 Nicotinic Agonist Development for Therapeutic Intervention

Nicotinic receptor agonists have been developed in an attempt to further characterize central nervous system cholinergic function and as potential candidates for the treatment of schizophrenia.

Drugs currently in development include a 1,4-Diaza-bicyclo[3.2.2]nonane-4-carboxylic acid 4-pyridin-2-yl-phenyl ester at Pfizer Inc., an (E)-*N*-methyl-5 (3-pyridinyl)-4-penten-2-amine at Targacept Inc., and a substituted heteroaryl-7-aza[2.2.1]bicycloheptane at Pharmacia and Upjohn Company. AR-R 17779, an Astra Arcus product, is an acetylcholine analog with full agonist properties at the α7-nicotinic receptor (Mullen et al., 2000). ABT-418, while primarily functioning as an α4-β2 agonist, also has some agonist properties at the α7-nicotinic receptor (Briggs et al., 1995). None of these drugs are related structurally. Only one is based on a natural alkaloid: the α7-selective agonist 3-(2,4-dimethoxybenzylidene)-anabaseine (DMXB-A). DMXB-A is one of a series of anabaseine compounds developed by Dr. William Kem at University of Florida during the past decade (*Figure 1.2-2*). Anabaseine, the initial compound, is an animal alkaloid found in marine worms (Kem et al., 1997). DMXB-A is a partial agonist at the human α7-receptor (De Fiebre et al., 1995; Briggs et al., 1996). It displays no significant binding at muscarinic receptors, but is a weak competitive antagonist at α4-β2-nicotinic and 5-HT3 receptors.

The efficacy of α7-nicotinic receptor agonists has been assessed in multiple animal paradigms of learning and memory. For example, DMXB-A improves monkey performance on a delayed matching to sample task, an effect that persists for 24 h after drug administration (Briggs et al., 1996). DMXB-A

Figure 1.2-2
Structures of nicotine and DMXB-A



improves eyeblink classical conditioning acquisition in older rabbits and in younger rabbits that have been impaired with mecamylamine treatment (Woodruff-Pak et al., 1994).

Tropisetron is marketed outside the U.S. as a 5HT3 receptor antagonist for nausea, but it has α7-nicotinic agonist effects in the same concentration range. Tropisetron normalizes P50 inhibition in schizophrenics, with the effect primarily occurring in the nonsmoking subjects (Koike et al., 2005).

Support for pursuing an α7-nicotinic agonist mechanism for the treatment of schizophrenia comes not only from consideration of the effects of various nicotinic agonists, but also from consideration of the unique effects of clozapine, possibly the most effective currently available drug for schizophrenia. Clozapine produces significantly more normalization of the P50 inhibition deficit than any other neuroleptics. First generation antipsychotics had little effect on P50 inhibition, and other second-generation antipsychotics have small, but generally nonsignificant effects, whereas clozapine produces in many patients nearly normal suppression of response (Adler et al., 2004). Clozapine increases release of acetylcholine in the hippocampus in animal models, possible through antagonism of presynaptic 5HT3 receptors that inhibit acetylcholine release. This effect normalizes hippocampal inhibition through an α7-nicotinic receptor mechanism (Simosky et al., 2003). Thus, clozapine, among its many actions, is an indirect α7-nicotinic receptor agonist. It may be significant that patients treated with clozapine significantly decrease their smoking and that smoking behavior is predictive of better clinical response to clozapine (George et al., 1997; McEvoy et al., 1999).

7.3 Phase 1 Proof-of-Concept Trial of DMXB-A for Neurocognitive and Neurophysiological Deficits in Schizophrenia

The aim of this study was to determine, as a proof of concept, if DMXB-A significantly improves neurocognition and to assess, by effects on P50 inhibition, whether its actions are consistent with activation of α7-nicotinic receptors. Because the proposed effect is agonism at a ligand-gated ion channel, biological effects were expected immediately, consistent with the results from animal models (Stevens et al., 1998). Twelve persons with schizophrenia consented to the study. They were concurrently treated with neuroleptic medications. Subjects who had not used nicotine or tobacco within the last month were selected to avoid possible interaction with chronic nicotine exposure. DMXB-A was administered orally (150 or 75 mg) followed 2 h later by a half dose (75 or 37.5 mg). The half dose, administered at the predicted half-life of the

first dose (Kitagawa et al., 2003), was chosen to extend the period of therapeutic drug levels during the behavioral measurements. The plasma levels obtained were consistent with the pharmacokinetic parameters established in the previous Phase 1 study in normals. Subjects received the high and low doses and identical-appearing placebos randomly on different days.

On each experimental day, the Repeatable Battery for Assessment of Neuropsychological Status (RBANS) was administered (Randolph, 1998). It assesses attention, immediate memory, visuospatial/construction, language, and delayed memory (Gold et al., 1999). Schizophrenics score significantly lower than normal subjects; the decrement is correlated with diminished psychosocial status. Compared to placebo, the DMXB-A significantly improved overall performance and specifically improved function on the Attention subscale. Inhibition of the P50 response to repeated stimulus, the sensory gating measure that led to identification of nicotinic receptor deficits in schizophrenia, also improved (Olincy et al., 2006).

These initial data support a positive effect of DMXB-A in schizophrenia, with a significant improvement in overall cognition and attention in particular. The clinical relevance of the neurocognitive change requires assessment after long-term administration. Symptomatic measures were not expected to change during a brief trial; therefore, clinical ratings on the Brief Psychiatric Rating Scale were considered exploratory pilot data. Ratings decreased in the course of the experimental day for all three treatments. Seven subjects had their lowest rating during DMXB-A, five during low-dose and an additional two during high dose, compared with one lowest during placebo and four identical during all treatments. Changes after DMXB-A administration were in blunted affect, somatic concerns, guilt, grandiosity, hallucinations, anxiety, and disorganization.

8 Conclusion

Cholinergic neurons are intimately involved in brain functions, with receptors ranging from G-protein-coupled muscarinic receptors to the ligand-gated ion channels formed by α7-subunits and other nicotinic receptor submits. Because of the role of cholinergic systems in modulating the brain's information processing and because of the brain's well-known deficits in this function in psychosis, it not surprising that there is pathology in these systems related to schizophrenia. It has been difficult to exploit this insight for therapeutic development using anticholinesterase drugs, because inhibition of cholinesterase contributes to the desensitization of the receptor populations, which may already be depleted because of variants in their genes. Instead, direct agonists seem to hold more promise. These agents selectively activate muscarinic or nicotinic receptor subclasses. Compared to nicotine, the nicotinic cholinergic agonist heavily abused by persons with schizophrenia, these new agonists do not produce as much tachyphylaxis. Some indication of clinically substantive effects has already been observed for both selective muscarinic and selective nicotinic agonists.

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1.3 Glutamatergic Approaches to the Conceptualization and Treatment of Schizophrenia

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_1.3,

Abstract: Although all current treatments for schizophrenia mediate their effects via blockade of the dopamine (D_2) receptor, limitations of the dopamine hypothesis have become increasingly apparent, as has the need for alternative neurochemical conceptualizations of schizophrenia.

Over the past 20 years, attention has turned increasingly to dysfunction of the brain glutamate system as a fundamental mechanism underlying brain dysfunction in schizophrenia. As opposed to dopamine which is present only in circumscribed brainstem nuclei - from which it projects widely in cortex and subcortical structures – glutamate is a widespread neurotransmitter in both cortex and subcortical systems and accounts for as many as 60% of brain synapses. Attention first turned to glutamatergic systems with the observation that phencyclidine (PCP) and similarly acting psychotomimetic compounds induced their unique behavioral effects by blocking neurotransmission at N-methyl-D-aspartate (NMDA)-type glutamate receptors. The ability of these compounds to transiently reproduce key symptoms of schizophrenia by blocking NMDA receptors led to the concept that symptoms in schizophrenia may reflect underlying dysfunction or dysregulation of NMDA receptor-mediated neurotransmission.

Since the original promulgation of the glutamate/NMDA hypothesis of schizophrenia in the late 1980's, significant progress has been made in delineating the potential role of NMDA dysfunction in the etiology of schizophrenia. Many of the most mystifying aspects of schizophrenia - such as why positive and negative symptoms fluctuate in severity in parallel rather than in opposition or why specific neurocognitive processes are impaired but others are paradoxically spared – become much more obvious when the disease is viewed from a glutamate/NMDA, rather than dopaminergic, perspective. Further, many of the findings from etiological investigations, such as genetic association studies, synergize much more with glutamatergic than dopaminergic models of the disorders.

Finally, viewing the disorder from a glutamatergic, rather than dopaminergic, perspective has opened new potential avenues for treatment that are only now making their way from preclinical to clinical investigation. These include interventions targeted at specific types of glutamate receptors, and include NMDA receptor modulators, glycine transport inhibitors and metabotropic agonists. To the extent that glutamatergic models of schizophrenia are correct, glutamate-based treatments should offer new opportunities for prevention and reversal of persistent symptoms and neurocognitive deficits of schizophrenia.

List of Abbreviations: AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; ASC, alanine-serine-cysteine; AX-CPT, AX continuous performance test; BPRS, brief psychosis rating scale; CDPPB, 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide; CHPG, 2-chloro-5-hydroxyphenylglycine; CSF, cerebrospinal fluid; D_2, dopamine type 2 receptor; DAAO, D-amino acid oxidase; DAOA or G72, D-amino acid oxidase activator; DISC-1, disrupted in schizophrenia-1; ERP, event-related potentials; GCPII, NAALADase, NAAG peptidase, glutamate carboxypeptidase II; GDA, glycine transport antagonist glycyl-dodecylamide; GLT-1, glutamate type I; GLYT1, glycine type I; LGN, lateral geniculate nucleus; LTD, long term depression; LTP, long-term potentiation; LY-379268, (-)-2-oxa-4-aminobicyclo[3.1.0.] hexane-4,6-dicarboxylate; mGluR, metabotropic glutamate; MMN, mismatch negativity; MPEP, 2-methyl-6-(phenylethynyl)pyridine; MTEP, 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine; NAAG, N-acetyl-aspartyl-glutamate; NAC, N-acetylcysteine; NFPS, N[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl] sarcosine; NMDA, N-methyl-D-aspartate; NRH, NMDA receptor-hypofunction; ODC, ornithine decarboxylase; PANSS, positive and negative symptom scale; PCP, phencyclidine; PK/PD, pharmacokinetic/pharmacodynamic; PN, processing negativity; PPI, prepulse inhibition; RBANS, repeatable battery for the assessment of neuropsychological status; (S)-3,4-DCPG, (S)-3,4-dicarboxyphenylglycine; SAMe, S-adenosylmethionine; SANS, scale for the assessment of negative symptoms; SAT1, spermidine/spermine N1-acetyltransferase; SNAT, small neutral amino acid transporters; SPECT, single photon emission; SSRI, serotonin reuptake inhibitors; VGLUT, vesicular glutamate transmitters; WCST, wisconsin card sorting task

Conflict of Interest: Dr. Javitt holds intellectual property rights for use of glycine, D-serine and glycine transport inhibitors in the treatment of schizophrenia.

1 Introduction

Schizophrenia is a severe mental disorder associated with both a specific profile of symptoms and a complex pattern of neurocognitive dysfunction. The first effective treatment for schizophrenia was discovered fortuitously in the mid-1950s (Delay and Deniker, 1955) and subsequently shown in the mid-1970s to mediate their effects at dopamine D_2 receptors (Seeman and Lee, 1975; Wong et al., 1986). The dopamine hypothesis has been the dominant neurochemical model of schizophrenia (Carlsson, 1988) and has proved heuristically valuable since that time. For example, all current treatments for schizophrenia mediate their effects via a blockade of the dopamine (D_2) receptor. Further, certain forms of cognitive dysfunction may relate to impaired dopaminergic function within the prefrontal cortex (see Chapter 1.1 this volume). Nevertheless, over recent years, limitations of the dopamine hypothesis have become increasingly apparent, as has the need for alternative neurochemical conceptualizations of schizophrenia.

Over the past 20 years, attention has turned increasingly to the dysfunction of the brain glutamate system as a fundamental mechanism underlying the brain dysfunction in schizophrenia. As opposed to dopamine, which is present only in circumscribed brainstem nuclei – from which it projects widely in the cortex and subcortical structures – glutamate is a widespread neurotransmitter in both the cortex and subcortical systems and accounts for as much as 60% of brain synapses. Because of its ubiquitous nature, the role of glutamate as a neurotransmitter in brain was not fully appreciated until the early-1980s, and glutamate receptor types were not fully cataloged until the mid-1980s.

Attention first turned to glutamatergic systems, with the observation that that phencyclidine (PCP) and similarly acting psychotomimetic compounds induced their unique behavioral effects by blocking neurotransmission at *N*-methyl-D-aspartate (NMDA)-type glutamate receptors (Javitt, 1987; Olney, 1989; Javitt and Zukin, 1991; Coyle, 1996). The ability of these compounds to transiently reproduce the key symptoms of schizophrenia by blocking NMDA receptors led to the concept that symptoms in schizophrenia may reflect the underlying dysfunction or dysregulation of NMDA receptor-mediated neurotransmission.

Since the original promulgation in the late 1980s of the glutamate/NMDA hypothesis of schizophrenia , significant progress has been made in delineating the potential role of NMDA dysfunction in the etiology of schizophrenia. Many of the most mystifying aspects of schizophrenia – such as why positive and negative symptoms fluctuate in severity in parallel rather than in opposition or why specific neurocognitive processes are impaired, but others are paradoxically spared – become much more obvious when the disease is viewed from a glutamate/NMDA, rather than a dopaminergic, perspective. Further, many of the findings from etiological investigations, such as genetic association studies, synergize much more with glutamatergic than with dopaminergic models of the disorders.

Finally, viewing the disorder from a glutamatergic, rather than a dopaminergic, perspective has opened new potential avenues for treatment that are only now making their way from preclinical to clinical investigation. These include interventions targeted at specific types of glutamate receptors, as well as reconceptualized remediational approaches. To the extent to which glutamatergic models of schizophrenia are correct, glutamate-based treatments should offer new opportunities for the prevention and reversal of persistent symptoms and neurocognitive deficits of schizophrenia.

2 Glutamatergic Physiology and Receptors

Current concepts of how and why neurotransmitter dysfunction may contribute to schizophrenia stem from decades of experience with modulatory neurotransmitters such as dopamine or serotonin. However, there are several major differences involved in viewing schizophrenia from a glutamatergic, rather than a dopaminergic, perspective.

First, while dopamine mediates its effects primarily by volume conduction across millimeters of tissue and tens of thousands of synapses (Smiley et al., 1994; Cragg and Rice, 2004), resulting in slow changes and tonic activation patterns, glutamatergic actions are specific to single synapses and induce rapid, phasic

depolarization. Thus, in addition to the issue of whether absolute glutamate levels are increased or decreased, the exact timing and localization of glutamate release at synapses is highly relevant. A concept of particular concern to glutamatergic functioning is signal-to-noise, e.g., the degree to which any single, phasic volley of glutamate release stands out against a background of tonic glutamatergic activity. Further, rapidity of release, which is controlled by presynaptic vesicular proteins, and of reuptake, which is controlled by glutamate transporters, becomes critical.

Second, while dopamine acts primarily at two receptor types, both of which produce slow potentials via G-protein-coupled mechanisms (i.e., metabotropic), glutamate mediates its effects at multiple postsynaptic receptors. While some glutamate receptors are metabotropic, the vast majority are coupled directly to ion channels (ionotropic). Different subtypes of glutamate receptors have different kinetic properties.

Finally, modulatory neurotransmitters such as dopamine, serotonin, or noradrenaline, arise from limited populations of neurotransmitters that innervate general brain regions, such as the prefrontal cortex or the striatum, but have little specificity of interaction within region due to the small number of neurons relative to the large scale of innervation. In contrast, over 60% of neurons in brain, including all cortical pyramidal neurons, are glutamatergic, most of the remaining neurons (primarily local circuit interneurons) being GABAergic. Thus, in many ways, glutamatergic and GABAergic neurons *are* the brain, and the critical issue is how the glutamatergic/GABAergic interplay across the brain is modulated by various transmitter processes. In other words, while neurotransmitters such as dopamine modulates selected synaptic transmission, the vast majority of information flow in the brain being mediated via glutamatergic synapses.

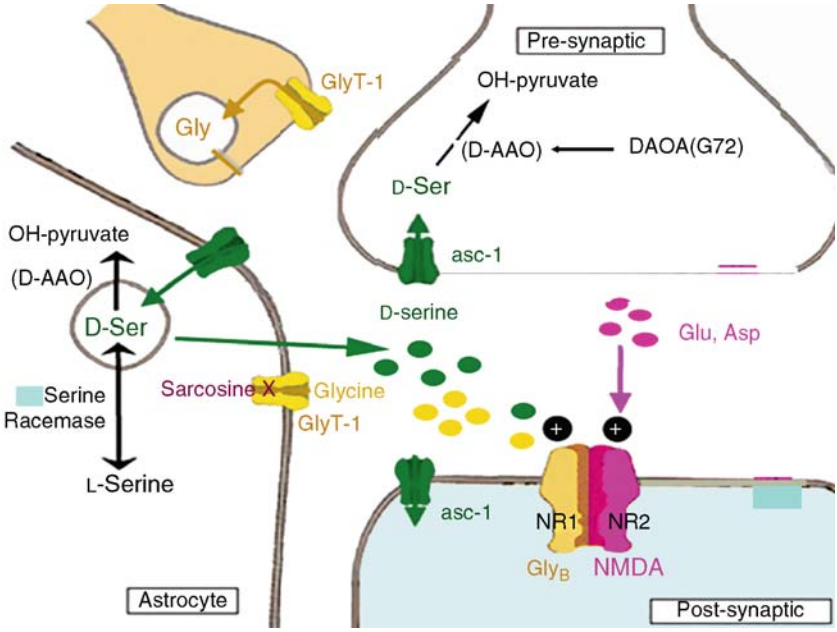
Because of the ubiquity of glutamatergic neurons, glutamatergic theories, by nature, imply a far more generalized pattern of dysfunction in brain process than do dopaminergic or other monoaminergic models. For example, dopaminergic models have typically focused on the dysfunction of specific brain regions that are considered to represent regionalized dopaminergic targets, such as prefrontal, cingulate, or limbic regions. In contrast, given that all parts of the brain receive glutamatergic afferents, glutamatergic models suggest that at least some degree of dysfunction should be evident across all brain regions. Because of the generalized nature of glutamatergic processes, however, it becomes critical to focus on specific subsets of glutamatergic mechanisms. The specificity of glutamatergic models depends upon the involvement of either specific receptors, such as the NMDA receptor, or specific aspects of glutamatergic function. Nevertheless, because of the convergence of actions operating on glutamatergic neurons, it is likely that multiple factors contribute to glutamatergic dysfunction within any one individual manifesting the clinical symptoms of schizophrenia, and moreover, that such mechanisms may differ across individuals with the disorder.

2.1 Presynaptic Glutamatergic Mechanisms

Glutamate is synthesized in the brain from glutamine, which is transported across the blood brain barrier with high affinity and present at high concentration in the extracellular brain fluid and the cerebrospinal fluid (CSF) (*Figure 1.3-1*). Following release, glutamate is reabsorbed by both neuronal and glial glutamate transporters via an energy-dependent transport process. Much of the brain glucose utilization is stoichiometrically coupled to glutamate recycling. Thus, functional brain imaging studies (e.g., PET, fMRI), while theoretically measures of local neuronal "activation," in fact, disproportionately detect local glutamate recycling (Magistretti and Pellerin, 1999; Riera et al., 2008). Overall, glutamate homeostasis is estimated to account for about 3% of the total brain energy utilization (Riera et al., 2008), whereas postsynaptic effects of glutamate, including the stimulation of action potentials, account for approximately 80% (Attwell and Laughlin, 2001). In hypoxic states, such as stroke, the recycling process breaks down, leading to an accumulation of external glutamate and excitotoxic damage (Hertz, 2008).

Glutamate synapses are specialized for rapid vesicle fusion/neurotransmitter release. Fusion is initiated by SNARE proteins such as syntaxin-1, synaptobrevin-2, and SNAP-25 (see Chapter 2.4 this volume). Presynaptic gene expression, in general, is widely disturbed in schizophrenia (Mirnics et al., 2000; Mirnics et al., 2001; Hemby et al., 2002), including reduced expression of SNAP-25 RNA(Hemby et al., 2002) and

Figure 1.3-1
Schematic model of the glutamate synapse. *Gly* glycine; *D-Ser* D-serine; *DAAO* D-amino acid oxidase; *DAOA* D-amino acid oxidase activator (aka, G72)



protein (Honer et al., 2002). SNAP-25 is expressed relatively selectively in presynaptic glutamatergic versus GABAergic terminals (Garbelli et al., 2008), and thus may serve as a marker of presynaptic glutamatergic dysfunction.

Synapsin proteins, which contribute to presynaptic glutamatergic vesicular development (Bogen et al., 2008), have also been shown to be expressed abnormally in a schizophrenia brain (Browning et al., 1993; Vawter et al., 2002). Further, polymorphisms of the synapsin II gene have been associated with schizophrenia (Chen et al., 2004a,b; Saviouk et al., 2007), indicating a potential pathogenic contribution. Dysbindin-1 (see Chapter 2.2 this volume), a well-replicated susceptibility gene for schizophrenia, is heavily expressed in presynaptic glutamatergic terminals (Talbot et al., 2006) and regulates the expression of SNARE proteins (Numakawa et al., 2004). Levels of dysbindin, like those of synapsin, are reported to be reduced in schizophrenia (Talbot et al., 2004).

Other presynaptic elements, such as complexins, that dock presynaptic vesicles (Brose, 2008) or syanptotagmin, which may couple Ca^{2+} entry to rapid vesicle fusion, permitting synchronous presynaptic glutamate release (Maximov and Sudhof, 2005), have not been heavily investigated in schizophrenia. One study has suggested an abnormal interaction between complexin-2 and synapsin-2 in schizophrenia, illustrating the complexity of the potential dysfunctional mechanisms (Lee et al., 2005).

Glutamate uptake into synapses is mediated by glutamate type I (GLT-1) transporters that are localized predominantly on astrocytes in the vicinity of asymmetric synapses (Melone et al., 2008). Vesicular glutamate transmitters (VGLUTs) transport glutamate into vesicles for release. Different vesicular glutamate transporters (VGLUT1-3) have been identified that may index subsets of glutamatergic terminals (Martin-Ibanez et al., 2006). The expression of vesicular glutamate transporters may be under dynamic control, permitting variable filling of presynaptic vesicles as a mechanism to maintain glutamate homeostasis (Erickson et al., 2006). As with SNARE proteins, glutamate transporters have been investigated in the etiology of schizophrenia only to a limited degree.

Astrocytes may also participate in glutamate release and homeostasis, possibly even through vesicular release (Bergersen and Gundersen, 2008). Although astrocytic abnormalities have been discussed in schizophrenia (Halassa et al., 2007; Kondziella et al., 2007), few postmortem studies have evaluated astrocytic function. For example, a reduced expression of glutamine synthetase has been demonstrated in some, but not all, brain regions in schizophrenia (Steffek et al., 2008), as has increased expression of the astocytic GLT-1 transporter (Matute et al., 2005).

2.2 Glutamate Receptors

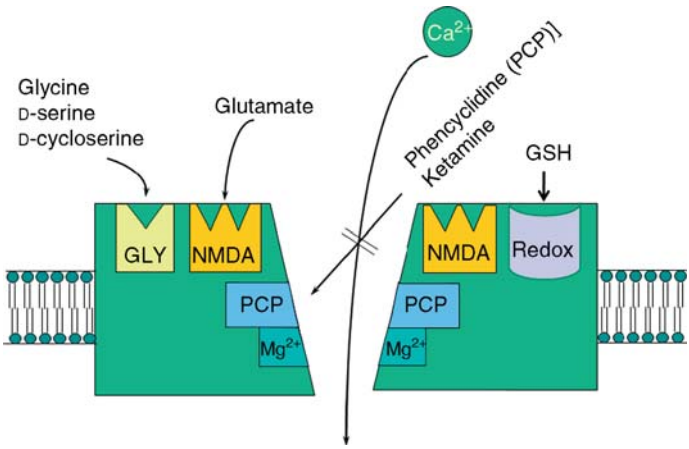
Receptors for glutamate are divided into two broad families. Ionotropic receptors are differentiated based upon their sensitivity to the synthetic glutamate derivatives NMDA, AMPA, and kainate. Metabotropic receptors, which are G protein coupled and mediate longer-term neuromodulatory effects of glutamate, are divided into groups on the basis of effector coupling and ligand sensitivity. Despite the differential sensitivity of these receptors to specific synthetic ligands, the endogenous neurotransmitter for all receptors is glutamate, and, to a much lesser extent, the closely related amino acid aspartate. Both types of families are potential treatment targets and have glutamate receptor modulators in various stages of development.

2.2.1 NMDA Receptors

NMDA receptors (*Figure 1.3-2*) are the most complex of the ionotropic receptors. In addition to the recognition site for glutamate, NMDA receptors contain several allosteric modulatory sites, including (1) a site for the endogenous brain amino acids glycine and D-serine, (2) a polyamine-sensitive site, and (3) a redox site that is sensitive to glutathione (see Chapter 2.5 this volume). All sites act primarily to facilitate activity at the receptor.

In addition to containing multiple modulatory sites, NMDA receptors are unique among ligand-gated channel-type receptors (both glutamatergic and nonglutamatergic) in that they are also voltage-dependent. The voltage dependency, which is subtype specific, is conferred by Mg^{2+}-induced blockade of the NMDA channel. At hyperpolarized membrane levels (e.g., −70 mV), NMDA receptors are blocked by Mg^{2+} so that no current flow occurs even when channels are opened by glutamate. In contrast, at depolarized membrane

Figure 1.3-2
Schematic model of the NMDA receptor complex



levels (e.g., −40 mV or above), the Mg^{2+} block is relieved, permitting current flow through open, unblocked NMDA channels. Thus, small changes in resting membrane potentials on NMDA-containing neurons can produce large changes in neuronal output. This permits NMDA receptors to function in nonlinear (multiplicative) gain mode, analogous to transistors, and thus, to participate in various logic operations.

Because NMDA receptors gate Ca^{2+} entry into the cell, they also serve as a trigger for the initiation of plastic events in the CNS, including long-term potentiation (LTP) and depression (LTD) (Cotman and Monaghan, 1988; Artola et al., 1990; Kirkwood et al., 1996). These processes are the primary physiological events underlying learning and new memory formation in the cortex and the hippocampus. NMDA dysfunction in schizophrenia would thus be consistent with the well-described mnemonic deficits associated with the disorder (Saykin et al., 1991; Bilder et al., 2000) (see Chapter 3.1 this volume).

NMDA receptors are blocked by "dissociative anesthetics," including PCP, ketamine, and MK-801 (dizocylpine) with rank order of potency MK-801 > PCP > ketamine. These compounds bind to a site located within the NMDA channel producing a noncompetitive (nonsurmountable) blockade of NMDA transmission. Several "designer" drugs that were popular in the late 1980s, such as TCP, also bind to the PCP receptor. NMDA transmission may be blocked as well by competitive antagonists that inhibit glutamate binding to its recognition site on the NMDA receptor. Although it was hoped at one time that NMDA inhibition via either the glutamate (competitive) or the PCP (noncompetitive) site might be beneficial in such hyperglutamatergic conditions as stroke or epilepsy, the approach has proved ineffective to date (reviewed in Javitt, 2004).

In addition to being expressed in neurons, NMDA receptors are expressed as well in growth cones of oligodendrocytes, and play a critical role both in development and in ischemia-induced damage (Karadottir et al., 2005; Salter and Fern, 2005).

Molecular composition: NMDA receptors are composed of multiple subunits including at least one NR1 subunit and one or more modulatory subunits from the NR2 (NR2A-NR2D) and/or NR3 (NR3A, NR3B) families. Further eight splice variants have been identified for the NR1 subunits. Each functional NMDA receptor is a heteromultimer, consisting of combinations of NR1, NR2 and/or NR3 subunits.

The different subunits and splice variants significantly alter the functional properties of native NMDA receptors, including their voltage sensitivity, peak conductance, and the degree to which they are influenced by the endogenous modulators such as glycine and D-serine. The glutamate binding site is located primarily on the NR2 subunits so that NMDA receptors vary in affinity for glutamate, based upon subunit composition. In contrast, glycine binds primarily to NR1, although NR2 subunits may modulate glycine affinity. Subunit composition also affects sensitivity to other agents. For example, NR2B-containing receptors are sensitive to the polyamine-site ligand ifenprodil (Lynch and Guttmann, 2001), while NR2A-containing receptors are highly susceptible to inhibition by Zn^{2+} (Lynch and Guttmann, 2001; Paoletti et al., 2008).

The NMDA subunit composition varies over space and time in the CNS. During development, NR2B subunits predominate in the forebrain, while both NR2A and NR2B are expressed in mature brain. In sensory systems, the switch from NR2B to NR2A is related to sensory experience and coincides with the timing of the critical period for sensory plasticity (Yashiro and Philpot, 2008). Conversely, light deprivation increases the ratio of NR2A to NR2B, which lowers the threshold for LTD and LTP in cortex, increasing the metaplasticity of the system (Philpot et al., 2007). NR2C is found predominantly in the developing forebrain (Pollard et al., 1993) and in the adult cerebellum, and may be responsible for the differential properties of cerebellar versus cortical NMDA receptors (Farrant et al., 1994). NR2D levels are typically low in adults, although upregulation has been reported in the prefrontal cortex in schizophrenia (Akbarian et al., 1996). In contrast, a more recent study found a selective downregulation of NR1, NR2A, and NR2C subunits in the frontal cortex, and no change in NR2B or NR2D (Beneyto and Meador-Woodruff, 2008), indicating the need for further investigation (see Chapter 4.2 this volume).

Interestingly, while the modulatory agents glycine and D-serine have similar excitatory effects on NMDA receptors containing NR2 subunits, they have opposite effects on receptors containing NR3 subunits, with glycine serving to activate NR3-containing receptors, and D-serine to inhibit them (Chatterton et al., 2002; Madry et al., 2008).

Modulation by glycine/D-serine: The glycine/D-serine site may be thought of as analogous to the benzodiazepine site of the $GABA_A$ receptor. Thus, the binding of glycine and/or D-serine regulates

the channel open time and the desensitization rate in the presence of agonist (glutamate), but does not, by itself, induce channel opening. Increasing glycine and D-serine levels potentiate NMDA transmission in a large number of neurophysiological models.

Levels of both glycine and D-serine are reported to be reduced in schizophrenia, although findings remain controversial. In the case of glycine, reduced plasma levels in schizophrenia correlate with the severity of negative symptoms (Sumiyoshi et al., 2004; Neeman et al., 2005) and response to clozapine (Sumiyoshi et al., 2005). In the case of D-serine, reduced serum (Hashimoto et al., 2003) and CSF (Hashimoto et al., 2005; Bendikov et al., 2007) levels have been reported, although contrary results have also been obtained (Fuchs et al., 2008).

Levels of synaptic glycine and D-serine must remain in equilibrium with those in the brain extracellular fluid and CSF. However, NMDA receptors are partially insulated from circulating glycine/D-serine level by the presence of amino acid transporters that are colocalized with NMDA receptors. In the case of glycine, NMDA receptors are protected by glycine type I (GLYT1) transporters that are colocalized with NMDA receptors, and play a key role in maintaining low, subsaturating glycine concentrations in the immediate vicinity of the NMDA receptor. Hippocampal slices form GLYT1 knockout mice do not show normal potentiation of NMDA response, following bath exposure to glycine or D-serine, suggesting that NMDA receptors are saturated by endogenous glycine in the absence of GLYT1 transporters. Further, GLYT1 knockouts show an enhanced spatial retention and decreased susceptibility to amphetamine, suggesting an increased NMDA function (Tsai et al., 2004).

D-serine is synthesized in brain primarily from L-serine via serine racemase, and degraded primarily by D-amino acid oxidase (DAAO). DAAO expression is high in cerebellum, leading to extremely low cerebellar D-serine levels, but low in cortex, where appreciable D-serine levels are observed. DAAO is regulated by the novel protein D-amino oxidase activator (DAOA), which is also known as G72 and is part of the G72/G30 gene complex. Linkages with schizophrenia have been reported for both DAAO (Chumakov et al., 2002), and more consistently, with DAOA (Chumakov et al., 2002; Li and He, 2007b; Shinkai et al., 2007; Maier, 2008; Shi et al., 2008) (see Chapter 2.1 this Volume). Further, variation in DAOA has been associated with cognitive dysfunction (Goldberg et al., 2006) and failures of hippocampal activation in schizophrenia (Hall et al., 2008), suggesting contribution to pathogenic mechanisms. Reduced serine racemase levels (Bendikov et al., 2007) and increased DAAO activity (Madeira et al., 2008) have been demonstrated in postmortem brain tissue in schizophrenia, perhaps accounting for reduced brain levels of D-serine.

In addition to GLYT1 transporters, small neutral amino acid (SNAT) transporters may also contribute to glycine transport (Javitt et al., 2005a). D-Serine is transported by system ASC (alanine-serine-cysteine) transporters (Rutter et al., 2007), although novel, D-serine-selective transporters have also been described (Javitt et al., 2002). Negative association with schizophrenia have been reported for both the GLYT1 (Tsai et al., 2006) and ASC-1 (Skowronek et al., 2006; Deng et al., 2008) transporters. Reduced expression of both ASC-1 and SNAT-2 genes have been reported in schizophrenia, although protein levels were increased only for ASC-1, potentially reflecting compensation for reduced D-serine levels (Burnet et al., 2008).

Additional modulatory sites: In addition to the glycine modulatory site, the NMDA receptor complex contains regulatory sites that are sensitive to polyamines, Zn^{2+}, protons, and redox agents such as glutathione due to exposure of free cysteines in the *N*-terminus. These sites have, in general, proved less amenable to pharmacological intervention than the glutamate and glycine binding sites, although recent findings support potential involvement of glutathione metabolism in schizophrenia (see Chapter 2.5 this Volume).

The role of the polyamine system in mental disorders has been recently reviewed (Fiori and Turecki, 2008). Primary polyamines include the compounds putrescine, spermidine, and spermine, which serve as agonists at the NMDA polyamine site, as well as the more recently discovered compound agmantine, which serves as an endogenous antagonist. Arcaine is a high-affinity synthetic antagonist commonly used in preclinical studies. Rate-limiting enzymes for polyamine synthesis are ornithine decarboxylase (ODC), S-adenosylmethionine (SAMe) decarboxylase (AMD1), and spermidine/spermine N^1-acetyltransferase (SAT1). Arginine serves as the major precursor via arginine decarboxylase. Interestingly, in one recent gene microarray study, only 5 of 71 metabolic pathways showed changes in postmortem schizophrenia brain. Of the 5 systems, one was related to ornithine and polyamine metabolism, suggesting a potential

involvement. At present, little is known about endogenous polyamine levels in schizophrenia cortex, and few ligands are available to modulate polyamine activity in vivo.

Zn^{2+} is localized in presynaptic vesicles at glutamatergic synapses and release in an activity-dependent manner (Paoletti et al., 2008). Zn^{2+}-containing fibers are found throughout the forebrain including the cortex, the striatum, and the hippocampus, but seem particularly involved in short-range cortical communication especially in the mossy fiber-CA3 pathway. NMDA receptors containing NR2A subunits are sensitive to nanomolar concentrations of Zn^{2+} similar to those seen in the extracellular brain fluid, suggesting that Zn^{2+} may exert a tonic inhibition of NMDA receptors (Erreger and Traynelis, 2008; Paoletti et al., 2008).

Abnormal Zn^{2+} metabolism is postulated to play a role in Parkinson's and Alzheimer's diseases (Barnham and Bush, 2008). However, to date there is little evidence linking alterations in zinc metabolism to schizophrenia. Although some early studies reported reduced brain Zn^{2+} levels in schizophrenia (Pfeiffer and Braverman, 1982), more recent studies have not found changes in plasma (Yanik et al., 2004) or brain (Kornhuber et al., 1994) levels, or in regional staining (Adams et al., 1995). Despite the fact that the zinc transporter (SLC30A4) is in a schizophrenia susceptibility region on chromosome 15q, linkage of the gene itself has been excluded (Kury et al., 2003; Chagnon, 2006).

The redox-sensitivity of NMDA receptors has been known since the late 1980s, although its functional significance has only recently been investigated. NMDA responses are inhibited in the presence of reducing agents and potentiated in the presence of oxidizing agents (Aizenman et al., 1989). In particular, glutathione has a potentiating effect on NMDA transmission, either directly or through modulation of brain redox state. There are, at present, no selective ligands for the NMDA redox site. Nevertheless, there is emerging evidence for reduced glutathione levels in schizophrenia, potentially related to genetic changes in enzymes responsible for glutathione synthesis (see Chapter 2.5 this Volume). In animals, effects of NMDA antagonists can be reversed by *N*-acetylcysteine (NAC), a metabolic precursor to glutathione, suggesting potential efficacy of such compounds in schizophrenia (see Clinical studies).

2.2.2 AMPA/kainate Receptors

AMPA/kainate receptors are a second class of ionotropic receptor for the neurotransmitter glutamate. AMPA receptors are composed of combinations of GluR1-4 subunits, while kainate receptors are composed of GluR5-7 and KA1 and KA2 subunits. Both receptor types interact closely with NMDA receptors, particularly in coordination with glutamatergic neurotransmission. Modulation of connection strength between neurons is initiated by Ca^{2+} flux through open, unblocked NMDA channels. Mature AMPA receptors containing the GluR2 subunit are Ca^{2+} impermeant (Tanaka et al., 2000), but do provide the primary depolarization necessary to unblock NMDA receptors and to permit calcium entry into the cell. Ca^{2+} entry through unblocked NMDA receptors, in turn, triggers AMPA insertion into the postsynaptic density and synaptic strengthening.

Moreover, if AMPA density falls below a critical threshold, levels of depolarization are insufficient to unblock NMDA channels, preventing postsynaptic depolarization or Ca^{2+} influx. The lack of Ca^{2+} influx prevents subsequent AMPA receptor insertion into the postsynaptic membrane. Thus, such synapses, despite containing intact NMDA receptors, are functionally silent and cannot be recovered by electrical stimulation alone (Isaac et al., 1999). This silencing of synapses could limit the degree of recovery to be expected even if normal glutamatergic functioning could be restored.

2.3 Metabotropic Receptors

In contrast to ionotropic receptors, the activation of metabotropic receptors triggers second messenger systems and affect neuronal metabolism. A crucial function of these receptors is regulation of presynaptic glutamate release and postsynaptic sensitivity. Metabotropic glutamate (mGluR) receptors are divided into three groups, based upon functional activity. Group I (types 1 and 5) receptors function

predominantly to potentiate both presynaptic glutamate release and postsynaptic NMDA neurotransmission. In contrast, group II (types 2 and 3) and Group III (types 4, 6, 7, and 8) receptors serve to limit glutamate release, particularly during conditions of glutamate spillover from the synaptic cleft. Thus, group I agonists would be expected to stimulate neurotransmission mediated by ionotropic glutamate receptors, whereas agonists for group II/III receptors would be expected to have opposite effects.

In addition to glutamate, mGluR3 receptors are activated by the dipeptide *N*-acetyl-aspartyl-glutamate (NAAG), which is released from presynaptic glutamate terminals and thus serves as an endogenous agonist (Coyle, 1997; Tsai, 2005). NAAG is degraded by glutamate carboxypeptidase II (GCPII, NAALADase, NAAG peptidase). A reduced expression of GCPII has been observed in one study in schizophrenia along with increased NAAG levels and reduced glutamate levels, leading to the hypothesis that NAAG increases may be pathogenic (Tsai et al., 1995). However, a second study did not find reduced GCPII in the brain overall, and found increased expression in hippocampal CA3, leaving the exact status of GCPII levels in schizophrenia to be determined (Olszewski et al., 2004).

High-affinity GCPII antagonists have been developed for clinical use in conditions such as stroke, neurodegenerative disorders, and neuropathic pain (Zhou et al., 2005; Thomas et al., 2006), permitting investigation of the role of NAAG in preclinical models of schizophrenia (Neale et al., 2005; Olszewski et al., 2008). The majority of GCPII is expressed in brain astrocytes, as are NAAG-sensitive mGluR3 receptors. NAAG receptors on astrocytes have also been proposed as a potential therapeutic target in schizophrenia (Baslow, 2008). Alterations in tissue levels of NAA and NAAG have been reported following chronic PCP treatment (Reynolds et al., 2005), suggesting bidirectional interactions between metabotropic and NMDA mechanisms.

3 Evidence for NMDA Dysfunction in Schizophrenia

The most robust evidence linking glutamate in general and NMDA receptors in particular to the pathophysiology of schizophrenia comes from studies of PCP and other "dissociative anesthetics" such as ketamine. PCP was first developed in the late 1950s as a potential anesthetic agent, along with the closely related compound ketamine (CI-581) (Chen and Weston, 1960; Domino et al., 1965). In preclinical testing, these compounds were found to produce a unique behavioral state in which animals were awake but seemingly "dissociated" from the environment. At higher doses, symptoms such as catatonia were observed in primates that were highly reminiscent of schizophrenia symptoms (Chen and Weston, 1960; Domino and Luby, 1981; Javitt and Zukin, 1991).

Interestingly, PCP-produced negative symptom like behaviors in monkeys, whereas it produced hyperactivity in rodents (Chen and Weston, 1960). This dissociation between the rodent and primate behavioral effects continues to complicate etiological research with PCP-like compounds, as one must always consider whether PCP- or ketamine-induced hyperactivity reflects homologous behaviors in humans such as agitation or other positive symptoms, or analogous behaviors, such as emotional withdrawal or other negative symptoms. The dissociation between effects in rodents and primates argues against the literal interpretation of rodent activity profiles as being related solely to positive symptoms.

In initial human testing, PCP and ketamine induced an abnormal mental state associated with psychosis. To investigators at the time, this state closely resembled the mental state associated with schizophrenia, leading to a series of controlled investigations to examine the similarity and differences between PCP-induced psychosis and schizophrenia, using low, subanesthetic doses of PCP (*Table 1.3-1*) (reviewed in Domino and Luby, 1981). These studies were discontinued when PCP was declared a controlled substance in the 1970s. In the 1970s, PCP became among the most popular drugs of abuse, providing further information regarding its psychotogenic potential.

Finally, there was a resurgence of interest in psychotogenic effects of ketamine in the early 1990s, following demonstrations that dissociative anesthetics such as PCP and ketamine induce their unique psychotomimetic effects by binding to a site located within the ion pore of the NMDA receptor (Anis et al., 1983; Javitt et al., 1987; Javitt and Zukin, 1991). Such studies remain ongoing and provide

critical information regarding similarities and differences between NMDA antagonist-induced states, and specific patterns of symptoms and cognitive dysfunction observed in schizophrenia.

Over recent decades, issues concerning glutamatergic dysfunction in schizophrenia have gone from "whether" to "how." Although most theories converge on NMDA receptors, the mechanism by which these receptors are involved in schizophrenia, as well as proposed models, vary across models. Three of the leading ones focus on (1) primary NMDA pathology, (2) NMDA-induced excitotoxicity, and (3) rebound hyperglutamatergia. These provide complementary approaches to the understanding of neurophysiological bases underlying symptoms and neurocognitive dysfunction in schizophrenia.

3.1 Symptom Patterns Following NMDA Antagonist Administration

In initial studies with PCP and ketamine in the early 1960s, it was noted that both agents produced what would now be considered positive, negative, and cognitive symptoms of schizophrenia (Luby et al., 1962; Javitt and Zukin, 1991). Similar results were observed in the clinical studies of PCP abusers in the early 1980s.

Symptomatic effects of NMDA blockade were better classified starting in the early 1990s in a series of ketamine challenge studies conducted in both normal volunteers and schizophrenia patients. In normal volunteers, significant increases in positive, negative, and cognitive symptoms were observed in schizophrenia, using scales such as the PANSS or BPRS/SANS.

Because PCP produces negative symptoms as prominently as, or more prominently than, positive symptoms, it has occasionally been argued that PCP psychosis should be seen primarily as a model for negative-symptom schizophrenia, whereas amphetamine- or LSD-psychosis should be seen as a model for positive symptoms (Gouzoulis-Mayfrank et al., 2005). However, in schizophrenia, negative and positive symptoms typically fluctuate in parallel, rather than opposition (Arndt et al., 1995), suggesting that a common underlying mechanism underlies both phases. Further, in PCP- or ketamine-induced psychosis, the relative proportions of positive and negative symptoms are highly similar to those observed in both acute and chronic schizophrenia, whereas in amphetamine- or LSD-induced psychosis, the level of positive versus negative symptoms is far in excess of the pattern observed even in acute schizophrenia stages (Gouzoulis-Mayfrank et al., 2005; Krystal et al., 2005b).

Similarly, although both amphetamine and ketamine produced increases in the cognitive factor score, the pattern of symptoms induced by the two compounds differed significantly. With the exception of worsening conceptual disorganization, amphetamine had no effect on cognitive symptoms. In contrast, ketamine significantly increased schizophrenia-like deficits not only in conceptual disorganization but also in abstract thinking, mannerisms, and poor attention. Although some additivity between amphetamine and ketamine effects was observed, the degree of interaction did not reach statistical significance (Krystal et al., 2005b).

Thus, the dichotomy between positive and negative/cognitive symptom schizophrenia does not appear warranted, although some patients may be found who have predominantly positive symptoms with no accompanying negative symptoms, but possibly with associated conceptual disorganization. Such patients typically are also highly responsive to antipsychotic treatment, suggesting that the appropriate dichotomy may be between patients having primarily a dopaminergic-type psychosis (robust positive symptoms, limited negative symptoms, and rapid treatment response to antipsychotic medication) and those having primarily an NMDA-pattern psychosis (balanced positive and negative symptoms, delayed/modest treatment response to antipsychotic medication). Unfortunately, while many schemata have been proposed to capture the dichotomy between such patient populations, such as process/reactive, paranoid/undifferentiated, or high/low HVA (Ottong and Garver, 1997), no objective measures have been developed that adequately differentiate the groups.

A potentially informative difference between ketamine-induced symptoms and those of schizophrenia is in the production of hallucinations. Thus, in established schizophrenia, auditory hallucinations consisting of voices of various types are common, whereas visual hallucinations are rare. In contrast, during ketamine-induced psychosis, visual perceptual distortions are common but organized auditory

Table 1.3-1

Data for controls and double-blind, placebo controlled crossover unless otherwise noted Neuropsychology

Domain	Test	Dose (bolus, route; drip)	N, design	Results	Reference
Speed of processing	Letter association task	Oral PCP; 5 vs. 10 mg	18, controlled	↔	Bakker and Amini (1961)
	Category Generation	IM; 0.25 vs. 0.5 mg/kg	31	↔	Ghoneim et al. (1985)
	Verbal fluency	IV; 0.1 mg/kg vs. 0.5 mg/kg/40 min	15	↓ (dose dependent)	Krystal et al. (1994)
	Trailmaking A and B	IM; 10 vs. 25 mg	10	↓ for 25 mg	Harborne et al. (1996)
	Verbal fluency	0.12 mg/kg, IV; 0.65 mg/kg/1 h	10	↓	Adler et al. (1998)
	Verbal fluency	0.26 mg/kg, IV; 0.65 mg/kg/1 h plus active/placebo lorazepam 2 mg	23	↓ not attenuated or worsened by lorazepam	Krystal et al. (1998)
	Benton's controlled Oral Word Association Test	IV, 0.97 mg/kg/2 h Measurements @ 0, 50, 100, 150, and 200 ng/mL	10, controlled	↔	Radant et al. (1998)
	Verbal fluency	0.26 mg/kg, IV; 0.65 mg/kg /1 h plus active/ placebo haloperidol 5 mg	19	↔	Krystal et al. (1999)
	Verbal fluency	0.27, 0.081, or 0.0243 mg/kg, IV; 150; 45; 13.5 ng/mL	15	↔	Newcomer et al. (1999)
	Verbal fluency (FAS); category instance generation (CIG)	0.23 mg/kg, IV; 0.5 mg/kg/1 h	20	CIG ↓ FAS ↔	Abel et al. (2003a)
	Trailmaking Part B; Verbal fluency	IV; 0.4 mg/kg vs. 0.8 mg/kg/80 min	54, no crossover	↓	Morgan et al. (2004a,b)
Working memory	Forward and Backward Digit Span; Word Digit Memory Task; Digit Comparison Task	Oral PCP; 5 vs. 10 mg	18, controlled	↓ on all tests	Bakker and Amini (1961)
	Digit span	0.4 mg/kg, IV; ½ doses were repeated every 15 min	11, crossover	↓ for forward digit span but not backward	Harris et al. (1975)
	N-back working memory	0.12 mg/kg, IV; 0.65 mg/kg/1 h	10	↓ on both 1 and 2 back	Adler et al. (1998)
	Spatial delayed response	0.27, 0.081, or 0.0243 mg/kg, IV; 150; 45; 13.5 ng/mL	15	↔	Newcomer et al. (1999)

	Backwards digit span	0.23 mg/kg, IV; 0.5 mg/kg/1 h	20	Near ↓	Abel et al. (2003a)
	Forward and backward digit span (WAIS-III); spatial span and spatial working memory tests (CANTAB)	IV; 50 vs. 100 ng/mL	11	↓ on verbal task (digit span), ↔ visual task (spatial)	Honey et al. (2003)
	N-back	IV; 0.4 mg/kg vs. 0.8 mg/kg over 80 min vs. placebo	54, double-blind controlled	↓	Morgan et al. (2004a,b)
	Working memory easy task	Placebo, then 0.26 mg/kg, IV; 0.65 mg/kg/ 100 min plus active/ placebo LY354740 (100 mg, 400 mg)	19	↓ ↑ with LY354740 Trend towards interaction	Krystal et al. (2005a)
	Working memory task	IV; 50 vs. 100 ng/mL	11	↔	Honey et al. (2004, 2005b)
	N-back	IM; vs. 0.4 mg/kg	18, double-blind controlled, crossover	↓ for accuracy	Lofwall et al. (2006)
	N-back	IV; 100 ng/mL	12	↓ for reaction time but not accuracy	Honey et al. (2008)
Executive functioning	Stroop test	Oral PCP; 5 vs. 10 mg	18, controlled	↓	Bakker and Amini (1961)
	WCST	IV; 0.1 mg/kg vs. 0.5 mg/kg/40 min	19	↔	Krystal et al. (1994)
	Stroop test	IM; 10 vs. 25 mg	10	↓ except on interference	Harborne et al. (1996)
	WCST	0.26 mg/kg, IV; 0.65 mg/kg/1 h plus active/placebo lorazepam 2 mg	24	↓ for categories completed and perseverative errors. No attenuation or worsening by lorazepam	Krystal et al. (1998)
	WCST	0.26 mg/kg, IV; 0.65 mg/kg/1 h plus active/placebo haloperidol 5 mg	37	↓ categories completed and perseverative errors, non-significantly attenuated by haloperidol.	(Krystal et al. 1999)
	Stroop test	0.27, 0.081, or 0.0243 mg/kg, IV; 150; 45; 13.5 ng/mL	15	↔	Newcomer et al. (1999)

continued

Table 1.3-1 (continued)

Domain	Test	Dose (bolus, route; drip)	N, design	Results	Reference
	Tower of London	IV; 50 vs. 100 ng/mL	11	↔	Honey et al. (2003)
	Stroop test	Placebo, then 0.26 mg/kg, IV; 0.65 mg/kg/100 min plus active/ placebo LY354740 (100 mg, 400 mg)	19	↔	Krystal et al. (2005a)
	Stroop test	0.27 mg/kg, IV; 0.12 mg/50 min	13	↓	Parwani et al. (2005)
	Stroop test	0.27 mg/kg, IV; 0.00225 mg/kg per minute for 2 h	10	↔	Rowland et al. (2005)
General	MMSE	IV; 0.1 vs. 0.5 mg/kg/40 min	15	↔	Krystal et al. (1994)
	MMSE	0.26 mg/kg, IV; 0.65 mg/kg/1 h plus active/ placebo haloperidol 5 mg	20	↓ ↔worsening of combination	Krystal et al. (1999)
	MMSE	IV; ketamine @ 50, 100, and 150 ng/mL vs. midazolam 30 ng/mL vs. ketamine + midazolam	11	↓ with both ketamine and midazolam and in combo	Suzuki et al. (2000)
	MMSE	0.23 mg/kg, IV; 0.5 mg/kg/1 h	20	↔	Abel et al. (2003a)
Attention/ vigilance	Reaction time	IV; 0.1 mg/kg/12 min (PCP)	10 controls and 10 patients	↓ with PCP, comparable to patients. LSD and amobarbital did not cause deficits	Rosenbaum et al. (1959)
	Digit symbol test	Oral PCP; 5 vs. 10 mg	18, controlled	↓	Bakker and Amini (1961)
	Serial sevens	IV; 0.1 mg/kg/12 min (PCP)	10 controls and 10 patients	↓ with PCP, comparable to patients. LSD and amobarbital ↔	Cohen et al. (1962)
	Continuous performance test	IV; 0.1 vs. 0.5 mg/kg/40 min	14	↓	Krystal et al. (1994)

	Visual search reaction time; 4-choice reaction time; Continuous attention	IM; 10 vs. 25 mg	10	↓ (dose dependent)	Harborne et al. (1996)
	Attention during recognition memory task	0.12 mg/kg, IV; 0.65 mg/kg/h	15	↓	Malhotra et al. (1996)
	N-back (0-back)	0.12 mg/kg, IV; 0.65 mg/kg/1 h	10	↔	Adler et al. (1998)
	Distractibility task continuous performance test	0.26 mg/kg, IV; 0.65 mg/kg/h vs. placebo plus active/placebo lorazepam 2 mg	22	↓ and lorazepam on both w/trend for interaction on CPT	Krystal et al. (1998)
	Continuous performance test	0.27, 0.081, or 0.0243 mg/kg, IV; 150; 45; 13.5 ng/mL	15	↔	Newcomer et al. (1999)
	Distractibility task continuous performance test	0.26 mg/kg, IV; 0.65 mg/kg/h plus active/placebo haloperidol 5 mg	18	↓ on both tasks. Haloperidol ↓ distractibility. No interaction	Krystal et al. (1999)
	AX-CPT	0.24 mg/kg, IV; 0.9 mg/kg/h	20	↓	Umbricht et al. (2000)
	Digit symbol substitution task; symbol cancellation task	IV; 0.5 mg/kg/h	24	↓ on both tests	Hetem et al. (2000)
	Two-choice visual reaction time task	IV; 0.5 mg/kg/h	8	↓	Guillermain et al. (2001)
	Continuous performance test	0.3 mg/kg/5 min	13, single-blind controlled, crossover	↓	Kreitschmann-Andermahr et al. (2001)
	Choice reaction time	IV; 0.5 mg/kg/h	8	↓	Micallef et al. (2002)
	Go/no go test Divided attention Vienna teaction time Signal detection task	5 mg, IV; 0.003 vs. 0.005 mg/kg/min	12	↓ only on signal detection task	Passie et al. (2005)
	Distractibility task	Placebo, then 0.26 mg/kg, IV; 0.65 mg/kg/100 min plus active/ placebo LY354740 (100 mg, 400 mg)	19 (approximately)	↓ with no interaction	Krystal et al. (2005a)

continued

Table 1.3-1 (continued)

Domain	Test	Dose (bolus, route; drip)	N, design	Results	Reference
	Distractibility task	0.23 mg/kg, IV; 0.5 mg/kg/h plus active/ placebo amphetamine sulfate (0.25 mg/kg)	27	↓ Amph↔	Krystal et al. (2005b)
	Serial digit learning test	IV; 0.3 mg/kg/1 min	23	↔	LaPorte et al. (2005)
	COVAT-inhibition of return (IOR)	All IM bolus followed by IV: (1) low DMT: (2) high DMT (3) low S-ketamine (4) high S-ketamine (does dependent on initial intensity of effects)	9 (6 dropouts)	↓ reaction times for both doses of DMT and low dose S-ketamine	Gouzoulis-Mayfrank et al. (2006)
	Digit symbol substitution task	IM; 0.2 vs. 0.4 mg/kg	18	↓	Lofwall et al. (2006)
	Continuous performance test	IV; 100 ng/mL	12	↓ for speed, but not accuracy	Honey et al. (2008)
	COVAT (IOR)	all IM bolus followed by IV: (1) DMT (2) S-ketamine	14	↓ for reaction times which was more pronounced for DMT	Daumann et al. (2008)
	AX-CPT	All IM bolus followed by IV: (1) low DMT: (2) high DMT (3) low S-ketamine (4) high S-ketamine (does dependent on initial intensity of effects)	9 (6 dropouts)	↓ for both drugs	Heekeren et al. (2008)
Verbal memory	Associative learning	0.4 mg/kg, IV; ½ doses repeated every 15 min	11, single blind, crossover	↔	Harris et al. (1975)
	Immediate and delayed recall	IV; 0.1 vs. 0.5 mg/ kg/40 min	16	↓	Krystal et al. (1994)
	Verbal memory test	IM; 10 vs. 25 mg	10	↓	Harborne et al. (1996)
	Wechsler memory scale	IV; 0.5 mg/kg/1 min	7 patients, double-blind controlled, crossover	↔	LaPorte et al. (1996)
	Recognition and recall memory task	0.12 mg/kg, IV; 0.65 mg/kg/1 h	15	↓	Malhotra et al. (1996)

Recognition and recall memory task	0.12 mg/kg, IV; 0.65 mg/kg/h	13 neuroleptic free patients, double blind, controlled, crossover	↓	Malhotra et al. (1997)
12-word memory task	IV, 0.97 mg/kg/2 h Measurements @ 0, 50, 100, 150, and 200 ng/mL	10, single-blind, controlled	↓ (dose-independent impairment)	Radant et al. (1998)
Immediate and delayed recall	0.26 mg/kg, IV; 0.65 mg/kg/1 h plus active/ placebo lorazepam 2 mg	23	↓ Lorazepam ↓. Possible interaction	Krystal et al. (1998)
Immediate and delayed recall	0.26 mg/kg, IV; 0.65 mg/kg/h plus active/ placebo haloperidol 5 mg	18	↓ Haloperidol ↔	Krystal et al. (1999)
Immediate and delayed paragraph recall; delayed match to sample	0.27, 0.081, or 0.0243 mg/kg, IV; 150; 45; 13.5 ng/mL	15	↓ on paragraph recall, and possible ↓ for nonverbal memory	Newcomer et al. (1999)
Episodic memory tasks	IV; 0.5 mg/kg/h	24	↓ free recall, particularly for words learned during infusion	Hetem et al. (2000)
Hopkins verbal learning task	0.26 mg/kg, IV; 0.65 mg/kg/1 h active/ placebo lamotrigine 300 mg	16	↓ Lamotrigine ↔	Anand et al. (2000)
Source memory task; prose recall sub-test of the rivermead behavioural memory test	IV; 0.4 vs. 0.8 mg/kg/80 min	54, double-blind controlled	↓ source memory and prose recall	Morgan et al. (2004a,b)
Episodic memory task	IV; 50 vs. 100 ng/mL	11	↔	Honey et al. (2004, 2005b)
Hopkins verbal learning test	Placebo, then 0.26 mg/kg, IV; 0.65 mg/kg/100 min plus active/placebo LY354740 (100 mg, 400 mg)	19	↓ LY354740 ↔	Krystal et al. (2005a)
Hopkins verbal learning test	IV; 0.3 mg/kg/1 min	23	Memory consolidation ↓ Recall ↔.	LaPorte et al. (2005)
Verbal delayed recall	0.27 mg/kg, IV; 0.12 mg/kg/50 min	13	↓ of words presented before, but not during infusion	Parwani et al. (2005)

continued

Table 1.3-1 (continued)

Domain	Test	Dose (bolus, route; drip)	N, design	Results	Reference
	Reality monitoring task	IV; 100 ng/mL	12	↓ on deeper vs. associative encoding	Honey et al. (2006)
	Episodic memory and metamemory	IM; 0.2 vs. 0.4 mg/kg	18	↓ encoding process	Lofwall et al. (2006)
Assorted	Word association test; progressive matrices	Oral PCP; 5 vs. 10 mg	18, controlled	↓	Bakker and Amini (1961)
	Goreham's proverb test	IV PCP; 0.1 mg/kg/ 12 min	10 controls and 10 patients	↓ with PCP, comparable to patients. LSD and amobarbital ↔	Cohen et al. (1962)
	Goreham's proverb test	0.26 mg/kg, IV; 0.65 mg/kg/1 h plus active/placebo lorazepam 2 mg	23	↓ Lorazepam ↓ concreteness. No interactions	Krystal et al. (1998)
	Gorham's proverb test	0.26 mg/kg, IV; 0.65 mg/kg/1 h plus active/ placebo haloperidol 5 mg	19	↓	Krystal et al. (1999)
	Speed of comprehension; word-stem completion; cued recall; the hayling task	IV; 0.4 vs. 0.8 mg/kg/80 min	54, double-blind controlled	↓ on all tests	Morgan et al. (2004a,b)
	Gorham's proverb test	Placebo, then 0.26 mg/kg, IV; 0.65 mg/kg/100 min plus active/placebo LY354740 (100 mg, 400 mg)	19	↔	Krystal et al. (2005a)
	Syndrome short test	HE LI160 750 mg tablets b.i.d. On day 7, a 4 mg S-ketamine IV/1 h	16	↔	(Murck et al. 2006)
	Semantic priming	IV; 113.4±56.7 vs. 236.7±65.3 ng/mL	48, double-blind controlled	↓	Morgan and Curran (2006)
	Quantitative computer-aided analysis of speech	0.40 mg/kg, IV; 0.21 mg/kg/h for 90 min	11 patients (no ketamine) and 9 controls, double-blind controlled, crossover	Repetitiousness↓ for controls and schizophrenia	Covington et al. (2007)
	Sentence completion and verbal self-monitoring tasks	IV; 100 ng/mL	12	↔	Honey et al. (2008)

Procedural memory	SRT task	IV; 0.4 vs. 0.8 mg/ kg/80 min	54, double-blind controlled	↓	Morgan et al. (2004a,b)
Visual memory	Picture recognition	IV; 0.05, 0.10, 0.15 and 0.20 mg/kg of (S)-ketamine or 0.20, 0.40, 0.60 and 0.80 mg/kg of (R)-ketamine/2 min	6	↓ by both S and R	Oye et al. (1992)
	Figural reproduction	IV; 0.5 mg/kg/1 min	7 patients, double-blind controlled, crossover	↔	LaPorte et al. (1996)
	Benton visual retention test	5 mg, IV; 0.003 vs. 0.005 mg/min/kg	12	↔	Passie et al. (2005)
Assorted visual	Eye movement tasks	IV, 0.97 mg/kg/2 h measurements @ 0, 50, 100, 150, and 200 ng/mL	10, single-blind, controlled	↓ pursuit tracking, visually guided saccades, and fixation	Radant et al. (1998)
	Closed loop gain and measures of gain during masking conditions	IV; 0.1 mg/kg/1 min	12	↓ closed loop gain, eye acceleration and mean mask gain ↔predictive pursuit in response.	Weiler et al. (2000)
	Smooth-pursuit eye movement task	IV; 0.1 mg/kg	12	↓ leading saccades # ↓ ratio for slowly moving targets	Avila et al. (2002)
	Binocular depth perception	5 mg, IV; 0.003 vs. 0.005 mg/min/kg	12	↔	Passie et al. (2003)
	Oculodynamic test	HE LI160 750 mg tablets b.i.d. On day 7, a 4 mg S-ketamine IV/1 h	16	↔	Murck et al. (2006)
	Contour integration task	Uncontrolled recreational ketamine use vs. poly-drug using controls	16 recreational ketamine users and 16 poly-drug using controls	↓ contour integration which normalized 3 days post ketamine	Uhlhaas et al. (2007)

Table Legend: ↓-significant impairment with ketamine; ↑-significant improvement; ↔-No change with ketamine

hallucinations are rare. Therefore, the pattern of hallucinations observed during ketamine challenge does not closely resemble the pattern observed in established schizophrenia. In contrast, however, the pattern of auditory and visual disturbance seen during ketamine administration does resemble the pattern observed early in the course in schizophrenia (McGhie and Chapman, 1961; Bowers, 1974) where both auditory and visual perceptual disturbances are common, and auditory hallucinations have not yet crystallized to the point of being identifiable as speech. Thus, acute ketamine challenge may be viewed best as a model of prodromal or acute incipient schizophrenia, rather than later, more chronic, phases.

In patients with established schizophrenia, increases in hallucinatory activity are observed during ketamine challenge (Malhotra et al., 1997; Lahti et al., 2001). Further, in primates, apparent hallucinatory behavior (i.e., threatening non-existent objects) is not observed during acute PCP treatment, but does emerge during chronic administration (Linn et al., 1999). In humans, for obvious ethical reasons, effects of chronic ketamine or PCP treatment are not well characterized. In a study that applied both amphetamine and ketamine challenge, additive effects were seen only in the case of hallucinations, suggesting that the circuitry underlying hallucinations may have unique sensitivity to both glutamatergic and dopaminergic dysfunction (Krystal et al., 2005b).

Recently, a relationship between ketamine-induced psychotic symptoms and NMDA receptor occupancy was reported (Stone et al., 2008). NMDA receptor binding in middle inferior frontal cortex showed a significant correlation with BPRS negative subscale, suggesting that ketamine may induce negative symptoms through direct inhibition of the NMDA receptor within this region.

3.2 Neurocognitive Effects of NMDA Antagonist Treatment

In addition to positive and negative symptoms, schizophrenia is associated with a pattern of neurocognitive dysfunction that represents a core feature of the disorder (Bilder et al., 1991; Gold et al., 1999). Based in part on the influence of dopaminergic models of schizophrenia, a great number of cognitive studies in schizophrenia have focused on the dysfunction of specific brain regions such as prefrontal dysfunction (see Chapter 1.1 this Volume). However, neurocognitive deficits in schizophrenia are not limited to prefrontal function. In studies that have utilized comprehensive neuropsychological batteries, similar levels of deficit have been observed across multiple neurocognitive domains, particularly in learning and declarative memory formation (Saykin et al., 1991; Bilder et al., 2000; Dickinson et al., 2006; Keefe et al., 2006) and not prefrontal "executive" dysfunction *per se*.

Glutamatergic models provide a framework from which to view the pattern of neuropsychological dysfunction associated with schizophrenia. Although glutamatergic systems are widespread, within each brain region, NMDA receptors participate in only a subset of processes. For example, in the hippocampus and cortex, NMDA receptor activation is required for the initiation, but not maintenance of LTP (Miyamoto, 2006). The observation that patients with schizophrenia (as opposed to those with the amnestic syndrome) show deficits in memory formation (Hartvig et al., 1995; Radant et al., 1998; Newcomer et al., 1999; Morgan et al., 2004a; Krystal et al., 2005b; Parwani et al. 2005; Rowland et al., 2005), but not retention, is thus consistent with an NMDA pattern of dysfunction within hippocampal regions, rather than structural damage to the hippocampus itself (see Chapter 3.1 in this Volume).

To date, a substantial literature has accumulated comparing effects of NMDA antagonists with those observed in schizophrenia, using paradigms sensitive to the cognitive aspects of information processing dysfunction in schizophrenia (*Table 1.3-1*). With ketamine administration, comparable neuropsychological deficits are reproduced in measures of executive functioning such as the Wisconsin Card Sorting Task (Krystal et al., 1994, 1998, 1999, 2000), attention/vigilance (Malhotra et al., 1996; Oranje et al., 2000; Krystal et al., 2005b; Passie et al., 2005), verbal fluency (Krystal et al., 1994; Adler et al., 1998; Radant et al., 1998), and visual and verbal working memory (Malhotra et al., 1996; Adler et al., 1998; Krystal et al., 1998, 1999, 2005b; Newcomer et al., 1999; Anand et al., 2000; Hetem et al., 2000; Ahn et al., 2003; Honey et al., 2003; Morgan et al., 2004a). Moreover, in monkeys treated with ketamine (Stoet and Snyder, 2006), characteristic, schizophrenia-like deficits are reproduced in a task switching paradigm (Kieffaber et al., 2006; Wylie et al., 2008).

Ketamine infusion also reproduces both the severity and the type of thought disorder seen in schizophrenia, both being associated, for example, with high levels of poverty of speech, circumstantiality and a loss of goal, and relatively low levels of distractive or stilted speech or paraphasias (Adler et al., 1999). Thus, a reduction in NMDA functioning within brain could serve as a single unifying feature to account for the otherwise complex pattern of deficit observed in the disorder.

Cognitive deficits in schizophrenia are reflected as well by reduced generation of cognitive event-related potentials (ERP) such as auditory P300 (Roth et al., 1981; Javitt et al., 1995) or processing negativity (PN) (Baribeau-Braun et al., 1983). Similar deficits are induced by ketamine challenge in normal volunteers, supporting the behavioral studies (*Table 1.3-2*). In fMRI studies, significant increases are observed in frontal and hippocampal activation (Honey et al., 2005a) even under circumstances where no overt effects are seen on behavior (*Table 1.3-3*). Thus, regions that have been implicated in cognitive dysfunction in schizophrenia are affected as well in ketamine challenge.

Table 1.3-2
Effect of ketamine on electrophysiological measures

	Modality/task	Results	Reference
Construct Gating	PPI and P50	↔	van Berckel et al. (1998)
	PPI	↑ in 1st block of the PPI session	Duncan et al. (2001)
	PPI and P50	↔	Oranje et al. (2002)
	PPI	PPI ↑ Startle amplitude ↓	Abel et al. (2003a)
	PPI	↔ following prolonged ketamine infusion	Boeijinga et al. (2007)
	PPI	PPI ↑ and startle ↓ after S-ketamine DMT ↓ both	Heekeren et al. (2007)
Sensory processing	Auditory MMN/visual AX-CPT	↓ MMN to pitch and duration deviants ↓ accuracy, increased BX errors	Umbricht et al. (2000)
	Auditory MMN/N100	MMN ↔ ↑ N100 amplitude to deviant stimuli	Oranje et al. (2000)
	Auditory neuromagnetic mismatch field (MMF)	MMF ↓	Kreitschmann-Andermahr et al. (2001)
	Auditory N100/P200	N100-P200 peak-to-peak amplitude ↓	Murck et al. (2006)
	Auditory MMN/Visual AX-CPT	MMN for ↓ relative to DMT. ↓ frontal MMN following ketamine. Signiciant decrease in hit rate, increased BX errors in AX-CPT	Heekeren et al. (2008)
Target detection	Dichotic listening, oddball/processing negativity (PN)	Performance ↔ PN and the P300 amplitude ↓	Oranje et al. (2000)
	Working memory/P300	Late positive complex (LPC) amplitude ↓ during memory scanning task	Ahn et al. (2003)
	Auditory oddball	↓ N2, P3a, P3b amplitude with both ketamine and thiopental. ↓ P3a latency with ketamine	Watson et al. (2008)

↓-significant impairment with ketamine; ↑-significant improvement; ↔-No change with ketamine

Table 1.3-3
Effect of ketamine on neuroimaging measures

Construct	Modality, ligand/task	Results	Reference
Resting blood flow	PET, [15O]regional cerebral blood flow (rCBF)	↑ rCBF in anterior cingulate cortex; ↓ rCBF in hippocampus and primary visual cortex	Lahti et al. (1995)
	PET, [18F] fluorodeoxyglucose (FDG)	Metabolic hyperfrontality, no psychotic symptoms	Vollenweider et al. (1997)
	PET, [18F]FDG	Ketamine produced bilateral ↑ in metabolic activity in the prefrontal cortex but no significant activation in other areas	Breier et al. (1997)
	PET, [15O]rCBF	Normal volunteers: ↑ rCBF within anterior cingulate, medial frontal cortex, right inferior frontal region Patients: total BPRS score correlated with rCBF in the two maxima located in the anterior cingulate cortex	Holcomb et al. (2005)
	fMRI, BOLD	↓ in ventromedial frontal cortex predicted dissociative effects of ketamine. ↑ activity in mid-posterior cingulate, thalamus, and temporal cortex	(Deakin et al. 2008)
D_2 receptor occupancy	PET, [11C]raclopride	↑ DA release in striatum; ↔ in cerebellum	Smith et al. (1998)
	PET, [11C]raclopride	↑ striatal dopamine release associated with symptoms	Breier et al. (1998)
	PET, [11C]raclopride	↑ DA release in ventral striatum, caudate nucleus and putamen	Vollenweider et al. (2000)
	PET, [11C]raclopride	↔ D_2 occupancy	Kegeles et al. (2002)
	SPECT, [123I]IBZ	↑ striatal dopamine release	Kegeles et al. (2000)
	PET, [11C]raclopride	↔ in D_2 occupancy	Aalto et al. (2005)
NMDA occupancy	SPECT [123I]CNS-1261	↓ [123I]CNS-1261 binding in all regions; significant correlation between negative symptoms and normalized binding in middle inferior frontal cortex	Stone et al. (2008)
Glutamate metabolism	4-T 1H proton magnetic resonance spectroscopy (1H-MRS), Stroop	Significant increase in anterior cingulate glutamate metabolism, no relationship to symptoms, marginally related to Stroop performance	Rowland et al. (2005)
Sensory processing	fMRI, repetitive AM tone (3 s ISI)	↓ activation in the left and right superior temporal gyrus	Rogers et al. (2004)
Face processing	fMRI, gender discrimination task	↓ activation in middle occipital and precentral gyrus	Abel et al. (2003b)
	fMRI, emotion vs. gender discrimination task	↓ activation in limbic and visual cortex	Abel et al. (2003c)
Inhibition of teturn	fMRI/COVAT (IOR)	↑ activation in the right superior frontal gyrus, the left superior temporal gyrus and the right midfrontal gyrus. ↔ with DMT	Daumann et al. (2008)
Verbal fluency	fMRI/verbal fluency task	Interaction of task demand with ketamine was observed in the anterior cingulate, prefrontal, and striatal regions	Fu et al. (2005)

Table 1.3-3 (continued)

Construct	Modality, ligand/task	Results	Reference
Memory encoding/ retrieval	fRMI/memory encoding and retrieval task	↑ activation in bilateral dorsolateral prefrontal regions, ventrolateral prefrontal regions, parietal areas, and the anterior cingulate gyrus	Honey et al. (2004)
	fMRI/ episodic memory retrieval task	↓ activation in anterior and posterior cingulate cortex	Northoff et al. (2005)
	fMRI/ episodic memory retrieval task	↑ activation of left and right prefrontal cortex and hippocampus	Honey et al. (2005a)
	fMRI/multiple tasks	Working memory: ↑ activation of basal ganglia and thalamus	Honey et al. (2008)

As opposed to ketamine, amphetamine does not induce neurocognitive deficits of schizophrenia during acute challenge (Krystal et al., 2005b). Further, several recent studies have assessed the ability of amphetamine to improve neurocognitive performance in schizophrenia, on tasks such as the Stroop test. In this test, patients showed a characteristic pattern of deficit characterized by an increased facilitation of response by stimulus congruence. Although amphetamine improved overall performance in this task in both normal and schizophrenia subjects, it failed to reverse the specific pattern of neurocognitive dysfunction associated with schizophrenia (Barch and Carter, 2005). Thus, dysfunction of dopaminergic systems appears to be neither necessary nor sufficient to account for the overall pattern of neuropsychological disturbance in schizophrenia, although interactions between dopaminergic and glutamatergic systems may occur.

3.3 NMDA Antagonist Effects on Sensory Processing

Although cortical dysfunction is studied most intensively in schizophrenia, using tasks sensitive to higher-order cognitive dysfunction, extensive deficits are observed as well in sensory processing (see Chapters 3.2 and 3.3 this Volume). For example, patients with schizophrenia show deficits in the generation of mismatch negativity (MMN), an ERP component that reflects dysfunction at the level of primary auditory cortex (Shelley et al., 1991; Javitt et al., 1995; Umbricht and Krljes, 2005). Similar deficits are observed following the intracortical administration of NMDA antagonists in monkeys (Javitt et al., 1996; Javitt, 2000), or systemic ketamine administration in normal volunteers (Umbricht et al., 2000; Kreitschmann-Andermahr et al., 2001) (*Table 1.3-2*). Deficits in MMN generation are accompanied by impaired auditory sensory processing (Strous et al., 1995; Wexler et al., 1998), with pattern of deficit indicating dysfunction within primary sensory regions (Rabinowicz et al., 2000). Similarly, ketamine induces impairments in proprioception and weight discrimination (Rosenbaum et al., 1959; Morgenstern et al., 1962; Oye et al., 1992) similar to those observed in schizophrenia (Ritzler, 1977; Javitt et al., 1999b).

In the visual system, patients show a characteristic pattern of neurophysiological impairment characterized by a decreased gain of visual responses within the magnocellular visual system (Butler et al., 2005). A similar pattern of result is seen following the microinfusion of an NMDA antagonist into cat LGN (Fox et al., 1990; Kwon et al., 1991). Similarly, patients show deficits in motion detection that reflect impaired motion processing within the magnocellular visual system (Chen et al., 1999; Kim et al., 2005). NMDA receptors play a critical role in the neurophysiological processes underlying motion detection at the neuronal level (Heggelund and Hartveit, 1990; Rivadulla et al., 2001), suggesting that basic deficits in motion processing likely reflect the underlying impairments in NMDA dysfunction within the early visual system (see Chapter 3.2, this volume). Ketamine challenge studies also show disrupted visual activation during facial gender (Abel et al., 2003b) and emotion recognition tasks (Abel et al., 2003c), suggesting contributions of low level visual deficits to higher cognitive function (*Table 1.3-3*).

NMDA antagonists reliably induce deficits in sensory gating measures, such as prepulse inhibition (PPI) that closely mimic the deficits seen in schizophrenia in both rodent (de Bruin et al., 1999;

Geyer et al., 2001) and primate (Linn et al., 2003) models. In contrast, ketamine appears to have little effect on either PPI or P50 gating in normal human volunteers (*Table 1.3-2*). The basis for the dissociation between animal and human studies is unknown. However, these findings suggest that gating deficits in schizophrenia may reflect primarily nonglutamatergic pathology.

3.4 Dopamine-Glutamate Interactions

Another feature of schizophrenia that cannot be explained well from a purely dopaminergic perspective is dysregulation of the dopamine system itself. Patients with schizophrenia show positive symptoms that are suggestive of underlying dopaminergic hyperactivity. Yet, despite decades of study, only few intrinsic deficits have been detected within the dopamine system that could account for this hyperactivity. For most patients, therefore, reasons for apparent dopaminergic hyperactivity remain unexplained.

The PCP/NMDA model provides two potential solutions to this problem. First, NMDA receptors play a critical role in the regulation of subcortical and cortical dopaminergic systems. Thus, for some subjects, failure of NMDA-based regulatory mechanisms might constitute the proximate cause of dopaminergic dysregulation. Second, because dopaminergic and glutamatergic fibers converge on the same striatal outflow neurons, symptoms reminiscent of dopaminergic hyperactivity may be induced by NMDA antagonists even in the absence of elevations in dopamine levels. Thus, the "dopaminergic hyperactivity" phenotype may reflect impaired NMDA function in the absence of specific alterations in dopamine levels. The lack of an increase in dopamine levels in some patients would potentially explain why antipsychotics are highly effective in reversing amphetamine-induced psychosis, yet remain ineffective for a significant proportion of patients.

Positron emission (PET) and single photon emission (SPECT) tomographic studies provide insights into patterns of neurochemical receptor dysfunction in schizophrenia (see Chapter 1.1 this Volume). Patients with schizophrenia, as a group, show enhanced striatal dopamine release to amphetamine challenge, consistent with endogenous dopaminergic hyperactivity/dysregulation (Laruelle, 1998). The enhanced release, however, is observed specifically during acute decompensation, and not during a remission phase, and is associated specifically with an increased severity of positive symptoms. Further, the degree of increase predicts the degree of responsiveness to antipsychotic medication (Laruelle et al., 1999).

In normal volunteers, pretreatment with ketamine leads to dopaminergic dysregulation similar to that observed in schizophrenia (Kegeles et al., 2000), even under conditions where no effect on basal DA release is observed (*Table 1.3-3*). Similar augmentation of amphetamine-induced dopamine release is observed in rodents treated subchronically with PCP or other NMDA antagonists (Balla et al., 2001a,b; Balla et al., 2003). A primary site of NMDA appears to be on local inhibitory interneurons within the striatum or other key brain regions (Javitt et al., 2005b). These serve as a "brake" on glutamate-stimulated dopamine release (Carlsson, 1993). Thus, failure of this brake may lead to dysregulated dopamine release of the type observed in schizophrenia.

Despite the increase in amphetamine-induced dopamine release observed during acute decompensations, no significant increases were observed during chronic stabilization in patients, despite significant residual symptoms. Moreover, the difference in activity observed between acute and chronic phases could not be attributed to medication, as changes were observed in both medicated and unmedicated patients during acute decompensation (Laruelle et al., 1999). Overall, therefore, these findings suggest that dopaminergic instability may account only for the increased severity of symptoms associated with acute decompensation.

Consistent with the absence of dopaminergic instability in chronic schizophrenia, dissociative effects of ketamine are observed even under administration conditions that do not acutely affect striatal dopamine levels (Kegeles et al., 2000). Similarly dopamine is not needed to mediate the behavioral effects of NMDA antagonists in rodents (Chartoff et al., 2005). A potential explanation for this finding is that in the striatum, glutamatergic and dopaminergic fibers converge on dendritic shafts and spines of striatal GABAergic medium spiny interneurons. At this site, NMDA and D_2 receptors produce opposite effects, NMDA receptors producing net stimulation of striatal interneurons and D_2 receptors producing net inhibition (Cepeda and Levine, 1998; Leveque et al., 2000; Onn et al., 2000). Thus, in the striatum, NMDA antagonists and dopamine agonists produce a similar inhibition of GABAergic outflow, the first by decreasing excitation

and the second by increasing inhibition. A primary deficit in NMDA transmission would just mimic effects of dopaminergic hyperactivity. Further, D_2 antagonists would be effective in balancing the effects of primary NMDA dysfunction, although the degree of effect would depend upon the tonic level of dopaminergic activity. Thus, persistent positive symptoms may reflect a lack of NMDA-mediated drive on inhibitory interneurons in the striatum rather than hyperactivity of dopaminergic innervation, and may respond preferentially to NMDA-based treatment approaches.

3.5 Neurochemical Effects of NMDA Antagonists

A final unexplained aspect of glutamatergic pathology concerns the downregulation of GABAergic neurotransmission, manifested as reduced expression of GAD67, a key synthesizing enzyme for GABA, as well as the downregulation of calcium binding proteins such as parvalbumin and calbindin localized within cortical GABAergic interneurons (Torrey et al., 2005). Deficits in GABAergic activity are considered to play a particular role in impaired gamma-wave generation in schizophrenia and in prefrontal neurocognitive dysfunction (Gonzalez-Burgos and Lewis, 2008). As with dopaminergic abnormalities, deficits in GABAergic transmission have yet to be explained based upon intrinsic GABAergic mechanisms (e.g., genetic polymorphisms of GAD67 or genes expressing the calcium binding proteins), and thus must be considered to reflect extrinsic dysfunction.

Also, as with dopaminergic dysfunction, GABAergic downregulation similar to that of schizophrenia is observed in animal models, following treatment with NMDA antagonists either in vitro (Kinney et al., 2006) or in vivo (Abdul-Monim et al., 2006; Behrens et al., 2007; Morrow et al., 2007). A parsimonious explanation for the finding is that the reduction of NMDA drive on these neurons leads to a decreased Ca^{2+} entry, leading to compensatory downregulation of binding proteins whose function is to sequester intracellular Ca^{2+} (Lisman et al., 2008). Such downregulation in turn leads to a prolongation of Ca^{2+} dynamics when Ca^{2+} does enter, leading to reduced high frequency activity.

Based upon GABAergic downregulation in schizophrenia, it has been suggested that GABAergic agonists targeting prefrontal GABA receptors may be particularly efficacious in schizophrenia (Lewis and Moghaddam, 2006). In a recent controlled, small scale study with MK-0777, a GABA modulator with selective activity at $GABA_A$ receptors containing alpha(2) or alpha(3) subunits significant improvement on several prefrontal tasks (the N-back, AX Continuous Performance Test (AX-CPT), and Preparing to Overcome Prepotency tasks was seen, although not with broader measures of cognition determined using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). In this small study, no significant improvement was observed in symptoms, although larger, multicenter studies are ongoing (Lewis et al., 2008). Overall, these findings support prefrontal GABAergic dysfunction as a potentially reversible downstream effect of primary glutamatergic dysfunction.

4 Glutamatergic Models of Schizophrenia

At present, the only brain manipulation that produces a clinical state behaviorally indistinguishable from schizophrenia is the blockade of brain NMDA receptors. The primary concept underlying glutamatergic models of schizophrenia, therefore, is that the dysfunction or dysregulation of NMDA receptor-mediated neurotransmission represents the common final mechanism underlying the symptom formation in schizophrenia. Although in some individuals, the NMDA dysfunction may be related to disturbances of the receptors themselves, for other individuals, these deficits may relate more to impairments of either upstream or downstream elements involving pre- and postsynaptic neural elements respectively.

In schizophrenia, identical twins show a concordance rate of approximately 50%, consistent with other presumed polygenic disorders. The fact that there is 50% concordance provides strong support for a genetic contribution. However, the 50% discordance rate indicates the important contribution of environmental factors as well. To date, significant genetic risk factors have been identified, which show convergence on glutamatergic pathways. There is also evidence for relationship between environmental factors and glutamatergic dysfunction.

4.1 Linkage/Association Studies in Schizophrenia

Candidate genes for schizophrenia have only recently been identified; however, considerable controversy continues to surround many of the targets (see Chapters 2.3, 2.2 and 2.1 this Volume). Nevertheless, a consistent finding from genetic studies in schizophrenia is that several of the identified genes interact closely with glutamatergic mechanisms in general and NMDA receptors in particular. As such, these studies provide additional support for glutamatergic theories of the disorder.

One of the best established candidate genes for schizophrenia is neuregulin, a brain transmitter that mediates its effects primarily through ErbB3 and ErbB4 receptors (see Chapter 2.3 this Volume). In initial studies, it was suggested that NRG1 might mediate its risk-enhancing effects, based upon interaction with NMDA receptors, based upon the observation that NRG1 hypomorphs had fewer functional NMDA receptors than wild-type mice (Stefansson et al., 2002). In a recent functional postmortem study, NRG1 stimulation was found to suppress NMDA receptor activation in prefrontal cortex tissue from schizophrenia patients to a greater extent than it did in tissue from matched comparison subjects (Hahn et al., 2006). Thus, an increased expression of NRG1 may increase risk for schizophrenia primarily by downregulating cortical NMDA receptor-mediated neurotransmission, and as given in the following section, may be a potential pharmacologic target.

Two other genes with strong relationship to schizophrenia, DAAO and G72 (aka DAOA) (see Chapter 2.1 in this Volume), have also been linked to schizophrenia in at least some studies. DAAO is the primary enzyme responsible for the degradation of D-serine in brain. G72 is a modulatory subunit for DAAO that appears to have arisen during late primate evolution. Initial studies (Chumakov et al., 2002; Korostishevsky et al., 2004; Schumacher et al., 2004) were suggestive of an association with schizophrenia. In more recent publications, however, no significant associations with either gene were found (Goldberg et al., 2006; Liu et al., 2006), although G72 was strongly associated with cognitive dysfunction and reduced hippocampal activation during an episodic working memory task. Thus, whether or not polymorphisms of DAAO and G72 explain the increased risk for schizophrenia, they may be associated with accompanying neurocognitive dysfunction.

There is also evidence implicating NMDA receptors directly in the genetics of schizophrenia. An early study in an African Bantu population found positive linkage between schizophrenia and markers near the NMDAR1 (GRIN1) gene on chromosome 9q34.3 and the NMDAR2B (GRIN2B) gene on chromosome 12p12 (Riley et al., 1997). Other studies have also reported linkages between the GRIN1 (Begni et al., 2003; Zhao et al., 2006; Galehdari, 2008) and GRIN2B (Di Maria et al., 2004) genes and schizophrenia. Abnormal interaction between GRIN1 and GRIN2B has also been reported (Qin et al., 2005). Polymorphisms of the GRIN2B and GRIN2A genes have also been associated with specific clinical features of the disorder such as clozapine resistance or chronic outcome (Chiu et al., 2003; Itokawa et al., 2003). A recent metaanalysis found support for the association between GRIN2B and schizophrenia (Li and He, 2007a), while other associations are less established.

Linkages to metabotropic glutamate receptor genes, including GRM3 (Harrison et al., 2008; Mossner et al., 2008) and GRM7 (Ohtsuki et al., 2008) have also been reported, with one risk SNP of GRM3 associated with alternative splicing of receptor and thus functional alteration (Sartorius et al., 2008). However, other studies of GRM3 have been negative (Schwab et al., 2008). Other potential risk genes for schizophrenia such as dysbindin (DTNBP1) (see Chapter 2.2 this Volume), disrupted in schizophrenia-1 (DISC-1), and RGS4 may also converge on glutamatergic systems (Moghaddam and Jackson, 2003; Harrison and Weinberger, 2005; Weinberger, 2005), although further clarification of the role of these genes in normal brain function is required.

4.2 Environmental and Neurochemical Factors

Environmental factors that contribute to the development of schizophrenia also converge on NMDA receptors. For example, it has been hypothesized that perinatal hypoxia, an important risk factor for schizophrenia, leads to the neurotoxic degeneration of NMDA-bearing cells, an effect that may only produce behavioral symptoms later in development (Olney et al., 1999).

Similarly, schizophrenia has recently been associated with decreased plasma levels of the NMDA agonists glycine (Sumiyoshi et al., 2004) and D-serine (Hashimoto et al., 2003), and increased levels of homocysteine (Susser et al., 1998; Levine et al., 2002), an agent that may act as a functional NMDA antagonist. Levels of kynurenic acid, an endogenous NMDA and nicotine receptor antagonist, may also be high in schizophrenia (Erhardt et al., 2001; Schwarcz et al., 2001) and lead to the inhibition of glutamatergic/NMDA function.

A final compound of potential etiological interest in schizophrenia is glutathione (see Chapter 2.5 in this Volume). In hippocampal slices, reduced glutathione levels are associated with reduced presynaptic glutamate release along with reduced postsynaptic NMDA activity, consistent with the phenotype observed in schizophrenia (Steullet et al., 2006). Although determinants of various neurochemical levels in brain are unknown at present, present findings suggest that alterations in metabolism or environmental exposure may explain significant variance in risk for developing schizophrenia, along with genetic factors.

4.3 Variants of the Glutamate Model

Although glutamatergic models are often referred to in the singular, in fact, multiple versions of the "glutamate model" of schizophrenia presently exist with subtle differences in presumed etiology, but potential profound differences in postulated pattern of deficit and therapeutic intervention. At present, three major variants exist, although subvariants of each can be considered.

4.3.1 PCP/NMDA Model

The first variant, termed originally the "PCP/NMDA model" is based primarily on the ability of drugs such as PCP and ketamine to induce ongoing psychotic symptoms and cognitive deficits by blocking NMDA receptors (Javitt, 1987; Javitt and Zukin, 1991). Since symptoms and cognitive deficits resolve following ketamine or PCP discontinuation, this model proposes that ongoing NMDA dysfunction is the key pathophysiological event in schizophrenia.

Because NMDA receptors are distributed throughout the brain, this model also posits a widespread, but process-specific, neural dysfunction in schizophrenia, involving widespread cortical and subcortical brain regions but only involving those processes that require active engagement of NMDA functions. Since NMDA receptors are blocked in many cells under physiological conditions, only a minority of processes within each brain region engage NMDA receptors. For example, in the hippocampus, NMDA receptors are engaged in initiation but not maintenance of LTP, and thus disrupt new learning but not maintenance of previously learned memories. Thus, the PCP/NMDA model would propose that only the initiation but not the maintenance of LTP should be impaired in schizophrenia (Javitt, 2007).

Because this model posits ongoing reduction in the integrity of NMDA receptor-mediated neurotransmission, this model predicts that the ideal treatment strategy would be to stimulate NMDA receptors either via one of the modulatory sites (e.g., glycine, redox) or via allosterically linked glutamate receptors (e.g., mGluR5) or converging NMDA systems (e.g., nicotine) (see Chapter 1.2 this Volume). Ideally, of course, causes of impaired NMDA transmission, such as reduced D-serine synthesis or NMDA gene expression, should also be identified and if possible reversed (Javitt, 2004). Among ketamine abusers, this model is most relevant to effects seen while patients are actively intoxicated but not post cessation.

4.3.2 NMDA Receptor-Hypofunction (NRH) Model

This alternative model of schizophrenia, based upon actions of PCP and ketamine, was also proposed in the late 1980s (Olney, 1989; Olney et al., 1989), but focuses on a separate aspect of action of NMDA antagonists. In addition to producing cognitive dysfunction, NMDA antagonists were observed to produce neurodegenerative changes in specific populations of cortical pyramidal neurons, particularly in frontal, posterior

cingulate, and retrosplenial brain regions, although delayed spread to larger brain networks (e.g., parietal, temporal, entorhinal cortex, hippocampus, and amygdala) (Olney and Farber, 1995; Olney et al., 1999). Neurotoxicity was marked by immediate vacuolization and heat shock protein expression, and was observed even after a single high dose of PCP, although it could also be seen after longer duration, lower dose administration (Ellison et al., 1999).

Several classes of drugs proved effective in blocking the neurotoxic effects of NMDA antagonists, including muscarinic antagonists; $GABA_A$ facilitators such as benzodiazepines and barbiturates; sigma receptor ligands; non-NMDA glutamate antagonists; α_2 receptor agonists; certain typical antipsychotics such as haloperidol, thioridazine, and loxapine; and atypical antipsychotics such as clozapine and olanzapine (Olney et al., 1999).

Pathogenically, it was proposed that the pattern of deficit would be related to alterations particularly in cingulate cortex, hippocampus, and related structures, and would involve damage particularly to local GABAergic interneurons. Preferred interventions include muscarinic antagonists, α_2 receptor agonists, and non-NMDA glutamate antagonists. To date, limited beneficial effects of such compounds have been observed in schizophrenia (Lindsley et al., 2006). Further, the pattern of regional brain change observed in this model does not match the pattern of more general neuropsychological dysfunction observed in schizophrenia.

In support of the NRH model, persistent ketamine abusers do appear to show frontotemporal deficits that persist even after ketamine cessation (Curran and Monaghan, 2001; Morgan et al., 2004c; Morgan and Curran, 2006). There may also be subgroups of schizophrenia patients with relatively constrained frontotemporal cognitive deficit patterns and little in the way of generalized dysfunction. To the extent to which such patients can be differentiated from the larger group of individuals with schizophrenia, they may represent an "NRH model" phenotype. Further, interventions suggested for the treatment of schizophrenia might prove useful in the prevention of neuropsychiatric sequelae of persistent ketamine abuse or overdose.

4.3.3 Glutamatergic Hyperfunction

The most recent variant of the basic PCP/NMDA model is based upon the observation that NMDA blockade leads to rebound hyperglutamatergia, presumably due to reduced excitatory drive within local GABAergic feedback loops. Because glutamatergic transmission is designed to be highly phasic, the elevation of tonic glutamate levels is pathological and leads to impairments in function over and above those induced by NMDA blockade itself.

As described earlier, glutamatergic neurons can fire in either irregular or burst firing mode. The application of NMDA antagonists at doses that impair working memory, alters the firing rate of glutamatergic neurons, leading to an increase in disorganized, irregular firing but a decrease in organized burst firing. Thus, while overall glutamatergic activity is increased, the salience of each particular burst is decreased, leading to reduced signal-to-noise and reduced effective information throughput (Jackson et al., 2004). Opposite effects are seen on activity within GABAergic interneurons, suggesting that alterations in firing rate of pyramidal neurons reflect reduced integrity of inhibitory feedback, leading to dysregulated pyramidal cell activity (Homayoun and Moghaddam, 2007).

Based upon this theory, the primary goal of glutamate-based treatments is neither to increase nor to decrease NMDA function per se, but rather to restore the balance between excitation and inhibition within cortical regions. This approach does not argue against the use of NMDA agonists to correct the fundamental deficit. However, it does suggest alternative approaches to compensate for a deficit that cannot otherwise be reversed. In particular, it suggests that reducing glutamatergic input to pyramidal neurons by modulating presynaptic release, can compensate for the failure of inhibitory drive.

Since this variant of the glutamate model was first proposed, significant supporting data have accumulated. In preclinical models, the effects of NMDA antagonists in rodent frontal cortex on presynaptic glutamate release and neuronal firing rates are reversed by metabotropic group 2/3 agonists, which also normalize behavior, supporting the concept that glutamatergic hyperactivity might be pathological (Moghaddam and Adams, 1998; Homayoun and Moghaddam, 2006). Further, in humans,

psychotomimetic effects of ketamine are prevented by pretreatment with a metabotropic group 2/3 agonist (Krystal et al., 2005a), and, as described in the following section, had promising preliminary results in a phase II trial (Patil et al., 2007).

More recently, it has been demonstrated that positive allosteric modulators of mGluR5 receptors, which are positively coupled with NMDA receptors, may also reverse the effects of NMDA antagonists on firing rate of prefrontal dopamine neurons (Homayoun and Moghaddam, 2006; Lecourtier et al., 2007; Darrah et al., 2008), while antagonists potentiate the effects of NMDA antagonists (Homayoun et al., 2004). Thus, metabotropic compounds that potentiate NMDA transmission (e.g., mGluR5 modulators) may be equally effective as those that inhibit presynaptic release (e.g., mGlu2/3 agonists), and further clinical studies are required to determine which, if any, of these approaches will ultimately prove successful in the treatment of schizophrenia.

5 Glutamate-Based Treatment Development

To date, all approved agents for the treatment of schizophrenia function by blocking neurotransmission at D_2-type dopamine receptors. These treatments are based upon the fortuitous discovery of the antipsychotic effects of chlorpromazine by Delay and Deniker in the mid 1950s (Delay and Deniker, 1955). The development of effective treatments for schizophrenia in the absence of further fortuitous discovery is a daunting task. Nevertheless, glutamatergic approaches to schizophrenia have stimulated significant drug development activities, including ongoing proof-of-concept studies that may lead to effective new treatments particularly for persistent negative and cognitive features of the disorder.

Treatment approaches have targeted not only NMDA receptors, which are postulated to mediate the psychotomimetic effects of PCP, ketamine, and other similar agents, but also other receptors that modulate NMDA function, including glycine site agonists, as well as AMPA and metabotropic receptors. Finally, indirect approaches to increasing the occupancy of modulatory transmitters at the glutamatergic receptors include the use of glycine transport and DAAO inhibitors, and precursors of glutathione are being studied.

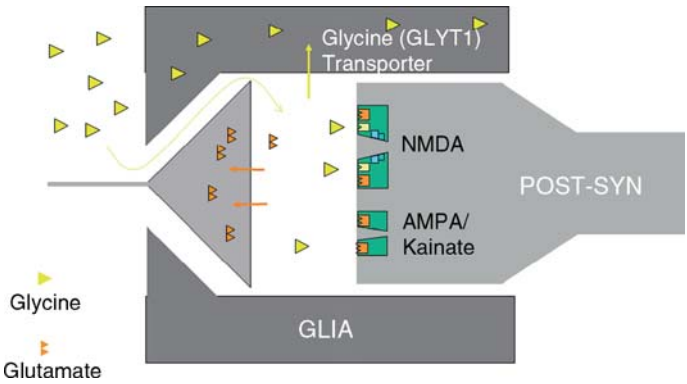
5.1 NMDA Receptor Glycine-site Agonists

Although the NMDA complex contains multiple agonist and/or modulatory sites, any one of which could be a target of drug development, the first-generation approach to glutamatergic treatment consists of targeting the allosteric glycine/ D-serine modulatory sites of the NMDA complex. Several factors underlie this approach.

First, the glutamate site, which is the main agonist site, cannot be targeted due to the risk of excitotoxicity. Further, as described earlier, during normal brain function, occupancy of the glutamate site is phasic, and therefore, persistent activation via this site is nonphysiological. Increases in tonic glutamate levels would be counterproductive, as they would lead to even further background "noise" that would interfere with the detection of the phasic glutamatergic signal.

Second, the glycine site was relatively amenable to intervention, because the endogenous amino acids that occupy the site – glycine and D-serine– when given at high dose will cross the blood–brain barrier and increase central CNS levels. Further, because glycine and D-serine are natural compounds, it has been possible for investigators to obtain FDA approval for their use without the extensive preclinical development usually required for new chemical entities. Research was also facilitated by the availability of D-cycloserine, an antituberculosis drug that fortuitously cross-reacts with the NMDA/glycine site, albeit with relatively low efficiency (Hood et al., 1989). The glycine site appears to be only partially occupied during physiological brain activity, providing potential for further stimulation via administration of exogenous agonist. Although extracellular levels of glycine and D-serine in brain are above the concentrations needed to fully saturate the NMDA/glycine site, the site appears to be "protected" by transporters that maintain low glycine and D-serine levels within the synaptic cleft. The synaptic cleft, in turn, is protected by tight junctions that restrict glycine and D-serine entry, permitting regulation of synaptic glycine/D-serine levels independently from levels within the extracellular space as a whole (*Figure 1.3-3*).

Figure 1.3-3
Schematic model of synaptic glycine regulation by glycine transport inhibitors



In addition to NMDA receptor modulation, a "second generation" approach has been the use of glycine (GLYT1) transport inhibitors, which, akin to serotonin reuptake inhibitors (SSRI), lead to increases in brain glycine levels by blocking its removal from the synaptic space. Finally, trials have been conducted with allosteric AMPA receptor modulators ("AMPAkines"), as well as metabotropic receptor agonists and antagonists, including a recent trial of the mGluR2/3 agonist LY-2140023 (Patil et al., 2007).

5.2 Early Studies – Glycine

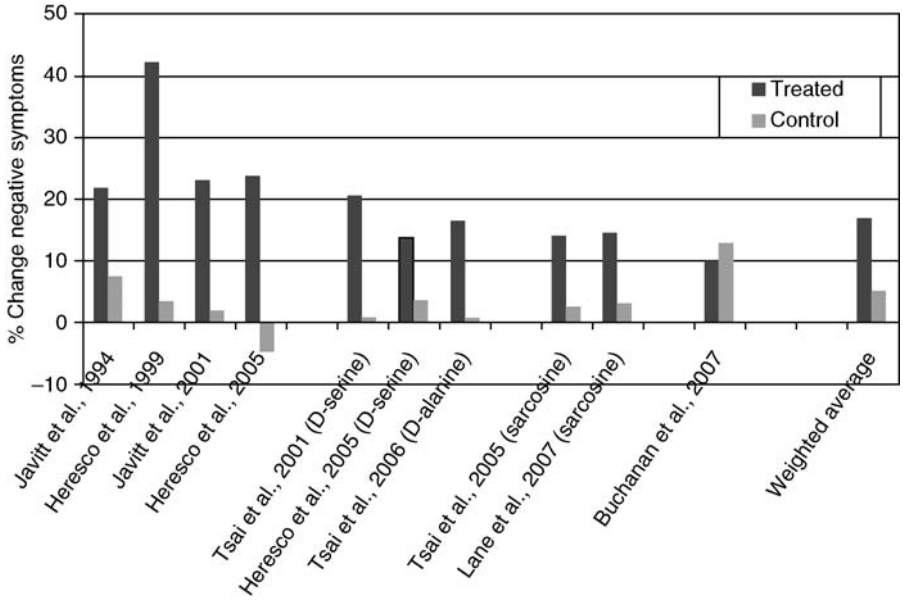
The first experimental use of glycine in preclinical models of schizophrenia was performed in the early-mid 1980s by Toth and Lajtha (Toth and Lajtha, 1981), who demonstrated first that large doses of nonessential amino acids do cross the blood–brain barrier when given at large doses, and second that, of a series of amino acids, only glycine reversed the behavioral effects of PCP (Toth and Lajtha, 1986). As the glycine effects on NMDA activation were not described until shortly thereafter (Javitt, 1987; Johnson and Ascher, 1987; Snell et al., 1987; Javitt and Zukin, 1988), the behavioral effects of glycine in mice were not readily interpretable when they were first observed. Similarly, the first clinical study performed with glycine (Waziri, 1988) – an open label study of 25 individuals – was based not on NMDA theory but on concepts of glycine/serine interconversion. Nevertheless, both early experiments provided retrospective support for NMDA-based treatment and prompted subsequent controlled investigation.

The first randomized, double blind clinical study to show unequivocal significant results was published in 1994 (Javitt et al., 1994) and showed significant, 17% reduction in negative symptoms in response to 30 g/D-glycine. Subsequent trials of higher dose, 60 g/day, glycine also demonstrated significant improvements (*Figure 1.3-4*). In some studies, the degree of negative symptom improvement has correlated significantly with baseline glycine levels, suggesting that patients with lowest pretreatment levels respond best to NMDA receptor agonist treatment (Heresco-Levy et al., 1999). With glycine, the critical plasma level for therapeutic response appears to be in the range of 600–1000 μM versus a basal level of approximately 200 μM. Similar levels have been observed in animal studies (Javitt et al., 2004). The mean percentage change associated with glycine treatment in these studies was approximately 30%, suggesting that if these findings could be replicated, results would be clinically meaningful.

In addition, studies performed over recent years have also documented reduced glycine and D-serine levels in plasma and CSF in schizophrenia (Hashimoto et al., 2003; Sumiyoshi et al., 2004; Neeman et al., 2005). Thus, rather than being drug treatments in the typical sense of the term, pharmacological use of glycine and D-serine may, in fact, simply be correcting an underlying neurochemical abnormality contributing to the pathophysiological development of schizophrenia.

Figure 1.3-4

Summary of negative symptom change across clinical trials in which *N*-methyl-D-aspartate (NMDA) full agonists (glycine, D-serine, D-alanine) or the glycine transport inhibitor sarcosine have been used in combination with antipsychotics other than clozapine (Originally published in Javitt (2008))



Despite these positive single site studies, a recent multicenter clinical trial (Buchanan et al., 2007) did not demonstrate significant overall results. In a subgroup analysis, however, significant efficacy of glycine was observed in inpatients, but not outpatients. This trial, however, was designed as an effectiveness rather than efficacy trial and was characterized by low achieved dose and serum amino acid levels, and large therapeutic responses to the chosen placebo. Thus, patients who did not tolerate full medication dose were allowed to remain enrolled on partial treatment. Further, the study did show significant improvements among inpatients and among patients receiving typical antipsychotics, suggesting potential treatment responsive subpopulations.

5.2.1 D-serine

The dosages using trials of glycine have been in the range of 60 g a day. Although glycine and D-serine bind to the NMDA/glycine site with similar potency, D-serine more easily crosses the blood–brain barrier and is less likely to be metabolized peripherally. Thus, practically, D-serine can be given effectively at lower doses than glycine.

An initial clinical trial with D-serine in schizophrenia was conducted by Tsai and colleges in 1998 (Tsai et al., 1998). The study involved 29 subjects treated for 6 weeks with either D-serine (30 mg/kg/day) or a placebo. In that study, all the patients met criteria for the Deficit Syndrome (see Chapter 5.1 this Volume). Moreover, patients were receiving treatment primarily with conventional antipsychotics. A highly significant ($p = 0.0004$ versus placebo), mean 20.6% reduction in negative symptoms was observed among D-serine patients, with significant improvements also. Significant improvement was noted in neuropsychological function as well, as reflected in WCST categories completed, and CGI.

These results were subsequently replicated in a double-blind, placebo-controlled crossover study (Heresco-Levy et al., 2005) in which D-serine or a placebo was added to atypical antipsychotics (risperidone

or olanzapine). Highly significant, large effect size (1.3 standard units) improvements were observed in SANS total score and in the negative factor score of the PANSS. Highly significant effects were observed for the PANSS cognitive and depression factors, and total BPRS score as well. Similar results were also obtained in a study using D-alanine, a lower affinity agonist at the glycine site, at a dose of 0.1 mg/kg (Tsai et al., 2005). An independent meta-analysis conducted in 2005 (Tuominen et al., 2005) concluded that in the first 132 patients studied with NMDA agonists including glycine, D-serine, and D-alanine, a highly significant ($p = 0.0004$), moderate effect size improvement in negative symptoms was observed across studies.

Since these published studies, preliminary negative results have been reported from a multicenter clinical study of 30 mg/kg (2 g/day) D-serine versus a placebo using negative symptoms and cognitive function as primary endpoints (Weiser et al., 2008). In contrast, a preliminary report from an open-label dose escalation PK/PD study found a significantly greater improvement in cognition with higher D-serine doses (60–120 mg/kg) than with 30 mg/kg. At high doses, large (>1.0 sd) pre versus post treatment improvements were observed in MATRICS cognition measures along with significant pre-post improvement in PANSS ratings (Kantrowitz et al., 2008), suggesting the need for further, double blind study of higher D-serine doses.

5.2.2 D-cycloserine

D-cycloserine (distinguished structurally from D-serine) is a synthetic compound approved for the treatment of tuberculosis that fortuitously cross-reacts with the NMDA-associated glycine-binding site. However, D-cycloserine is a mixed agonist/antagonist, showing agonist activity only in the presence of low brain glycine/D-serine concentrations and which is only approximately 50% as effective as glycine or D-serine in the potentiation of NMDA receptor-mediated neurotransmission (Hood et al., 1989).

Compared with full glycine site agonists, D-cycloserine has proved less efficacious across clinical sites and across studies within clinical site (Heresco-Levy et al., 1998), and doses in excess of 100 mg cause symptom exacerbation due to emergent NMDA receptor antagonist effects, producing a narrow therapeutic window (van Berckel et al., 1999). A meta-analysis of clinical studies performed through 2004 found evidence of significant beneficial effect of full glycine site agonists across studies, but not of D-cycloserine (Tuominen et al., 2005). Despite its overall lack of efficacy, however, D-cycloserine was reported to increase temporal lobe activation during a word recall task, with effects correlating with the degree of reduction in negative symptoms (Yurgelun-Todd et al., 2005). Significant effects have also recently been observed during once weekly, as opposed to daily, treatment, suggesting that tolerance may be an issue (Goff et al., 2008a). More recently, D-cycloserine has been reported to be useful for the facilitation of fear extinction during acute dosing (Norberg et al., 2008). Thus, specific beneficial effects may occur over short-term treatment, although it is postulated that tolerance may occur during longer term trials.

5.2.3 NMDA Agonists in Combination with Clozapine

Relative to effects in combination with typical or newer atypical antipsychotics, glycine site agonists have proved less effective when combined with clozapine. In double blind, placebo-controlled studies in which glycine (Evins et al., 2000) or D-serine (Tsai et al., 1999) has been added to clozapine, no significant beneficial response has been observed, while D-cycloserine is reported to lead to the worsening of symptoms when used in combination with clozapine (Goff et al., 1996). A parsimonious explanation for the differential effects of NMDA agonists in combination with clozapine versus other antipsychotic agents, therefore, is that clozapine may already increase synaptic glycine levels through as-yet-unknown mechanisms. Recently, clozapine has been shown to block glycine and glutamine transport mediated by SNAT2-like synaptosomal transporters, providing a potential mechanism for both the differential therapeutic effects of clozapine and the differential effects of NMDA receptor modulators in the presence of clozapine versus other antipsychotics (Javitt et al., 2005a). This finding may also account for the reported ability of clozapine to increase serum glutamate levels (Evins et al., 1997), and downregulate central glutamate transport (Pietraszek et al., 2002; Melone et al., 2003).

5.2.4 Use of Glycine in the Schizophrenic Prodrome

The majority of studies with NMDA agonists have focused on individuals well advanced in their illness. Recently, however, glycine was used in an open label monotherapy study in 10 individuals showing prodromal signs of schizophrenia. Although the number of subjects was limited, three met early remission criteria, one other showed substantial improvement and two showed moderate improvement (Woods et al., 2004). Across all subjects, large effect size changes were observed across both positive and negative domains. Effects of glycine were more pronounced than those that had been observed in a double blind study of olanzapine (McGlashan et al., 2006). These data, if confirmed, would indicate that NMDA agonists might have a primary role in the earliest stages of schizophrenia psychosis, with potential impact across symptomatic domains.

5.3 Glycine Transport Inhibitors

Both glycine and D-serine appear to be effective when used in treatment resistant schizophrenia. However, both must be given at gram-level doses in order to significantly elevate CNS levels. An alternative approach to increasing CNS levels is the use of glycine transport inhibitors (GTIs), which raise synaptic glycine levels by preventing its removal from the synaptic cleft. The use of GTIs to augment NMDA functioning is analogous to the use of selective SSRIs to raise synaptic serotonin levels in depression.

Initial preclinical studies were performed using the relatively nonselective glycine transport antagonist glycyldodecylamide (GDA) (Javitt and Frusciante, 1997; Javitt et al., 1997; Javitt et al., 1999a; Harsing et al., 2001). This drug was shown to inhibit glycine transport in cortical (Javitt and Frusciante, 1997) or hippocampal (Harsing et al., 2001) synaptosomes, and inhibit amphetamine-induced dopamine release (Javitt et al., 2000) and PCP-induced hyperactivity in rodents (Toth et al., 1986; Javitt et al., 1997; Javitt et al., 1999a). More recent studies have been performed with selective, high-affinity glycine transport inhibitors such as *N*[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl] sarcosine (NFPS) (Aubrey and Vandenberg, 2001), Org 24598 (Brown et al., 2001), CP-802,079 (Martina et al., 2004), or SSR504734 (Depoortere et al., 2005), or SSR103800 (Boulay et al., 2008).

As with GDA, high affinity glycine transport inhibitors have been found to reverse PCP-induced hyperactivity (Harsing et al., 2003) and dopaminergic hyperreactivity (Javitt et al., 2004) in rodents, and to potentiate NMDA responses in hippocampal slices in vitro (Bergeron et al., 1998; Kinney et al., 2003b; Martina et al., 2004; Depoortere et al., 2005; Boulay et al., 2008) and prefrontal cortical neurons in vivo (Chen et al., 2003). Glycine transport inhibitors also reverse PPI abnormalities in DBA/2J mice (Kinney et al., 2003b; Depoortere et al., 2005; Boulay et al., 2008) and rats with neonatal hippocampal lesions (Le Pen et al., 2003), supporting a role of glycine transport inhibitors in the treatment of schizophrenia. In striatal dopamine assays, GLYT1 inhibitors reduce amphetamine-induced dopamine release in vivo (Javitt et al., 2004) and NMDA-stimulated release in vitro (Bennett and Gronier, 2005; Javitt et al., 2005b), suggesting a likely effect on positive, as well as negative, symptoms of schizophrenia.

At saturating doses, effective GLYT1 inhibitors produce approximately twofold to threefold increases in extracellular glycine concentrations (Martina et al., 2004; Depoortere et al., 2005; Boulay et al., 2008). Significantly, however, positive effects on NMDA receptor-mediated neurotransmission occur at concentrations 2–3 orders of magnitude lower than those needed to significantly increase extracellular glycine levels. Further, an inverted U-shape curve has been observed in several studies where beneficial effects of GLYT1 antagonists on NMDA function may be diminished or lost at the highest doses used. These findings are consistent with a model in which GLYT1 inhibitors primarily affect glycine concentrations within the synaptic cleft (*Figure 1.3-3*), which represents an effectively separate brain compartment from the overall extracellular space or CSF. Increases in extracellular glycine levels would occur only as a consequence of the diffusion of glycine from the synaptic to the general extracellular space, a process that would occur only at very high glycine concentrations. At extremely high concentrations, glycine may induce the internalization of NMDA receptors, leading to a loss of facilitatory glycine effects on NMDA transmission (Martina et al., 2004). In rodents, effects of GLYT1 inhibitors have been found to be similar to those of clozapine (Lipina et al., 2005), suggesting potential overlap of cognition enhancing mechanisms.

Further support for the use of GLYT1 antagonists comes from studies of GLYT1 knock-down mice. GLYT1$^{-/-}$ mice cannot be developed due to neonatal lethality (loss of breathing) (Tsai et al., 2004). Nevertheless, alternative strategies have been employed. For example, GLYT1$^{+/-}$ heterozygote mice show a significant decrease in GLYT1 expression throughout the brain and an enhancement of hippocampal NMDA activity consistent with GLYT1 inhibitor studies (Martina et al., 2004; Tsai et al., 2004; Gabernet et al., 2005). Similarly, selective forebrain knockouts show reductions in frontal glycine transport and potentiation of hippocampal NMDA responses as well as procognitive ability on several learning/memory paradigms (Yee et al., 2006). As with animals treated with GLYT1 inhibitors, GLYT1$^{+/-}$ heterozygote mice show some evidence of NMDA internalization, particularly behavioral hypersensitivity to the NMDA antagonist MK-801 (Tsai et al., 2004). Nevertheless, on balance, the phenotype supports a net potentiation of NMDA neurotransmission.

Sarcosine (*N*-methylglycine), a naturally occurring glycine transport inhibitor, has been used in several clinical trials in Taiwan. Although no significant safety concerns have emerged in limited trials conducted to date, this compound is not yet available for investigational use in the US. Initial studies with sarcosine showed efficacy similar to that of direct glycine-site agonists (i.e., glycine, D-serine and D-alanine) when added on to either typical or nonclozapine atypicals in chronic stabilized inpatients (*Figure 1.3-4*). Sarcosine was also found to be relatively ineffective in combination with clozapine, consistent with prior studies using direct agonists. However, when administered in combination with risperidone to acutely decompensated patients, the sarcosine + risperidone combination showed superior efficacy to risperidone alone (Lane et al., 2005). In all these studies, medications were used at single, nonoptimized doses, raising the possibility that greater efficacy and different comparative effects might be observed at higher doses. Both safety and dose-finding studies are needed to permit clinical studies to be performed with sarcosine in the US.

Most recently, in a small double blind trial (Lane et al., 2008), sarcosine showed efficacy as monotherapy in acutely decompensated patients. Despite the small sample size, the monotherapy study represents a critical evolution of the NMDA-based therapeutic approach, which had up until then been limited to add-on studies. In the study, no true placebo was used, although a lower dose of sarcosine was used as partial control and in order to evaluate the dose-dependence of findings. Obviously, more extensive clinical trials, as well as the introduction of high affinity, selective GLYT1 inhibitor compounds, are needed.

5.4 Other Ionotropic Targets

Because AMPA receptors function in concert with NMDA receptors, they have been proposed as alternative therapeutic targets in schizophrenia. AMPAkines function as positive allosteric modulators of AMPA receptor-mediated neurotransmission, and facilitate learning and memory in both human (Ingvar et al., 1997) and animal (Hampson et al., 1998) models. Further, these drugs act synergistically with antipsychotics to reverse amphetamine-induced hyperactivity (Johnson et al., 1999).

In a pilot study, the AMPAkine CX-516 induced significant improvements in memory and attention when added to clozapine, despite the lack of symptomatic improvement (Goff et al., 2001). However, these results were not confirmed in a larger confirmation study (Goff et al., 2008b) nor were beneficial effects observed in a small monotherapy trial (Marenco et al., 2002). Although the downregulation of AMPA receptors is less with AMPAkines than with direct agonists, there is some concern that downregulation may occur and may limit long-term treatment strategies (Jourdi et al., 2005).

5.5 Metabotropic Receptors

As described in section 2.3, metabotropic receptors are divided into families, based upon second messenger mechanisms and functional effect. Group I receptors potentiate presynaptic glutamate release and NMDA receptor-mediated neurotransmission. Therapeutic effectiveness of group I agonists is therefore

predicted based upon models that postulate low NMDA receptor activity and/or glutamate levels as being pathophysiological in schizophrenia. In contrast, Group II/III agonists inhibit glutamate release. The use of these agents follows models that postulate that glutamatergic hyperactivity may be pathophysiological.

5.5.1 Group I Receptors

Group I includes both mGLUR1 and mGLUR5 receptors, both of which stimulate NMDA receptors via differential second messenger cascades (Benquet et al., 2002; Heidinger et al., 2002). Preclinical studies have evaluated the ability of Group I antagonists to induce schizophrenia-like behavioral effects, and Group I agonists to reverse the effects of amphetamine, PCP, and other psychotomimetics. The most widely used mGluR5 antagonist, 2-methyl-6-(phenylethynyl)pyridine (MPEP), does not affect locomotor activity or PPI by itself but potentiates PCP-induced increases in locomotor activity and disruption of PPI (Henry et al., 2002; Kinney et al., 2003a). Similar effects have been observed with the more recently developed compound 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP) (Cosford et al., 2003). Finally, mGluR1 (Brody et al., 2003) and mGluR5 (Kinney et al., 2003a) knock-out mice show disruptions of PPI that respond poorly to the known treatments for schizophrenia (Brody et al., 2003), supporting a potential role of Group I receptors as therapeutic targets in schizophrenia. Group I antagonists also produce anxiolytic-like effects in several animal models of anxiety, suggesting that they may be independent targets for the treatment of anxiety disorders (Chojnacka-Wojcik et al., 2001).

Studies with Group I agonists have also been supportive of potential therapeutic effectiveness, but are more limited. For example, the mGluR5 agonist 2-chloro-5-hydroxyphenylglycine (CHPG) has been found to reverse PPI-disruptive effects of amphetamine in rodents (Kinney et al., 2003a). Similarly, both nonselective and Group I selective agonists inhibit PCP-induced dopamine release in rodent prefrontal cortex (Maeda et al., 2003).

An issue in the use of direct agonists is rapid receptor desensitization preventing chronic use. An alternative approach is the use of positive allosteric modulators, which do not bind directly to the agonist-binding site. Positive modulators, in general, have proved to be lipophilic and centrally acting, and henceattractive as potential pharmacological agents (Pin and Acher, 2002; Rodriguez and Williams, 2007). Pro therapeutic effects have been reported in animal models of schizophrenia with the mGlu5 positive allosteric modulators 3-cyano-*N*-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) (Lecourtier et al., 2007), and ADX47273 (Liu et al., 2008), supporting the utility of this approach.

Despite encouraging preclinical results with Group I agonists, clinical data remain lacking. Further, group I receptors have a markedly different cellular distribution in primates than rodents (Muly et al., 2003; Paquet and Smith, 2003). Primate studies and eventual clinical trials therefore will be needed to validate this target for the treatment of neuropsychiatric disorders. Nevertheless, the preclinical efficacy of these compounds remains encouraging for eventual therapeutic utility.

5.5.2 Group II Metabotropic Agonists

Group II and III metabotropic receptors are negatively linked to glutamate release, and may limit endogenous release under conditions of glutamate excess. The use of Group II/III agonists in schizophrenia is therefore based upon the hypothesis that increased glutamate levels may be pathophysiological. Several high-affinity agonists have been developed over recent years, including (-)-2-oxa-4-aminobicyclo[3.1.0.] hexane-4,6-dicarboxylate (LY-379268) and the related compound LY-354740, permitting characterization of the effects of Group II agonists in both preclinical and clinical studies (Schoepp, 2002).

Potential utility of mGlu2/3 agonists in schizophrenia was first suggested based upon a preclinical study demonstrating the ability of LY-379268 to block PCP-induced increases in prefrontal glutamate, along with PCP-induced impairments in working memory (Moghaddam and Adams, 1998; Lorrain et al., 2003). Similarly, the compound LY-3279268 was shown to inhibit PCP-induced hyperactivity during both acute (Cartmell et al., 2000; Clark et al., 2002) and repeated (Cartmell et al., 2000) administration, and reverse PCP-induced behaviors in monoamine-depleted mice (Swanson and Schoepp, 2002). Finally,

the compound LY-354740 has been found to reverse the effects of NMDA antagonists in rodents (Moghaddam and Adams, 1998) and primates (van Berckel et al., 2006), as well as in humans (Krystal et al., 2005a).

The first mGlu2/3 agonist to be entered into clinical efficacy trials for schizophrenia is the compound LY-2140023, which is the prodrug of the mGlu2/3 receptor agonist LY-404039 (Patil et al., 2007). This compound was tested against a placebo and active olanzapine in a randomized, three-armed, double-blind, placebo-controlled study. Treatment with LY-2140023, like treatment with olanzapine, was safe and well tolerated; treated patients showed statistically significant improvements in both positive and negative symptoms of schizophrenia compared with those treated with a placebo ($p < 0.001$ at week 4) and was statistically similar to olanzapine. If replicated, this trial would be the first double-blind trial to demonstrate clinical efficacy of a nondopaminergic treatment in schizophrenia.

To date, compounds that have been used in clinical trials have been nonselective for mGluR3, relative to mGluR2, receptors. Given linkages between the mGluR3 receptor (GRM3) gene and schizophrenia, it was tempting to speculate that compounds might target a receptor that is already dysfunctional. However, several recent preclinical studies suggest that preclinical potency of compounds such as LY404039 (Fell et al., 2008) or LY379268 (Woolley et al., 2008) may be more related to their effect on mGluR2, rather than mGluR3, receptors. Overall, further clinical studies and more selective compounds are required.

5.5.3 Group III Metabotropic Agonists

mGluR8 receptors, which like mGluR2/3 receptors may function as presynaptic autoreceptors, have also been proposed as potential therapeutic targets in schizophrenia (Robbins et al., 2007). Like other mGluRs, mGluR8 receptors have widespread CNS distribution with expression in key brain areas associated with schizophrenia, such as the hippocampus. (S)-3,4-dicarboxyphenylglycine ((S)-3,4-DCPG) is a relatively selective agonist for mGluR8. In preclinical models, 3,4-DCPG reversed the behavioral effects of amphetamine but not PCP (Robbins et al., 2007). Further, the compound induced catalepsy at doses similar to those at which it reversed amphetamine-induced hyperactivity, suggesting that it may function closer to a typical, rather than atypical, antipsychotic (Ossowska et al., 2004).

5.6 Redox Site

A further potential target site of the NMDA receptor is the glutathione or redox site (see Chapter 2.5 this Volume). NMDA receptors possess a well-characterized redox/glutathione sensitive site (Lipton et al., 2002) that may exert physiological activity over NMDA activation (*Figure 1.3-2*). In addition, glutathione has been found to modulate dopamine release in striatal slices via both NMDA and non-NMDA receptors (Janaky et al., 2007). In schizophrenia, reduced glutathione levels have been reported in both cortex and CSF (Do et al., 2000), leading potentially to an NMDA hypofunction state (Steullet et al., 2006). The glutathione modulator (NAC) has recently been shown to improve the generation of MMN in schizophrenia (Lavoie et al., 2007). Further, in a double-blind multicenter study, NAC treatment (1 g BID) added on to placebo led to significant improvement in PANSS total, negative, and general symptoms, relative to placebo, along with significant improvement in global function. Overall effect size was moderate, although the study was limited by relatively high rates of noncompletion (Berk et al., 2008). Thus, as with other NMDA-based treatments, preliminary results are encouraging, although larger scale studies are needed.

6 Future Research and Treatment Implications

Over the last 40 years, the dopamine model has been the leading neurochemical hypothesis of schizophrenia. This model has proved heuristically valuable, with all current medications for schizophrenia

functioning primarily to block dopamine D_2 receptors. Yet it remains unlikely that dopaminergic dysfunction, on its own, can fully account for the wide range of symptoms and neurocognitive deficits seen in schizophrenia. Glutamatergic models provide an alternative approach for conceptualizing the brain abnormalities associated with schizophrenia. As opposed to dopaminergic agonists, NMDA antagonists produce negative and cognitive symptoms of schizophrenia, along with positive symptoms, and induce neuropsychological deficits that are extremely similar to those observed in schizophrenia. At present, there are no approved medications for the treatment of either negative symptoms or neurocognitive dysfunction. New treatment approaches aimed at potentiating glutamatergic neurotransmission, particularly at NMDA- and metabotropic-type receptors, however, offer some new hope for future clinical development.

Acknowledgements

Preparation of this manuscript was supported in part by USPHS grants K02 MH01439, R01 DA03383, and R37 MH49334.

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Section 2

Molecular Targets

2.1 *G72/G30* in Neuropsychiatric Disorders

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_2.1,

Abstract: *G72* and *G30* constitute a pair of primate-specific genes on complementary strands of human chromosomal 13q33. G72 is proposed to encode a protein that can activate D-amino acid oxidase (DAAO), therefore, named as D-aminoacid oxidase activator (DAOA) (Chumakov et al., 2002). This hypothesis about G72 and DAAO requires substantial further proof (see discussion later), and the "neutral" name G72 will be used here, although DAOA is widely used in the literature now. The *G72*/*G30* gene is, at this time, one of the best supported loci for both schizophrenia (SZ) and bipolar disorder (BD) by independent datasets (Craddock et al., 2006; Detera-Wadleigh and McMahon, 2006). Here, we will describe the discovery of gene complex G72/G30, association studies with SZ and BD with a meta-analysis, as well as brief information about the biology of G72/G30.

List of Abbreviations: CD/MRV, Common Disease/Multiple Rare Variants; DAAO, D-amino acid oxidase; DAOA, D-aminoacid oxidase activator; EST, expression sequence tag; GEO, Gene Expression Omnibus; MAF, minor allele frequency; NIMH, National Institute of Mental Health; NMDA, *N*-methyl D-aspartate; RT-PCR, reverse transcription-PCR

1 The Discovery of *G72*/*G30* and Initial Schizophrenia Association Studies

Several linkage analyses support a broad region on 13q32-34 as linked to both schizophrenia (SZ) and bipolar disorder (BD) (for a comprehensive summary of these linkage studies, see review (Detera-Wadleigh and McMahon, 2006)). In 2002, Badner and Gershon published a meta-analysis method for linkage results, which considered the distribution of *P*-values on each chromosome in all published genome-wide scans (Badner and Gershon, 2002). This meta-analysis found that 13q32-33 had significant linkage evidences across all published studies, for both BD and SZ (Badner and Gershon, 2002). Similar conclusions had been reached earlier by Wildenauer et al. (1999), Berrettini (2000), and Gershon (2000). However, subsequent meta-analyses of linkage studies did not pick out the same region (Lewis et al., 2003; Segurado et al., 2003; McQueen et al., 2005), which may be due to different analytical methods used, different although overlapping linkage studies included, and/or genetic heterogeneity across different populations.

In a study of SZ, Chumakov et al. (Chumakov et al., 2002) from the Genset Corporation in France interrogated a 5-Mb region on 13q33 using 191 SNPs in 213 unrelated cases and 241 unrelated controls originating from Quebec (French Canadian). Two clusters of SNPs (spanning 1,400- and 65-kb regions, respectively) showed significant association with disease. These SNPs mapped to a region devoid of known genes. Thirteen SNPs selected from the 65-kb region, which harbors the most significant signals, were tested for replication in a second sample set from Russia. Several SNPs in this distal cluster revealed significant association. Gene discovery was performed in the two associated regions. Three genes, *G90*, *G72*, and *G30* were identified by computational analysis, followed by experiments of reverse transcription-PCR (RT-PCR) and rapid amplification of cDNA ends (RACE). *G90* was mapped to the proximal 1,400-kb region, though the *G90* gene sequence has not yet been released to a public database. *G72* and *G30* were mapped to the distal 65-kb region. Using artificially expressed *G72* C-terminal fragment as bait, D-amino acid oxidase (DAAO) was identified as a protein that can bind to G72 in a yeast two-hybrid experiment. In vitro experiment further detected increased D-serine oxidation by DAAO in the presence of increasing concentrations of recombinant G72 protein. G72 was subsequently renamed as D-aminoacid oxidase activator (DAOA). DAAO is a trophic factor for the NMDA class glutamate receptors. The popular NMDA receptor hypofunction hypothesis in SZ (See "Glutamate and schizophrenia: Phencyclidine, N-mathyl-D-aspartate (NMDA) receptors: etiology and treatment" also (Coyle et al., 2003; Moghaddam, 2003; Lindsley et al., 2006)) makes the DAOA–DAAO hypothesis appealing.

2 Association and Meta-Analysis of *G72*/*G30* in Schizophrenia and Bipolar Disorder

The finding of *G72*–SZ association (Chumakov et al., 2002) was quickly followed by a report of association with BD from our laboratory (Hattori et al., 2003). In this report, there were two familial samples: the

Clinical Neurogenetics (CNG) pedigrees and the National Institute of Mental Health (NIMH) Genetics Initiative for BD pedigrees waves 1 and 2. In the CNG samples, 13q linkage has been demonstrated; 6 of 16 SNPs showed significant association with BD. The NIMH samples showed only near-significant association ($P = 0.055$) with disease for one of the SNPs (rs1935058, also the strongest signal in CNG). Most importantly, one haplotype consisting of seven SNPs across the region showed significant association with BD in both samples, with global *P*-values of 0.0004 in the CNG sample and 0.008 in the NIMH sample, respectively. These data provided the initial evidence of association between *G72*/*G30* and BD.

A sizeable number of replication studies in BD and SZ followed upon these two publications, with multiple reports of nominally significant associations. Repeatedly, these findings have been reviewed as the most promising current associations in SZ (McGuffin et al., 2003; Maier et al., 2006), BD (DePaulo, 2004; Craddock and Forty, 2006), or both (Craddock et al., 2005; Maier et al., 2005; Craddock et al., 2006). In a meta-analysis using the Fisher's method (combining *P*-values across studies), Detera-Wadleigh and McMahon (Detera-Wadleigh and McMahon, 2006) reviewed ten studies of SZ and BD, and found that there was no individual SNP at which multiple studies were significantly associated with both SZ and BD, although the overlap in SNPs between studies was very spotty. In a detailed meta-analysis by Ma et al. (Ma et al., 2006), raw data of three SNPs in SZ case-control studies could be pooled. There were four European samples totaling 1,974 individuals and two Asian samples totaling 2,251 individuals. The European data were significantly heterogeneous and less consistent in showing association than the Asian data. The Asian data had consistent association at rs778293 (no significant heterogeneity in Asians).

We performed our own meta-analysis for this chapter. *Table 2.1-1* summarizes all published *G72/G30* association studies on SZ and/or BD before December 2006 (searched in PubMed), in a table formatted similar to that of Detera-Wadleigh and McMahon (2006). This table lists all SNPs that showed nominally significant allelic or haplotypic association with SZ or BD in at least one study. Different studies genotyped different SNPs and reported analyses of different haplotypes. So far, only five SNPs were studied in more than half of the 28 sample sets, including both BD (6 sample sets) and SZ (22 sample sets). On top of this difference, as shown in the table, the associated SNPs vary across studies. None of the SNPs has been consistently associated with either disease. Five SNPs (rs3916965, rs1341402, rs2391191, rs778293, and rs3918342) show "flip-flop" associations (significant association with the same SNP, but with opposite risk alleles). It is thought that opposite effects of the same allele may be found in populations characterized by different LD patterns. A genuine risk allele may appear to be a relatively protective allele (and vice versa) when a multilocus effect is not considered (Lin et al., 2007). However, the possibility of spurious association cannot be excluded since most of the follow-up studies did not perform statistical correction for multiple testing and/or there would be a low statistical power in a small-size sample.

We used the program Meta-analysis with Interactive eXplanations (MIX) (Bax et al., 2006) to perform an updated meta-analysis of five SNPs that have been frequently analyzed in schizophrenia associations. Odds ratios (ORs) for individual case-control studies were calculated from two by two tables. The 95% confidence intervals (CIs) were calculated using Woolf's method. ORs and 95% CIs for family-based studies were calculated using Cho et al.'s method (2005). Logarithm of the ORs and their associated standard errors (se) were used for subsequent meta-analyses (Kazeem and Farrall, 2005). Data was initially analyzed using the fixed-effects model, which assumes constant effects of the "risk" allele across studies and between-study variation due to random variation. Cochran's *Q* test revealed significant heterogeneity ($P < 0.05$) between studies (among all populations, European or Asian) for all five SNPs with or without nominal significant association except for rs3916965 in Asian (*P* for *Q* test is 0.12, *P* for *Z* test is 0.02) (*Table 2.1-2*, fixed-effects model). Thus, we employed the DerSimonian and Laird's random-effects model in the meta-analysis (DerSimonian and Laird, 1986), which assumes that between-study variation is due to both random variation and an individual study effect. Pooled ORs were calculated using the random-effects approach and examined for significance using *Z* test. We did not find any disease association with these five SNPs under the random-effects model (data not shown).

Using a similar approach, we analyzed five SNPs in BD. Significant heterogeneity between studies was found, and no significant association revealed under either fixed-effects (*Table 2.1-3*) or random-effects models (data not shown) though all existing studies are from Caucasian samples.

Table 2.1-1
Updated summary of results of DAOA/G30 association studies[a]

Ref.	n[b]/Disease/ethnic/design	db SNP ID/Alias/Hg17 position/alleles							
		rs3916965/ M12[i]/ 104901361/ A/G	rs3916966/ M13/ 104908896/ C/A	rs1935058/ –/ 104909351/ C/T	rs1341402/ –/ 104913510/ C/T	rs3916967/ M14/ 104915349/ T/C	rs2391191/ M15/ 104917447/ A/G	rs1935062/ –/ 104926137/ A/C	–/ DAOA_3′UTR-SNP 12/ 104927538/ C/A
1	213/SZ[c]/French Canadian/CC	G/.007[j]	ns			T/.038	A/.032		
	183/SZ/Russian/CC	ns[k]	ns			ns			
2	22 Extended pedigrees/BD[d]/ White US/TDT		ns	C/.00077	T/.0075		ns	A/.0078	
	152 Pedigrees/BD/White US/TDT			ns	ns		ns	ns	
3	98/SZ. P-NOS[e]/mixed US/TDT			ns		T/.015	G/.031	ns	
4	139/BD/White US/CC			ns			ns	A/.0072	
5	210/SZ/White US/TDT	ns	ns			ns	ns		
	169/SZ/South African/TDT	ns	ns			ns	0.045		
	233/SZ/South African/TDT[l]	ns	ns			ns	ns		
6	60/SZ/Ashkenazim/CC	ns	ns			ns	ns		
7	299/SZ/German/CC	A/.048			ns		A/.037	ns	
	300/BD/German/CC	ns			ns		ns	ns	
8	537/SZ/Han Chinese/CC	A/.0019				ns	A/.001	ns	
9	159/SZ/mixed US/TDT	ns	ns			ns	ns		
10	337/BD/Ashkenazi Jewish/TDT						ns	ns	
	274/SZ/Ashkenazi Jewish/TDT						ns	ns	
11	233/SZ/Han Chinese/TDT	A/.0018				ns	A/.0108		
12	588/SZ/Han Chinese/CC	ns				ns	ns		
	183/SZ/Scotich/CC	ns				ns	ns		
13	217 Pedigrees/SZ/Caucasian US/TDT					ns	ns		
	236/SZ-cognition[f]/Caucasian US/CC								
	10/SZ-memory[g]/Caucasian US/CC								

14	709/SZ/White UK/CC	ns		ns	ns		ns		ns
	706/BPI/White UK/CC	A/.047		ns	C/.03		ns		A/.01
	818/Mood[h]/White UK/CC	A/.02		ns	C/.01		A/.025		A/.002
15	218 Pedigrees/SZ/Taiwan Chinese/TDT	ns	ns			ns	ns		
16	216/SZ/Taiwan Chinese/TDT								
17	216/SZ/Chinese/CC						ns		

		db SNP ID/Alias/Hg17 position/alleles							
	n[b]/Disease/ethnic/design	rs947267/ M18/ 104937663/ A/C	rs778294/ M19/ 104940236/ A/G	rs954581/ –/ 104950267/ C/T	rs3916971/ M21/ 104960002/ C/T	rs778293/ M22/ 104967200/ A/G	rs3918342/ M23/ 104983750/ C/T	rs1421292/ M24/ 104996236/ A/T	Global *P*-value for haplotype analysis
1	213/SZ[c]/French Canadian/CC	ns	ns		ns	A/.003	T/.019	T/.019	
	183/SZ/Russian/CC	ns			ns	ns	T/.017	ns	
2	22 Extended pedigrees/BD[d]/ White US/TDT	ns	G/.018	ns			ns		0.0004
	152 Pedigrees/BD/White US/TDT	ns	ns	ns					0.008
3	98/SZ. P-NOS[e]/mixed US/TDT		ns			ns			0.004
4	139/BD/White US/CC	A/.0403		ns					0.004
5	210/SZ/White US/TDT						ns		
	169/SZ/South African/TDT						ns		
	233/SZ/South African/TDT[l]						ns		
6	60/SZ/Ashkenazim/CC	ns	ns		ns	ns	T/0.001		
7	299/SZ/German/CC		ns				T/.033	T/.036	0.032
	300/BD/German/CC		ns				T/.013	ns	0.08
8	537/SZ/Han Chinese/CC					ns	ns		0.00001
9	159/SZ/mixed US/TDT				ns	ns	ns	ns	
10	337/BD/Ashkenazi Jewish/TDT	ns	ns						
	274/SZ/Ashkenazi Jewish/TDT	ns	ns						
11	233/SZ/Han Chinese/TDT								0.000012
12	588/SZ/Han Chinese/CC		ns			G/.0013	ns		0.006
	183/SZ/Scotich/CC		G/.025			G/.022	C/.0005		0.0003

continued

Table 2.1-1 (continued)

		db SNP ID/Alias/Hg17 position/alleles							
	n[b]/Disease/ethnic/design	rs947267/ M18/ 104937663/ A/C	rs778294/ M19/ 104940236/ A/G	rs954581/ –/ 104950267/ C/T	rs3916971/ M21/ 104960002/ C/T	rs778293/ M22/ 104967200/ A/G	rs3918342/ M23/ 104983750/ C/T	rs1421292/ M24/ 104996236/ A/T	Global *P*-value for haplotype analysis
13	217 Pedigrees/SZ/Caucasian US/TDT	ns	ns			ns	ns	ns	
	236/SZ-cognition[f]/Caucasian US/CC		ns				/.007	TT/<.05	
	10/SZ-memory[g]/Caucasian US/CC							TT/.01	
14	709/SZ/White UK/CC		ns	Ns		ns		ns	
	706/BPI/White UK/CC		ns	Ns		ns		ns	
	818/Mood[h]/White UK/CC		ns	Ns		ns		ns	
15	218 Pedigrees/SZ/Taiwan Chinese/TDT	ns	ns	Ns		ns	ns		
16	216/SZ/Taiwan Chinese/TDT	A/.016							
17	216/SZ/Chinese/CC	A/0.006	ns						0.006

SNPs within haplotype showing most significant association in individual study are highlighted in gray. Key: (1) Chumakov et al., 2002, (2) Hattori et al., 2003, (3) Addington et al., 2004, (4) Chen et al., 2004, (5) Hall, Gogos, and Karayiorgou, 2004, (6) Korostishevsky et al., 2004, (7) Schumacher et al., 2004, (8) Wang et al., 2004, (9) Mulle et al., 2005, (10) Fallin et al., 2005, (11) Zou et al., 2005, (12) Ma et al., 2006, (13) Goldberg et al., 2006, (14) Williams et al., 2006, (15) Liu et al., 2006, (16): Hong et al., 2006, (17) Yue et al., 2006

[a]Summarized data of SNPs with either individual allelic or haplotypic association

[b]Number of cases (case-control design) or trios (family-based transmission/disequilibrium test)

[c]Schizophrenia

[d]Bipolar disorder

[e]Schizophrenia and psychosis not otherwise specified

[f]Cognition related to SZ, including N back working memory, continuous performance, and verbal paired associate learning

[g]Working memory or episodic memory examined with BOLD fMRI

[h]SZ or BD patients with lifetime co-occurrence of one or more episodes of major mood disorder (mania or depression)

[i]M-, SNP notation by Chumakov et al., 2002

[j]Associated allele/genotype with *P* value

[k]Not significant at $P < 0.05$

[l]Including 169 trios and 64 extended families

Table 2.1-2

Meta-analysis of association studies between *DAOA* and schizophrenia under fixed-effects model

	Number of			rs3916965 (G)[a]		rs2391191 (A)		rs778293 (A)		rs3918342 (T)		rs1421292 (T)	
Study	Cases	Controls	Ethnicity	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI
Chumakov et al.	213	241	European	1.46	1.12–1.91	0.74	0.57–0.97	1.48	1.13–1.95	1.38	1.06–1.79	1.38	1.06–1.79
Chumakov et al.	183	183	European	0.92	0.69–1.24	–	–	1.09	0.81–1.48	1.44	1.07–1.93	1.27	0.95–1.70
Korostishevsky et al.	60	130	European	1.02	0.65–1.58	0.77	0.49–1.20	1.52	0.98–2.36	2.16	1.39–3.37	–	–
Schumacher et al.	299	300	European	0.81	0.64–1.02	1.24	0.98–1.56	–	–	0.76	0.60–0.95	1.27	1.01–1.60
Wang et al.	537	538	Asian	0.81	0.68–0.96	1.33	1.12–1.58	0.88	0.74–1.04	1.15	0.96–1.35	–	–
Ma et al.	588	588	Asian	1.01	0.86–1.20	1.01	0.86–1.20	0.76	0.64–0.90	0.89	0.76–1.05	–	–
Ma et al.	183	182	European	0.83	0.61–1.13	1.10	0.81–1.49	0.71	0.53–0.95	0.59	0.44–0.80	–	–
Williams et al.	709	1416	European	1.08	0.95–1.23	0.93	0.81–1.06	1.06	0.93–1.21	–	–	0.97	0.85–1.10
Yue et al.	216	321	Asian	–	–	1.11	0.87–1.43	–	–	–	–	–	–
Addington et al.	98 families	Mixed	–	–	1.80	1.04–3.11	1.19	0.74–1.92	–	–	–	–	
Mulle et al.	159 families	Mixed	1.00	0.71–1.40	0.91	0.65–1.28	0.80	0.57–1.12	1.03	0.72–1.47	1.03	0.73–1.46	
Zou et al.	233 families	Asian	0.65	0.49–0.85	0.71	0.54–0.92	–	–	–	–	–	–	
Liu et al.	218 families	Asian	0.84	0.57–1.24	1.92	1.57–2.35	1.82	1.49–2.22	1.15	0.95–1.39	–	–	
$\text{Pooled}_{\text{All}}$	2772	3578	All	0.96	0.90–1.03	1.09	1.02–1.16	1.05	0.97–1.12	1.03	0.96–1.12	1.11	1.00–1.22
Significance				$Z^b = 1.15$	$P_Z = 0.25$	$Z = 2.64$	$P_Z = 0.008$	$Z = 1.22$	$P_Z = 0.22$	$Z = 0.83$	$P_Z = 0.41$	$Z = 2.03$	$P_Z = 0.04$
Heterogeneity				$Q^c = 24.55$	$P_Q = 0.006$	$Q = 67.36$	$P_Q < 0.0001$	$Q = 65.78$	$P_Q < 0.0001$	$Q = 46.09$	$P_Q < 0.0001$	$Q = 8.70$	$P_Q = 0.07$
$\text{Pooled}_{\text{Asian}}$	1125	1,126	Asian	0.89	0.80–0.98	1.28	1.16–1.40	1.05	0.95–1.16	1.07	0.97–1.17	–	–
Significance				$Z = 2.28$	$P_Z = 0.02$	$Z = 5.15$	$P_Z < 0.0001$	$Z = 0.91$	$P_Z = 0.37$	$Z = 1.37$	$P_Z = 0.17$	–	–
Heterogeneity				$Q = 7.40$	$P_Q = 0.12$	$Q = 36.09$	$P_Q < 0.0001$	$Q = 50.28$	$P_Q < 0.0001$	$Q = 15.30$	$P_Q = 0.004$	–	–
$\text{Pooled}_{\text{European}}$	1647	1,036	European	1.02	0.93–1.13	0.96	0.87–1.06	1.08	0.98–1.20	1.03	0.91–1.17	1.11	1.01–1.23
Significance				$Z = 0.53$	$P_Z = 0.60$	$Z = 0.82$	$P_Z = 0.41$	$Z = 1.52$	$P_Z = 0.13$	$Z = 0.48$	$P_Z = 0.63$	$Z = 2.07$	$P_Z = 0.04$

[a]The risk allele reported in the first study by Chumakov et al.

[b]Z test was used to determine the significance of the overall odds ratio; $P_Z < 0.05$ are in bold

[c]Cochran's Q test was used to assess the heterogeneity between samples; $P_Q < 0.1$ indicates significant heterogeneity

Table 2.1-3

Meta-analysis of association studies between *DAOA* and bipolar disorder

	Number of			rs1935058 (C)[a]		Rs1341402 (T)		rs2391191 (A)		Rs1935062 (A)		rs778294 (G)	
Study	Cases	Controls	Ethnicity	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI
Chen et al.	139	113	Caucasian	0.96	0.67–1.36	–	–	0.81	0.57–1.16	1.70	1.18–2.46	–	–
Schumacher et al.	300	300	Caucasian	–	–	1.12	0.86–1.45	1.14	0.90–1.44	1.05	0.82–1.33	1.16	0.90–1.48
Williams et al.	706	1,416	Caucasian	0.93	0.82–1.06	0.84	0.72–0.97	0.89	0.78–1.01	–	–	0.93	0.81–1.07
Hattori et al.	174 families[b]	Caucasian	2.22	0.44–0.96	1.54	1.04–2.28	1.62	1.10–2.38	0.57	0.38–0.84	1.84	1.23–2.74	
Pooled				1.02	0.90–1.15	0.95	0.84–1.08	0.97	0.87–1.08	1.03	0.86–1.23	1.03	0.92–1.16
Significance				$Z^c = 0.28$	$P_Z = 0.78$	$Z = 0.77$	$P_Z = 0.44$	$Z = 0.55$	$P_Z = 0.58$	Z = 0.29	$P_Z = 0.77$	$Z = 0.54$	$P_Z = 0.59$
Heterogeneity				$Q^d = 16.97$	$P_Q = 0.0002$	$Q = 9.71$	$P_Q = 0.008$	$Q = 10.93$	$P_Q = 0.012$	$Q = 15.93$	$P_Q = 0.0003$	$Q = 11.17$	$P_Q = 0.0038$

[a]The risk allele reported in the first study by Hattori et al.

[b]Combined sample

[c]*Z* test was used to determine the significance of the overall odds ratio

[d]Cochran's *Q* test was used to assess the heterogeneity between samples; $P_Q < 0.10$ indicates significant heterogeneity

We also noticed that 11 out of 28 sample sets showed significant association for different haplotypes, using global *P* values, as evidence of association (*Table 2.1-1*). One possibility is that multiple causal variants are contained in different haplotypes in different populations or subpopulations. Another possibility is that a single disease mutation has occurred on multiple haplotypic backgrounds. This could contribute to the allelic heterogeneity that we observed in the meta-analysis.

In summary, different SNPs and haplotypes in the *G72/G30* region showed BD and/or SZ association in different studies. Strong between-study heterogeneities were observed for almost all the five SNPs that were analyzed in the meta-analysis. All these data suggest potential strong allelic heterogeneity at the *G72/G30* locus. As usual, the conclusions from meta-analyses must be read with caution because of possible publication bias toward positive reports.

3 Resequencing the *G72/G30* Region May Identify Potential Rare Susceptibility Alleles

The published association studies of the *G72/G30* region focus on the Common Disease/Common Variants (CD/CV) hypothesis (Lander, 1996; Cargill et al., 1999; Chakravarti, 1999). This hypothesis predicts that one or a few predominant susceptibility alleles account for most of the disease-causing variation at each gene underlying a common disease (Reich and Lander, 2001). Reich and Lander (Reich and Lander, 2001) modeled the modern allelic spectrum at a disease locus assuming a low mutation rate and a very recent large expansion in the human population. They concluded that a gene contributing to a common disease would generally have a single ancestral disease allele at a common frequency prior to the expansion, resulting in the disease allele being frequent among current patients (e.g., minor allele frequency (MAF) > 0.1), and contrfibuting to the most risk for disease. There has not been sufficient time since the expansion for a rare disease allele to become frequent in the population or a common allele to become infrequent, unless it has been subject to severe selection. This gives theoretical support to the CD/CV model, and has received genetic epidemiological support by multiple meta-analyses of genetic association data as reviewed elsewhere (Lohmueller et al., 2003; Bertram et al., 2007).

Pritchard et al. (Pritchard, 2001; Pritchard and Cox, 2002) proposed an alternative, but not mutually exclusive Common Disease/Multiple Rare Variants (CD/MRV) hypothesis. They proposed that under conditions of higher, yet plausible, mutation rates and moderate "purifying" selection, considerable allelic and locus heterogeneity could be generated. These alleles may have a total frequency at intermediate effects, which could include many mutations at low frequencies and several variants at intermediate to common frequencies. Considerable allelic heterogeneity can be expected in a gene for a common disease, which leads to several implications for detecting variants conferring susceptibility. First, allelic heterogeneity will considerably reduce the power of current association methods, which test whether a common SNP or haplotype is found in cases more often than controls. Association methods using haplotypes may not be powerful enough to identify rare variants, because most susceptibility alleles would have been generated on different haplotypes through new mutations, and the haplotypes would cancel each other's signals. Second, a candidate gene approach for an association study is looking for a different class of variants (rare alleles), than an approach looking for variants underlying a significant linkage signal (alleles at intermediate frequency) (Pritchard and Cox, 2002). It logically follows that candidate gene studies need to perform extensive sequencing in large number of individuals to identify low-frequency variants that may contribute to disease liability.

On the basis of a similar model, Lin et al. (Lin et al., 2004) proposed that long haplotypes constructed from common polymorphisms spanning a large genetic distance could be used to detect association with rare variants, since rare mutations are much younger in age and would result in long tracts of linkage disequilibrium around recent mutations. Common polymorphisms derived from the HapMap project (http://www.hapmap.org), with MAF ≥ 0.05 are the basis of current proposed genome-wide association platforms, such as the Affymetrix 500K, Illumina 317K, and 550K SNP genotyping panels. The power of such a data set to detect association with lower frequency disease alleles is modest (Wang et al., 2005; Zeggini et al., 2005). Analysis of common polymorphisms for association with rare alleles requires

exhaustive analysis of all possible haplotypes over a given distance (Lin et al., 2004). Zeggini (Zeggini et al., 2005) has shown, however, that it is not statistically efficient to screen for association with rare variants using common haplotypes, and that direct identification of the rare variants by resequencing prior to association testing is statistically more efficient. For biological investigation of the function of such variants, resequencing would also be required.

Recently, the CD/MRV hypothesis has received experimental support by the discovery of multiple rare variants in a few genes associated with several common disorders/quantity traits, including colorectal ademonas (Fearnhead et al., 2004) and low plasma high-density lipoprotein levels (Cohen et al., 2004, 2006), and MRV in single gene such as angiotensinogen levels with its coding gene *AGT* (Zhu et al., 2005) and bipolar disorder with the solute carrier family 12, member 6 gene *SLC12A6* (also named *KCC3*) (Meyer et al., 2005). Thus, the CD/MRV hypothesis may require a design reorientation toward extensive resequencing for fine mapping studies of susceptibility to common disease.

It is reasonable to resequence all exonic fragments of all alternative splicing forms in the *G72* gene in hundreds of unrelated patients with SZ or BD and a comparative number of unrelated controls.

4 Biological Studies of G72/G30

Apart from the BD and SZ diagnoses, the human phenotypes associated with *G72*/*G30* are not well delineated. Goldberg et al. (2006) found that *G72* SNPs increased risk of cognitive impairment in SZ.

G72 and *G30* are very low abundance transcripts in most human tissues (Hattori et al., 2003). We performed *G72* and *G30* expression study in cDNAs from 16 human tissues (heart, whole brain, placenta, lung, whole liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral leukocytes, and placenta), fetal brain, five brain subregions (amygdala, substantia nigra, thalamus, cerebral cortex, hippocampus), and RNA from human fetal brain, adult whole brain. By using RT-PCR and TaqMan real-time quantification PCR, we can only detect *G72* expression at very low level in human testis and brain. No *G30* expression can be detected in all human tissues. Northern blot and dot blot of *G72* gave no signal in multiple human tissues in our experiments (data not shown). Bioinformatics analyses also support that *G72* and *G30* are low abundance genes. No *G72* or *G30* expression sequence tags (ESTs) were found in any known cDNA libraries. The Stanley Medical Research Institute Online Genomics Database (https://216.55.162.172/) contains microarray data from multiple platforms on samples of SZ, BD, and normal control brain tissues. No *G72* probes give hybridization signal in the database. Similarly, Gene Expression Omnibus (GEO) database analysis (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gds) indicated that no *G72* hybridization signals were found in studies of brain or neuron cell lines. But, GEO data revealed relatively high expression of *G72* in various prostate cell types, normal endometrium throughout the menstrual cycle, CD34+ cell, breast normal and cancer tissues, papillary renal cell carcinoma, normal and tumor thyroid, head and neck squamous cell carcinoma. We further confirmed the relatively high G72 expression by real-time PCR in breast cancer cell lines. The biological implication of low abundance transcripts in brain but relatively high in a few other apparently mental-unrelated tissue types is not clear.

Low abundance may contribute to the variability of splice variants observed in different PCR clone experiments. *G72* has numerous splicing variants in human tissues. Chumakov (Chumakov et al., 2002) reported two alternative splicing forms of *G72*: the longest possible coding form *G72* (*LG72*) (Genbank accession number: NM_172370; AY138546) expressed in amygdale, caudate nucleus, spinal cord, and testis. It encodes a 153-amino acid (aa) protein referred to as *pLG72*. Another short *G72* variant (*SG72*, AY138547) that encodes a 16-aa peptide, was detected in testis, different parts of brain, and spinal cord. In the following study, our collaborators (Hattori et al., 2003) characterized several additional splicing variants of *G72* by RACE PCR, including three new alternative splicing forms (AY170469, AY170470, AY170471) from human testis and one from adult/fetal brain and amygdala (AY223901). Thereafter, we identify four new alternative splicing forms of *G72* from human, including two from testis (DQ386869 and DQ386870), two from brain (one from substantia nigra and amygdale (DQ343761), and one from adult whole brain (DQ357223)). Different splice variants defined a total of nine exons.

G72 is a primate-specific gene. Transcripts of *G72* have been demonstrated only in primates (Chumakov et al., 2002). Although, all exons of *G72* have homologs in other primate species, they contain stop codons or mutated splice sites in nonhumans. Therefore, if they are active, *G72* orthologs in other primates could encode much shorter and significantly different proteins than what could be predicted in humans. *G72* could represent a rare case of a primate-specific gene with a rapidly changing protein structure presumably connected with a rapid evolution of underlying brain function (Johnson et al., 2001).

No homolog could be found for the putative G72 and G30 proteins. *G72* is a novel gene with no recognizable functional motifs in protein sequence. Chumakov (Chumakov et al., 2002) reported an in vitro artificial transcription and translation assay of pLG72, but not G30 protein, suggesting that only the *G72* gene can be artificially translated. Transient transfection of COS-7 cells with *pLG72* gene showed that pLG72 is mainly localized in the Golgi apparatus. Molla (Molla et al., 2006) reported overexpression of wild-type and His-tagged pLG72 in Escherichia coli. Both variants form inclusion bodies and have been refolded and purified to homogeneity. Far-UV CD spectra and secondary structure prediction suggest a high content of alpha-helices for pLG72, a structural feature compatible with an intrinsic membrane protein. So, pLG72 could undergo posttranslational modifications in Golgi apparatus (e.g., glycosylation) that could increase its solubility in vivo.

5 DAOA–DAAO–NMDA Hypothesis

Chumakov reported yeast two-hybrid screening using the carboxyterminal 89-aa peptide of LG72 as "bait," which captured a clone encoding DAAO (Chumakov et al., 2002). DAAO is an important modulator of *N*-methyl D-aspartate (NMDA) receptors via regulation of D-serine levels. Physical binding between LG72 and DAAO was confirmed in vitro by column binding and glutathione *S*-transferase pull down. Further, it was shown that addition of increasing amounts of pLG72 stimulated an increase in the oxidation of D-serine by DAAO. Chumakov's study suggested the participation of pLG72 in the regulation of NMDA-type glutamate receptors in human brain. Since DAAO can oxidize D-serine, an endogenous ligand of the NMDA-type glutamate receptor, if some individuals were to overproduce pLG72, they could exhibit a lower NMDA-type glutamate receptor activity predisposing them to schizophrenia, which could result in glutamate signaling hypofunction, a mechanism proposed in schizophrenia (Carlsson et al., 1999; Mohn et al., 1999; Javitt, 2001).

However, the hypothesis about the DAOA–DAAO–NMDA hypothesis remains questionable. After observing all the transcripts, we still cannot determine the dominant *G72* splicing form in human tissues because of the low abundance. We did not observe the "*LG72*" splicing form in our lab so far, while we have obtained many others. The potential encoded protein products can vary significantly depending on which exons are included. Therefore, the validity of the artificial translation of *LG72* used in the study by Chumakov et al. remains unclear. The yeast two-hybridization results using natural G72 protein are to be confirmed. No endogenous G72 protein expression can be confirmed in human brain so far. No data shows the native G72 and G30 protein in human brain tissue or other nontransfected cells. Thus, the DAOA–DAAO–NMDA hypothesis that established on a peptide fragment of LG72 requires further evidence. It is speculated that *G72* and *G30* may even be noncoding RNA genes.

6 Future Directions for *G72/G30* Study

Despite multiple association studies, no systematic analysis of *G72* gene region has been performed in SZ or BD. For example, only 1–19 different markers in the 220-kb region have been published in different studies. Using recently released HapMap genotype data with MAF > 0.1 in Caucasian, Tagger in the Haploview program (http://www.broad.mit.edu/mpg/haploview/) picked up 99-tag SNPs with pairwise $r^2 > 0.85$. It is necessary to test the variant(s) under both CD/CV and CD/MRV models consistently in different sample sets to obtain comparable results. Data sharing is a major obstacle in the fields preventing thorough use of the information. Test of the CD/MRV model in neuropsychiatric diseases is in its infancy stage. Sample size

for achieving appropriate statistical power, high-throughput sequencing technologies, and efficient statistical methods will be needed for the CD/MRV approach. Other genetic variants such as copy number variation in the *G72* region should be investigated (Feuk et al., 2006; Redon et al., 2006). In addition, potential functional variants in the *G72* gene region need to be identified. When more is known about the biology of the *G72* gene, its interaction with other gene(s) or environmental risk factors deserves investigation for susceptibility to psychiatric diseases such as SZ and BD. Endophenotype is thought to be very important in psychiatric genetics because it may help to distinguish genetic homogeneous subgroups of patients and thus provide greater statistical power for identifying vulnerability genes (Gottesman and Gould, 2003; Meyer-Lindenberg and Weinberger, 2006). More association studies of *G72* with endophenotypes particularly psychosis as suggested by linkage analyses (Potash et al., 2003, 2004; Goes et al., 2007) are expected. It may facilitate to identify susceptibility genes (alleles) (Goldberg et al., 2006; Williams et al., 2006). More biological studies will be needed to clarify whether *G72* is a real protein-coding gene and which biological pathway it impacts or involves in the pathology of psychiatric diseases.

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2.2 Dysbindin-1 and Its Protein Family

With Special Attention to the Potential Role of Dysbindin-1 in Neuronal Functions and the Pathophysiology of Schizophrenia

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_2.2,

Abstract: Variation in the gene encoding dysbindin-1 (i.e., dystrobrevin-binding protein 1: DTNBP1) has frequently been associated with schizophrenia. Several studies have also found that dysbindin-1 gene and protein expression are altered in two affected brain areas in that disorder (i.e., the dorsolateral prefrontal cortex and the hippocampal formation). To provide the context and information needed for further study of these phenomena, this chapter provides the first comprehensive review of the dysbindin protein family. The family has three paralogs (dysbindin-1, -2, and -3), each of which is encoded by a different gene and expressed in more than one isoform. There are at least eight family members in humans (dysbindin-1A, -1B, -1C, -2A, -2B, -2C, -3A, and -3B).

Dysbindin-1 is distinguished from other paralogs of the dysbindin family by the presence of a coiled coil domain important in interactions with other proteins. We focus on this paralog since it is the only one associated with schizophrenia thus far. Its gene, DTNBP1, has three major transcripts encoding isoforms running on western blots at approximately 50, 37, and 33 kDa (=dysbindin-1A, -1B, and -1C, respectively). The ~37 kDa isoform cannot be studied in mice, which appear to lack an ortholog of human dysbindin-1B. While present in neuronal cell bodies throughout the central nervous system, dysbindin-1 is prominently enriched only in certain synaptic fields, mainly those known to be dopaminergic, glutamatergic, and/or GABAergic. In synaptic tissue of the human brain, dysbindin-1A is mainly concentrated in postsynaptic density fractions, dysbindin-1B in synaptic vesicle fractions, and dysbindin-1C in both those fractions. It is unknown if the isoforms differ in binding partners, but they are collectively known to bind a large number of proteins, including several proteins belonging to the biogenesis of lysosome-related organelles complex 1 (BLOC-1).

An animal model of dysbindin-1's functions is available in the sandy (sdy) mouse, which has a naturally occurring deletion mutation in DTNBP1 that leads to loss of dysbindin-1 in homozygous mice, which also shows a loss or reduction in other BLOC-1 binding partners. Among the many abnormalities of homozygous sdy mice are increased dopamine transmission in limbic tissue, decreased glutamate release and NMDA-mediated postsynaptic currents in prefrontal cortex, smaller excitatory evoked responses and loss of inhibitory responses after stimulation in the hippocampal formation, and severe deficits in spatial learning and memory processes. While the homozygous sdy mouse shares behavioral and biological features of schizophrenia, it is currently unclear if it serves an animal model of that disorder. It may, however, model cognitive aspects of schizophrenia.

Dysbindin-1 may have diverse functions. Among these are the promotion of cell growth and proliferation, protection against neuronal apoptosis, facilitation of axon, dendrite, and dendritic spine growth, regulation of AP-3 cargo transport to lysosome-related organelles (including reserve pool synaptic vesicles), facilitation of glutamate release and inhibition of dopamine release, regulation of constitutive D2R cell surface expression, and promotion of cognitive processes.

While the association between genetic variation in DTNBP1 and schizophrenia has been questioned recently, there is mounting evidence that the associations found reflect actual susceptibility variants in the gene. Even in the absence of such variants, altered dysbindin-1 gene and protein expression have been found in the dorsolateral prefrontal cortex and hippocampal formation of schizophrenia cases. These changes may contribute to the pathophysiology of the disorder by altering brain development, dopaminergic and glutamatergic transmission, functional connectivity of neuronal populations in the cerebral cortical and the hippocampal formation, and cognition.

List of Anatomical Abbreviations: Ac, anterior commissure; AC, auditory cortex; AHA, anterior hypothalamic area; AM, amygdala; AON, anterior olfactory nucleus; AT, axon terminal; BG, basal ganglia; BLA, basolateral amygdala; BMA, basomedial amygdala; Br, brain; BST, bed nucleus of the stria terminalis; CA, central amygdala; CA1, cornu ammonis (hippocampus) field 1; CA3, cornu ammonis field 3; Cbl Ctx, cerebellar cortex; cc, corpus callosum; cERC, caudal entorhinal cortex; CG, central gray; CgC, cingulate cortex; CPu, caudate-putamen complex (striatum); D, dendrite; DCN, deep cerebellar nuclei; DG, dentate gyrus; DGg, dentate gyrus granule cell layer; DGh, dentate gyrus hilus (= polymorph layer); DGiml, dentate gyrus inner molecular layer; dhc, dorsal hippocampal commissure; Dk, darkschewitsch nucleus; DLPFC, dorsolateral prefrontal cortex; ERC, entorhinal cortex; f, fornix; fi, fimbria; FWM, frontal cortex white matter; G, granule cell layer; Gi, gigantocellular reticular formation; GPe, globus pallidus, external segment;

GPi, globus pallidus internal segment; H, heart; Hi, hilus of dentate gyrus; HF, hippocampal formation; IML, inner molecular layer of dentate gyrus; ION, inferior olivary nucleus; K, kidney; L, stratum lucidum of CA2 and CA3; Li, liver; LA, lateral amygdala; LH, lateral hypothalamic area; LP, lateral posterior thalamic nucleus; LS, lateral septal nucleus; Lu, lung; M, molecular layer; MA, medial amygdala; MC, motor cortex; MD, mediodorsal nucleus; MG, medial geniculate; MH, medial habenular nucleus; MmN, mammillary nucleus; MPA, medial preoptic area; Mt, mitochondrion; Mu, muscle (skeletal); NAc, nucleus accumbens; O, stratum oriens; OFC, orbitofrontal cortex; OML, outer molecular layer of dentate gyrus; ot, optic tract; OT, olfactory tubercle; P, purkinje cell layer of cerebellar cortex; P, stratum pyramidal; PLC, prelimbic cortex; pc, posterior commissure; PC, piriform cortex; PCA, posterior cortical amygdaloid nucleus; pp, perforant path; Pr, prepositus nucleus; PSD, postsynaptic density; Put, putamen; Pv, parvocellular medullary reticular formation; PVH, paraventricular hypothalamic nucleus; pyr, pyramidal tract of medulla; R, stratum radiatum; Re, reuniens nucleus; rERC, rostral entorhinal cortex; RF, reticular formation; RN, red nucleus; RSC, retrosplenial cortex; SC, schaffer collaterals; SCo, superior colliculus; SFi, septofimbrial nucleus; SG, substantia gelatinosa; Sk, skin; SI, small intestine; SNc, substantia nigra, pars compacta; SNl, substantia nigra, pars lateralis; SNr, substantia nigra, pars reticulata; soc, supraoptic commissure; Sol, solitary nucleus; Sp, dendritic spine; Spl, spleen; SpV, spinal nucleus of trigeminal nerve; Ssc, somatosensory cortex; St, stomach; str, stria terminalis; Sub, subiculum; SuM, supramammillary nucleus; SV, synaptic vesicle; ta, temporoammonic tract; Te, testes; Th, thymus; TL, temporal lobe; VMH, ventromedial hypothalamic area; VN, vestibular nuclear complex; VP, ventral pallidum; VPN, ventroposterior thalamic nuclei; VTA, ventral tegmental area; XII Nuc, 12th cranial nucleus (hypoglossal nucleus)

1 Introduction

The first member of the dysbindin protein family, dysbindin-1, was discovered in the search for protein interactions whose disruption may contribute to cognitive deficits in patients with Duchenne and Becker muscular dystrophy (DMD and BMD, respectively). Between 18-63% of DMD cases and 3-25% of BMD cases exhibit mild, nonprogressive deficits in tasks dependent on verbal working memory (cf. Rapaport et al., 1991, Billard et al., 1992; Nicholson et al., 1993; Bardoni et al., 1999, and Kumagai et al., 2001; D'Angelo and Bresolin, 2006). DMD and BMD are caused by mutations in the gene encoding dystrophin (Blake et al., 2002; Darras et al., 2003), resulting in loss of full-length dystrophin (Dp427) in DMD and usually in reduced and/or abnormal Dp427 in BMD (Darras et al., 2003). The consequences are debilitating because dystrophin is the core component of the dystrophin glycoprotein complex (DGC), which is important in stabilizing muscle membranes and in transducing various extracellular signals to the cytoskeleton of muscle cells (Biggar et al., 2002; Blake et al., 2002; D'Angelo and Bresolin, 2006). DGCs containing Dp427 are present not only in muscle, but also in the brain (Blake et al., 1999; Brünig et al., 2002; Haenggi and Fritschy, 2006). Most of their component proteins, one of which is dystrobrevin, are concentrated in postsynaptic densities (PSDs) of the cerebral cortex, hippocampal formation, and cerebellum (Lidov et al., 1990; Kim et al., 1992; 1995; Blake et al., 1999; Brünig et al., 2002). Brain DGCs containing Dp427 are associated with GABAergic (Knuesel et al., 1999; Brünig et al., 2002) and probably also with glutamatergic (Haenggi and Fritschy, 2006) synapses, both of which play important roles in cognition (Chapouthier and Venault, 2002; Myhrer, 2003; Riedel et al., 2003).

Disruption of brain DGCs in DMD and BMD might thus contribute to cognitive deficits in those disorders, consistent with learning and memory deficits in mouse models of DMD (Vaillend et al., 2004), though the deficits in such models are less severe than in DMD itself (D'Angelo and Bresolin, 2006). As demonstrated by Derek Blake and his colleagues, postsynaptic DGCs differ from muscle and glial DGCs in the type of dystrobrevin they contain. Muscle and glial cells only express α-dytrobrevin, whereas nerve cells in the adult brain express only β-dystrobrevin (Blake et al., 1998, 1999; Reese et al., 2007). Like other DGC components in the brain, β-dystrobrevin is concentrated in PSDs and, unlike α-dystrobrevin, binds full-length brain dystrophin (Blake et al., 1999). Identifying new binding β-dystrobrevin binding partners in the brain might, then, suggest how disruption of postsynaptic DGCs in DMD and BMD contribute to cognitive deficits in those disorders. To find novel candidates for β-dystrobrevin binding partners, Matthew Benson in Derek Blake's laboratory performed a yeast two-hybrid screen of cDNA libraries for the mouse brain and

H2K myotubes with the entire β-dystrobrevin coding region as bait. In 1999, the screen identified four clones from the brain cDNA library and nine clones from the muscle cDNA library that derived from the same gene, which was subsequently named dystrobrevin binding protein 1 (DTNBP1) by the Human Genome Organization (HUGO). The protein it encodes was named "dysbindin" by Benson (2001). We refer to it as dysbindin-1, because it was just the first characterized member of a novel protein family. The name "dysbindin-1" is preferable to "DTNBP1" used as a synonym in the literature, because the protein in question may not actually bind dystrobrevins in vivo (Nazarian et al., 2006) and was not the first dystrobrevin binding partner known. We use "DTNBP1" only to designate the encoding gene, a practice that is too well established to change.

In the same year dysbindin-1 was discovered, 1999, Richard Straub and his colleagues discovered that certain single nucleotide polymorphisms (SNPs) in DTNBP1 and combinations of them (haplotypes) were significantly associated with schizophrenia. When published in 2002, the discovery drew considerable attention as the first genetic susceptibility locus for schizophrenia identified by positional cloning (Straub et al., 2002). While there have been negative studies (e.g., Peters et al., 2008; Sanders et al., 2008; Sullivan et al., 2008), many other studies on diverse populations also found genetic variation in DTNBP1 to be associated with schizophrenia (e.g., Williams et al., 2005; Duan et al., 2007; Vilella et al., 2008), as well as with bipolar disorder (Fallin et al., 2005; Breen et al., 2006; Joo et al., 2007; Pae et al., 2007b). Indeed, two recent analyses conclude that DTNBP1 remains among the top candidates for genes affecting risk of the disorder (Allen et al., 2008; Sun et al., 2008). Interest in dysbindin itself increased with the subsequent discovery that levels of DTNBP1 gene and protein expression were reduced in brains of schizophrenia cases (cf. Straub et al., 2004; Talbot et al., 2004; Tang et al., 2009 and Weickert et al., 2004, 2008).

In 2003, Straub presented evidence that SNPs in DTNBP1 associated with schizophrenia are also associated with impaired cognitive performance in those cases (Straub et al., 2003). Though recently contested (Peters et al., 2008), later reports have confirmed that there are SNPs in DTNBP1 associated with impaired cognition in schizophrenia populations (Fanous et al., 2005; Burdick et al., 2007; Donohoe et al., 2007; Zinkstock et al., 2007). Even those who are simply carriers of such SNPs without overt symptoms of schizophrenia show cognitive deficits in comparison to non-carriers (Burdick et al., 2006; Fallgatter et al., 2006; Luciano et al., 2009). It would appear, then, that dysbindin is involved in the cognitive impairments which have been recognized as a core feature of schizophrenia by an increasing number of investigators (e.g., Elvevåg and Goldberg, 2000; Barch, 2005; Kraus and Keefe, 2007).

Given its potential roles in cognition and psychosis, interest in the biology of dysbindin continues to increase. In the few years since its discovery, much has been learned about this novel protein. Yet there has been no comprehensive analytical review of dysbindin, which is a necessary aid in generating informed hypotheses about the functions of this novel protein and its potential contributions to the pathophysiology of schizophrenia. This chapter provides such a review based not only on published literature, but on relevant data available in bioinformatic databases.

2 Members of the Dysbindin Protein Family

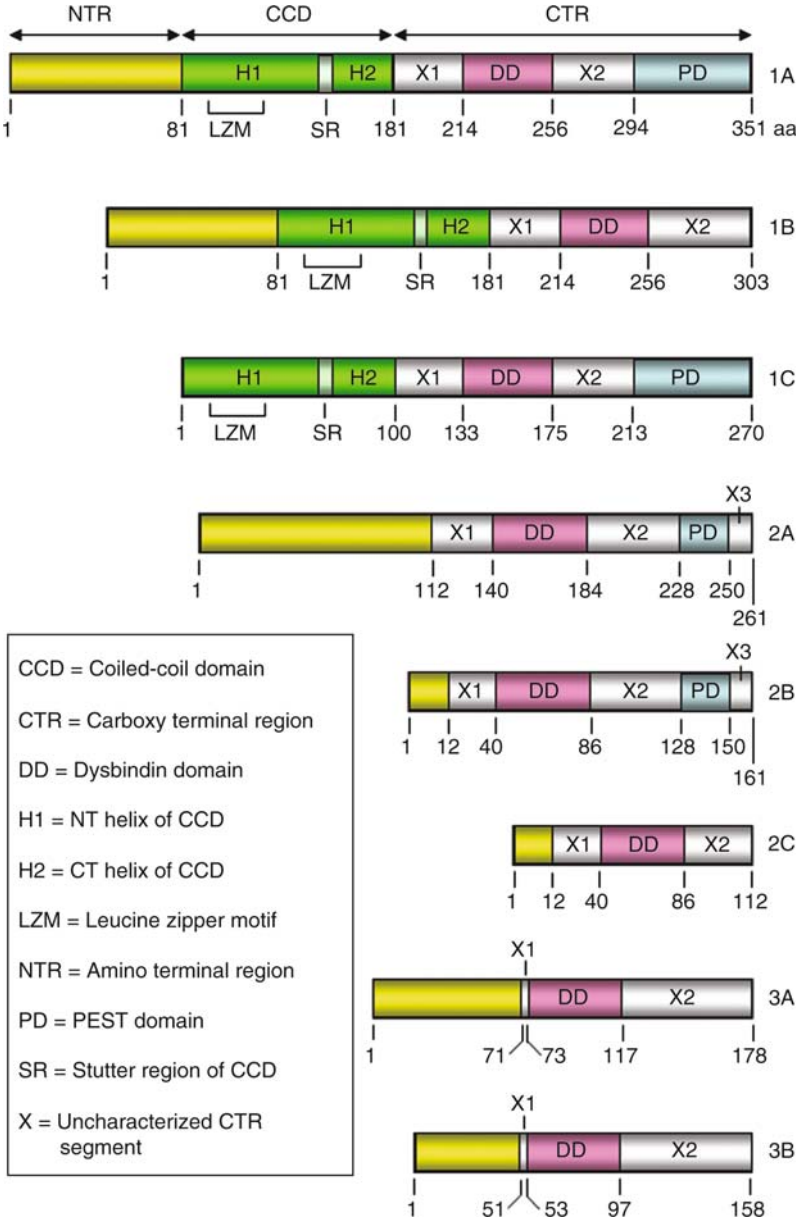
The protein originally named dysbindin belongs to a family of animal proteins sharing a region of significant sequence homology called the dysbindin domain (DD). The family is a group of evolutionarily conserved proteins of relatively small size (112–362 amino in length) expressed widely in the body, including the central nervous system (CNS). Their amino acid (aa) sequences suggest that they are neither secreted nor integral membrane proteins, but are rather cytosolic and often nuclear proteins associating secondarily with membranes (see *Section 2.2.4.2*).

2.1 Family Membership Criteria

More members of the dysbindin protein family are known in humans than in any other species at present. In articular, there are eight human proteins with dysbindin domains listed by the National Center for Biotechnology Information (NCBI: http://www.ncbi.nlm.nih.gov/) and/or by the Universal Protein Resource (UniProt: http://www.pir.uniprot.org/index.shtml). They are schematically shown in *Figure 2.2-1*, which

Figure 2.2-1

Dysbindin family paralogs and isoforms known in humans. See text *Section 2.2.3* for explanation of segmentation of the proteins into an amino terminal region (NTR), coiled-coil domain (CCD) divided into two helices (H1 and H2), and a carboxy terminal region (CTR) consisting of the dysbindin domain (DD), PEST domain (PD), and uncharacterized areas X1–X3. Dysbindin-1B is not found in the mouse



indicates our nomenclature for them and for their structural components. *Table 2.2-1* provides basic information about each protein and their orthologs in other species. The location of the dysbindin domain in human, mouse, rat, and frog proteins is specified in *Table 2.2-2*.

Both of the bioinformatic databases mentioned above list more proteins as dysbindin family members than we recognize in *Table 2.2-1*. We limit the family to those proteins that (1) share significant aa sequence homology with human full-length dysbindin (accession number Q96EV8 in NCBI and UniProt), (2) are not simply hypothetical or fragmentary proteins, and (3) have known and complete mRNA coding sequences indicating tissue expression of complete proteins. Such criteria lead us to exclude several proteins identified by NCBI and/or UniProt as dysbindin family proteins. One is the mouse protein AAH46622 (NCBI, listed by UniProt as Q80XJ9), which is not significantly related in aa sequence to human (or mouse) full-length dysbindin. We also exclude the mouse protein Q91WZ8–2 (UniProt) for which there is no identified transcript and may be an incomplete protein as explained below (*Section 2.2.2.2.1*). Other proteins are only regarded here as candidate dysbindin family members until their transcripts are identified, namely the puffer fish (*Tetraodon nigroviridis*) proteins Q4S9B5, Q4SIK3, and Q4SS33 (NCBI and UniProt).

2.2 Paralogs and Their Isoforms

The dysbindin protein family in humans, mice, and rats includes at least three paralogs designated as dysbindin-1, -2, and -3, which are all expressed in the brain (Benson et al., in preparation). As summarized here, each paralog differs from the others in its encoding gene (*Table 2.2-1*), secondary structure, predicted location and types of phosphorylation sites, and sometimes cellular location in the nervous system. At this early stage in research on the dysbindin protein family, identification of its paralogs and their isoforms derives from discovery of mRNAs encoding them as specified in *Table 2.2-1*. The aa sequences of dysbindin proteins are thus extrapolations from the mRNA nucleotide sequences.

2.2.1 Dysbindin-1 (=DTNBP1 Protein)

2.2.1.1 Basic Information Dysbindin-1 is encoded by the DTNBP1 gene at chromosome locus 6p22.3 in humans, 13 A5 in mice, and 17 in rats. It has been found in both invertebrates and all vertebrates studied to date (*Table 2.2-1*). As detailed below (see *Section 2.2.6.3.2*), its localization in the brain is mainly, if not exclusively, neuronal. The recent claim that it is located in astroglial endfeet (Iijima et al., 2009) is questionable, because the specificity of the dysbindin-1 antibody used was not established. While dysbindin-1 can belong to several protein complexes, it is best established as a member of BLOC-1 (biogenesis of lysosome-related organelles complex) as described later in this section.

As illustrated in *Figure 2.2-1*, dysbindin-1 consists of an amino terminal region (NTR) of variable length, a coiled coil domain (CCD) not found in other dysbindin paralogs, and a relatively lengthy carboxy terminal region (CTR). This region consists of the DD (*Table 2.2-2*), two uncharacterized CTR segments (X1 and X2), and in some isoforms a terminal PEST domain (PD, *Table 2.2-3*), which promotes rapid degradation via conformational changes induced by factors such as phosphorylation state (Rechsteiner and Rogers, 1996; García-Alai et al., 2006). We discuss the various segments of dysbindin proteins in *Section 2.2.3.3.2*.

There are at least three isoforms of dysbindin-1 in primates (Yin et al., 2006; Oyama et al., 2009), which may be characterized as follows:

Dysbindin-1A is the full-length isoform, the mouse ortholog of which was the first dysbindin protein identified (Benson et al., 2001). This is the protein meant when "dysbindin" is used without qualification. Dysbindin-1A is a 351 aa protein in humans; orthologs in other species are 339–362 aa in length. Such orthologs have been identified not only in humans and mice, but also in the cow, pig, rat, chicken, frog, zebrafish, and amphioxus (*Table 2.2-1*). There is also some evidence for dysbindin-1A in sheep (NP_01119821) and the chimpanzee (XP_001169961) in the NCBI database. All known dysbindin-1A orthologs have one or more PEST domain at or near the end of the CTR (e.g., see *Table 2.2-3*).

Table 2.2-1
Members of the dysbindin protein family based on NCBI and UniProt databases[a]

Paralogs and their isoforms	Protein ID[b]	Common synonym(s)	Encoding Gene[c]		mRNA ID (NCBI)	# Amino acids	MM[d] (kDa)	Coiled-Coil domain[e]	Leucine zipper motif[f]
			Symbol (Gene ID)	Chromosome location					
DYSBINDIN-1									
1) Isoform A									
Human	Q96EV8	1) Dysbindin 2) DTNBP1 3) DTNBP1, isoform 1 or A 4) Dysbindin-1A 5) Hermansky-Pudlak Syndrome 7 (HPS7) homolog	DTNBP1 (84062) [17328]	6p22.3	NM_032122	351	39.49	aa 81-181	aa 94-121
Cow (*Bos taurus*)	Q2HJA5	1) Dysbindin 2) Dtnbp1	DTNBP1 (506612)	23	NM_001045947	342	38.21	aa 81-181	aa 94-121
Pig (*Sus scrofa*)	(NP_001092070) [A5A770]	DTNBP1	DTNBP1 (100049697)	?	NM_001098600	342	38.42	aa 81-181	aa 94-121
Mouse (*Mus musculus*)	(Q91WZ8) [Q91WZ8-1]	1) Dysbindin 2) HPS7 homolog 3) Dtnbp1, isoform 1 4) Dysbindin-1A	Dtnbp1 (94245)	13 A5	NM_025772	352	39.65	aa 81-181	aa 94-121
Rat (*Rattus norvegicus*)	Q5M834	1) Dysbindin 2) Dtnbp1	Dtnbp1 (641528)	17	NM_001037664	352	39.74	aa 81-181	aa 94-121

Chicken (*Gallus gallus*)	Q5ZKM0	1) Dysbindin 2) Dtnbp1	DTNBP1 (420840)	2	NM_001006372	351	39.59	aa 80-180	aa 93-120
Frog (Xenopus laevis)	(AAH81232) [Q66IR4]	MGC85477 protein	MGC85477 (447615)	?	BC081232.1	354	40.11	aa 81-181	aa 94-121
Frog (Xenopus tropicalis)	(AAI66106)	MGC181093 protein	LOC100158494 (100158494)	?	BC166106	362	40.83	aa 81-181	aa 94-121
Atlantic Salmon (*Salmo salar*)	(NP_001134077.1) [B5X5M4-1]	Dysbindin	dtnbp1 (100195576)	?	BT046343.1	369	41.54	aa 81-181	aa 93 - 120
Zebra Fish (*Danio rerio*)	(AAI52673) [Q7ZWE6]	1) Dtnbp1a 2) Dtnbp1	dtnbp1 (394109)	16	NM_201134	362	40.86	aa 81-181	aa 94-121
Amphioxus (*Branchiostoma belcheri tsingtaunese*)	(AAQ18148) [Q6WLC0]	Dystrobrevin binding protein	?	?	AY280671	339	38.31	aa 112-155	aa 114- 141
2) Isoform B									
Human	(NP_898861) [Q96EV8-2]	1) DTNBP1, isoform B 2) DTNBP1, isoform 2	DTNBP1 (84062)	6p22.3	NM_183040	303	34.83	aa 81-181	aa 94-121
Frog (Xenopus laevis)	(NP_001086586) [Q6DF99]	Dtnbp1	dtnbp1 (446421)	?	NM_001093117	316	36.08	aa 81-181	aa 94-121
Fruit Fly (*Drosophila melanogaster*)	Q9VVT5	CG6856-PA	CG6856 (40052)	3L	NM_140807	288	32.36	aa 153-239	aa 156- 180
3) Isoform C									
Human	(NP_898862)	DTNBP1, isoform C	DTNBP1 (84062)	6p22.3	NM_183041	270	30.39	aa 1-100	aa 13-40
Mouse (*Mus musculus*)	(AAP91871) [Q91WZ8-3]	DTNBP1, isoform 3	Dtnbp1 (94245)	13 A5	AY265461	271	30.58	aa 1-100	aa 13-40

continued

Table 2.2-1 (continued)

Paralogs and their isoforms	Protein ID[b]	Common synonym(s)	Encoding Gene[c]		mRNA ID (NCBI)	# Amino acids	MM[d] (kDa)	Coiled-Coil domain[e]	Leucine zipper motif[f]
			Symbol (Gene ID)	Chromosome location					
DYSBINDIN-2									
1) Isoform A									
Human	Q9BQY9	1) Dysbindin domain-containing protein 2 (DBNDD2) 2) Hypothalamic protein HSMNP1	DBNDD2 (55861)[15881]	20q13.12	AF220191	261	27.86	(None)	(None)
2) Isoform B									
Human	(NP_001041690) [Q331S6]	1) DBNDD2 2) Casein kinase-1 binding partner (CK1BP) 3) Stem cell factor apoptosis response protein-1 (SCF ARP1), isoform a	DBNDD2 (55861)[15881]	20q13.12	BC012818	161	17.52	(None)	(None)
Cow (*Bos taurus*)	(AAI03173) [Q3ZBQ3]	DBNDD2	DBNDD2 (507590)	13	BC103172	161	17.37	(None)	(None)
Mouse (*Mus musculus*)	Q9CRD4Q330P7	1) Dbndd2 2) SCF ARP1	Dbndd2 (52840)	2 H3	NM_026797	158	17.19	(None)	(None)
Rat (*Rattus norvegicus*)	(NP_001040576) [Q331S7]	SCF ARP1	LOC499941 (499941)	3q42	NM_001047111	158	17.19	(None)	(None)

3) Isoform C									
Human	Q5QPV4 (NP_001041687)	1) DBNDD2 2) SCF ARP1, isoform b	DBNDD2 (55861)[15881]	20q13.12	NM_001048222	112	12.61	(None)	(None)
DYSBINDIN-3									
1) Isoform A									
Human	(NP_076948) [Q9BW25]	DBNDD1, isoform 2	DBNDD1 (79007) [28455]	16q24.3	NM_024043	178	19.64	(None)	(None)
2) Isoform B									
Human	(NP_001036075) [Q9H9R9]	Dysbindin domain-containing protein 1 (DBNDD1), isoform 1	DBNDD1 (79007)[28455]	16q24.3	NM_001042610	158	17.03	(None)	(None)
Cow (*Bos taurus*)	(AAI46183) [A6H7B4]	1) DBNDD1 2) MGC166260	DBNDD1 (518157)	18	BC146182	158	17.02	(None)	(None)
Mouse (*Mus musculus*)	(NP_082422) [Q9CZ00]	Dbndd1	Dbndd1 (72185)	8 E2	NM_028146	160	17.48	(None)	(None)
Rat (*Rattus norvegicus*)	Q5M831	Dbndd1	RGD1310008 (361437)	19q12	BC088277	160	17.33	(None)	(None)
Frog (Xenopus laevis)	Q6DJE5	MGC84427 protein	MGC84427 (444829)	?	BC075234.1	160	17.42	(None)	(None)

[a]NCBI = National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov); UniProt = Universal Protein Resource (http://www.pir.uniprot.org)

[b]Protein IDs not in parentheses or brackets are common to both NCBI and UniProt. IDs in parentheses are unique to NCBI; those in brackets are UniProt

[c]Gene symbols and loci are from NCBI Entrez Gene. Gene IDs in parentheses are from NCBI Entrez Gene; those in brackets are from the HUGO Gene Nomenclature Committee (HGNC)

[d]Predicted molecular mass (MM) from amino acid sequence

[e]Predicted using the PCoils program (http://toolkit.tuebingen.mpg.de/pcoils)

[f]The beginning and ending amino acid (aa) are the first "a" and and final "g" positions in the leucine zipper motif sequence

Table 2.2-2
Dysbindin domain (DD) locations in humans, mice, rats, and frogs[a]

	Amino acid numbers			
Dysbindin paralog	Human	Mouse	Rat	Frog
1A	214–256	214–268	214–268	212–261
1B	214–256	NA	NA	212–261
1C	133–175	133–187	?	?
2A	140–184	?	?	?
2B	40–86	26–90	40–84	?
2C	40–80	?	?	?
3A	73–117	?	?	?
3B	53–97	53–105	55–99	51–100

[a]Based on BLAST 2 comparisons (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi). Mouse, rat, and frog DDs can only be estimated, because some dysbindin isoforms have not been detected yet in those species as indicated by question marks

NA = not applicable, because dysbindin-1B is not expressed in the mouse or rat

Table 2.2-3
Clear PEST domains in dysbindin proteins[a] (i.e., aa sequences with PEST scores > 5.0)

	Amino acid numbers			
Dysbindin paralog	Human	Mouse	Rat	Frog
1A	294–308 (13.75) 308–336 (7.42) 339–351 (18.62)	334–352 (10.48)	334–352 (10.48)	293–352 (12.31)
1B	None	NA	NA	None
1C	213–227 (13.75) 227–255 (7.42) 258–270 (18.62)	253–272 (10.48)	?	?
2A	228–250 (20.34)	?	?	?
2B	128–150 (20.34)	126–158 (16.58)	126–158 (14.57)	?
2C	None	?	?	?
3A	None	?	?	?
3B	None	None	None	None

[a]Based on PESTFind (https://emb1.bcc.univie.ac.at/toolbox/pestfind/pestfind-analysis-webtool.htm). PEST scores are given in parentheses

NA = not applicable, because dysbindin-1B is not expressed in the mouse or rat

None indicates absence of an aa sequence with a PEST score below 5.0. Question marks indicate the absence of aa information on certain dysbindin paralogs in non-human species

Dysbindin-1B is a 303 aa protein in humans and a 316 aa protein in frogs (*Table 2.2-1*). It is essentially a CT-truncated version of dysbindin-1A with the added distinction of a markedly different C-terminal sequence (aa 272–303 in humans and 272–316 in frogs) that lacks a PEST domain (*Table 2.2-3*). This does not describe a mouse protein of similar length (300 aa) identified by UniProt as dysbindin isoform 2 (Q91WZ8–2), which is merely mouse dysbindin-1A missing amino acids 170 through 220 and should thus not be mistaken for an ortholog of human dysbindin-1B. We exclude it from the dysbindin

protein family because it has no known transcript and no known orthologs in other species. There is no known mouse ortholog of human dysbindin-1B. A chimpanzee ortholog has been predicted from DNA in that species (XP_001169936 on NCBI).

The dysbindin protein found in the fruit fly *Drosophila melanogaster* known as CG6856-PA is tentatively classified here as an ortholog of dysbindin-1B. The aa sequence of its CCD is significantly related to that of CCDs in all known orthologs of dysbindin-1A and -1B. But the CTR of CG6856-PA is short (50 aa versus 161–181 aa in dysbindin-1A orthologs), and it lacks a PEST domain like dysbindin-1B. Moreover, consistent with the nearly exclusive synaptic vesicle localization of dysbindin-1B in synaptosomes of the human brain (see *Section 2.2.6.3.1*), CG6856-PA has been found to play a pre-synaptic, but not a post-synaptic, function at glutamatergic neuromuscular junctions in *Drosophila* (Shao et al., 2008). CG6856-PA is nevertheless quite different from other all other dysbindin-1 orthologs except that of amphioxus (= the lancelet, an invertebrate chordate), because neither its NTR nor its CTR are significantly related to corresponding regions in any vertebrate dysbindin-1 ortholog.

Dysbindin-1C is a 270 aa protein in humans and a 271 aa protein in mice (*Table 2.2-1*); in both species it lacks an NTR and thus begins with the CCD (*Figures 2.2-1* and *2.2-3*). It is otherwise identical to dysbindin-1A, but not a degradation product of that isoform since it is the product of a different mRNA. While the first promoter in DTNBP1 probably drives transcription for dysbindin-1A, the second promoter in that gene may drive transcription for dysbindin-1C (see *Figure 2.2-8b*). At the end of the CTR in both these isoforms is a clear PEST domain (*Table 2.2-3*).

Using the NetPhos 2.0 program (http://www.cbs.dtu.dk/services/NetPhos/) based on the work of Blom et al. (1999), we have identified many predicted phosphorylation sites in dysbindin family proteins (*Table 2.2-4*). As mapped in *Figure 2.2-2*, those sites are mainly serine residues with a lesser number of threonine and tyrosine residues. The vast majority of these amino acids have a probability of phosphorylation estimated to be 80–99%, consistent with recent evidence that dysbindin-1A and -1B in cells are predominantly phosphorylated proteins (Oyama et al., 2009). Using the sister NetPhosK 1.0 program (http://www.cbs.dtu.dk/services/NetPhosK/) based on the work of Blom et al. (2004), we found that many of the predicted phosphorylation sites are predicted targets of diverse serine/threonine kinases, although only those for protein kinase B (PKB = Akt) and DNA protein kinase have been tested thus far (both confirmed). All the predicted kinase targets in humans, mice, and rats are listed in *Table 2.2-5* and mapped in humans in *Figure 2.2-2*. The only predictions omitted are rare casein kinase 1 (CK1) targets in isoforms of dysbindin-1, because Yin et al. (2006) have shown that CK1 does not bind dysbindin containing a CCD. As described below, dysbindin-2 (Yin et al., 2006) and probably also dysbindin-3 is a substrate for CK1 (*Figure 2.2-2*).

As evident in *Figure 2.2-2*, dysbindin-1 differs from other dysbindin paralogs not only in having a CCD, but also in its set of predicted phosphorylation sites. It is the only paralog with predicted tyrosine phosphorylation sites, which are located in its DD and neighboring X1 segment (*Figure 2.2-2* and *Table 2.2-4*). It is unknown which kinase might target these predicted tyrosine phosphorylation sites. Dysbindin-1 is also the only dysbindin paralog with a predicted PKB/Akt phosphorylation target, located in its NTR (*Table 2.2-5*).

Additional dysbindin-1 isoforms are likely to exist. As discussed in *Section 2.2.6.1.2*, there are many known and proposed transcripts of the DTNBP1 gene in humans. In addition to those encoding dysbindin-1A, -1B, and -1C, there are five for which we know the complete exon coding sequence that would encode both a CCD and a DD. They are transcripts *b*, *c*, *f*, *g*, and *h* in the nomenclature of the NCBI gene database AceView. The proteins encoded by these transcripts are predicted to be 338, 334, 219, 212, and 208 aa in length. In our nomenclature, they would be dysbindin-1 isoforms D, E, F, G, and H, respectively. In the human dorsolateral prefrontal cortex, mRNA for dysbindin-1A, -1B, -1C, and -1E has been found (i.e., AceView human DTNBP1 transcripts *a*, *d*, *e*, and *c*, respectively: R. Straub, personal communication).

2.2.1.2 Relationship to Lysosome-Related Organelles (LROs) As noted earlier, dysbindin-1 is a member of BLOC-1, which is one of at least three functionally related complexes contributing to the biogenesis of lysosome-related organelles or LROs (i.e., BLOC-1, -2, and -3: see Dell'Angelica, 2004, Di Pietro and Dell'Angelica, 2005; Wei, 2006, Li et al., 2007). LROs share several properties of conventional lysosomes:

Table 2.2-4
Predicted amino acid phosphosphorylation sites in dysbindin proteins[a]

Dysbindin paralog	Amino acid type	Amino acid numbers		
		Human	Mouse	Rat
1A	Serine	11, 24, 27, 34, 53, 71, 93, 124, 227, 230, 258, 297, 299, 300, 305, 311, 316, 321, 335	11, 24, 27, 53, 93, 124, 157, 161, 227, 230, 258, 267, 287, 291, 293, 298, 315, 335, 343, 348	11, 24, 27, 34, 53, 93, 124, 161, 227, 230, 247, 258, 277, 291, 293, 298, 335, 343, 348
	Threonine	4, 58, 168, 337, 342	4, 123, 178, 336	4, 58, 123, 331, 336
	Tyrosine	210, 215	210, 215	158, 210
1B	Serine	11, 24, 27, 34, 53, 71, 93, 124, 227, 230, 258, 267, 288	NA	NA
	Threonine	4, 58, 168	NA	NA
	Tyrosine	210, 215	NA	NA
1C	Serine	12, 43, 146, 149, 177, 216, 218, 219, 224, 230, 235, 240, 254	12, 43, 76, 80, 146, 149, 177, 186, 206, 210, 212, 217, 234, 254, 262, 267	NA
	Threonine	87, 256, 261	42, 97, 255	NA
	Tyrosine	129, 134	129, 134	
2A	Serine	22, 27, 32, 50, 57, 154, 192, 208, 213, 216–221, 223, 224, 229, 244	NA	NA
	Threonine	188, 207, 212, 239	NA	NA
	Tyrosine	None	NA	NA
2B	Serine	54, 92, 108, 113, 116–121, 123, 124, 129, 144	54, 92, 108, 113, 116, 117, 119, 120, 123, 127, 131, 142	54, 92, 108, 113, 116, 117, 119, 120, 123, 127, 131, 142
	Threonine	88, 107, 112, 139	112	88, 112
	Tyrosine	None	None	None
2C	Serine	54, 104	NA	NA
	Threonine	88	NA	NA
	Tyrosine	None	NA	NA
3A	Serine	83, 84, 87, 104, 115, 139, 141, 150	NA	NA
	Threonine	56, 164, 171	NA	NA
	Tyrosine	None	NA	NA
3B	Serine	63, 64, 67, 84, 95, 119, 121, 130	26, 65, 66, 69, 86, 97, 117, 121, 123, 139	24, 65, 66, 69, 86, 97, 117, 121, 123
	Threonine	36, 144, 151	36, 146, 153	13, 36, 137, 146, 153
	Tyrosine	None	None	None

[a]Predicted using NetPhos 2.0 (http://www.cbs.dtu.dk/services/NetPhos/). Data not available (NA) reflect absence of information on rodent orthologs of some human dysbindin proteins (see *Table 2.2-2* and text)

a lumen with acid-dependent hydrolases enabling macromolecular degradation, lysosomal proteins (LAMP-1, -2, and/or -3), and an ability to fuse with cell membranes (cf. Dell'Angelica et al., 2000, Blott and Griffiths, 2002; Luzio et al., 2007, and Raposo et al., 2007). In contrast to lysosomes, however, LROs are cell-specific organelles displaying considerable diversity in structure, content, and function (Dell'Angelica et al., 2000; Blott and Griffiths, 2002; Raposo et al., 2007). With few exceptions, LROs are further distinguished from lysosomes by their secretory ability. Lysosome fusion with cell membranes appears to

be a form of nonsecretory exocytosis which repairs (Andrews, 2002; McNeill, 2002) or expands (Jaiswal et al., 2002; Arantes and Andrews, 2006) the cell membrane. Most LROs instead secrete material for activation of other cells or for transfer to them (Blott and Griffiths, 2002; Holt et al., 2006). Consequently, LROs are frequently called secretory lysosomes, which aptly describes most of these

Figure 2.2-2

Predicted phosphorylation sites in dysbindin family proteins. The specific locations of these sites are given in *Table 2.2.4*. The types of kinases likely to phosphorylate a given site (if known) are indicated by coded flags attached to the vertical markers at that site. See *Section 2.2.3* for further information and *Figure 2.2-1* for other abbreviations

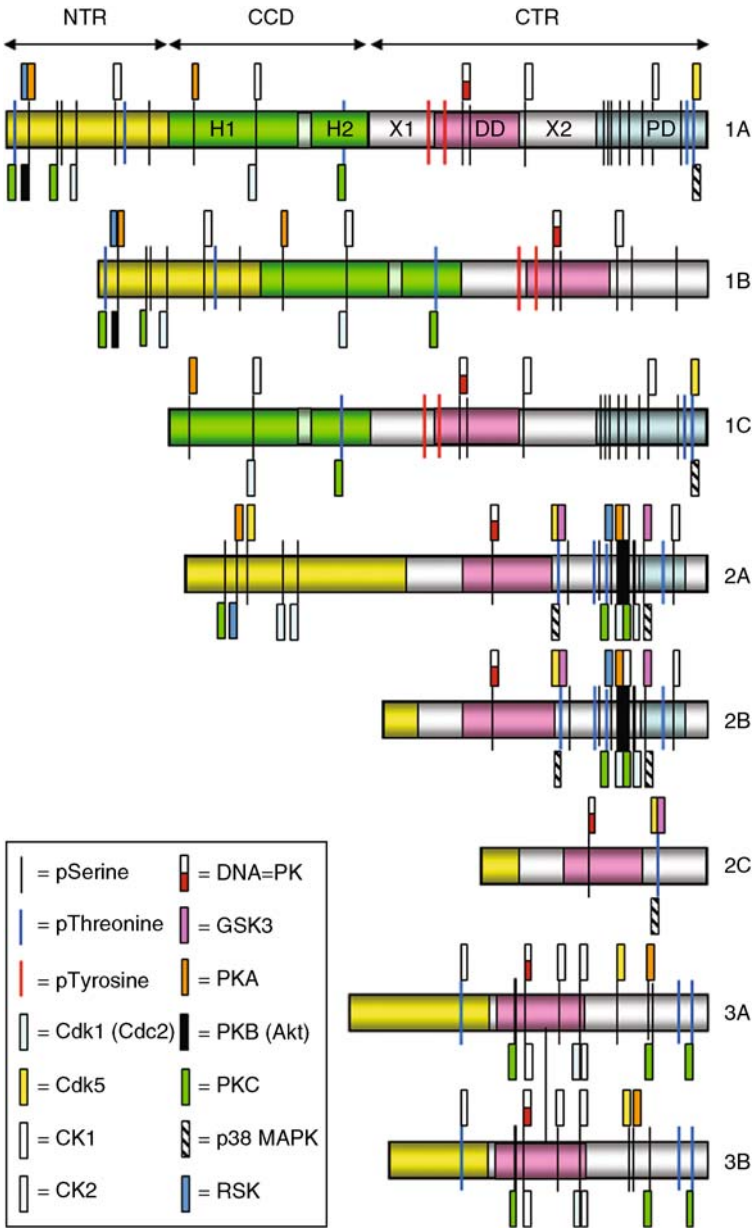


Table 2.2-5
Predicted serine/threonine kinase sites in dysbindin proteins[a]

Dysbindin paralog	Kinase type[b]	Amino acid numbers of serine (S) and threonine (T) kinase targets		
		Human	Mouse	Rat
1A	Cdk1	S-34, S-124	S-53, S-291	S-247, S-291
	Cdk5	T-342	T-336	T-336
	CK2	S-53, S-124, S-258, S-321	S-53, T-123, S-124, S-258, S-315, S-343	S-53, T-123, S-124, S-258, S-343
	DNA PK	S-227	T-178	T-277
	PKA	S-11, S-93	S-11, S-93	S-11, S-93
	PKB	S-11	S-11	S-11
	PKC	T-4, S-24, T-168	T-4, S-24, S-157, S-161	T-4, S-24, S-161
	p38 MAPK	T-342	T-336	T-336
	RSK	S-11	S-11	S-11
1B	Cdk1	S-34, S-124	NA	NA
	CK2	S-53, S-124, S-258	NA	NA
	DNA PK	S-227	NA	NA
	PKA	S-11, S-93	NA	NA
	PKB	S-11	NA	NA
	PKC	T-4, S-24, T-168	NA	NA
	RSK	S-11	NA	NA
1C	Cdk1	S-43	S-210	NA
	Cdk5	T-261	T-255	NA
	CK2	S-43, S-177, S-240	T-42, S-43, S-177, S-234, S-262	NA
	DNA PK	S-146	T-97	NA
	PKA	S-12	S-12	NA
	PKC	T-87	S-76, S-80	NA
	p38 MAPK	T-261	T-255	NA
2A	Cdk1	S-50, S-57, S-216, S218, S-220, S-221, S-224	NA	NA
	Cdk5	S-32, T-188	NA	NA
	CK1	S-219, S-220	NA	NA
	CK2	S-244	NA	NA
	DNA PK	S-154	NA	NA
	GSK3	T-188, S-229	NA	NA
	PKA	S-27, S-217	NA	NA
	PKC	S-22, T-212, S-216, S-218, S-220	NA	NA
	p38 MAPK	T-188, S-229	NA	NA
	RSK	S-27, S-213, S-217	NA	NA
2B	Cdk1	S-116, S-118, S-120, S-121, S-124	S-116, S-120	S-116, S-120
	Cdk5	T-88	S-127	T-88, S-127
	CK1	S-119, S-120	S-131	S-131
	CK2	S-144	S-142	S-142
	DNA PK	S-54	S-54, S-117	S-54, S-117
	GSK3	T-88, S-129	S-127	T-88, S-127

continued

Table 2.2-5 (continued)

Dysbindin paralog	Kinase type[b]	Amino acid numbers of serine (S) and threonine (T) kinase targets		
		Human	Mouse	Rat
	PKA	S-117	S-113, S-117	S-113, S-117
	PKC	T-112, S-116, S-118, S-120	T-112, S-117, S-120, S-123	T-112, S-117, S-120, S-123
	p38 MAPK	T-88, S-129	S-127	T-88, S-127
	RSK	S-113, S-117	S-113, S-117	S-113, S-117
2C	Cdk5	T-88	NA	NA
	DNA PK	S-54	NA	NA
	GSK3	T-88	NA	NA
	p38 MAPK	T-88	NA	NA
3A	Cdk1	S-115	NA	NA
	Cdk5	S-139	NA	NA
	CK1	S-87, S-115	NA	NA
	CK2	T-56, S-104, S-115	NA	NA
	DNA PK	S-87	NA	NA
	PKA	S-141	NA	NA
	PKC	S-83, S-150, T-171	NA	NA
3B	Cdk1	S-95	S-97	S-97
	Cdk5	S-119	S-121	S-121
	CK1	S-67, S-95	S-69	S-69
	CK2	T-36, S-84, S-95	T-36, S-86, S-97, S-139, T-153	S-86, S-97, T-153
	DNA PK	S-67	S-69	S-69
	GSK3	None	S-121	S-121
	PKA	S-121	S-123	S-123
	PKC	S-63, S-130, T-151	S-65	S-24, S-65
	p38 MAPK	None	T-36	T-36, T-137

[a]Specifically those kinase sites predicted using NetPhosK 1.0 (http://www.cbs.dtu.dk/services/NetPhosK/) which are also predicted to be amino acid phosphorylation sites using NetPhos 2.0 (http://www.cbs.dtu.dk/services/NetPhos/) as listed in *Table 2.2-4*. Data not available (NA) reflect absence of information on rodent orthologs of some human dysbindin proteins (see *Table 2.2-2* and text)

[b]*Cdk1*= cyclin-dependent kinases 1 (Cdc2), *Cdk5* = cyclin-dependent kinase 5, *CK1 and CK2* = casein kinases 1 and 2, respectively, *DNA PK* = DNA-dependent protein kinase, *GSK3* = glycogen synthase kinase 3, *PKA* = cAMP-dependent kinase, *PKB* = protein kinase B (Akt), *PKC* = protein kinase C, *p38 MAPK* = p38 mitogen-activated protein kinase, *RSK* = ribosomal S6 kinase

organelles identified to date: acrosomes in sperm cells; azurophil, basophil, and eosinophil granules in white blood cells (leukocytes); δ (dense) and λ (lysosomal) granules in blood platelets; lamellar bodies in lung epithelial type II cells; lytic granules in cytotoxic lymphocytes; major histocompatibility complex (MHC) class II compartments in antigen presenting cells; melanosomes in cutaneous melanocytes; secretory lysosomes in renal tubular cells; multivesicular bodies in osteoclasts; and the Weibel–Palade bodies in vascular endothelial cells (cf. Dell'Angelica et al., 2000, Rendu and Brohard-Bohn, 2001; Blott and Griffiths, 2002; Sun-Wada et al., 2004, Moreno and Alvarado, 2006, and Raposo et al., 2007).

While none of the many established LROs listed above are known to exist in neural tissue, there are two candidate LROs in the brain. The first is an atypical lysosome in axons and axon terminals secreting Ca^{2+} intracellularly (McGuiness et al., 2007). The second is a type of synaptic vesicle derived from endosomes by

a mechanism dependent on adaptor protein complex 3 (AP-3) (Salazar et al., 2004a). This type of vesicle probably populates reserve pool of synaptic vesicles released under strong stimulation (Voglmaier and Edwards, 2007). It is similar to the AP-3 derived synaptic vesicle-like microvesicles (SVLM) in neuroendocrine pheochromocytoma-12 (PC-12) cells (cf. Salazar et al., 2004a, 2004b, 2005a, 2005b, 2006). Among the proteins closely associated with such synaptic vesicles and SVLMs are the lysosomal markers LAMP-1 and LAMP-2 and four members of BLOC-1, specifically dysbindin-1, muted, pallidin, and snapin (cf. Salazar et al., 2004a, 2005b, 2006). On membranes, BLOC-1 can bind AP-3 (Di Pietro et al., 2006) and thereby regulate the functions of AP-3 in neurons and peripheral tissues as explained in *Section 2.2.6.5.5*.

For further discussion of dysbindin-1, see *Section 2.2.6*.

2.2.2 Dysbindin-2 (=Dysbindin Domain-Containing Protein 2, DBNDD2)

Dysbindin-2 is encoded by gene DBNDD2 at chromosome locus 20q13.12 in humans, 13 in the cow, 2 H3 in mice, and 3q42 in rats. It has not been reported in invertebrates. Among vertebrates, dysbindin-2 may have appeared first in fish given a fragmentary 152 aa pufferfish (*Tetraodon nigroviridis*) protein (UniProt Q4TJ04) significantly related in aa sequence to human dysbindin-2B of similar length. In mammals, dysbindin-2 is expressed in neurons and especially in glia of white matter tracts (Benson et al., in preparation), most likely oligodendrocytes.

As illustrated in *Figure 2.2-1*, dysbindin-2 consists of an NTR of variable length and a relatively long CTR. The latter consists of the DD (*Table 2.2-2*), X segments 1–3, and a PEST domain (*Table 2.2-3*). As noted above, we discuss the various segments of dysbindin proteins later in *Section 2.2.3*.

Dysbindin-2 has three isoforms in humans. ***Dysbindin-2A*** is the full-length isoform, which is a 261 aa protein not yet identified in other species. It is also known as hypothalamic protein HSMNP1 and less commonly as chromosome 20 open reading frame 35 (C20orf35), isoform CRA_g. Dysbindin-2A is the only known member of the dysbindin protein family that contains a signal peptide domain, which is located within its first 30 aa as determined using the SignalP 3.0 program (http://www.cbs.dtu.dk/services/SignalP/) of Bendtsen et al. (2004b) and the iPSORT program (http://biocaml.org/ipsort/iPSORT/) of Bannai et al. (2002). Upon emerging from ribosomes, a signal peptide binds a signal recognition particle which essentially guides the signal peptide and its parent protein to a channel (i.e., the translocon) across the membrane of the endoplasmic reticulum (ER) and hence into the gateway of the classical secretory pathway (see Walter and Johnson, 1994; Martoglio, 2003; Hegde and Bernstein, 2006). Once in the ER lumen, the signal peptide is cleaved from the parent protein. For that reason, dysbindin-2A is probably the precursor of a secretory protein at least 30 aa shorter than the precursor itself.

Dysbindin-2B is a shorter, NT-truncated version of dysbindin-2A lacking the first 100 aa of that isoform; it is a 161 aa protein in humans and a 158 aa protein in mice and rats (*Table 2.2-1*). The mouse protein has simply been called dysbindin-2 by Benson et al. (in preparation), because dysbindin-2A and -2C have not been identified in the mouse yet. It is unlikely that dysbindin-2B is a cleavage product of dysbindin-2A, because mRNAs have been identified for both isoforms. Moreover, cleavage of the signal peptide in dysbindin-2A still yields a protein exceeding the length of dysbindin-2B by at least 70 aa.

Dysbindin-2C is a 112 aa protein thus far identified only in humans. It is a CT-truncated version of dysbindin-2B with a distinctly different terminal sequence over the last 18 aa. Unlike dysbindin-2A and -2B, dysbindin-2C lacks a clear PEST domain. It also differs from other dysbindin-2 isoforms in having a high probability of being a nonclassical secretory protein as determined using the Secretome 2.0 program (http://www.cbs.dtu.dk/services/SecretomeP/) of Bendtsen et al. (2004a). Such proteins are exocytosed independently of the ER and Golgi apparatus (Nickel, 2003, 2005).

As mentioned earlier, dysbindin-2 and -3 differ from dysbindin-1 in their lack of a CCD and predicted tyrosine and PKB phosphorylation sites. The lack of a CCD probably accounts for the failure of dysbindin-2B, unlike dysbindin-1, to bind β-dystrobrevin (Nazarian et al., 2006). Dysbindin-2 and -3 differ from each other in several respects. First, dysbindin-2 has a higher degree of sequence identity to dysbindin-1 of the same species. In humans, for example, the sequence identity to dysbindin-1 is 60% for dysbindin-2 isoforms, but only 46–51% for dysbindin-3 isoforms. Second, unlike both dysbindin-1 and -3,

dysbindin-2 is a predicted glycogen synthase kinase 3 (GSK-3) target across mammalian species (Table 2.2-5). Third, dysbindin-2A and -2B isoforms, but not dysbindin-3 isoforms, share a PEST domain and predicted p38 MAPK and ribosomal S6 kinase (RSK) targets.

Dysbindin-2B has been identified as both stem cell factor apoptosis response protein 1 (SCF ARP1) by Lucas et al. (2005) and as a casein kinase 1 binding partner (CK1BP) by Yin et al. (2006). SCF ARP1 is expressed in bone marrow-derived myelomonocytic stem cells upon induction of apoptosis by withdrawal of stem cell growth factor SCF (Lucas et al., 2005). Programmed cell death via apoptosis controls stem cell numbers during hematopoietic cell development (Domen 2001). Since apoptosis is similarly important in development of neurons (Kuan et al., 2000; Buss and Oppenheim, 2004), dysbindin-2B may play a role in nervous system development (see also Section 2.2.3.3.1).

As CK1BP, dysbindin-2B may also play a role in the adult nervous system. The highly acidic CTR of CK1BP binds the δ (but not α) isoform of CK1 (i.e., CKiδ), probably at the catalytic domain of that kinase (Yin et al., 2006). Such binding inhibits substrate phosphorylation by (and autophosphorylation of) CKiδ (Yin et al., 2006). CKiδ phosphorylates tau and thereby inhibits the ability of tau to bind microtubules (Li et al., 2004a; Yin et al., 2006), disruption of which is prominent in Alzheimer's disease (Mi and Johnson, 2006). CKiδ phosphorylates α-synuclein (Yin et al., 2006) at serine 129 (Okochi et al., 2000), which promotes abnormal aggregation of that protein (Smith et al., 2005; Anderson et al., 2006), as occurs in Parkinson's disease (Wood-Kaczmar et al., 2006). Elevated CKiδ may thus contribute to the etiology of neurodegenerative disorders, consistent with the finding that CKiδ is a major marker of granulovacuolar degeneration bodies in such disorders (Ghosal et al., 1999). It may also contribute to cognitive decline in neurodegenerative disorders, because CKiδ immunoreactivity levels in neurofibrillary tangles of Alzheimer cases correlates strongly with their cognitive status (Ghoshal et al., 2002). As an inhibitor of CKiδ-mediated phosphorylation of tau and α-synuclein (Yin et al., 2006), dysbindin 2B warrants investigation as a target for treatment strategies in Alzheimer's and Parkinson's disease.

2.2.3 Dysbindin-3 (=Dysbindin Domain-Containing Protein 1, DBNDD1)

Dysbindin-3 is encoded by gene DBNDD1 at chromosome locus 16q24.3 in humans, 8 E2 in mice, and 19q12 in rats. It is not been reported in invertebrates. Among vertebrates, the first appearance of dysbindin-3 may be in fish given a 156 aa protein in the pufferfish *Tetraodon nigroviridis* listed by UniProt (Q4SIK3) that is significantly related to human dysbindin-3B of similar length. In mammals, dysbindin-3 is expressed in neurons but apparently not in glia (Benson et al., in preparation).

As illustrated in Figure 2.2-1, dysbindin-3 consists of an NTR and a CTR made up of the DD and X segments 1 and 2. This paralog has no clear PEST domain as discussed in Section 2.2.3.3.2. Dysbindin-3 differs from both dysbindin-1 and -2 in several ways identified in Section 2.2.2.2.2.

Dysbindin-3 has two isoforms. ***Dysbindin-3A*** is a 178 aa protein in humans; no orthologs have been identified yet in other species. ***Dysbindin-3B*** is a 158 aa protein in humans, but a 160 aa protein in the mouse, rat, and frog (Table 2.2-1). The mouse ortholog has simply been designated as dysbindin-3 by Benson et al. (in preparation) since dysbindin-3A has not been identified the mouse. In humans, dysbindin-3A and -3B differ only in the NTR: the initial 10 aa differ in sequence and the next 20 aa in dysbindin 3A are missing in dysbindin 3B. Like dysbindin-2C, but unlike other dysbindin family proteins, dysbindin-3 isoforms have a high probability of being nonclassical secretory proteins.

3 Components of Dysbindin Family Members and Their Predicted Phosphorylation Sites

The structural and functional divisions of dysbindin family proteins are still poorly understood. What can be said on that topic is summarized below.

3.1 Amino Terminal Region (NTR)

We use the term NTR to designate the segment of a dysbindin protein that extends from the first aa to the start of the CCD in dysbindin-1 isoforms and to the start of X1 in dysbindin-2 and -3 isoforms (*Figure 2.2-1*). The NTR is missing in dysbindin-1C and severely truncated in dysbindin-2B and -2C. In all but those cases, the NTR has one or more predicted serine and/or threonine kinase sites (*Figure 2.2-2*), which are most numerous in dysbindin-1A, -1B, and -2A. A predicted PKB/Akt binding site in dysbindin-1A and -1B is distinctive, because it is not found in other dysbindin proteins. Although that is the only phosphorylation site for which there is direct evidence (Blake et al., in preparation), additional kinases are predicted to phosphorylate other sites in the NTR, namely cyclin-dependent kinase 1 (Cdk1 = Cdc2), casein kinase 2 (CK2), cAMP-dependent kinase (PKA), protein kinase C (PKC), and ribosomal S6 kinase (RSK). As in other segments of dysbindin proteins, no classic protein–protein interaction motifs are found in the NTR. Phosphorylation of that region in dysbindin-1 isoforms may nevertheless influence protein binding by the CCD as explained below.

3.2 Coiled Coil Domain (CCD) and Its Leucine Zipper (LZ) Motif

A coiled coil is a protein bundle of 2–5 alpha helices wrapped around each other into a superhelix, also called a supercoil (Lupas, 1996a; Mason and Arndt, 2004; Lupas and Gruber, 2005). In the simplest form of coiled coil, helical domains of two proteins wind around one another and bind via a distinctive knobs-into-holes pattern whereby an amino acid side chain of one helix (knob) inserts into a space surrounded by four side chains of the facing helix (hole) as first suggested by Francis Crick in 1952 (Lupas, 1996a; Lupas and Gruber, 2005).

A CCD is not synonymous with a coiled coil. It is an alpha helical region of a protein that can form a supercoil with an alpha helical region of at least one other protein molecule. A supercoil is thus the intermeshing of two or more CCDs. The formation of a supercoil requires the primary structure of the CCDs to be a series of seven-residue repeats (heptads) in each of which there is a particular order of hydrophobic and hydrophilic residues (see Lupas, 1996a; Mason and Arndt, 2004; Lupas and Gruber, 2005). The seven letters, "a" to "g," designate the position of amino acids in a heptad. Hydrophobic residues are in the "a" and "d" positions, while hydrophilic residues are in the "e" and "g" positions. Corresponding amino acids on the second member of a two-helix coil are designated by letters followed by an apostrophe (e.g., a'). The helices are bound together by hydrophobic and van der Waals interactions between corresponding "a" and "d" amino acids (a-a' and d-d') and by electrostatic interactions between corresponding "e" and "g" amino acids (e-g' and e'-g: Cohen and Parry, 1990; Mason and Arndt, 2004). Such interactions occurring repetitively in the heptads of CCDs explain why they are stable surfaces for protein–protein interactions. They are accordingly rather common: about 6.4% of all eukaryotic proteins contain CCDs (Rose et al., 2005). Though not entirely apt, they have been described as "cellular Velcro" holding together molecules, subcellular structures, and even tissue (O'Shea et al., 1993; Rose et al., 2005). They serve many functions: as zippers in membrane fusion proteins, as adaptors between soluble molecules and cytoskeletal proteins, and as integral portions of molecular motors, including those based on actin and microtubules (Burkhard et al., 2001; Rose et al., 2005).

In the initial report on dysbindin proteins, Benson et al. (2001) predicted a CCD in dysbindin-1 of the fruit fly and mouse using the Coils program of Lupas (1996b, http://www.ch.embnet.org/software/COILS_form.html). That is confirmed using the more recent and more reliable PCoils program of Gruber et al. (2006, http://toolkit.tuebingen.mpg.de/pcoils). Both programs predict CCDs in dysbindin-1 isoforms of all species where the aa sequences are known; neither program makes such predictions for dysbindin-2 or -3 isoforms of any species (*Table 2.2-1*; *Figures 2.2-3* and *2.2-4*). In vertebrates, the predicted CCD is 101 aa in length, beginning at aa 80 or 81 and ending at aa 180 or 181 (*Table 2.2-1*). About 58% of the 24 known or candidate-binding partners of dysbindin-1 (see *Tables 2.2-6* and *2.2-7*) are predicted to have one or more CCD (see *Table 2.2-8*). It is likely, then, that dysbindin-1 interacts with many of its binding partners via its CCD. For a discussion of all the proposed dysbindin-1 binding partners, see *Section 2.2.6.5*.

Figure 2.2-3

Coiled-coil domains (CCDs) are predicted in dysbindin-1, but not in dysbindin-2 or -3. Graphs show the probability (Y axis) of a coiled-coil forming at each amino acid (X axis) in the specified dysbindin family member using the PCoils program. The *green, blue*, and *red* lines graph that probability using a 14, 21, or 28 residue window, respectively. The red line is considered the best predictor of a CCD (assumed to exist when the probability is greater than 0.5). The blue line is considered the best predictor of the beginning and end of the CCD. A clear CCD is predicted for dysbindin-1 isoforms, but not for isoforms of dysbindin-2 or dysbindin-3. The dip in probability of a coiled-coil forming in the dysbindin-1 CCD is due to a short stutter region that separates helix 1 (H1) from helix 2 (H2). The abrupt beginning of the predicted CCD in dysbindin-1C reflects the absence of the uncoiled NTR of dysbindin-1A and -1B

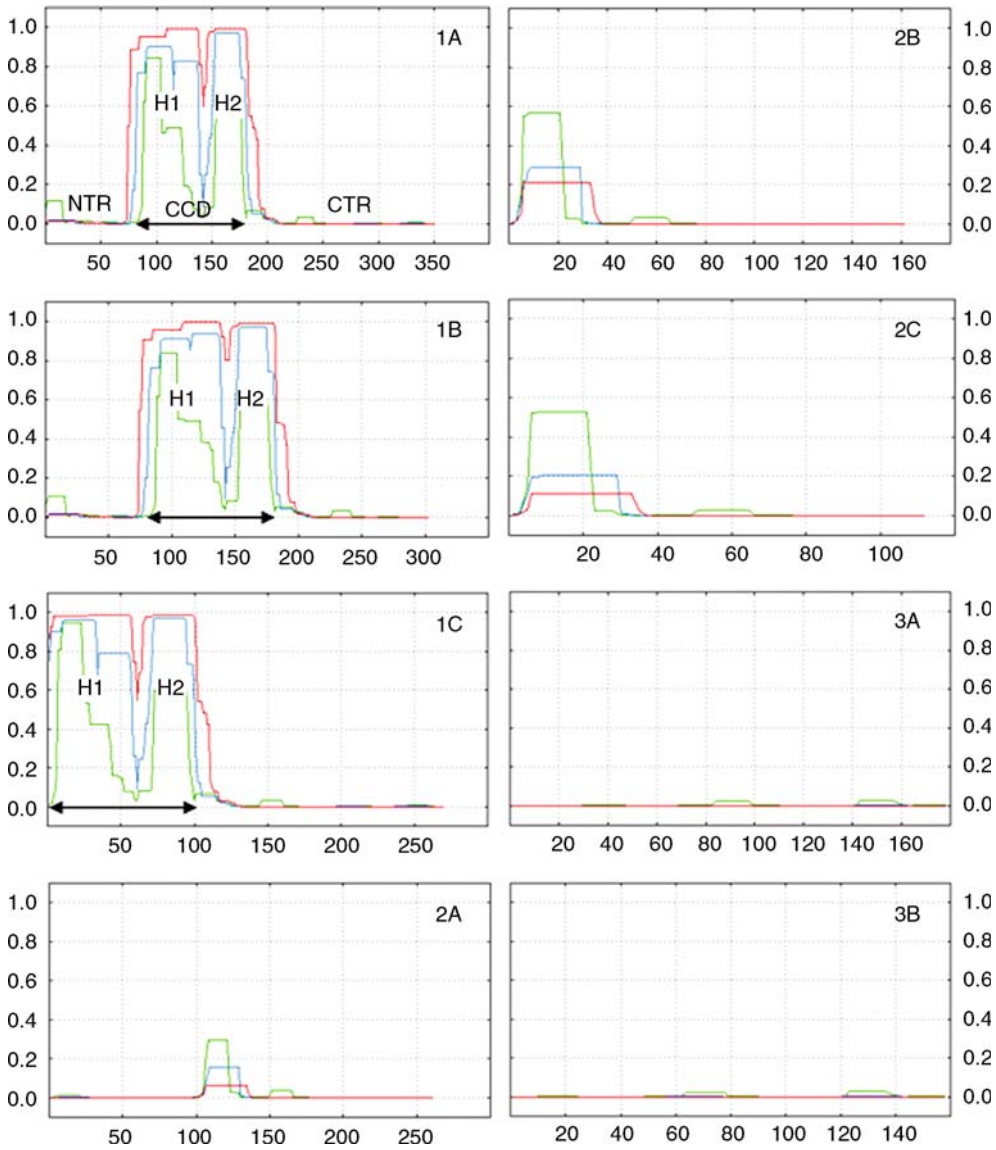
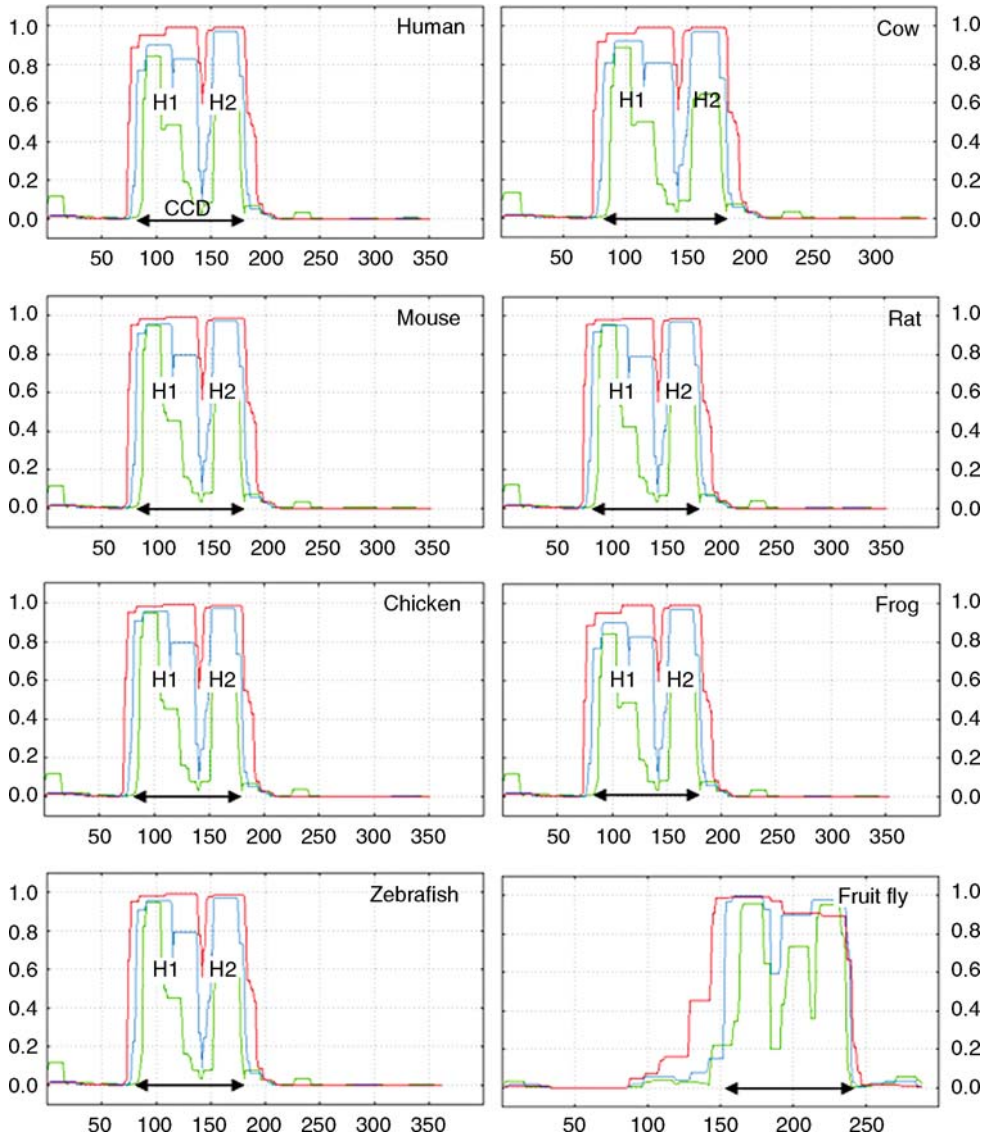


Figure 2.2-4

Evolutionary conservation of predicted coiled-coil domains (CCDs) in dysbindin-1 orthologs of diverse vertebrate species and an invertebrate (i.e., *Drosophila melanogaster*). For explanation of the graphs, see *Figure 2.2-3* legend



About two thirds of the distance into the CCD of vertebrate dysbindin-1, there is a sequence of about 4–6 aa in which the probability of forming a coiled coil drops sharply (*Figures 2.2-3* and *2.2-4*). Those amino acids appear to constitute what is called a stutter in an alpha helix, which may actually facilitate alpha helix formation and stable coupling with other such helices (Cohen and Parry; Brown et al., 1996; Lupas and Gruber, 2005). Using the terminology of Nazarian et al. (2006), we designate the CCD before the stutter as helix 1 (H1) and the remainder of the CCD after the stutter as helix 2 (H2)

Table 2.2-6
Established dysbindin-1 binding partners[a]

Binding partners	Protein Id[b]	Common synonyms(s)	Encoding Gene[c]		# Amino acids	Evidence[d]				References
			Symbol	Location		Y2H	IP cells	IP tissue		
Adaptor protein complex 3, subunit μA[f]	P53676 (r)	AP-3μA	Ap3m1 (r)	15p16 (r)	418 (r)			✓	Brain[e]	Taneichi-Kuroda et al. (2009)
Dystrobrevin α	Q9Y4J8 (h)	α dystrobrevin	DTNA (h)	18q12 (h)	743 (h)	✓				Nazarian et al. (2006)
	Q9D2N4 (m)		Dtna (m)	18 A2 (m)	746 (m)	✓	✓			Benson et al. (2001)
	[XP_001054] (r)		Dtna (r)	18p12	749 (r)			✓	SkM + Brain	Benson et al. (2001)
Dystrobrevin β	O60941 (h)	β dystrobrevin	DTNB (h)	2p24 (h)	627 (h)	✓				Nazarian et al. (2006)
	O70585 (m)		Dtnb (m)	12 A1.1 (m)	700 (m)	✓	✓			Benson et al. (2001);Li et al. (2003)
	P84060 (r)		Dtnb (r)	6q14 (r)	654 (r)			✓	Brain	Benson et al. (2001)
Muted	Q8TDH9 (h)	Muted mouse homolog	MUTED (h)	6p25.1-6p24.3 (h)	187 (h)	✓				Starcevic and Dell'Angelica (2004);Nazarian et al. (2006)
	Q8R015 (m)		Muted (m)	13 A3.3	185 (m)		✓			Li et al. (2003)
Mysospryn	Q70KF4 (m)	Cardiomyopathy associated protein 5	Cmya5 (m)	13 C3 (m)	3739 (m)	✓	✓	✓	SkM	Benson et al. (2004)
Pallidin	Q9UL45 (h)	Syntaxin13-interacting protein	PLDN (h)	15q21.1 (h)	172 (h)	✓				Starcevic and Dell'Angelica (2004);Nazarian et al. (2006)
	Q9R0C0 (m)		Pldn (m)	2 E5 (m)	172 (m)	✓	✓	✓	SkM + Brain	Li et al. (2003); Nazarian et al. (2006)

continued

Table 2.2-6 (continued)

Binding partners	Protein Id[b]	Common synonyms(s)	Encoding Gene[c]		# Amino acids	Evidence[d]				References
			Symbol	Location		Y2H	IP cells	IP tissue		
Ring Finger Protein 151	Q2KHN1 (h)	RNF151	RNF151 (h)	16p13.3 (h)	245 (h)		✓			Nian et al. (2007)
	Q9CQ29 (m)		Rnf151 (m)	17 A3.3	239 (m)	✓				Nian et al. (2007)
Snapin	O95295 (h)	SNARE-associated protein	SNAPAP (h)	1q21.3 (h)	136 (h)	✓				Starcevic and Dell'Angelica (2004);Nazarian et al. (2006)
	Q9Z266 (m)		Snapap (m)	3 F1 (m)	136 (m)		✓	✓	Brain	Talbot et al. (2006)
Tripartite Motif-Containing Protein 32	Q13049 (h)	1) TRIM32 2) HT2A	TRIM32 (h)	9q33.1 (h)	653 (h)		✓			Locke et al. (2009)
	Q8CH72 (m)		TRIM32 (m)	4 C1 (m)	655 (m)	✓				Locke et al. (2009)

[a]In addition to the listed binding partners, dysbindin-1 binds the DNA protein kinase complex (Oyama et al., 2009). However, it has not been conclusively established which elements of that complex directly bind dysbindin-1

[b]Protein IDs are common to NCBI and UniProt, except for bracketed IDs unique to NCBI. The letters in parentheses indicate species: h = human, m = mouse, and r = rat

[c]NCBI Entrez Gene

[d]Y2H = yeast two-hybrid. IP cells = immunoprecipitation in vitro. IP tissue = IP in tissue lysates. SkM = skeletal muscle

[e]In vitro assays with GST-dysbindin-1 and His-AP-3μA confirmed direct interaction of these proteins (Taneichi-Kuroda et al., 2009)

[f]Dysbindin-1 may also bind AP-3βB (Oyama et al., 2009), but not AP-3βA (Li et al., 2003)

Table 2.2-7

Candidate dysbindin-1 binding partners suggested by yeast two-hybrid studies[a]

Binding partners	Protein ID[b]	Common synonym(s)	Encoding Gene[c]		# Amino acids
			Symbol	Location	
A-Kinase Anchor Protein 6	Q13023 (h)	1) Protein kinase A-anchoring protein 6 (AKAP6) 2) Muscle A-kinase anchor protein (mAKAP) 3) A-Kinase anchor protein 100 kDa (AKAP100)	AKAP6 (h)	14q13.1 (h)	2319 (h)
BLOC-1, subunit 2	Q9CWG9 (m)	1) BLOS2 2) Centrosome-associatedprotein (Ceap)	Bloc1s2 (m)	19 C3 (m)	143 (m)
Cyclin A2	P20248 (h)		CCNA2 (h)	4q25-q31(h)	432 (h)
Dystonin-1	(NP_899236) (h)	1) Dystonin isoform 1 2) Bullous pemphigoid antigen 1 (BPAG1), isoform 1	DST (h)	6p12-p11 (h)	5497 (h)
Microtubule-actin cross-linking factor 1, isoform b	Q96PK2 (h)	1) MACF1b 2) MACF1, isoform 4 3) Actin cross-linking family protein 7 (ACF7)	MACF1 (h)	1p32-p31 (h)	5938 (h)
Nesprin-1β2	(AAO27774) (h)	(NOT synaptic nuclei expressed protein 1)	SYNE1 (h)	6q25 (h)	3212 (h)
Neurobeachin	Q8NFP9 (h)	1) Protein neurobeachin 2) Lysosomal-trafficking regulator 2 3) Protein BCL8B	NBEA (h)	13q13 (h)	2946 (h)
Proteasome activator 28, gamma subunit	P61289 (h)	1) PA28γ 2) Proteasome activator complex, subunit 3 (PSME3) 3) Ki nuclear autoantigen 4) Activator of multicatalytic protease, subunit 3 5) 11S regulator complex, subunit gamma (REG gamma)	PSME3 (h)	17q21 (h)	254 (h)

continued

Table 2.2-7 (continued)

Binding partners	Protein ID[b]	Common synonym(s)	Encoding Gene[c]		# Amino acids
			Symbol	Location	
Rabenosyn-5	Q9H1K0 (h)	1) FYVE finger-containing Rab5 effector protein rabenosyn-5 2) Zinc finger FYVE domain-containing protein 20	ZFYVE20 (h)	3p24.3 (h)	784 (h)
RAN Binding Protein 5	(NP_002262) (h)	1) RanBP5 2) RAN GTP binding protein 5 3) Importin beta-3 subunit (IMB3) 4) Karyopherin (= importin) beta 3	RANBP5 (h)	13q32.2 (h)	1115 (h)
Ras-related protein 11A	P62491 (h)	RAB11A	RAB11A (h)	15q21.3-q22.31 (h)	216 (h)
Secretory protein 8, isoform a	Q96A65 (h)	1) Sec8a 2) Sec8-like protein 1 (Sec8L1) 3) Exocyst complex component 4	EXOC4 (h)	7q31 (h)	974 (h)
Syntabulin, isoform 1	Q9NX95 (h)	1) Syntaxin-1 binding protein 2) Golgi-localized syntaphilin-related protein (Golsyn)	FLJ20366 (h) GOLSYN (h)	8q23.2 (h)	663 (h)
Transcription initiation factor IIIB, isoform 3	(NP_663718) (h)	General transcription factor IIIB, 90 kDa subunit	BRF1 (h)	14q (h)	473 (h)
Zinc finger protein protein 490	Q9ULM2 (h)	1) ZNF 490 2) KIAA1198	ZNF490 (h)	19p13.2 (h)	529 (h)

[a]Reported by Camargo et al. (2007), except for BLOS2, which is based on work of M.A. Benson and D.J. Blake (in preparation)

[b]Protein IDs not in parentheses or brackets are common to NCBI and UniProt. IDs in parentheses are unique to NCBI; those in brackets are unique to UniProt. The letters in parentheses indicate species: h = human and m = mouse

[c]According to NCBI Entrez Gene

Table 2.2-8

Leucine zipper (LZ) motifs in predicted CCDs of established and candidate dysbindin-1 binding partners[a]

Binding partners[b]	Species	LZ motif location(aa-aa)	LZ motif type[c]	LZ motif sequence (From first aa in "a" position to last aa in "g" position)
Adaptor protein complex 3, subunit μA (AP-3μA)	Rat	(None)	(None)	(None)
A-Kinase Anchor Protein (AKAP6 or mAKAP)	Human	847-874	I-L-I-L	LEL**I**ASHKAG**L**KDMLRM**I**ASQWKE**L**QRQ
BLOC-1, subunit 2 (BLOS2)	Mouse	92-119	L-L-I-V	LKD**L**NQKYAE**L**QPYLDQ**I**NMIEEQ**V**AAL
Cyclin A2	Human	(None)	(None)	(None)
Dystonin-1	Human	459-486	L-L-L-V	ADQ**L**VQRVAK**L**RDEIMA**L**RNECSS**V**YSK
		1757-1784	L-V-I-V	QEN**L**NQQYQK**V**KAQHEK**I**ISQHQA**V**IIA
		3557-3584	L-L-I-V	TEG**L**WKQQSE**L**RVLQED**I**LLRKQN**V**DQA
		4002-4029	V-L-L-M	NKN**V**SVDMEK**L**QPLYET**L**KQRGEE**M**IAR
		4937-4971	L-L-L-L-L	QQR**L**ASALAG**L**IAKQEL**L**EALLAW**L**QWAETT**L**TDK
Dystrobrevin α	Human	510-552	L-L-L-L-L-L	NPT**L**LAELRL**L**RQRKDE**L**EQRMSA**L**QESRRE**L**MVQLEG**L**MKL
	Mouse Rat	508-548	L-L-L-L-L-L	NPT**L**LAELRL**L**RQRKDE**L**EQRMSA**L**QESRRE**L**MVQLEG**L**MKL
Dystrobrevin β	Human	475-516	L-L-L-L-L-L	NPT**L**LAELRL**L**RQRKDE**L**EQRMSA**L**QESRRE**L**MVQLEE**L**MKL
	Mouse	475-516	L-L-L-L-L-L	NPM**L**LAELRL**L**RQRKDE**L**EQRMSA**L**QESRRE**L**MVQLEG**L**MKL
Microtubule-actin cross-linking factor 1, isoform b (MACF1b)	Human	1917-1937	V-V-L-L	QNA**V**EIEKTK**V**LNQHTQ**L**EGRLQD**L**RAW
		3428-3455	M-L-L-L	NQN**M**DAVTEE**L**QAKTGS**L**EEMTQR**L**REF
		3592-3619	L-V-I-I	TDS**L**QSQIED**V**RLFLNK**I**HVLKLD**I**EAS
		4591-4618	I-L-I-L	AET**I**KEETDG**L**HEELEF**I**RILGAD**L**IFA
		5414-5448	L-L-L-I-L	QQR**L**ETALSE**L**VANAEL**L**EELLAW**I**QWAETT**L**IQR
Muted	Human + Mouse	(None)	(None)	(None)
Mysospryn	Mouse	3202-3250	V-I-L-L-I-I-I	DHE**V**SALDTA**I**SAVKVQ**L**GEFLEN**L**QEKSLR**I**EAFVSE**I**ESFFNT**I**EEK
Nesprin-1β2	Human	53-73	M- L-L-L	AKD**M**KKFEAE**L**KKLQAA**L**EQAQAT**L**TSP
		1578-1605	V-L-L-L	LEA**V**QVQVDN**L**QNLQDD**L**EKQERS**L**QKF
		3000-3027	L-L-L-V	AEI**L**QDHHKQ**L**MQIKHE**L**LESQLR**V**ASL
Neurobeachin	Human	(None)	(None)	(none)
Proteasome activator 28, gamma subunit (PA28γ)	Human	112-139	L-V-L-V	NQQ**L**VDIIEK**V**KPEIRL**L**IEKCNT**V**KMW

continued

Table 2.2-8 (continued)

Binding partners[b]	Species	LZ motif location(aa-aa)	LZ motif type[c]	LZ motif sequence (From first aa in "a" position to last aa in "g" position)
Pallidin	Human	115-142	I-L-L-L	LVN**I**RKEMLM**L**HEKTSK**L**KKRALK**L**QQK
	Mouse	115-142	I-L-L-L	LVT**I**RKEMLL**L**HEKTSK**L**KKRALK**L**QQK
Rabenosyne-5	Human	(None)	(None)	(None)
RAN Binding Protein 5 (RanBP5)	Human	(None)	(None)	(None)
Ras-related protein 11A (RAB 11A)	Human	(None)	(None)	(None)
Ring Finger Protein 151 (RNF151)	Human + Mouse	(None)	(None)	(None)
Secretory protein 8, isoform a (Sec8a)	Human	286-313	L-V-L-I	LAK**L**KKIPET**V**KAIIER**L**EQELKQ**I**VKR
Snapin	Human	44-71	V-L-L-I	VHA**V**RESQVE**L**REQIDN**L**ATELCR**I**NED
	Mouse	44-71	V-L-L-I	VHA**V**RESQVE**L**REQIDN**L**ATELCR**I**NED
Syntabulin-1	Human	275-309	V-L-L-I-L	EVT**V**RHLKTK**L**KESERR**L**HERESE**I**VELKSQ**L**ARM
		324-351	L-I-I-L	QLA**L**KEARKE**I**KQLKQV**I**ETMRSS**L**ADK
Transcription initiation factor IIIB, isoform 3	Human	(None)	(None)	(None)
Tripartite Motif-Containing Protein 32 (TRIM 32)	Human	160-187	L-L-L-V	MGE**L**QRRKAA**L**EGVSKD**L**QARYKA**V**LQE
	Mouse	161-188	L-L-L-V	TGE**L**QRRKAA**L**EGVSRD**L**QARYKA**V**LQE
Zinc Finger Protein 490	Human	(None)	(None)	(None)

[a]Coiled coil domains (CCDs) were predicted using the PCoils program (http://toolkit.tuebingen.mpg.de/pcoils). LZ motifs here include hybrid motifs including not only leucine (L), but also isoleucine (I), methionine (M), and/or valine (V)

[b]Protein IDs and other basic information about the listed binding partners are given in *Tables 2.2-6* and *2.2-7*

[c]LZ motif type refers to the sequence of amino acids at "d" positions. Those amino acids in bold and underlined in the motif sequence

(see *Figures 2.2-1* and *2.2-2*). The division of the CCD into two helixes potentially allows it greater flexibility in interaction with other proteins.

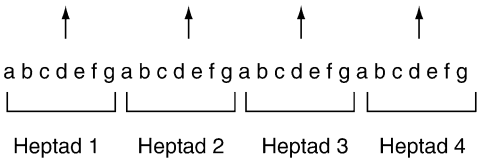
Only the H1 segment of the dysbindin-1 CCD contains a leucine zipper (LZ) motif (*Table 2.2-1* and *Figure 2.2-5*), which is a well-established dimerization surface in CCDs first discovered in transcription factors (Landschulz et al., 1988; O'Shea et al., 1989; Alber, 1992; Hodges, 1992). In the strict sense of the term, an LZ motif is an amino acid sequence in a CCD with leucine in the "d" position of at least four successive heptads. While coiled coils formed by interaction of LZ motifs on opposing protein molecules are most stable with leucine in the "d" (Alber, 1992; Hodges, 1992; Moitra et al., 1997), substitution of one or more of those leucines with other hydrophobic amino acids (e.g., isoleucine, methionine, or valine) yield hybrid LZ motifs still able to form stable coiled coils (Ransone et al., 1989; Hu et al., 1990; van Heeckeren et al., 1992). Such hybrid LZ motifs are found in many naturally occurring proteins (Cohen and Parry, 1990; see also *Table 2.2-8*), where they are often the critical binding sites for interactions between molecules of the same or different protein (Leung and Lassam, 1998; Richie-Jannetta et al., 2003; Surks and Mendelsohn, 2003; Köhler et al., 2005).

The classic LZ motifs common to all vertebrate dysbindin-1 orthologs (*Figure 2.2-5*) are known to bind classic and hybrid LZ motifs of the same or different protein (Rodrigues and Park, 1993; Surks and Mendelsohn, 2003; Liu et al., 2006). We have seen no clear evidence in Western blots that dysbindin-1 ever forms homodimers, but it is capable of forming heterodimers with many of its known and candidate-binding partners with LZ (or hybrid LZ) motifs. Six of the nine established dysbindin-1 binding partners (i.e., 66%) listed in *Table 2.2-6* have one or more LZ (or hybrid LZ) motifs, as do 7 out of 15

Figure 2.2-5

Evolutionary changes in the amino acid sequence of leucine (or hybrid) leucine zipper motifs in the CCD of dysbindin-1 orthologs. The amino acid (aa) numbers specify the beginning and end of the zipper motif in each species. These motifs consist of 28 amino acids broken into four sets of 7 aa sequences (i.e., four heptads). Attention is called to the amino acids in the "d" position within each heptad, the identity of which is especially important to the stability of interactions with leucine zippers on other protein molecules

Human	aa 94	LVE **L** QEQLQQ **L** PALIAD **L** ESMTAN **L** THL	aa 121
Cow	aa 94	LRD **L** QQQLQQ **L** PGLIAD **L** ESLTAS **L** THL	aa 121
Pig	aa 94	LLE **L** QQQLQQ **L** PGLIAD **L** ESMTAN **L** THL	aa 121
Mouse	aa 94	LNE **L** QGQLQQ **L** PALLQD **L** ESLMAS **L** AHL	aa 121
Rat	aa 94	LAE **L** QEQLQQ **L** PALLQD **V** ESLMAS **L** AHL	aa 121
Chicken	aa 93	LVE **L** QDQLQQ **I** PGFLAD **L** ECLTAS **L** ARL	aa 120
Frog	aa 94	LVE **L** QEQLQQ **V** PGFVAD **L** ESVTAK **L** VAL	aa 121
Zebrafish	aa 94	VLE **L** QEQLQQ **I** PTFLSD **L** ETITSR **I** AHL	aa 121
Amphioxus	aa 114	LSS **I** TEMVAG **L** SSDFEQ **L** EYLLNE **L** EDV	aa 141
Fruit fly	aa 156	LNS **L** EEMGKQ **L** EIELEK **L** EDLREE**C** ELQ	aa 180



candidate-binding partners (i.e., 47%) listed in *Table 2.2-7*. The specific location and sequences of those motifs are given in *Table 2.2-8*. Their importance is suggested by the finding of Nazarian et al. (2006) that the dysbindin-1 CCD segment containing a LZ motif (i.e., H1), but not that lacking such a motif (i.e., H2), interacts with several known binding partners of dysbindin-1. These partners are pallidin, snapin, and dystrobrevins.

Benson et al. (2001) argue, however, that the CCD of β-dystrobrevin is not involved in binding dysbindin-1, because deletion of that CCD has no effect on the normal ability of dysbindin-1 to alter localization of β-dystrobrevin in COS-7 cells. But the β-dystrobrevin amino acids deleted (384–486) would not eliminate the predicted CCD (aa 431–522) of the mouse β-dystrobrevin which Benson et al. (2001) transfected their COS-7 cells to express. Indeed, later studies indicated that β-dystrobrevin does have the ability to interact with dysbindin-1 via supercoil formation. In particular, in vitro tests have shown that a deletion in the CCD of dysbindin-1 prevents interaction of that protein with β-dystrobrevin (Li et al. 2003, see their *Figure 2.2-4*) and that isolated CCDs of the two proteins can interact directly (Nazarian et al., 2006). Results of in vivo tests nevertheless argue against the possibility that any interaction occurs between these proteins under physiological conditions (see introduction to *Section 2.2.6.5*).

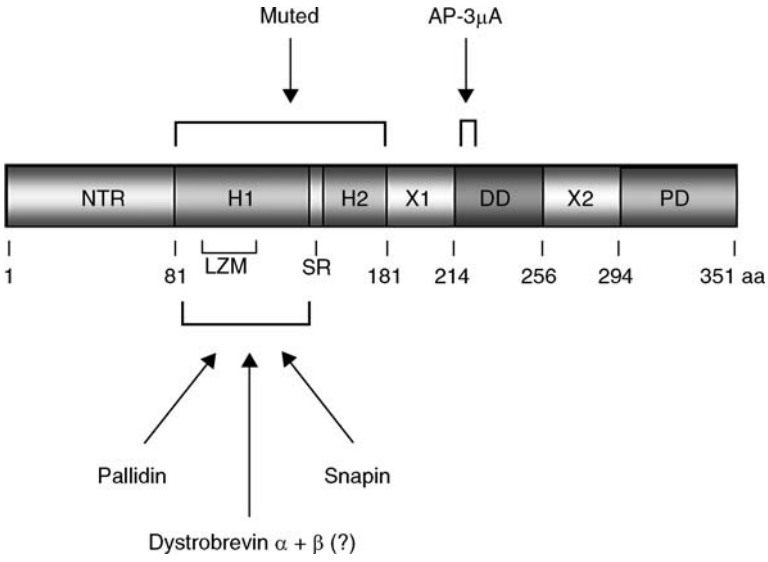
While dystrobrevins may be an exception, the observations mentioned above suggest that the conserved LZ motif found in the H1 segment of the dysbindin-1 CCD plays a major role in binding other proteins. It will nevertheless be necessary to test this hypothesis for each known and candidate dysbindin-1 binding partners with an LZ (or hybrid LZ) motif, because it cannot be predicted from the aa sequences of LZ motifs on two different proteins whether they will actually form a stable coiled coil, as is true for CCD–CCD binding in general (see Arndt et al., 2002; Mason and Arndt, 2004). Tests common in LZ research (e.g., Hodges, 1992; Moitra et al., 1997; Richie-Jannetta et al., 2003; Surks and Mendelsohn, 2003; Köhler et al., 2005) are needed to determine if interaction of dysbindin-1 with a binding partner is impaired when one or more of the leucines in the "d" positions of the LZ motif in H1 are substituted with less hydrophobic amino acids such as alanine or glutamate that destabilize a coiled coil formed with another LZ (or hybrid LZ) motif.

A second hypothesis is necessary to explain dysbindin-1 interactions with proteins whose CCDs lack LZ (or hybrid LZ) motifs. These include four known or candidate dysbindin-1 binding partners, namely muted, proteasome activator 28 gamma (PA28γ), and transcription factor IIIB, isoform 3. As shown by Nazarian et al. (2006), both segments of the CCD in dysbindin-1 are necessary to bind muted (*Figure 2.2-6*). The same may be true for other dysbindin-1 binding partners with CCDs lacking an LZ (or hybrid LZ) motif.

The CCDs of mammalian dysbindin-1 isoforms contain three predicted sites of serine/threonine phosphorylation: (1) aa 93 phosphorylated by PKA in H1, (2) aa124 phosphorylated by Cdk1 and CK2 also in H1, and (3) aa 161 or 168 phosphorylated by PKC in H2 (cf. *Figure 2.2-2* and *Table 2.2-5*). The first two sites flank the LZ motif (aa 94–121), the significance of which is not clarified by the literature on such motifs. It has been found, however, that phosphorylation of sites in or flanking CCDs in other proteins either inhibits or facilitates interaction with binding partners. Cdk1 (= Cdc2) phosphorylation of sites near the CCD of lamin inhibits CCD-mediated polymerization of that protein, allowing disassembly of the lamin network below the nuclear membrane at the start of mitosis (Heitlinger et al., 1991; Peter et al., 1991; Stuurman et al., 1996). Similarly, phosphorylation in the CCD of myosin II prevents the CCD-mediated polymerization of that protein into thick filaments required for dynamic cytoskeletal functions (Liang et al., 1999). In contrast, CK2 phosphorylation of a site near the CCD of vesicle tethering protein p115 has been found to promote CCD-mediated vesicle docking with the Golgi membrane (Dirac-Svejstrup et al., 2000). Similarly, PKC phosphorylation of sites within 61 residues of the CCD in insulin receptor substrate p53 (IRSp53) promotes CCD-dependent translocation to postsynaptic densities in hippocampal neurons (Hori et al., 2005). Given these precedents, predicted phosphorylation sites in CCDs of dysbindin-1 isoforms or in the flanking sectors (NTR and X1) of those isoforms may regulate CCD interactions with their binding partners.

Finally, it should be noted that interactions of dysbindin-1 with other proteins are not always mediated by the formation of coiled coils. This is apparent from several observations. (1) AP-3μA, which lacks a CCD, binds dysbindin-1 outside its CCD, specifically in an area of the CTR covering aa 215 (Taneichi-Kuroda,

Figure 2.2-6
Loci of interactions between dysbindin-1 and several of its established binding partners based on the work of Nazarian et al. (2006) and Taneichi-Kuroda et al. (2009). Apart from AP-3uA, all these binding partners interact with the CCD of dysbindin-1. Except for muted and AP-3μA, the leucine zipper-containing H1 segment of the CCD is sufficient for binding of the proteins listed. Binding of muted requires interaction with both H1 and H2. See text (*Section 2.2.3.2*) for further information



2009: see *Figure 2.2-6*). That area contains an AP-3 binding motif (YXXΦ: Bonifacino and Traub, 2003) present at virtually the same location in all vertebrate orthologs of dysbindin-1, where the motif consists of a YLQI sequence at the start of the DD. (2) Dysbindin-1 may bind the C-terminus, not the CCD, of β-dystrobrevin (Benson et al., 2001). (3) Many known or candidate dysbindin-1 binding partners lack predicted CCDs, including cyclin A2, rabenosyne-5, ras-related protein 11A (rab 11A), ras-related nuclear (RAN) binding protein 5 (RanBP5), ring finger protein 151 (RNF 151), and zinc finger protein 490. Cyclin A2 does have a sequence consistent with a hybrid LZ motif but lacks a predicted CCD, which suggests the sequence is simply a hybrid leucine repeat that does not form an extended alpha helix. Such a repeat could still serve as a binding site, as was unintentionally shown by Marx et al. (2001), who found that leucine/isoleucine repeats in ryanodine receptor 2 (RyR2) are critical in binding other leucine/isoleucine repeats in muscle A-kinase anchor protein (mAKAP = AKAP6), protein phosphatase 2A, 130 kDa subunit (PR130), and spinophilin. But CCDs are not known or predicted in RyR2 or in any of its binding partners other than mAKAP. What Marx et al. (2001) demonstrated, then, were interactions occurring between hybrid leucine repeats outside CCDs. With a single exception, however, such repeats are unlikely binding sites of dysbindin-1, because only one ortholog of the protein (i.e., amphioxus dysbindin-1A) contains a hybrid leucine repeat outside the CCD. We thus lack clues to the nature or location of many dysbindin-1 binding sites outside the CCD.

3.3 Carboxy Terminal Region (CTR)

The CTR extends from the end of the CCD in dysbindin-1 isoforms and from the end of the NTR in dysbindin-2 and -3 isoforms (*Figure 2.2-1*). It includes the DD, a PEST domain in dysbindin-1A, -1C, -2A, and -2B, and two or three zones of indeterminate nature we designate uncharacterized segments of the CTR (X1–3).

3.3.1 Dysbindin Domain (DD)

Conservatively defined, the DD is the segment of a dysbindin protein showing significant sequence homology to all other known members of the dysbindin protein family of the same species. As that implies, the DD need not be an aa sequence identical across family members. Its extent in a given family member is more accurately estimated when more family members of the same species are available for comparison. Since all eight family members are known only in humans, the length of the DD is known with greatest confidence in the human orthologs, where it is estimated to span 41–47 aa (*Figure 2.2-1*). *Table 2.2-2* gives our best estimates of the DD's extent and location in all known dysbindin family members in the human, mouse, rat, and frog.

The only distinctive and consistent feature of the DD across all dysbindin family proteins is a phosphorylation site for DNA-dependent protein kinase (DNA-PK: *Figure 2.2-2* and *Table 2.2-5*) predicted using the NetPhosK 1.0 program of Blom et al. (2004). This is true for all species in which multiple dysbindin family members are known and thus for all species in which the location of the DD can be estimated (i.e., humans, mice, rats, and frogs: see *Table 2.2-2*). In other species, the location of the DD cannot be estimated, because those species are known to express only one dysbindin family member (i.e., dysbindin-1A). In that one family member, however, there is always at least one predicted DNA-PK site, which (apart from the fruit fly) is located in or near the area of the CTR occupied by the DD in human, mouse, rat, and frog dysbindin-1A. While mammalian dysbindin family proteins each have just one predicted DNA-PK site, all nonmammalian members of that family for which we have aa sequence data, including the fruit fly, have 2–3 predicted DNA-PK sites. These observations taken together suggest that the dysbindin protein family evolved from nuclear proteins (see also *Section 2.2.5.1*).

DNA-PK is a serine/threonine kinase belonging to the superfamily of phosphatidylinositol 3-kinase-like protein kinases (PIKKs; Burma and Chen, 2004; Collis et al., 2005). It is present in glial and neuronal nuclei, where it is expressed at high levels prenatally and at much lower levels postnatally, though levels may rise during advanced aging (Oka et al., 2000), which directly or indirectly binds dysbindin-1 and phosphorylates its three major isoforms (Oyama et al., 2009). DNA-PK serves as both a sensor of DNA damage and a transducer of adaptive reactions to such damage (Burma and Chen, 2004). It is activated by double-strand breaks in DNA (Burma and Chen, 2004; Collis et al., 2005). Under conditions causing excessive or unrepairable DNA damage, DNA-PK can trigger apoptosis (Chakravarthy et al., 1999; Woo et al., 2002; Mukherjee et al., 2006). Apoptosis in bone marrow-derived myelomonocytic stem cells induces gene expression of dysbindin-2B (= stem cell factor apoptosis response protein 1), which may mediate later events in apoptosis upon phosphorylation by DNA-PK.

Under less extreme conditions, DNA-PK plays a central role in repairing double-strand breaks in DNA (Burma and Chen, 2004; Collis et al., 2005). This repair function probably accounts for known anti-apoptotic effects of DNA-PK on embryonic and early postnatal neurons (Chechlacz et al., 2001, Vermuri et al., 2001) and on early postnatal and adult neurons subject to excitotoxicity and oxidative stress (Culmsee et al., 2001; Neema et al., 2005). We will consider later the possibility that these anti-apoptotic effects may be mediated in part by DNA-PK phosphorylation of dysbindin-1 (see *Section 2.2.6.5.3*).

3.3.2 PEST Domain (PD)

A motif that often marks a protein for rapid degradation is a PEST sequence, a hydrophilic segment at least 12 aa in length flanked by arginine, lysine, or histidine residues and including at least one proline (P) residue, one glutamate (E) or aspartate residue, and one serine (S) or threonine (T) residue (Rechsteiner and Rogers, 1996; Singh et al., 2006). The lesser the hydrophobic index and the greater the number of P, E, S, and T residues, the greater the probability that the sequence is a proteolytic signal. That probability is indicated by the score for the sequence obtained using the PEST-Find algorithm of Mathog and Rechsteiner (https://emb1.bcc.univie.ac.at/toolbox/pestfind/pestfind-analysis-webtool.htm). A score greater than zero indicates a possible PEST sequence, but it is commonly agreed that only scores greater than 5.0 indicate

a highly probable degradation target (Rechsteiner and Rogers, 1996; Kakkar et al., 1999; Singh et al., 2006). Sequences of dysbindin-1A, -1C, -2A, and -2B at or near their C-termini have strongly positive PEST-FIND scores (i.e., 13.75–20.34: *Table 2.2-3*). Those sequences are designated here as PEST domains (PDs, *Figures 2.2-1* and *2.2-2*). No such domains are evident in other dysbindin family proteins. The PEST-FIND program yields only negative scores for dysbindin-1B and scores below 5.0 for dysbindin-2C, -3A, and -3B. These proteins would thus be expected to have lower turnover rates than dysbindin-1A, -1C, -2A, and -2B.

While some PEST sequences may be constitutive proteolytic signals, most of them are conditional signals dependent on activating factors such as phosphorylation (Rechsteiner and Rogers, 1996; García-Alai et al., 2006). As shown in *Figure 2.2-2*, the PDs of dysbindin proteins have multiple predicted phosphorylation sites. In fact, a large number of such sites are concentrated in the PDs of dysbindin-1A and -1C. Phosphorylation of PEST sequences in other proteins appear to cause a conformational change (García-Alai et al., 2006) that is recognized and acted upon by the ubiquitin-26S proteasome system leading to rapid degradation of the parent protein (Rechsteiner and Rogers, 1996). It is currently unknown, however, if the PD is the site binding the E3 ubiquitin ligase TRIM32 (tripartite motif-containing protein 32), a recently discovered binding partner of dysbindin-1 that promotes its degradation (Locke et al., 2009).

3.3.3 Uncharacterized CTR Segments (X1–3)

Those portions of the CTR outside the DD and PD are undefined segments of dysbindin proteins. They have no known features clearly distinguishing them from other segments of those proteins across all paralogs and isoforms. Each member of the dysbindin protein family has two to three such uncharacterized zones that we simply call X segments (*Figure 2.2-1*).

X1 is the closest of the uncharacterized CTR segments to the NTR. In dysbindin-1 proteins, it lies between the CCD and the DD and contains one of the two predicted tyrosine phosphorylation sites found in those proteins (*Figure 2.2-2*). The beginning of X1 is less obvious in dysbindin-2 and -3, because those proteins lack a CCD. Nevertheless, BLASTp comparisons with dysbindin-1 isoforms yield a clue to the starting aa. Those comparisons show that significant aa sequence homology of dysbindin-1 isoforms with both dysbindin-2 and -3 is restricted to the CTR. The beginning of the homologous sequence in dysbindin-2 and -3 can be used as the start of their X1 segments, which is indicated in *Figure 2.2-1*. The end of X1 in all dysbindin proteins is simply the aa preceding the start of the DD.

X2 lies between the DD and either the PD or (if that is absent) the CT of the protein. It has an unusually high density of predicted phosphorylation sites in dysbindin-2A, -2B, -3A, and -3B (*Figure 2.2-2*). In other dysbindin proteins, however, very few such sites are found in X2.

X3 is only present in dysbindin-2A and -2B, where it is adjacent to the end of the PD. It is short and lacks any predicted phosphorylation sites.

4 Intracellular Localization of Dysbindin Family Members and Their Functional Implications

While immunohistochemical studies have clarified the intracellular localization of dysbindin-1 (Benson et al., 2001; Talbot et al., 2004, 2006; Di Pietro et al., 2006) and to some extent dysbindin-2B (Lucas et al., 2005), there are no comparable studies on other dysbindin family members. We thus lack the empirical data to compare their subcellular sites of action. But their most likely intracellular loci can be predicted from their aa sequence using programs developed for that purpose in response to the explosion of newly discovered proteins over the last decade. There are many such programs, the most recent of which are highly validated (Lu et al., 2004; Bulashevska and Eils, 2006; Höglund et al., 2006; Pierleoni et al., 2006; Yu et al., 2006) and yield results consistent with immunohistochemical findings on the few dysbindin family proteins localized to date.

4.1 Unlikely Sites

Dysbindin family proteins lack motifs indicative of direct binding to actin, DNA, RNA, or ribosomes. They also lack motifs indicative of sorting to, or retention in, the endoplasmic reticulum (ER), Golgi apparatus, mitochondria, or peroxisomes. The absence of such motifs is easily determined with online programs: PSORT2 (http://psort.ims.u-tokyo.ac.jp/form2.html) of Horton and Nakai (1997), iPSORT (http://bio-caml.org/ipsort/iPSORT/) of Bannai et al. (2002), the predictor of Golgi transmembrane proteins (http://ccb.imb.uq.edu.au/golgi/golgi_predictor.shtml of Yuan and Teasdale (2002), the peroxisome targeting signal predictor PTS1 (http://mendel.imp.ac.at/mendeljsp/sat/pts1/PTS1predictor.jsp) of Neuberger et al. (2003), and the current mitochondrial protein predictor program MitoProt2 (http://ihg.gsf.de/ihg/mito-prot.html) based on Claros and Vincens (1996; see also Prokisch et al., 2006). It is unclear if the slight dysbindin-1 immunoreactivity seen on or alongside mitochondria at the electron microscopic level (Di Pietro et al., 2006; Talbot et al., 2006) is actually specific to the antigen.

As explained in ❯ *Section 2.2.2.2.2* above, dysbindin family proteins other than dysbindin-2A lack a signal peptide. Such an N-terminal peptide is necessary for entry into the classical secretory pathway beginning in the ER and continuing in the Golgi apparatus. It is thus unlikely that dysbindin family proteins other than dysbindin-2A are secreted via the ER-Golgi network. That is consistent with the lack of ER membrane retention motifs in dysbindin family proteins noted above and with the rarity of N-terminal glycosylation sites in those proteins found using the NetNGlyc program (http://www.cbs.dtu.dk/services/NetNGlyc/). Indeed, sites for post-translational N-terminal glycosylation, which occur primarily in the lumen of the ER and Golgi apparatus (Roth, 2002), are absent in vertebrate members of the dysbindin protein family with the possible exception of human dysbindin-1C and mouse dysbindin-3B. An additional indicator that dysbindin family proteins are not part of the classical secretory cycle is the absence of motifs for transport from the cell membrane to the Golgi apparatus found with the PSORT2 program.

Dysbindin family proteins are also unlikely to be integral components of membranes or membrane-binding proteins. Transmembrane domains are not predicted in any of the family members in any species using either the HMMTOP program (http://www.enzim.hu/hmmtop/index.html) of Tusnády and Simon (2001) or the TMHMM program (http://www.cbs.dtu.dk/services/TMHMM-2.0/) of Krogh et al. (2001). Nor are any dysbindin family members predicted to contain sites promoting direct membrane binding, i.e., sites for N-terminal myristoylation, using the NMT-MYR program (http://mendel.imp.ac.at/myristate/SUPLpredictor.htm) of Maurer-Stroh et al. (2002) or for glycosylphosphatidylinositol (GPI) lipid anchors using the GPI program 3.0 (http://mendel.imp.ac.at/sat/gpi/gpi_server.html) of Eisenhaber et al. (2003). N-terminal myristoylation is a co-translational or posttranslational modification that prepares a protein for association with membranes (Boutin, 1997; Farazi et al., 2001). GPI anchors are posttranslationally modified lipids that anchor proteins to the lumenal surface of intracellular membranes in the classical secretory pathway and to the external surface of the cell membrane (Chatterjee and Mayor, 2001). The apparent absence of GPI anchoring sites in dysbindin family proteins reinforces the conclusion reached above that such proteins are not present in classical secretory pathways and indicate that they are also not bound to the outer leaflet of the plasma membrane.

Completing the list of sites at which dysbindin family proteins are unlikely to be integral components are lysosomes. None of the family members are predicted to be lysosomal by any of the many subcellular localization programs available online. Since they are also not predicted to be peroxisomal as indicated earlier, the family members are not likely to be directly involved in cellular catabolism or degradation. Via its binding partner myospryn, which in turn binds the muscle-specific intermediate filament protein desmin, dysbindin-1 may instead indirectly influence the intracellular location of conventional lysosomes (Kouloumenta et al., 2007). As a component of BLOC-1, dysbindin-1 appears to play a different function with respect to the secretory lysosomes, specifically promoting trafficking of cargo proteins such as tyrosinase-related protein-1 to melanosomes (Di Pietro et al., 2006; Setty et al., 2007) and vesicle associated membrane protein 7 (VAMP7 = TI-VAMP, tetanus neurotoxin-insensitive VAMP) to synaptic-like microvesicles in PC12 cells (Salazar et al., 2006).

4.2 Likely Sites: Cytoplasmic, Cytoskeletal, and Synaptic

What, then, are the most likely intracellular sites of dysbindin family proteins? Either a cytoplasmic or nuclear location is predicted by the major subcellular localization programs: BaCelLo of Pierleoni et al. (2006), Localizome of Lee et al. (2006), MultiLoc of Höglund et al. (2006), Proteome Analyst of Lu et al. (2004), and PSORT of Horton and Nakai (1997). Except for PSORT, they all predict a predominantly cytoplasmic site for most dysbindin family proteins, including dysbindin-2A, which could be found in the ER and Golgi apparatus due to its N-terminal signal sequence as explained previously. Dysbindin-2A could thus be both a classical secretory protein and a cytoplasmic protein, especially given the great variability in efficiency of translocation into the ER by signal peptide-containing proteins, which depends on the specific signal sequence and the intracellular environment (Levine et al., 2005).

While the findings of Benson et al. (2001) and our own light and electron microscopic (EM) studies are consistent with the view that dysbindin-1 is at least partially cytosolic, most of it is membranous (Talbot et al., 2006) or nuclear (see *Section 2.2.4.3*). Given multiple indications that dysbindin family proteins are neither integral membrane proteins nor directly linked to membranes (see *Section 2.2.4.1*), the concentration of dysbindin-1 in membranous tissue fractions probably reflects *indirect* membrane linkage, making it a peripheral membrane protein. This is not surprising given that most dysbindin-1 binding partners listed in *Tables 2.2-6* and *2.2-7* are themselves cytosolic proteins binding intracellular membranes. Indeed, all of dysbindin-1's BLOC-1 binding partners (i.e., muted, pallidin, snapin, and BLOC-1 subunit 2 [i.e., BLOS2]) are associated with clatherin-coated vesicles in HeLa cells (Borner et al., 2006), three of which (muted, pallidin, and snapin) are also associated with non-clathrin-coated SVLMs in PC-12 cells and synaptic vesicles in neurons (Salazar et al., 2005b, 2006). Six other candidate dysbindin-1 binding partners are associated with membranes: (1) AP-3μA with AP-3 derived SVLMs and synaptic vesicles (Salazar et al., 2005b, 2006; Danglot and Galli, 2007), (2) neurobeachin with Golgi-associated vesicles and postsynaptic membranes (Wang et al., 2000), (3) rab 11A with recycling endosomes (Seachrist and Ferguson, 2003), (4) RanBP5 with the nuclear pore complex (Deane et al., 1997), (5) rabenosyne-5 with early endosomes (De Renzis et al., 2002), and (6) sec8 with secretory vesicles (Hsu et al., 2004; Wang and Hsu, 2006) and the postsynaptic density (PSD) anchored to the postsynaptic membrane (Riefler et al., 2003; Sans et al., 2003). These binding partners may account for dysbindin-1A and -1B in synaptic membrane fractions of the mouse brain (Louneva et al., 2007).

Dysbindin-1 binding partners probably account as well for the sites at which the protein is found immunohistochemically at the EM level (immunoEM). In human melanoma cells, those sites are mainly early endosomes, peripheral vesicles, and the limiting membrane of melanosomes (Di Pietro et al., 2006; see also *Section 2.2.6.3.2.1*). In mouse hippocampal formation neurons, dysbindin-1 is primarily found on microtubules, synaptic vesicles, and PSDs and to a lesser degree at the internal face of dendritic and presynaptic membranes perhaps in association with recycling endosomes (Talbot et al., 2006; see *Section 2.2.6.3.2.3*). We noted above those binding partners that may account for the localization of dysbindin-1 to endosomes, secretory vesicles, and PSDs. Other binding partners could explain its localization to the cytoskeleton even though it lacks actin binding motifs (see *Section 2.2.4.1*). These are proteins that directly bind the cytoskeleton, specifically the plakins dystonin-1 (= BPAG1) and MACF1 (= ACF7), which bind actin, intermediate filaments, and microtubules (Leung et al., 2002), and three other proteins known to bind actin, namely pallidin (Falcón-Pérez et al., 2002), syntabulin-1 (Su et al., 2004a), and sec8 (Wang et al., 2004). The functions of dysbindin-1 suggested by these and other findings are specified in *Section 2.2.6.5*.

The only immunohistochemical data available on other dysbindin family proteins concern dysbindin-2B. Consistent with the predictions made earlier, Lucas et al. (2005) found this family member in rat myelomonocytic stem cells to be a cytosolic protein associated with vesicles, albeit of unspecified type. It may thus be a peripheral membrane protein like dysbindin-1.

A cytoplasmic locus for dysbindin-2C, -3A, and -3B as predicted by recent subcellular localization programs might seem inconsistent with the previously noted finding (see *Sections 2.2.2.2.2* and *2.2.2.2.3*)

that they have a high probability of being nonclassical secretory proteins. But cytosolic proteins can be excreted from the cytoplasm without packaging in vesicles, specifically via membrane transporters or membrane blebbing (Nickel, 2003, 2005).

4.3 Nuclear Localization

In addition to the cytoplasm, it appears likely that most dysbindin family proteins are also present in the nucleus of many cell types, as suggested by the predicted DNA-PK kinase site(s) found in all those proteins (see *Section 2.2.3.3.1*). Although zebrafish dysbindin-1A is the only dysbindin family member containing a classic nuclear localization signal according to the PredictNLS program based on Cokol et al. (2000), vertebrate orthologs of dysbindin-1 are likely to spend time in the nucleus according to the NucPred program (http://sbcweb.pdc.kth.se/cgi-bin/maccallr/nucpred/multi.pl) of Heddad et al. (2004), which makes predictions from aa sequences using an algorithm not dependent on finding that they contain a classic nuclear localization signal. The NucPred predictions agree with the original study on dysbindin-1 by Benson et al. (2001), with our own studies on human and mouse brain using multiple antibodies (see Talbot et al., 2004, 2006), and with recent findings of Oyama et al. (2009). Indeed, strong dysbindin-1 immunoreactivity is found in neuronal cell nuclei of most brain areas with all validated dysbindin-1 antibodies (e.g., *Figure 2.2-15c,d*, see also *Section 2.2.6.3.2.3*). Such immunoreactivity is seen at the EM level in association with chromatin and is diminished by antibody preadsorption with the antigen. As a more definitive test, we used the well characterized dysbindin-1 antibody PA3111 (Talbot et al., 2004, 2006) to immunoblot nuclear extracts from brains of C57BL/6 mice and of sandy mice lacking dysbindin-1 due to a mutation in the DTNBP1 gene (see *Section 2.2.6.4.1*). Full-length mouse dysbindin-1 served as the positive control, while nuclear extracts of sandy mouse brains served as negative controls. Brain cell nuclei in the mouse, which lacks dysbindin-1B, proved to be highly enriched in dysbindin-1A and -1C. In contrast, we find that human brain cell nuclei are enriched in dysbindin-1B with little, if any, dysbindin-1A or -1C (Louneva et al., in preparation). The nuclei of COS-7 cells (transformed cells derived from monkey kidney) contain both dysbindin-1A and -1B (Oyama et al., 2009).

There is a very simple explanation for the presence of dysbindin-1 in cell nuclei. Six of the known or candidate dysbindin-1 binding partners (*Tables 2.2-6* and *2.2-7*) are either found in the nucleus or at its limiting membranes. Those within the nucleus are cyclin A2 (Pines and Hunter, 1994), transcription factor IIIB, isoform 3 (Geiduschek and Kassavetis, 2001), and PA28γ (= Ki antigen; Murata et al., 1999). The binding partners at the nuclear envelope are A-kinase anchor protein 6 (AKAP6: Dodge-Kafka and Kapiloff, 2006), nesprin-1β (Zhang et al., 2001; Padmakumar et al., 2004), and RanBP-5 (Deane et al., 1997).

It should be recognized, however, that dysbindin-1 is not detectable in the nuclei of all cell types. Our immunohistochemical work with the PA3111 antibody noted above finds little if any dysbindin-1 immunoreactivity in the nuclei of certain neuronal populations (see *Section 2.2.6.3.2.3*). Using a similar antibody, Di Prietro et al. (2006) likewise found no dysbindin-1 in nuclei of cell of the melanoma cell line MNT-1.

Support for dysbindin-2 or -3 in cell nuclei is currently limited to predictions by subcellular localization programs, because there are no antibodies known to be specific for those dysbindin proteins at present. Human and mouse orthologs of dysbindin-2B are predicted to be mainly nuclear by three programs: PSORT2, BaCelLo, and MultiLoc. Mouse and rat orthologs of dysbindin-3B are predicted to be primarily nuclear by PSORT2 and BaCelLo. PSORT2 also predicts a mainly nuclear localization for dysbindin-2A and -2C, but all other subcellular localization programs predict them to be primarily cytoplasmic. Dysbindin-3A is consistently predicted to be mainly cytoplasmic. Apart from these exceptions, then, 5 of the 8 known dysbindin family members are commonly predicted to be nuclear by multiple subcellular localization programs. That proves an accurate prediction for dysbindin-1, as noted above, even though a substantial amount of the protein is present in cytoplasm associated with cell membranes and the cytoskeleton as described in *Section 2.2.4.2*.

5 Evolutionary Considerations: Molecular Phylogenetics

5.1 Origins

There is no evidence of the dysbindin protein family in unicellular organisms or plants. The family appears to originate instead from a common ancestor of both invertebrates and vertebrates, perhaps as long as 600 million years ago in the hypothesized *Urbilateria* (De Robertis and Sasai, 1996). A centralized nervous system may have originated in such an animal given studies on the "living fossil" *Platynereis dumerilii*, a primitive marine sand worm (Denes et al., 2007). The earliest phylogenetic evidence of the dysbindin protein family is an apparent dysbindin-1B ortholog in the arthropod *Drosophila melanogaster* (see *Section 2.2.2.2.1*). The nucleotide sequence of the encoding gene CG6856, however, is not significantly similar to that of non-arthropod genes encoding dysbindin family proteins.

The dysbindin family thus appears to have arisen independently in the arthropods and in later animals. The most ancient of the latter is the invertebrate chordate amphioxus (i.e., the lancelet: see also Guo et al., 2009), in which we find the first evidence of dysbindin-1A (*Table 2.2-1*). Other dysbindin family members cannot be traced back as far. The earliest phylogenetic evidence we have for an unambiguous dysbindin-1B is in the frog, that for dysbindin-1C is in the mouse, and that for dysbindin-2 and -3 is in fish (see *Sections 2.2.2.2.1–2.2.2.2.3*). While we lack sufficient information to determine the likely evolutionary relationships among all eight family members, it is possible that they are all derived from nuclear proteins since one or more predicted DNA-PK sites occur in all family members (including *Drosophila* dysbindin-1) regardless of species, almost invariably in or near the segment of the protein with significant sequence homology across all family members (i.e., in the defining dysbindin domain [DD], see *Section 2.2.3.3.1*).

5.2 Vertebrate Evolution of Dysbindin-1A

Of the three dysbindin paralogs and their isoforms, only dysbindin-1A is known in a sufficiently broad range of species to identify evolutionary patterns (see *Table 2.2-9*). As already noted, the earliest known dysbindin-1A ortholog is found in the invertebrate chordate amphioxus, but has only a relatively low degree of aa sequence homology with the human ortholog (i.e., 36%). As seen in zebrafish, however, a relatively high degree of sequence homology with the human ortholog (i.e., 67%) was attained early in vertebrate evolution. The degree of homology is slightly greater in the frog (72%) and reaches high levels before the radiation of mammalian species judging from the degree of homology in birds represented by the chicken (79%). The degree of homology is no greater in mice, but is distinctly higher in the cow (87%) and chimpanzee (95%). This shows that dysbindin-1A as a whole has been highly conserved in vertebrate evolution, as graphically shown in a seed alignment of the dysbindin-1A aa sequence from the zebrafish, mouse, and human (*Figure 2.2-7*). Even in chimpanzees, however, the protein is not identical to that in humans, especially in the NTR (see *Table 2.2-9*), indicating that dysbindin-1 continued to evolve in the human lineage. This is consistent with evidence that allelic variation in the encoding gene, DTNBP1, has changed significantly in the last 10,000 years, especially in European populations (Crespi et al., 2007).

5.2.1 Changes in the CCD and its LZ Motif

The CCD of dysbindin-1, which is virtually identical in all isoforms of the same species, changed considerably in the course of evolution. The oldest of all known dysbindin-1A orthologs (i.e., that in amphioxus) has a CCD significantly similar in aa sequence to the CCD of zebrafish and frog orthologs, but not to the CCD in less phylogenetically related chicken or human orthologs (e.g., *Table 2.2-1*). This may reflect evolutionary pressure for changes in the structure of the CCD. The same pressure may have driven

Table 2.2-9

Similarity of nonhuman to human dysbindin-1A (% amino acid sequence homology to human dysbindin-1A)[a]

Species in descending phylogenetic order	Complete protein	N-terminus region	Coiled-Coil domain	C-terminus region
Chimpanzee (*Pan troglodytes*)[b]	95	83	100	98
Cow (*Bos taurus*)	87	94	87	83
Pig (*Sus scrofa*)	86	93	91	84
Mouse (*Mus musculus*)	79	93	77	73
Rat (*Rattus norvegicus*)	78	91	75	73
Chicken (*Gallus gallus*)	79	83	73	80
Frog (*Xenopus laevis*)	72	72	71	73
Zebrafish (*Brachydanio rerio*)	67	71	67	66
Amphioxus (*Branchiostoma belcheri tsingtaunese*)	36	40	NS[c]	39

[a]Specifically degree of identical sequence alignment based on NCBI Blast 2 comparisons

[b]Based on the predicted chimpanzee dysbindin-1A (XP_001169961 in NCBI database)

[c]Non-significant (NS) amino acid sequence identity, though the coiled coil domain in amphioxus dysbindin-1A shares significant aa sequence homology with the same domain of zebrafish and frog dysbindin-1A

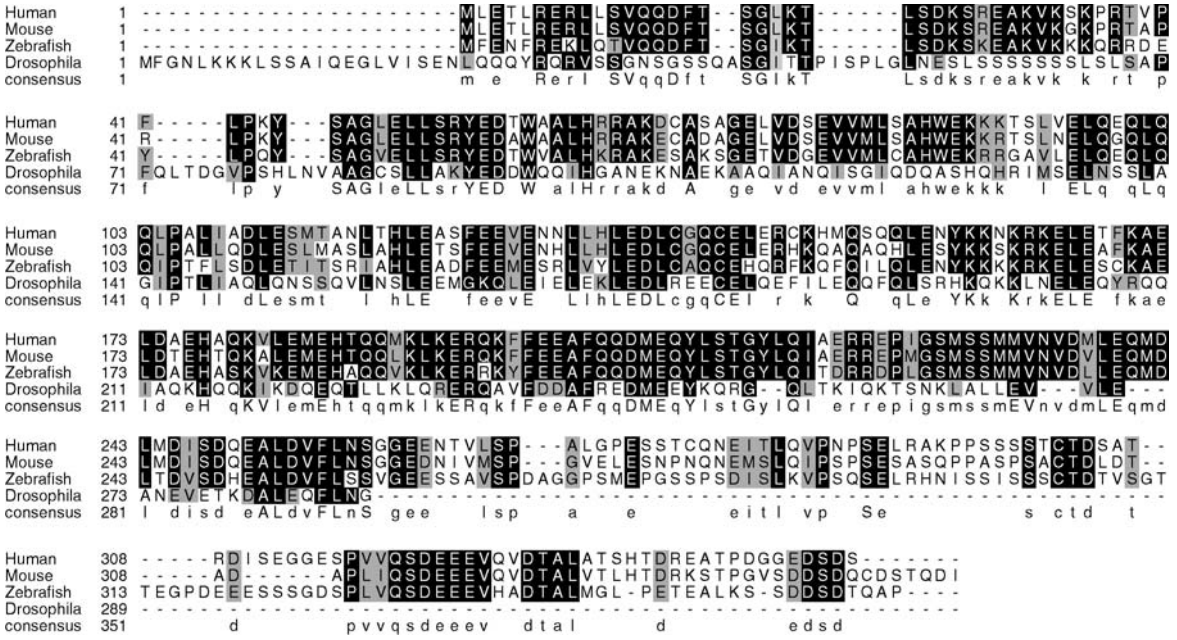
convergent evolution of the CCDs in dysbindin-1B of *Drosophila* and humans, which share significant sequence homology. That would not be expected without convergent evolution since there is no significant similarity in the aa sequence of CCDs in dysbindin-1 isoforms of *Drosophila* and amphioxus, which are more closely related phylogenetically than *Drosophila* and humans. We are admittedly comparing an apparent dysbindin-1B in *Drosophila* to dysbindin-1A in amphioxus (because those are the only dysbindin-1 isoforms known in those species), but that should not account for the lack of significant similarity in their CCDs, because the CCDs of dysbindin-1A and -1B are virtually identical in a given species judging from those in which both isoforms are known (i.e., the frog and human).

One source of evolutionary pressure that drove change in dysbindin-1 CCDs may have been the adaptive advantage of establishing LZ motifs (see *Section 2.2.3.2*) capable of forming more stable, durable coiled coils with binding partners. In *Drosophila*, the CCD has a rare hybrid LZ motif consisting of four successive heptads with leucine in the "d" position of the first three heptads and cysteine in the "d" position of the fourth heptad (*Figure 2.2-5*). Replacement of a "d" position leucine with cysteine in a LZ motif need not impair the dimerization function of such a motif (van Heeckeren et al., 1992). Nevertheless, a cysteine in the "d" position of LZ motifs is rarely observed (see Cohen and Parry, 1990), probably because it contributes little stability to a coiled coil (Moitra et al., 1997). In amphioxus, zebrafish, frog, chicken, and rat, the CCD has a more common LZ motif, but it is still a hybrid in which the "d" positions are occupied by leucine, isoleucine, or valine (*Figure 2.2-5*). In the mouse, pig, cow, and human, a classic LZ motif is seen in the CCD with leucine in all the "d" positions (*Figure 2.2-5*). Since leucine is the most stabilizing of amino acids common in the "d" positions of LZ motifs (Moitra et al., 1997), the progressive shift toward classic LZ motifs in dysbindin-1 evolution should have enabled progressively more stable interactions with binding partners.

Closer inspection of the LZ motifs in dysbindin-1 orthologs supports the conclusion just reached. The degree to which LZ motifs form stable coiled coils is determined mainly by the amino acids in their hydrophobic "d" *and* "a" positions and only to a lesser degree by the amino acids in their hydrophilic "e" and "g" positions (Hu et al., 1993; Moitra et al., 1997; Wagschal et al., 1999; Acharya et al., 2002; Mason and Arndt, 2004). Thermodynamic studies have determined contributions of specific "a" and "d" position amino acids to coiled coil stability by calculating the amount of energy transferred upon induced unfolding of the coiled coil. The contribution of an amino acid in an "a" or "d" position in a coiled coil is expressed as $\Delta\Delta G_A$, which is the kcal/mol change in free energy (ΔG) transfer upon unfolding induced thermally or chemically compared with that observed with coiled coils of the same proteins in which the amino acid under study is replaced by the weakly hydrophobic amino acid alanine. Wagschal et al. (1999) provides such data on many amino acids in the "a" position, while Moitra et al. (1997) does so on fewer amino acids in the

Figure 2.2-7

Seed alignment of dysbindin-1 orthologs identified in human, mouse, zebrafish, and fruit fly (*Drosophila melanogaster*). The isoform represented is dysbindin-1A with the exception of that in the fruit fly. The only dysbindin isoform identified in the fruit fly appears to be an ortholog of dysbindin-1B (see text *Section 2.2.2.2.1*)



"d" position. With such thermodynamic data, we can estimate the fitness of a LZ motif to form stable coiled coils by adding the $\Delta\Delta G_A$ for each of amino acids in the "a" and "d" positions of that motif. The total $\Delta\Delta G_A$ or $\Sigma\ \Delta\Delta G_A$ for each LZ motifs of dysbindin-1 shown in *Figure 2.2-5* is thus easily calculated. The rare hybrid LZ motif in *Drosophila* has the lowest $\Sigma\ \Delta\Delta G_A$ score (19.09 kcal/mol). Higher scores ($\Sigma\ \Delta\Delta G_A$ = 22.13–24.57 kcal/mol) are found for the more common hybrid LZ motifs in the amphioxus, zebrafish, frog, and chicken. The highest scores ($\Sigma\ \Delta\Delta G_A$ = 27.44–30.94 kcal/mol) are found only in mammals, which (apart from the rat) have classic LZ motifs. These calculations reinforce the view that evolution of dysbindin-1 was marked by progressive adaptation of its LZ motif for more durable interaction with binding partners.

5.2.2 Changes in the CTR and NTR

Unlike the CCD, the NTR and CTR segments of dysbindin-1A orthologs were more conserved in the course of vertebrate evolution. That is especially true for the NTR (see also Guo et al., 2009). In that protein segment, the degree of sequence homology of non-human to human dysbindin-1A reaches 84% in the chicken and 93% in the mouse (*Table 2.2-9*). This suggests that the NTR was optimized early in vertebrate evolution for a major role in dysbindin-1A functions, which may be regulated by kinases such as PKB/Akt that could affect the adjacent CCD's ability to interact with binding partners as speculated in *Sections 2.2.3.1* and *2.2.3.2*. During primate evolution, however, further changes occurred in the NTR, which is less similar to the human NTR in the chimpanzee (83%) than in the cow (94%). In contrast, the rest of the chimpanzee dysbindin-1A is virtually identical to the human ortholog (*Table 2.2-9*). Evolution of dysbindin-1A in the human lineage thus appears to have altered primarily the NTR, perhaps reflecting the relatively recent changes in allelic variation in DTNBP1 discovered by Crespi et al. (2007).

6 A Closer Look at Dysbindin-1

Of the three dysbindin paralogs, dysbindin-1 has attracted the most attention to date because variations in its encoding gene, DTNBP1, have been repeatedly associated with schizophrenia (see *Section 2.2.6.6.1*) and with cognitive impairments (see *Section 2.2.6.6.3.5*). As a result, there is a considerably larger body of literature on dysbindin-1 than on dysbindin-2 or -3, our knowledge of which was covered fully in preceding sections. The much more extensive literature on dysbindin-1 thus warrants more detailed examination.

6.1 The Encoding Gene and Its Transcription

Apart from SNP analyses, there is still a paucity of published information on the gene encoding dysbindin-1 and on its transcripts. Much of the information below derives instead from bioinformatic sources, especially the NCBI gene database AceView (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/index.html) and to a lesser extent ECgene (http://genome.ewha.ac.kr/ECgene).

6.1.1 The DTNBP1 Gene

As noted in *Section 2.2.2.2.1*, dysbindin-1 is encoded by the dystrobrevin binding protein 1 gene (abbreviated as DTNBP1 in humans and as Dtnbp1 in mice). In humans, the gene covers 140.26 kb on the petite (p) arm of chromosome 6 at cytogenic band 22.3 (i.e., 6p22.3). In mice, it covers a smaller, 80.07 kb, segment on chromosome 13 at cytogenic band A5 (i.e., 13A5). In both those species, the gene is on the minus (reverse) strand of the double helix in NCBI's Map View. What little is known about orthologous genes in other species is provided by Guo et al. (2009), namely in the zebra fish, chicken, opossum, rat, cow, pig, dog, marmoset and rhesus monkeys, orangutan, and chimpanzee.

The exonic–intronic structure of the dysbindin-1 gene is complex and not well established since the cDNA clones available for determining that structure (165 in humans and 207 in the mouse: see AceView) include many that derive only from partially expressed sequence tags or ESTs. Using conservative criteria,

studies in the literature estimate 10 exons in the mouse (Li et al., 2003, Guo et al., 2009) and either 10 (Guo et al., 2009) or 13 (Williams et al., 2004) exons in humans. We consider the higher number more likely in humans. In both species, however, there are even more variants proposed on AceView. For the sake of simplicity, we present the diagramatic form of only the published exonic–intronic structures (see *Figures 2.2-8b* and *2.2-9c*).

As illustrated in *Figure 2.2-8d*, it is possible to deduce which exons encode each component of human dysbindin-1 taking into account the size of coding elements and other factors. The NTR appears to be encoded by exons 1 and 3–5, which are protein coding elements in transcripts for dysbindin-1A and -1B, but not in the transcript for dysbindin-1C, which lacks the NTR (see *Section 2.2.2.2.1* and *Figures 2.2-1* and *2.2-3*). Exon 2 cannot encode part of the NTR, because it is not encoded by transcripts of the three major isoforms of dysbindin-1 (see ECgene and *Figure 2.2-8c*). The CCD common to all dysbindin-1 isoforms appears to be encoded by exons 6–8 and part of exon 11, which are protein coding elements in all those isoforms. A deletion mutation in exons 6 and 7 of Dtnbp1 in the sandy mouse is likely to cause loss of about half the CCD (see *Section 2.2.6.4.1* for details). Exons 9 and 10 cannot encode part of the CCD, because neither exon is represented in transcripts of dysbindin-1A, -1B, or -1C (see ECgene and *Figure 2.2-8c*). The dysbindin domain (DD) and the flanking protein segments X1 and X2 appear to be encoded by part of exon 11 and by exon 12, which are at least partial coding elements in all dysbindin-1 isoforms. The PEST domain (PD) is encoded by exon 13, which is a coding element in transcripts for dysbindin-1A and -1C, but is not encoded in dysbindin-1B, which lacks a PD (see *Section 2.2.2.2.1* and *Figure 2.2-1*).

On the same chromosome as DTNBP1 are a number of other genes expressed in the brain (see *Figure 2.2-8a*). Among these is the gene encoding the myosin regulatory light chain interacting protein (MYLIP), a neuronal protein inhibiting neurite outgrowth (Olsson et al., 1999, 2000; Bornhauser et al., 2003) and genes encoding dystonin-1 (DST) and nesprin-1β2 (SYNE1), two candidate dysbindin-1 binding partners (*Table 2.2-7*). In the mouse, Dst and Syne1 are not on the same chromosome as Dtnbp1.

Of greater interest, however, are genes flanking DTNBP1 that share with it an association with schizophrenia according to one or more studies. They have the same position relative to the dysbindin-1 gene in humans and mice. In the human gene nomenclature, they are JARID2, MUTED, and ATXN1 (see *Figure 2.2-8a*). JARID2 is immediately adjacent to the telomeric end of DTNBP1; it encodes jumonji, which is a transcriptional repressor important in both neural and cardiac development (Takeuchi et al., 2006). A tetranucleotide repeat polymorphism in JARID2 near its junction with DTNBP1 has been associated with schizophrenia (Pedrosa et al., 2007). MUTED is farther than JARID2 from DTNBP1, but still on its telomeric side. It encodes the protein muted, which is a known binding partner of dysbindin-1 (see *Table 2.2-7*) and of other BLOC-1 proteins (see Starcevic and Dell'Angelica, 2004). Straub et al. (2005) have reported that multiple SNPs in MUTED are associated with schizophrenia (Straub et al., 2005). While a later study did not confirm that finding, it did find that an epistatic interaction between MUTED and DTNBP1 adds to risk for schizophrenia (Morris et al., 2008). Finally, on the centromeric side of DTNBP1, though not adjacent to it, is the gene ATXN1 encoding ataxin 1. Autosomal dominant mutations (specifically CAG

Figure 2.2-8

(shown on next page). The human dysbindin-1 gene (DTNBP1), its major transcripts, and the likely relationship of exons to segments of the translated protein. (a), Cytogenic representation of chromosome 6 showing the location of DTNBP1 at 6p22.3 and of other genes that (1) encode known or candidate binding dysbindin-1 binding partners (i.e., MUTED, DYST, and SYNE1), (2) influence neuronal development (i.e., MYLIP), and/or (3) are associated with schizophrenia (i.e., ATXN1, JARID, and MUTED). The proteins encoded by these genes are identified in the text (see text *Section 2.2.6.1.1*). (b) Basic exonic-intronic structure of DTNBP1 deduced by Williams et al. (2004), who hypothesize four promoters (P1 – P4) though only P1 can be considered highly likely at present (see Pedrosa et al., 2009). The rectangular blocks represent exons, the width of which is roughly proportional to the number of nucleotides in each exon. (c) The three established transcripts of DTNBP1 (variants 1–3) with their NCBI accession numbers. Variants 1 and 3 encode 10 exons, while variant 2 encodes only 9 exons. The encoded, but untranslated exons (or parts of exons) are unshaded. (d) Deduced relationship between translated exons and segments of dysbindin-1 (see text *Section 2.2.6.1.1* for details)

Figure 2.2-8 (continued)

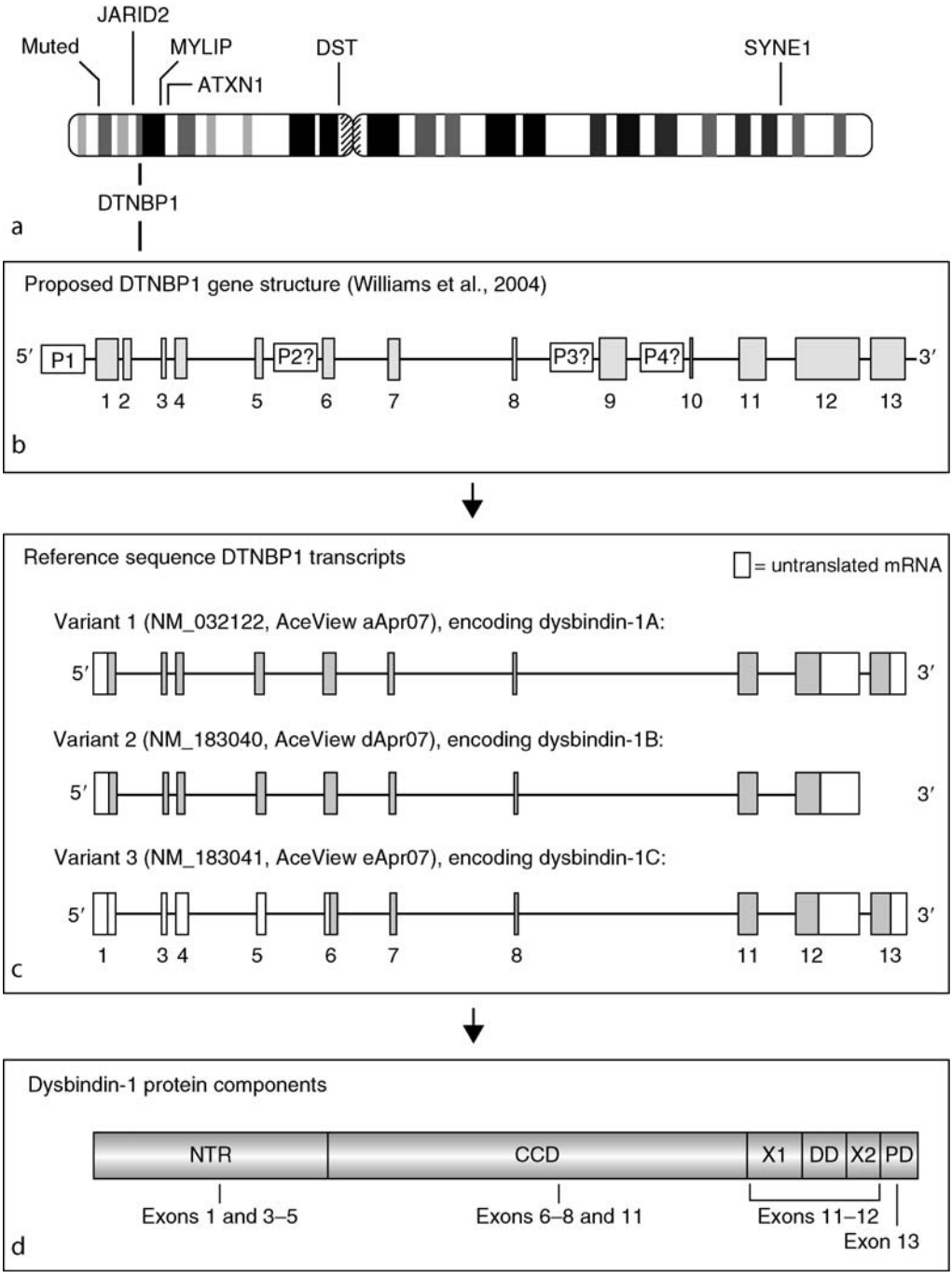
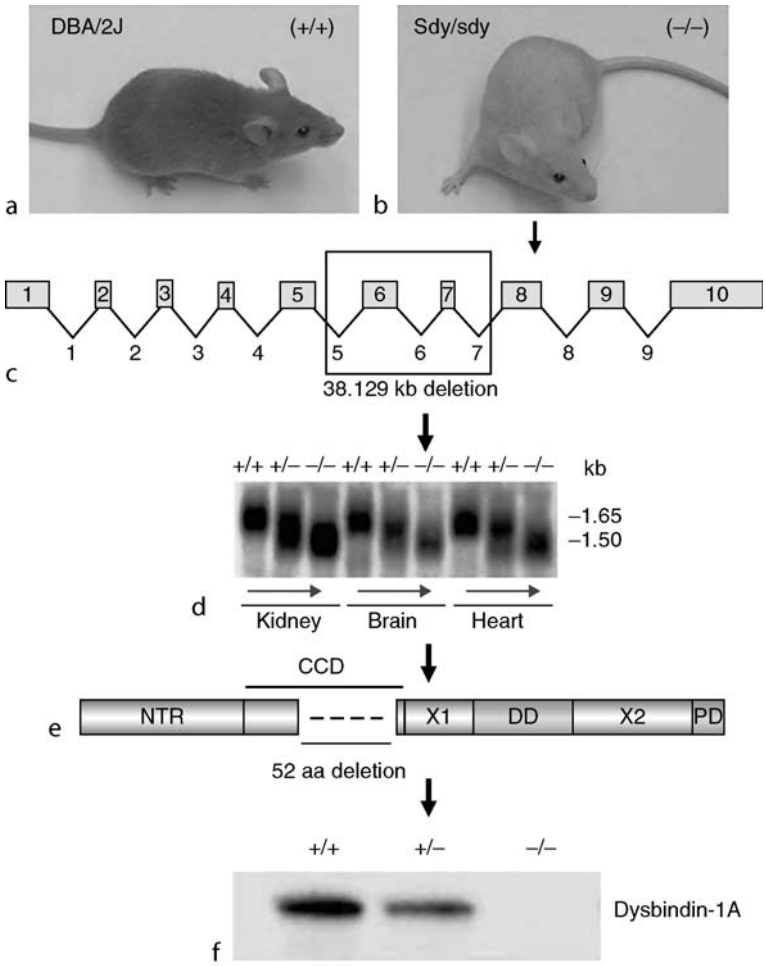


Figure 2.2-9

The mouse dysbindin-1 gene (Dtnbp1) and its mutation in sandy (sdy) mice. (a) and (b) Coat-color difference between wild type and sdy homozygous mice due to defective melanosome formation (see text *Section 2.2.6.4.2.3*). (c) Basic exonic-intronic structure of mouse Dtnbp1 gene showing large deletion mutation of exons 6 and 7 in sdy mice reported by Li et al. (2003). (d) Northern blots on the kidney, brain, and heart showing relative size of Dtnbp1 transcripts in wild type (+/+), heterozygous (+/−), and homozygous (−/−) mice (Li et al., 2003). Note the progressive size reduction from wild type to homozygous animals, presumably due to mixture of normal and truncated transcripts in the heterozygous animals. (e) Schematic of dysbindin-1 lacking 52 aa in the CCD due to mutation-induced truncation of transcript. (f) Dysbindin-1A decrease and loss in kidney tissue of heterozygous and homozygous animals, respectively (Li et al., 2003), probably due to rapid degradation as a result of failure to form stable interactions with BLOC-1 binding partners in the absence of an intact CCD (see text *Section 2.2.6.4.1* and *Figure 2.2-19*). We thank Richard T. Swank for panels (a) and (b). Panels (c), (d), and (f) are adapted with permission from Li et al. (2003)



repeats) in ATXN1 cause spinocerebellar ataxia type 1, a progressive neurodegenerative disorder marked by ataxia of gait and by cognitive deficits (Bürk et al., 2003; Dueñas et al., 2006). The CAG repeats in ATXN1 have been associated with schizophrenia in some studies (Wang et al., 1996; Joo et al., 1999) but not in others (Ohara et al., 1997; Pujana et al., 1997). While association of ATXN1 with schizophrenia is not certain, there are clearly multiple susceptibility loci for that disorder in the vicinity of DTNBP1.

6.1.2 Transcripts

Transcription of the human DTNBP1 gene can generate at least 16 different mRNAs (*Table 2.2-10*). Fourteen of these are alternatively spliced variants (AceView transcripts *a*-*m* and *p*); the remainder are unspliced forms (AceView transcripts *n*-*o*). The alternatively spliced variants include transcripts of dysbindin-1A, -1B, and -1C (i.e., AceView transcripts *a*, *d*, and *e*, respectively). These appear to be the most commonly expressed transcripts based on the number of cDNA clones available for their reconstruction (see *Table 2.2-10*). As noted earlier (see *Section 2.2.2.2.1*), however, there is evidence for at least five other DTNBP1 transcripts in humans (i.e., AceView transcripts *b*, *c*, *f*, *g*, and *h*). Their complete exon coding sequences are known and are predicted to contain both a CCD and DD. They would encode proteins 338, 334, 219, 212, and 208 aa in length, designated here as dysbindin-1D, -1E, -1F, -1G, and -1H, respectively. These isoforms may be expressed only in certain cell types under certain developmental or physiological conditions. For example, *b* has been detected only in neuroblastomas, *c* only in dorsolateral prefrontal cortex, lymphomas, the placenta, T cell leukemia, and uterine sarcomas, and *f* only in small cell lung carcinomas.

Caution is advised with respect to the remaining eight human DTNBP1 transcripts listed by AceView. As noted in that database, transcripts *o* and *p* are unlikely to encode complete proteins. Transcripts i, *k*-*m*, and *n* are suspect. Transcript *j* does not encode a DD, which is the defining feature of all dysbindin paralogs; it may not actually be a DTNBP1 transcript. A similar, though less confident assessment can be given for transcripts *i*, *m*, and *n*. These do not appear to encode a CCD, which is a central feature of currently known dysbindin-1 isoforms. There is also no apparent CCD encoded by transcripts *k* and *l*, but we only have partial nucleotide sequences for these mRNAs.

Transcription of the mouse Dtnbp1 gene can generate as many as 10 different mRNAs (*Table 2.2-11*). Eight are alternatively spliced variants (AceView transcripts *a*-*f*, *h*, and *i*), while the remaining two are unspliced forms (AceView transcripts *g* and *j*). The alternatively spliced variants include those for dysbindin-1A and -1C (i.e., AceView transcripts *a* and *b*, respectively). There is no known mouse transcript encoding a protein orthologous to human dysbindin-1B. While it has been predicted that all 10 mouse transcripts are translated; this has been verified only for transcripts *a* and *b*. They are the only ones known to encode both a CCD and a DD. Two of the other transcripts, *d* and *e*, encode a DD but no clear CCD. Three other transcripts, *c*, *g*, and *i*, do encode a CCD, but apparently not a DD. In the mouse, then, the only proposed transcripts encoding proteins clearly orthologous to human dysbindin-1 are those for dysbindin-1A and -1C (see *Section 2.2.6.3.1*).

The mouse Dtnbp1 transcript identified as *a* on AceView is predicted to encode a protein of 408 aa, which is 56 aa longer than the largest mouse dysbindin-1A isoform reported in the literature (i.e., the 352 aa isoform of Benson et al., 2001). If we accept the first ATG in transcript *a* as the start codon, the predicted protein matches the 352 aa isoform. AceView instead lists the longer possibility for two reasons. Near the 5′ end of the transcript is a less common start codon sequence (CTG). Between the 5′ end and the first ATG sequence are 168 nucleotides potentially encoding an arginine–proline rich N-terminal extension that may serve as a nuclear localization signal of functional interest. Indeed, the 408 aa variant of dysbindin-1A has been predicted in mouse undifferentiated limb mesenchyme (NCBI accession no. AAH48682). But it is not predicted elsewhere. In most tissues, then, transcript *a* is probably translated as the 352 aa isoform.

6.1.3 Transcriptional Regulation

Differential allele expression assays show that cis- and trans- acting sequences regulate transcription of DTNBP1 (cf. Bray et al., 2003, 2005, 2008). One or more cis-acting sequence occurs at chromosomal

Table 2.2-10

Known and proposed human DTNBP1 transcripts according to AceView[a]

Transcript	Number of cDNA clones used to reconstruct	Complete mRNA sequence known?	mRNA size (nucleotide number)	cDNA clones fully supporting exonic structure	Number of exons encoded	Protein coding likely?	Predicted number of AA encoded	CCD encoded?	DD encoded?	Normal brain expression? (specific areas)
aApr07 (Dysbindin-1A)	70	Yes	1403	AY265460,	10	Yes	351	Yes	Yes	Yes[b] (DLPFC, HF+Hyp)
bApr07	1	Yes	1516	BX394616 + BX394617	11	Yes	338	Yes	Yes	Unknown
cApr07	4	No	1273	BQ440940 + BX343622	9	Yes	334	Yes	Yes	Yes (DLPFC)
dApr07 (Dysbindin-1B)	33	Yes	1955	AL136637	9	Yes	303	Yes	Yes	Yes (Caudate + Pit)
eApr07 (Dysbindin-1C)	15	Yes	1443	AK054593	10	Yes	270	Yes	Yes	Yes (DLPFC)
fApr07	1	No	808	BE793298	7	Yes	219	Yes	Yes	Unknown
gApr07	7	Yes	1518	CR623452	9	Yes	212	Yes	Yes	Yes (HF)
hApr07	4	Yes	1306	CR599171	9	Yes	208	Yes	Yes	Unknown
iApr07	7	No	1012 (CDS)[c]	AA443420 + BP202776	4	Yes	195	?	Yes	Yes (Am + Hyp)
jApr07	3	Yes	677	BG699807	6	Yes	163	Yes	No	Yes[b] (HF)
kApr07	1	No	499	AL711802	4	Yes	142	?	Yes	Unknown
lApr07	2	No	425	Al299674 + Al299675+	3	Yes	141	?	Yes	Unknown
mApr07	1	No	824 (CDS)[c]	CD172391	2	Yes	120	?	Yes	Yes
nApr07	4	No	696 (CDS)[c]	CA450471	1	Yes	91	?	Yes	Unknown
oApr07	2	No	620	Bl752857 + DB465987	1	No	39	?	?	Yes (HF)
pApr07	1	No	568	CR737548	3	No	20	?	?	Unknown

[a]As of April, 2007: see http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi?c=geneid&org=9606&l=84062

[b]Specific brain areas in parentheses are abbreviated as follows: *Am* amygdala, DLPFC dorsolateral preprontal corter, *HF* hippocampal formation, *Hyp* hypothalamus, *Pit* pituitary

[c]Nucleotide number for the complete mRNA sequence encoding exons (i.e., its complete coding sequence or CDS)

Table 2.2-11
Known and proposed mouse Dtnbp1 transcripts according to AceView[a]

Transcript	Number of cDNA clones used to reconstruct	Complete mRNA sequence known?	mRNA size (nucleotide number)	cDNA clones fully supporting exonic structure	Number of exons encoded	Protein coding likely?	Predicted number of AA encoded	CCD encoded?	DD encoded?	Normal brain expression? (specific areas)
aSep07 (Dysbindin-1A)	181	Yes[b]	1361	BC048682	10	Yes	352 or 408[c]	Yes	Yes	Yes[d]
bSep07 (Dysbindin-1C)	3	Yes	1082 (CDS)[e]	AK017400	6	Yes	271	Yes	Yes	Yes
cSep07	1	No	>1229	AK150306	8	Yes	218	Yes	?	Unknown
dSep07	6	Yes	840 (CDS)[e]	CX238878	4	Yes	196	?	Yes	Yes (wall of LV + Vis Ctx)[f]
eSep07	4	Yes	879 (CDS)[e]	TI_158613972	4	Yes	169	?	Yes	Yes (wall of LV)[f]
fSep07	2	No	>913	DT903910	2	Yes	120	?	?	Unknown
gSep07	2	No	>300	CG518916	1	Yes	99	Yes	?	Unknown
hSep07	1	No	>357	BY016106	2	Yes	89	?	?	Unknown
iSep07	1	Yes	558 (CDS)[e]	BE631597	4	Yes	82	Yes	?	Unknown
jSep07	2	Yes	1041 (CDS)[e]	AW048926	1	Yes	71	?	?	Unknown

[a]As of September, 2007: see http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi?exdb=AceView&db=mm_37&term=dtnbp1&submit=Go

[b]The 5′-end of this mRNA may be incomplete

[c]The known start codon predicts a 352 aa protein, but the nucleotide sequence before that predicts an additional 56 aa (see text *Section 2.2.6.1.2*)

[d]This transcript has been found in many brain areas, namely the area postrema, diencephalon (thalamus + hypothalamus), pituitary, subfornical organ, visual cortex, and the wall of the lateral ventricle. It has also been found in the retina

[e]Nucleotide number for the complete mRNA sequence encoding exons (i.e., its complete coding sequence or CDS)

[f]LV lateral ventricle, Vis Ctx visual cortex

locus 6p22 containing DTNBP1, with one or more trans-acting sequence occurring at chromosome locus 8p22–12 that contains several other genes associated with schizophrenia, including neuregulin (Bray et al., 2008). These sequences may regulate different DTNBP1 promoters, the diversity of which is needed to explain the known diversity in the first exons of DTNBP1 transcripts. Williams et al. (2004) proposed four promoters in DTNBP1 near the 5′ ends of what they call exons 1, 6, 9, and 10 (see *Figure 2.2–2.8b*). These areas contain typical promoter sequences except that near the 5′ end of exon 9.

While the specific location of cis- and –trans-acting sequences regulating DTNPB1 gene expression remain to be established, one of the former may be in the first putative promoter. In that location, Pedrosa et al. (2009) identified a likely regulatory sequence between nucleotides 15,770,998 and 15,771,876 on the minus strand of chromosome 6p. That sequence is of special interest, because it contains a SNP location associated with schizophrenia in multiple studies (i.e., rs2619538: see *Table 2.2-14*).

In the core of the first putative promoter region, Liao and Chen (2004) found consensus binding sites for several transcription factors. Only two were identified unambiguously: nuclear factor 1 (NF-1) and specificity protein 1 (Sp1). NF-1 is a transcriptional activator of cis-acting control sequences and promoters regulating expression of genes for cell growth and proliferation, embryogenesis, and neural development (Gronostajski, 2000; Plachez et al., 2008). Sp1 is also a transcriptional activator of cis-acting control sequences and promoters regulating expression of diverse genes (Li et al., 2004b), including those regulating (1) cell growth and proliferation (cf. Karlseder et al., 1996, Lin et al., 1996, and Yoon et al., 1999), (2) embryogenesis (Zhao and Meng, 2005), (3) expression of dynamin 1 (Yoo et al., 2002), NMDA receptor subunit 1 (Okamaoto et al., 2002; Liu et al., 2004), and SNAP-25 (Cai et al., 2008), and (4) adaptive responses to oxidative stress (Ryu et al., 2003; Lee et al., 2006). Sp1 regulates gene expression with other transcription factors it binds (Li et al., 2004b), such as E2F1 (Karlseder et al., 1996; Lin et al., 1996). Bound to gene promoters, E2F1 can – depending on cellular conditions – activate a number of processes, including cell growth and proliferation, apoptosis, or DNA repair (cf. Crosby and Almasan, 2004, Rogoff and Kowalik, 2004, Stevens and La Thangue, 2004, and Pützer, 2007). In the nervous system, E2F1 is highly expressed in proliferating neuronal precursors (Dagnino et al., 1997) and is important in adult neurogenesis (Cooper-Kuhn et al., 2002).

It is likely that at least one of the DTNBP1 promoters binds E2F1 given the finding of Iwanaga et al. (2006) that serum deprivation of embryonic fibroblasts transfected to ectopically express E2F1 causes a 10 fold increase in expression of a dysbindin-1 transcript. Unlike the case for the great majority of E2F1-induced genes, DTNBP1 expression was unaffected by serum alone, indicating that the effect observed upon serum-deprivation was cell-cycle independent. The same cell-cycle independent effect of E2F1 on gene expression was observed for the gene encoding cyclin A2, which is a candidate binding partner of dysbindin-1 (*Table 2.2-7*). It seems probable, then, that E2F1-induced expression of DTNBP1 is related to post-mitotic functions, the most likely ones being apoptotic and DNA repair functions of E2F1 (Stevens and La Thangue, 2004; Pützer, 2007). It must be noted, however, that the DTNBP1 transcript found to be regulated by E2F1 by Iwanaga et al. (2006) is uncommon. It encodes all but the first exon of dysbindin-1A (K. Ohtani, personal communication) and may thus be a novel dysbindin-1C transcript. Further study is needed to determine if expression of other DTNBP1 transcripts are regulated by E2F1 under different circumstances and in different cell types than tested by Iwanaga et al. (2006).

6.2 Gene Expression in Mammalian Tissues

Only a few studies provide information about dysbindin-1 gene expression in tissue. They provide little information about the distribution of specific DTNBP1 transcripts, but they do indicate the distribution of the transcripts as a group.

6.2.1 Expression in Normal Tissues

According to AceView, dysbindin-1 gene expression is ubiquitous in human and mouse tissues (see *Table 2.2-12*). That was first discovered in mice by Benson et al. (2001) using northern blotting with a

Table 2.2-12
Gene expression of dysbindin-1 in normal human and mouse tissues[a]

	Type of transcript				
	For dysbindin-1A		For dysbindin-1B[b]	For dysbindin-1C	
Type of tissue	Human	Mouse	Human	Human	Mouse
Bone marrow		•		•	
Brain	•	•	•	•	
Area postrema		•	•		
Caudate nucleus			•		
Diencephalon	•	•			
Hippocampus	•				
Hypothalamus	•				
Subformical organ		•			
Visual cortex		•			
Wall of lateral ventricle		•			
White matter			•		
Breast	•	•			
Cervix			•		
Colon	•		•		
Embryonic tissue					
Endoderm		•			
Limb menchyme		•			
Rathke's pouch (anlage of anterior pituitary)		•			
Stem cells		•	•		
Epithelial cells of vasculature	•				
Eye					
Choroid	•				
Cornea			•		
Retinal nerve cells		•			
Retinal pigment epithelium	•	•			
Heart	•	•	•		
Aorta	•				
Intestine, small		•	•		
Kidney	•	•			
Liver	•	•	•		
Lung	•	•			
Lymphatic system					
Lymphocytes	•				
Germinal center B cells	•				
Macrophages					
In blood		•			
In lung alveoli	•				
Muscle, skeletal		•			
Olfactory epithelium	•				
Pancreas		•			
Islets			•		
Pituitary		•	•		
Placenta	•	•			
Prostate	•	•			
Salivary gland		•			

continued

Table 2.2-12 (continued)

Type of tissue	Type of transcript				
	For dysbindin-1A		For dysbindin-1B[b]	For dysbindin-1C	
	Human	Mouse	Human	Human	Mouse
Skin		•			
Spleen	•	•	•		
Stomach	•	•			
Testes		•			
Tonsil primary beta cells			•		
Thymus	•	•			
Uterus	•	•			•
Vesicular (seminal) gland		•			

[a]According to AceView and Benson et al. (2001). For further information on DTNBP1 expression in brain, see text (*Section 2.2.6.2.1*). Dots indicate positive findings. Blanks indicate unknown expression of transcripts

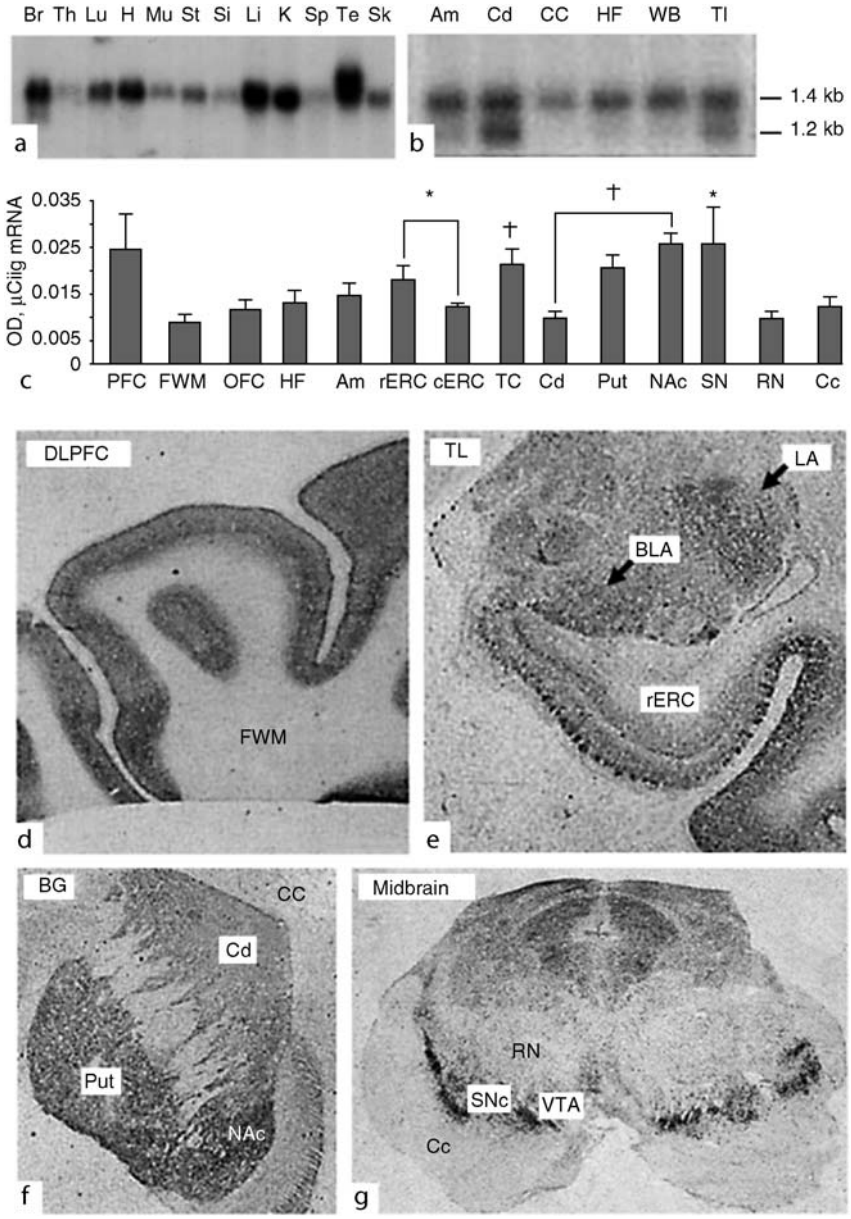
[b]Dysbindin-1B is not expressed in the mouse

cDNA probe for dysbindin-1A mRNA, which should hybridize with all dysbindin-1 mRNAs (*Figure 2.2-10a*). Among the tissues they studied, the highest gene expression was found in the testes, kidney, liver, heart, and brain. Moderate expression occurred in lung, skin, and stomach. Only low expression was detected in skeletal muscle, small intestine, spleen, and thymus. Similar findings were made in humans by Straub et al. (2002), who noted gene expression in heart, lung, and many brain areas (i.e., frontal and temporal lobes, caudate nucleus, amygdala, thalamus, hypothalamus, substantia nigra, pons, medulla, and cerebellum). The most widely and most strongly expressed of all dysbindin-1 transcripts in the body is the one encoding dysbindin-1A (see *Table 2.2-12* and *Figure 2.2-10a*). Many tissues also express the transcript, which appears to encode dysbindin-1B, but only a few are known to express the transcript for dysbindin-1C (*Table 2.2-12*). Transcripts for both dysbindin-1A and -1B have been found in at least two human brain areas: the dorsolateral prefrontal cortex (Tang et al., 2009) and the hippocampal formation (Tang et al., unpublished).

Dysbindin-1 gene expression in the developing mouse brain has been studied by Kumamoto et al. (2007). They examined DBA/2J mice at gestational (G) days 10.5, 12.5, 14.5, 16.5, 18.5 and at postnatal (P) days 0, 3, and 7. Tissue sections were prepared for in situ hybridization with riboprobes for the full-length mRNA that would recognize all dysbindin-1 mRNAs. As early as G12.5, gene expression was detectable with all the riboprobes. At that time point, expression was strong in (1) the ventricular zone from whose radial glial cells all pyramidal neurons of the cerebral cortex develop directly or indirectly (Hevner at al., 2006) and in (2) the ganglionic eminence from which develop the projection neurons of the striatum and all interneurons of the mouse cerebral cortex, basal ganglia, and hippocampus (Marin and Rubenstein, 2001; Brazel et al., 2003; Wonders and Anderson, 2005). In these two proliferative zones, Dtnbp1 expression remained strong to moderate from G12.5 to G16.5, which coincides with peak periods of neuronal generation and especially of programmed cell death in the mouse brain (Blaschke et al., 1996, 1998). At G14.5, Dtnbp1 expression was strong in the cerebellum and basal midbrain (i.e., substantia nigra and ventral tegmental area), which decreased at G16.5 and then again progressively from G18.5 to P7. At G18.5 (i.e., just before the end of gestation), Dtnbp1 expression remained very low in the hippocampal formation, increased somewhat at birth in CA1, and was strong between P3 and P7, when signal was seen in all CA fields and in the hilus of the dentate gyrus. At the latest times studied, P3 through P7, gene expression was visible in the cortical plate of the cerebral cortex, mainly in layers II and III. As noted by the authors, these findings suggest that dysbindin-1 plays a role in brain development and that disruption of that role due to genetic factors could help explain the association of certain DTNBP1 haplotypes with schizophrenia (see *Section 2.2.6.6.3.1*). This is consistent with the finding that postnatal DTNBP1 gene expression in humans peaks during adolescence (C. Weickert, personal communication), an age period of heightened risk for schizophrenia onset (Jablensky, 2003).

Figure 2.2-10

Dysbindin-1 gene expression in mammalian tissues. (a) Northern blot of diverse mouse tissues (reprinted with permission from Benson et al., 2001). (b) Northern blot of different areas of the human brain (adapted from Weickert et al., 2004). (c) Optical density (OD) of in situ hybridization signal with a DTNBP1 riboprobe in diverse areas of the human brain (adapted from Weickert et al., 2004). * indicates $p < 0.05$ differences (rostral ERC vs. caudal ERC, substantia nigra [SN] vs. red nucleus [RN], and SN vs. superior colliculus [Co]). † indicates $p < 0.01$ differences (temporal neocortex [TN] vs. cERC and caudate [Cd] vs. nucleus accumbens [NAc]). (d–g): Distribution of hybridization signal in the dorsolateral prefrontal cortex (DLPFC), temporal lobe (TL), basal ganglia (BG), and midbrain of normal human adults (adapted from Weickert et al., 2004). For explanation of anatomical abbreviations not specified here, see List of Anatomical Abbreviations (pp. 109–110)



Dysbindin-1 gene expression in the adult brain is known mainly from the report of Weickert et al. (2004). As part of their study on altered gene expression of DTNBP1 in schizophrenia, they ran northern blots and prepared tissue for in situ hybridization on 15 normal human males and females between 18 and 67 years of age. They used a riboprobe designed to recognize mRNA that encodes exons 6–8 of dysbindin-1A. Because those exons are encoded by all three major transcripts of DTNBP1 (see *Figure 2.2-8b*), the riboprobe would hybridize with mRNAs of all those transcripts. As shown in *Figure 2.2-10b–g* gene expression of dysbindin-1 was found in all tissues tested. Two mRNA species were detected: a 1.4 kb species in all structures studied that probably corresponds to AceView DTNBP1 transcript *a* encoding dysbindin-1A and an additional 1.2 kb mRNA species in the amygdala, caudate nucleus, and thalamus possibly corresponding to AceView DTNBP1 transcript *d* encoding dysbindin-1B (*Figure 2.2-10b*). As noted above, use of transcript-specific probes has since allowed detection of all three major DTNBP1 transcripts in the hippocampal formation and dorsolateral prefrontal cortex. Weak northern and in situ signals in the corpus callosum and frontal white matter (*Figure 2.2-10b,c*) may reflect a low level of expression in glia, especially oligodendrocytes common in such tissue, but might also reflect expression by rare neurons present in that tissue. Much higher gene expression of DTNBP1 was found in neuron-rich tissue (i.e., gray matter: see *Figure 2.2-10b–g*). The highest levels occurred in dopamine-rich areas of the brain, namely the substantia nigra-ventral tegmental area (SN-VTA: see also Kumamoto et al., 2006), two striatal areas (nucleus accumbens and the putamen), a limbic area (the amygdala), and two cerebrocortical areas (dorsolateral prefrontal cortex and rostral entorhinal cortex (*Figure 2.2-10c–g*). High gene expression also occurred in the temporal neocortex. Moderate levels were seen in the caudal entorhinal cortex and the hippocampal formation. Our work (Talbot et al., 2004) and later work by Weickert et al. (2008) demonstrated, however, that DTNBP1 gene expression is particularly high in some hippocampal areas, specifically CA2 and CA3 of the hippocampus and the hilus of the dentate gyrus (see *Figure 2.2-11c–e*). Neighboring areas display lower expression (i.e., CA1 and dentate gyrus granule cells *Figure 2.2-11b,f*).

6.2.2 Expression in Cancer Tissues

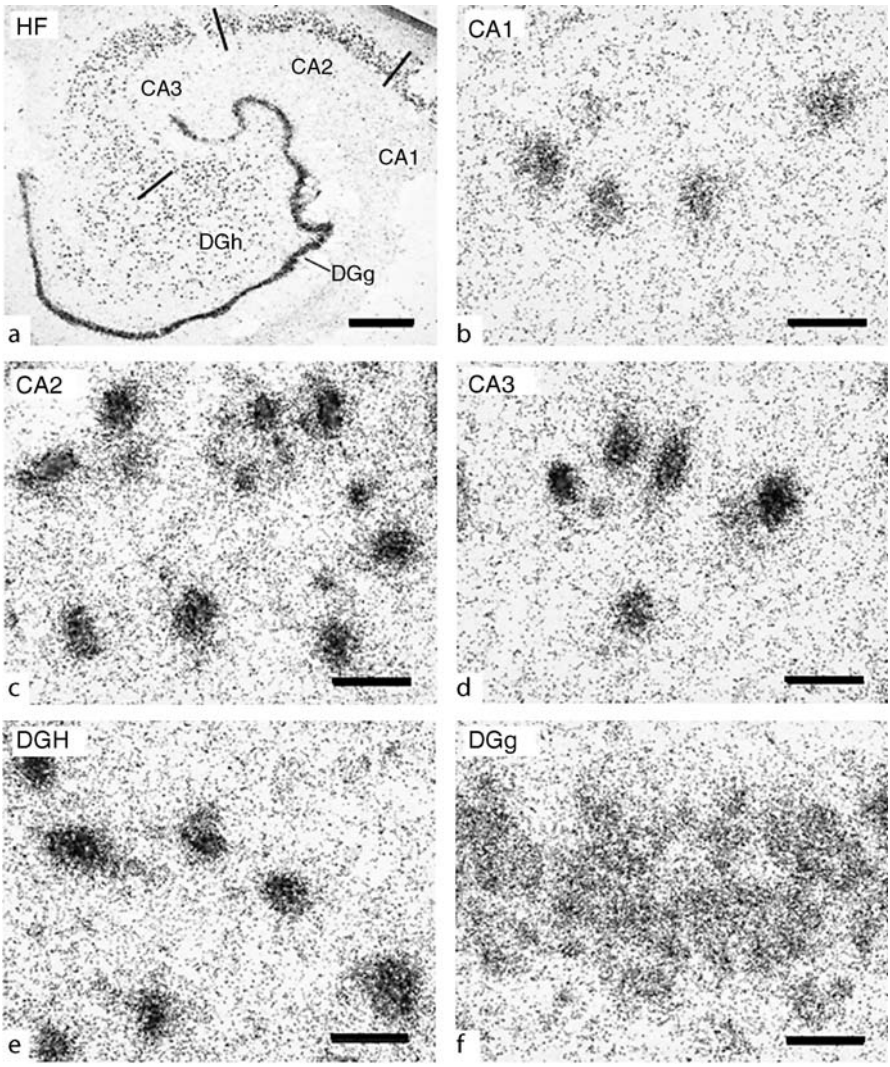
DTNBP1 is overexpressed in many cancerous tissues (*Table 2.2-13* and ECgene). The affected transcripts are nearly always those normally expressed in the tissue types involved (cf. *Tables 2.2-12* and *2.2-13*). The DTNBP1 transcript most commonly identified in cancerous tissue is that encoding dysbindin-1A, but transcripts for dysbindin-1B and -1C have also been detected (*Table 2.2-13*). Overexpression of DTNBP1 in cancer may play a role in cell proliferation, because its occurrence has been noted in germinal cells of mouse embryos (*Table 2.2-12*; see also *Section 2.2.6.2.1*) and is known to promote phosphorylation of PKB/Akt (Numakawa et al., 2004), which can stimulate cell proliferation via multiple signaling pathways (Manning and Cantley, 2007). Cell proliferation may also result from an effect of dysbindin-1 on one of its candidate binding partners, specifically an isoform of transcription initiation factor IIIB (Camargo et al., 2007), which is highly activated by several oncogenic proteins and promotes the sustained cell growth needed for proliferation (White, 2004).

6.3 Protein Expression in Mammalian Tissues

Efforts to localize and quantify dysbindin-1 protein in tissue have been hampered by problems in generating antibodies which are specific for the protein. Like most other BLOC-1 proteins, it is only weakly immunogenic. Candidate antibodies are often partially or wholly nonspecific. It is thus critical that they be validated with carefully chosen positive and negative controls. The best positive control is, of course, purified, full-length protein. The ideal negative control for antibodies raised against mouse isoforms would be tissue from Dtnbp1 knockout mice. Since such mice are not currently available, the best alternative is tissue derived from homozygous sandy mice, which lack detectable dysbindin-1 due to a Dtnbp1 mutation (see *Section 2.2.6.4.1* and *Figure 2.2-12b*). Unfortunately, dysbindin-1 antibodies currently available commercially are not marketed with the results of such controls and must consequently be treated cautiously. The commercial antibodies we have tested failed positive and/or negative control tests.

Figure 2.2-11

Dysbindin-1 gene expression in the human hippocampal formation shown seen with in situ hybridization. As seen at low magnification in (a), all major cell layers express the gene, but the level of expression is highest in the pyramidal layer of CA3 (c) and the polymorph layer of the DGh (d). Labeling with the antisense probe versus the sense probe is shown in (e) and (f), respectively. The scale bar in (a) is 1 mm, while those in (b–f) are 20 μm. Reprinted with permission from Talbot et al. (2004)



While an increasing number of laboratories have generated dysbindin-1 antibodies, the only ones validated with both positive and negative controls to date are products of laboratories at the University of Oxford (Benson et al., 2001; Talbot et al., 2004, 2006), the University of California at Los Angeles (Starcevic and Dell'Angelica, 2004), and the University of Pennsylvania (Tang et al., 2009; Talbot et al., submitted). These are affinity purified rabbit polyclonal antibodies raised against recombinant dysbindin-1A. Each recognizes not only dysbindin-1A, but at least one other dysbindin-1 isoform. Among the best characterized of these antibodies are UPenn 329 and 331 respectively raised against the CTR and NTR of human

Table 2.2-13
Gene expression of dysbindin-1 in cancerous human tissues according to AceView

	Type of transcript		
Type of tissue	For dysbindin-1A	For dysbindin-1B	For dysbindin-1C
Blood			
T-cell leukemia	•		
Bone			
Chondrosarcoma	•		
Brain			
Anaplastic oligodendroglioma	•		
Glioblastoma	•		
Neuroblastoma		•	•
Breast adenocarcinoma		•	
Gonads			
Germ cell tumor	•		
Ovarian carcinoma	•		
Kidney clear cell tumor	•		
Lung			
Primary cystic fibrosis	•		
Carcinoid tumor	•		
Lymphatic system			
Chronic lymphatic leukemia	•		
Follicular lymphoma			•
Lymphoma cell line		•	
Pancreas insulinoma	•		
Parathyroid gland tumor	•		
Retinoblastoma		•	
Skeletal muscle			
Rhabdomyosarcoma		•	
Skin			
Melanoma		•	
Squamous cell carcinoma[a]	•		•
Uterus			
Leiomyosarcoma	•		
Endometrial adenocarcinoma		•	

[a]See also Higo et al. (2005)

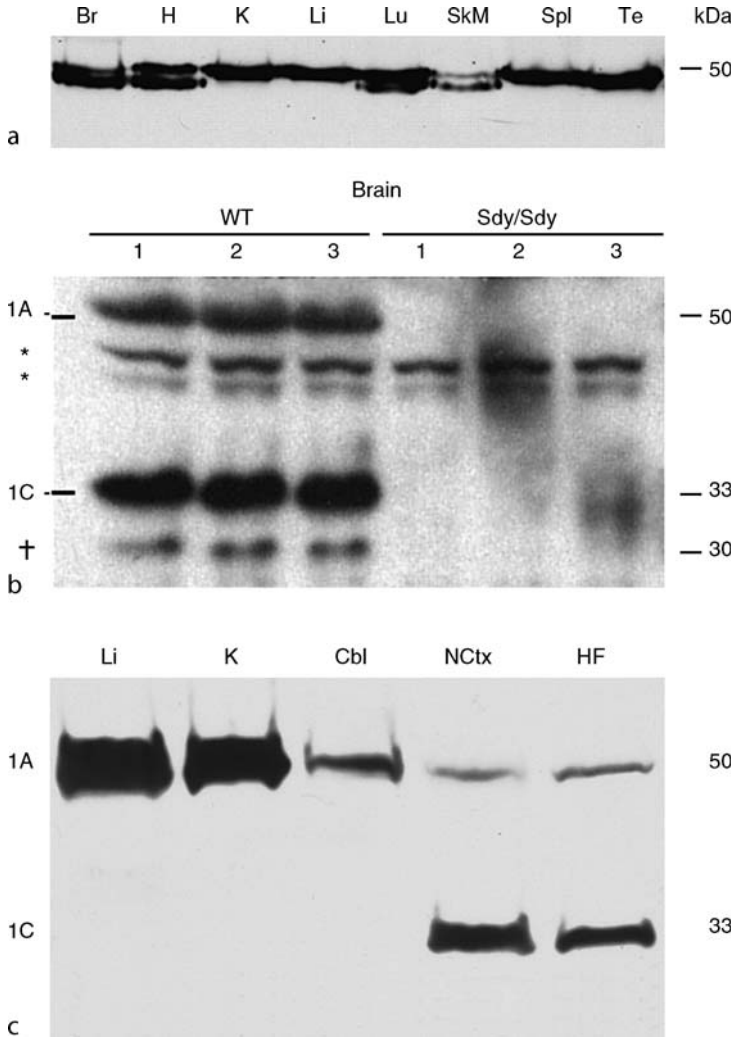
dysbindin-1A and the series of Oxford antibodies: m10FLA against full-length mouse dysbindin-1A, m10CT-FP and PA3111 against its CTR, NTm10A against its N-terminal half, and Hdys764 against the CTR of human dysbindin-1A. The most specific and sensitive of these antibodies are Oxford PA3111, which preferentially recognizes dysbindin-1A and -1C, and UPenn 331, which preferentially recognizes dysbindin-1B (Talbot et al., 2004; 2006; Tang et al., 2009; Talbot et al., submitted). Our description of dysbindin-1 distribution in tissue below thus relies mainly on findings with Oxford PA3111 and UPenn 331.

6.3.1 Western Blotting

Using m10CT-FP and PA31111, dysbindin-1 protein has been found in all tissues studied (*Figure 2.2-12a*). It thus appears to be as ubiquitous as DTNBP1 gene expression (see above). In WESTERN blots, multiple bands can be seen, the exact number depending on species and tissue type (*Figure 2.2-12b* and *c*). In human brain

Figure 2.2-12

Western blots of mouse tissues using the dysbindin-1 antibodies m10CT (a) and PA3111 (b and c). (a) Documents the ubiquitous presence of the protein in diverse organs: brain (Br), heart (H), kidney (K), liver (Li), lung (Lu), skeletal muscle (SkM), spleen (Spl), and testes (Te) as shown by Benson et al. (2001). The existence of multiple isoforms in some tissues is only suggested in the relatively high density gel used, (b) documents two dysbindin-1 isoforms (1A and 1C) in whole mouse brain lysates. These isoforms run at about 50 and 33 kDa; neither is detected in homozygous sandy (sdy) mice. Another band at about 30 kDa (†) is seen in wild type (WT) mice, but is probably a degradation product of the 33 kDa band. Two bands marked by asterisks are cross-reacting proteins, because they are just as strong in the homozygous sdy mice lacking dysbindin-1, (c) shows that some tissues express only the 50 kDa isoform (e.g., kidney, liver, and cerebellum), while others express both the 50 and 33 kDa bands (e.g., the neocortex, NCtx, and hippocampal formation, HF). Dysbindin-1B is not detected in the mouse



tissue, especially in synaptic fractions, PA3111 recognizes up to three bands at molecular masses near 50, 37, and 33 kDa, roughly the calculated masses of the major dysbindin-1 isoforms (dysbindin-1A, -1B, and -1C, respectively), taking into account their acidic nature (pI = 4.35–5.17) and many possible phosphorylation sites (see *Figure 2.2-2* and *Tables 2.2-4* and *2.2-5*). The specificity of the observed bands is indicated by their absence when PA3111 is first bound (i.e., preadsorbed) with recombinant dysbindin-1 before exposure to the samples tested in western blots. In the mouse brain, PA3111 reveals only the ~50 and ~33 kDa bands, which are known to be specific not only because they are absent after preadsorption with recombinant mouse dysbindin-1, but because they are also absent in tissue from homozygous sandy mice (*Figure 2.2-12b*) lacking dysbindin-1 due to a Dtnbp1 deletion mutation (see *Section 2.2.6.4.1*). A specific band at ~37 kDa band is also absent in mouse tissues. A 30 kDa (*Figure 2.2-12b*) band does pass specificity tests, but is not consistently seen across blots run with the same samples and may thus be a degradation product of the 33 kDa isoform. Two nonspecific bands at ~45 kDa (*Figure 2.2-12b*) are seen in some tissues (e.g., mouse whole brain, spinal cord, and heart), but not in any individual brain area we have studied (i.e., prefrontal cortex, hippocampal formation, striatum, or cerebellum).

The ~50 kDa band (48–53 kDa) is identified as dysbindin-1A in our WESTERNS, because it runs close to the molecular mass of our histidine-tagged recombinant mouse dysbindin-1A. Its identity is confirmed by the fact that it is recognized by antibodies we have recently generated to amino acid sequences in the CTR of human dysbindin-1A, but not found in dysbindin-1B, -2, or -3. The ~50 kDa band is the most consistently observed dysbindin-1 band across tissues. We find it in all tissues examined to date: the adrenal gland, heart, kidney, liver, lung, spleen, skeletal muscle, testes, spinal cord, cerebellum, striatum, hippocampus, and cerebral cortex (e.g., *Figure 2.2-12a* and *c*). In mouse and human synaptosomes, the ~50 kDa isoform is heavily concentrated in the PSD fraction with a much lesser amount in the presynaptic membrane and no detectable amount in the synaptic vesicle fraction (Talbot et al., in preparation).

The ~37 kDa band (36–38 kDa) is close in molecular mass to dysbindin-1B, which cannot be a degradation product of dysbindin-1A because of its unique C-terminus (see *Section 2.2.2.2.1*). We cannot detect this band in the mouse brain with any of the dysbindin-1 antibodies we have tested, including one (UPenn 331) which has a high affinity for the ~37 kDa band in human tissue. (UPenn 331 also recognizes the ~50 kDa band in humans and mice, albeit with much less affinity, and hence can recognize both dysbindin-1A and -1B, but not dysbindin-1C or other dysbindin paralogs as expected from the fact that it was raised against an aa sequence in the NTR of human dysbindin-1A and -1B absent in dysbindin-1C, -2 or -3.) In synaptosomes of the human brain, the ~37 kDa isoform is heavily concentrated in synaptic vesicle fractions with much lesser amounts in the PSD fractions and very little in presynaptic membrane fractions (Talbot et al., submitted).

The ~33 kDa band (32–34 kDa) is close in molecular mass to dysbindin-1C, which lacks the NTR of dysbindin-1A but is otherwise identical to dysbindin-1A (see *Section 2.2.2.2.1*). It is consequently difficult to determine if the ~33 kDa band represents (1) dysbindin-1C itself, (2) a degradative product of dysbindin-1A missing the NTR, or (c) some combination of the first two possibilities. The first possibility seems most likely, however, because the ~33 kDa band is absent in some tissues (e.g., cerebellum, kidney, and liver) where the ~50 kDa band (i.e., dysbindin-1A) is present and hence subject to degradation (*Figure 2.2-12c*). Moreover, the ~33 kDa band is often stronger than the 50 kDa band even in fresh tissue. We thus believe that the ~33 kDa band does represent dysbindin-1C. It is seen in the heart, lung, skeletal muscle, striatum, hippocampal formation, and cerebral cortex (e.g., *Figure 2.2-12a and c*). In synaptosomes of the mouse and human brain, the ~33 kDa isoform is concentrated in synaptic vesicle and PSD fractions with very little, if any, present in the presynaptic membrane fraction (Talbot et al., in preparation).

It should be noted that the band we identify as dysbindin-1C does not correspond to the band of the same name recognized by the dysbindin-1 antibody of Oyama et al. (2009). In COS-7 cells transfected to express human dysbindin-1C, their antibody recognizes two proteins. They interpret the upper band as dysbindin-1C and the lower one as a degradation product. This identification requires further confirmation, because the upper band has a molecular mass greater than that for dysbindin-1B, which is 100 aa longer than dysbindin-1C.

6.3.2 Immunohistochemistry

Dysbindin-1 has been localized immunohistochemically in a human melanoma cell line (MNT-1), mouse skeletal muscle, and the mouse and human CNS.

6.3.2.1 MNT-1 Melanoma Cells In these cells, Di Pietro et al. (2006) localized dysbindin-1 at the EM level with the UCLA antibody. The bulk of the nanogold-labeled antibody (i.e., 70% of the nanogold particles) was found on tubulovesicular elements identified as early endosomes based on their morphology and co-labeling with transferin (Tf) and early endosome antigen 1 (EEA1). Less nanogold labeling was observed on unidentified vesicles scattered in the cell periphery (13% of the labeling), on the limiting membrane of melanosomes (11%), and on mitochondria (6%). There was no labeling of cell nuclei or the cell membrane, the latter suggesting that labeled tubulovesicular elements did not include recycling endosomes that derive from early endosomes and carry cargo to the cell membrane (Sachse et al., 2002; Schmidt and Haucke, 2007).

6.3.2.2 Skeletal Muscle Benson et al. (2001) localized dysbindin-1 in mouse skeletal muscle using an early Oxford antibody (m10CT-FP). They found the protein concentrated near the plasma membrane (sacrcolemma) of the muscle fibers. It is co-localized there with α-dystrobrevin, a dysbindin-1 binding partner, which is bound to the dystrophin glycoprotein complex, stabilizing the sarcolemma. Dysbindin-1 near the sarcolemma was diminished at neuromuscular junctions, suggesting that the protein does not play a postsynaptic role at such cholingergic synapses. Benson et al. (2001) also found dysbindin-1 immunoreactivity in large blood vessels and capillaries of skeletal muscle. That is doubtful, however, because we have never observed vascular immunoreactivity in muscle or any other tissue at the light or EM level using any of a large number of dysbindin-1 antibodies.

Localization of dysbindin-1 near the sarcolemma is not dependent upon its association with α-dystrobrevin or myospryn. Benson et al. (2001) found that sarcolemmal levels of dysbindin-1 are *increased* in the mdx mouse, an animal model of Duchenne muscular dystrophy. Yet mdx mice also display *decreased* sarcolemmal levels of α-dystrobrevin (Benson et al., 2001) and myospryn (Reynolds et al., 2008). The sarcolemmal association is more likely due to other known or candidate-binding partners of dysbindin-1, specifically pallidin, snapin, and rab 11, which are all increased along with dysbindin-1 in mdx muscle (Nazarian et al., 2006). But the increases in pallidin and dysbindin-1 are not related to muscular dystrophy since there are no apparent abnormalities in the muscles or motor behavior of pallid mice (Nazarian et al., 2006), which are deficient in pallidin and dysbindin-1 (Li et al., 2003).

6.3.2.3 Central Nervous System (CNS) The literature offers brief accounts of dysbindin-1's distribution in the brain as a whole (Benson et al., 2001; Arnold et al., 2003) and more detailed accounts of its distribution in specific brain areas (Sillitoe et al., 2003; Talbot et al., 2004, 2006). These accounts are not fully consistent, however. For example, Benson et al. (2001) report that the protein is detected almost entirely in axons, whereas Talbot et al. (2004, 2006) report that it is present in many neuronal cell bodies and only in certain axons and axon terminals. Such discrepancies appear to stem from the use of different dysbindin-1 antibodies, all of which recognize one or more nonspecific bands in some tissues. In order to overcome that problem, we have used several validated dysbindin-1 antibodies to map the protein in the CNS of adult mice, macaques, and humans (Talbot et al., in preparation). The results were virtually the same in all the species studied, but do not differentiate among major isoforms of dysbindin-1 since the antibodies used (i.e., m10FLA, m10CT-FP, PA3111, and the UCLA antibody) recognize all of them. We summarize here the results consistently obtained with multiple dysbindin-1 antibodies at the light microscopic level and with PA3111A at the EM level.

Dysbindin-1 proves to be a ubiquitous neuronal protein in the CNS under optimal immunohistochemical conditions. Those conditions include heat-induced epitope retrieval in EDTA (Pileri et al., 1997) and signal amplification using nickel sulfate (Cullen, 1994) or silver-gold (Teclemeriam-Mesbah et al., 1997). Virtually all neuronal populations appear to be immunoreactive for dysbindin-1 in the brain and the spinal cord (*Figure 2.2-13*). As noted earlier, Iijima et al. (2009) reported that dysbindin-1 is found in

Figure 2.2-13

Immunohistochemical distribution of dysbindin-1 in the mouse brain visualized with PA3111 at low magnification. Location of cellular and neuropil Immunoreactivity on the left-hand side of each section is indicated by atlas-style drawings on the right-hand side. While cellular localization is not easily detected at the magnification shown, the ubiquitous distribution of dysbindin-1 in the brain is evident, as is its high concentration in neuropil of the hippocampal formation (including DGiml, arrowed in d), caudate-putamen complex (CPU), substantia nigra, pars reticulata, cerebellum, and substantia gelatinosa (SG) of the spinal trigeminal nucleus (SpV). Strong immunoreactivity near the surface of the cerebral cortex is largely an edge artifact. (Asterisks mark areas damaged during sectioning.) For further details, see *Section 2.2.6.3.2.3***. Labeling is explained in List of Anatomical Abbreviations (pp. 109–110)**

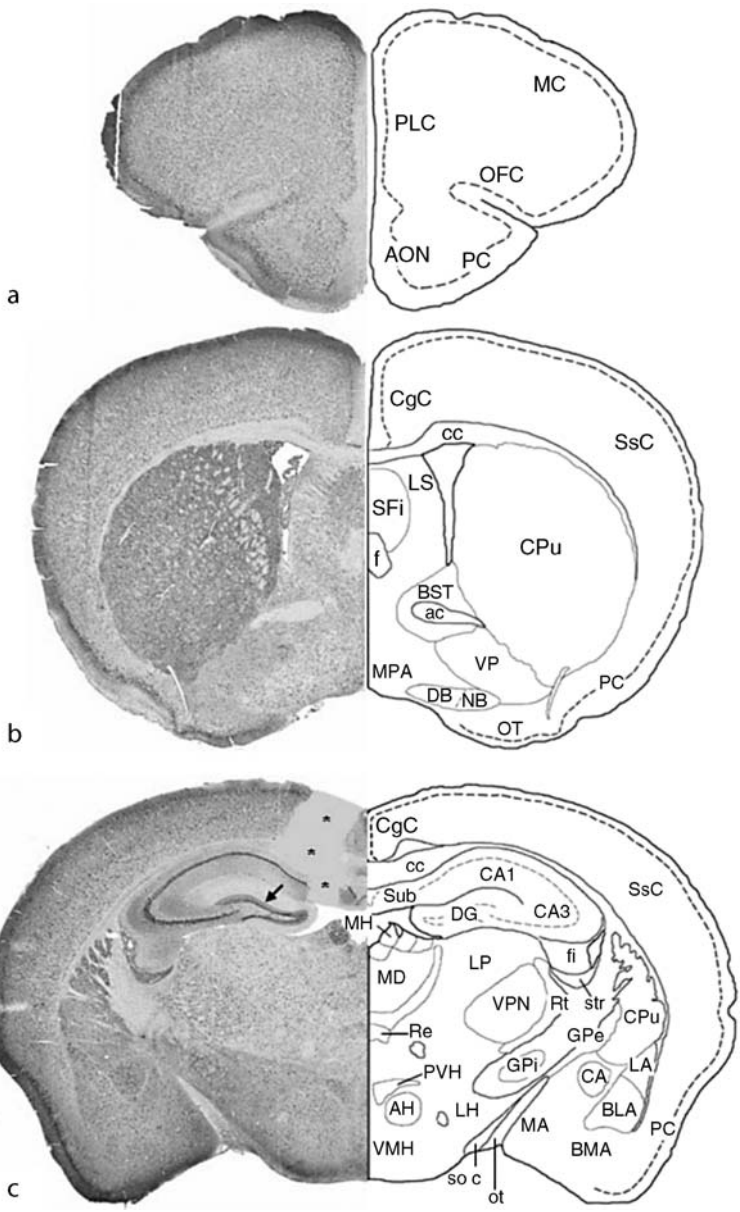
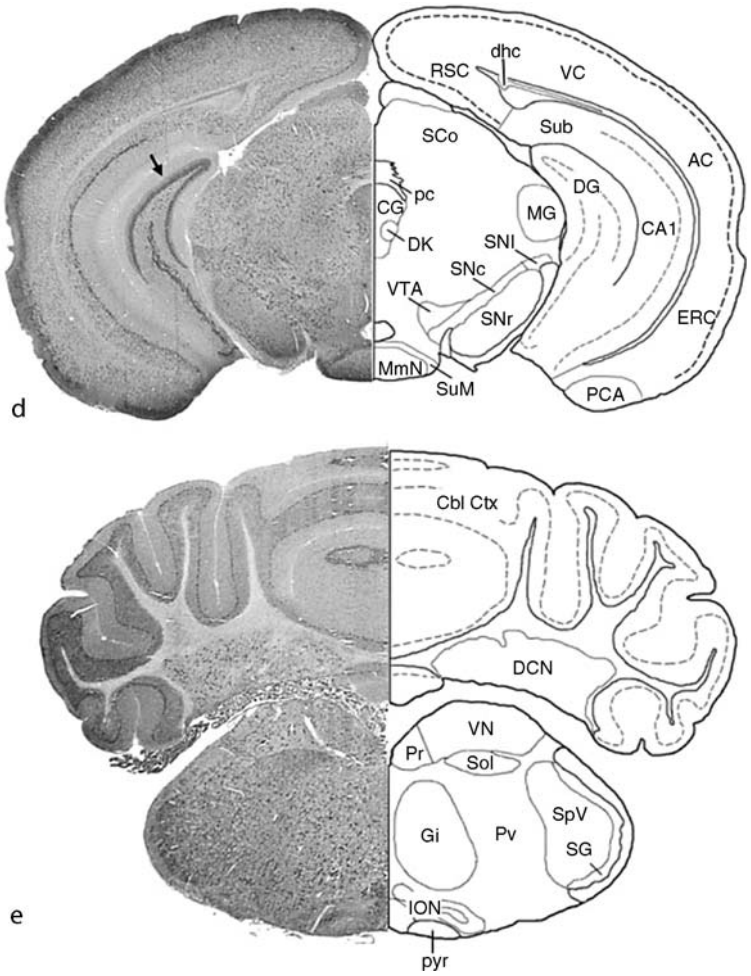


Figure 2.2-13 (continued)

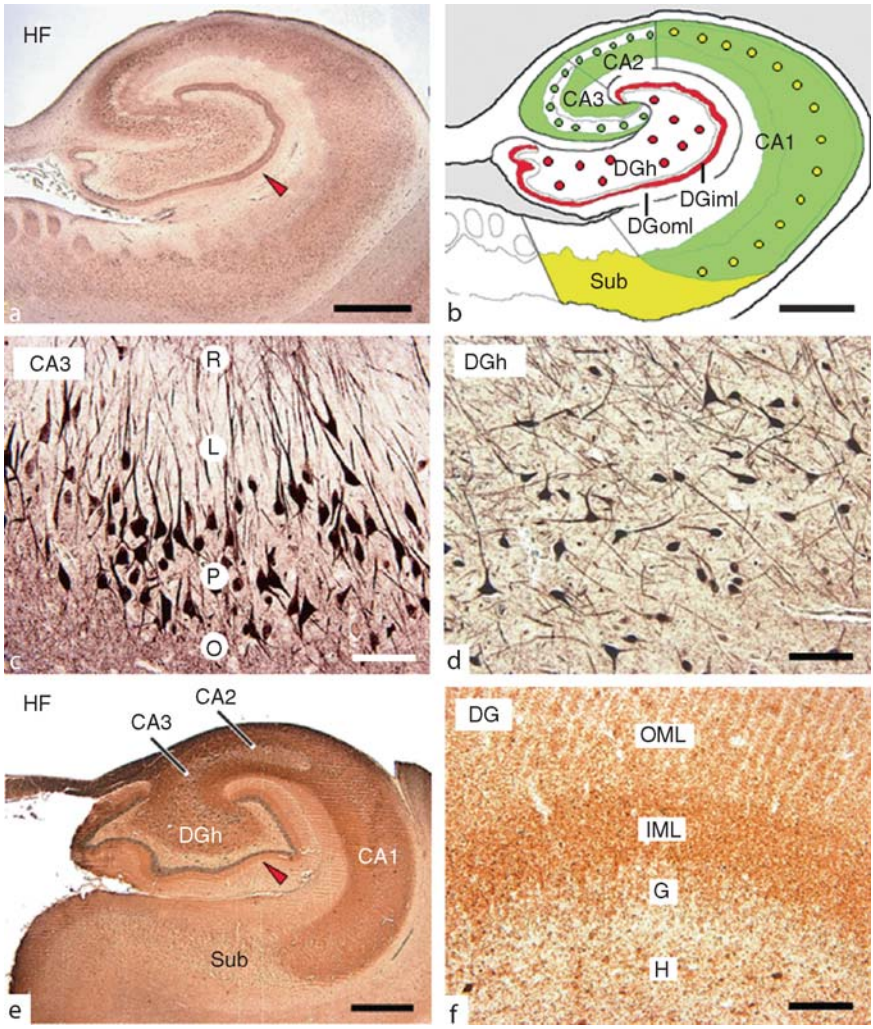


astroglial endfeet around brain capillaries (Iijima et al., 2009), but this was based on observations with a dysbindin-1 antibody whose specificity they did not establish conclusively. Using validated dysbindin-1 antibodies, no consistently detectable immunoreactivity is seen in glia cells of the gray or white matter. In contrast, such antibodies show that dysbindin-1 is spread throughout the cell body of neurons, consistent with cytoplasmic localization. In many cases, the protein extends into at least proximal portions of dendrites (e.g., *Figure 2.2-14c,d*). Moderate or high levels of reaction product are often seen in neuronal nuclei (e.g., *Figure 2.2-14c,d*; see also Oyama et al., 2009 and Figure 3e,f in Salazar et al., 2006), which is not due to a nonspecific reaction (see *Section 2.2.4.3*). This is consistently seen with antibodies against the NTR of dysbindin-1, which may possess a nuclear localization signal. With antibodies against its CTR (e.g., PA3111), some neuronal populations show conspicuously low immunoreactivity in cell nuclei (i.e., primary sensory areas of neocortex, hippocampal field CA1, septal and magnocellular preoptic areas of the basal forebrain, paraventricular and supraoptic nuclei of the hypothalamus, substantia nigra pars reticulata, deep cerebellar nuclei, and motor neurons).

At the EM level, our work indicates that dysbindin-1 in neuronal cell bodies is often found on the chromatin of the nucleus, along membranes of the endoplasmic reticulum, and over the cisterna and

Figure 2.2-14

Dysbindin-1 is concentrated in hippocampal formation neuropil. (a) Shows the distribution in humans with antibody PA3111. Note the high neuropil immunoreactivity in DGiml (indicated by *red* arrowhead) as well as in CA3. Compare the neuropil distribution with that of associational and commissural terminals in the HF as shown in (b), which marks the cellular origin and termination of those pathways in the DG (*red* cells and *red* terminal field) and CA3 (*green* cells and *green* terminal field). The origin and termination of CA1 output (*yellow*) to the subiculum (*yellow* terminal field) is also indicated, (c) and (d) show the high levels of dysbindin-1 in the origin of association and commissural pathways (i.e., CA3 pyramidal cells and DG hilar cells), (e) and (f) show the distribution of dysbindin-1 in the HF of the macaque monkey with antibody Hdys746. Note that the neuropil distribution is very similar to that in humans. Labeling is explained in List of Anatomical Abbreviations (pp. 109–110). The scale bar in (a), (b), and (e) is 1 mm; that in (f) is 100 μm; that in (c) and (d) is 50 μm

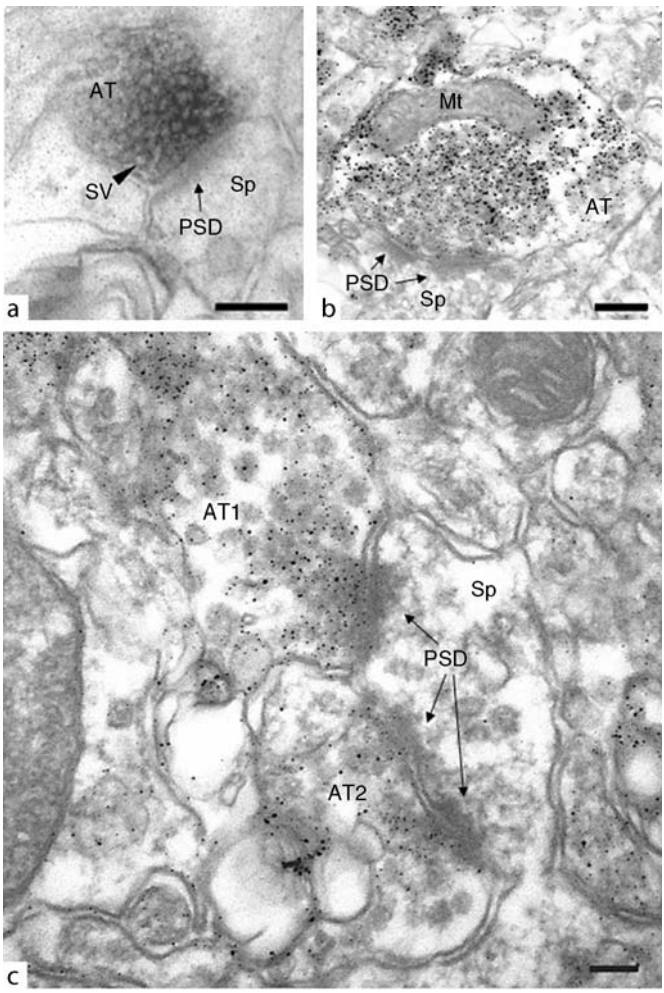


tubulovesicular elements of the Golgi apparatus, including the trans Golgi network (TGN). Some of the protein in the latter area may be in recycling endosomes given that these tubulovesicular elements are often located close to the TGN (Schmidt and Haucke, 2007). Such endosomes cycle cargo to the plasma membrane, where at least a small amount of dysbindin-1 is found intermittently in the cell body. Cell membrane localization is more

common in dendrites, dendritic spines, and axon terminals (*Figures 2.2-15c*, *2.2-17b–d*). As noted earlier (see *Section 2.2.6.3.2.1*), dysbindin-1 is not seen in the cell membrane of MNT-1 cells, which are not highly polarized in structure and thus do not need the specialized endosomal trafficking of epithelial or neuronal cells (see Schmidt and Haucke, 2007). Dysbindin-1 in neuronal recycling endosomes would be consistent with evidence that it can bind rab11A (Camargo et al., 2007), which is a marker of recycling endosomes (Trischler et al., 1999). An association with rab11A may explain the dysbindin-1 labeling seen in the core of dendritic spines on hippocampal pyramidal neurons (*Figure 2.2-17d,e*). We originally considered the core labeling a continuation of the clear microtubule labeling in the dendritic stalk from which the spines arose (Talbot et al., 2006). But its nonlinear pattern and its sporadic presence along spine

Figure 2.2-15

Presynaptic localization of dysbindin-1 in DGiml of macaque monkeys (a) and DBA/2J mice (b–c). ImmunoEM labeling with PA3111 was visualized with DAB reaction product enhanced with nickel in (a) and with silver-gold treatment in (b-c), where labeling of dysbindin-1 appears as dense, black grains. Virtually all labeling in DGiml is closely associated with synaptic vesicles (SV) in axon terminals (AT) on spines (Sp) with unlabeled postsynaptic densities (PSD). Mt, mitochondrion. Scale bars = 200 nm. Reprinted from Talbot et al. (2006)



membranes suggest that dysbindin-1 may be on the recycling endosomes responsible for spine growth (Park et al., 2006).

Dysbindin-1 protein expression levels vary across neuronal populations in the CNS independent of the transmitters they use. Very high concentrations of dysbindin-1 occur in certain glutamatergic neurons of the hippocampal formation (*Figure 2.2-14c,d*). Many glutamatergic neurons elsewhere display moderate to high concentrations of dysbindin-1, including large pyramidal cells of neocortical layer III and/or V, stellate cells of entorhinal cortex layer II, large neurons of the deep cerebellar nuclei, and small cells of the inferior olivary complex in the medulla. Moderate to high concentrations of the protein also occur in (1) cholinergic cells of the basal forebrain, medial habenula, cranial motor nuclei, and spinal motor nuclei, (2) dopaminergic cells of the substantia nigra pars compacta and the adjoining ventral tegmental area, (3) diverse GABAergic cell populations (e.g., striatal medium spiny cells, thalamic reticular nucleus cells, cerebellar Purkinje cells, and gigantocellular neurons of the reticular formation), (4) noradrenergic cells of the locus ceruleus in the pons, and (5) serotonergic cells of brainstem raphe nuclei. As this indicates, dysbinin-1 is not selectively enriched in neurons using any one type of transmitter.

Despite expression in neurons throughout the CNS, dysbindin-1 is visibly enriched in only a relatively small number of synaptic fields, which are most often dopaminergic, glutamatergic, and/or GABAergic. Viewed at the light microscopic level, these fields are areas of diffuse immunoreactivity denser than background levels in tissue known for its high density of synapses (e.g., *Figures 2.2-13–2.2-14*). Where dysbindin-1 has been localized in such fields with immunoEM, the protein occurs mainly in PSDs of dendrites, along microtubules in dendrites and axons, and/or around synaptic vesicles of axon terminals (Talbot et al., 2006; see also Taneichi-Kuroda et al., 2009). Such localization could be mediated by known or candidate dysbindin-1 binding partners, namely (1) AP-3μA, which (with other AP-3 components) binds AP-3 derived SVLMs and synaptic vesicles (Salazar et al., 2005b, 2006; Danglot and Galli, 2007), (2) exocyst subunit 8 (sec8), which binds both microtubules and PSD proteins (see Wang and Hsu, 2006), (3) pallidin, which via syntaxin-13 (Huang et al., 1999; Moriyama and Bonifacino, 2002) binds synaptic vesicles (Takamori et al., 2006) and recycling endosomes (Trischler et al., 1999), (4) snapin, which binds to synaptic vesicle proteins (i.e., synapsin I and synaptotagmin I: Krapivinsky et al., 2006; see also Kao, this volume), as well as the PSD (Talbot et al., 2006), and (5) syntabulin, which attaches syntaxin-1 containing vesicles to microtubules via kinesin 1 (see Su et al., 2004a).

Axon terminal fields enriched in dysbindin-1 at the light microscopic level occur in the following locations: sensory relay nuclei, somatosensory nociceptive interneurons, the cerebellar-inferior olivary complex, the medial habenula and its interpeduncular target, the basal ganglia and its output to the substantia nigra, the central amygdaloid nucleus, the hippocampal formation and its lateral septal target, and to a lesser degree the neocortex. The areas just mentioned probably account for much of the dysbindin-1 found in pre- and post-synaptic fractions of the mouse brain described above.

6.3.2.3.1 Sensory Relay Nuclei

While it is unknown if sensory neurons express dysbindin-1, many CNS neurons relaying auditory, somatosensory, visceral, or visual information to the neocortex are moderately to strongly immunoreactive for the protein. The relay nuclei with such immunoreactivity in the auditory system are the cochlear nuclei of the pons, the inferior colliculus of the midbrain, and the medial geniculate body of the thalamus. Such relay nuclei in the somatosensory system are the dorsal horn of the spinal cord, the cuneate, gracile, and spinal trigeminal nuclei of the medulla, and the ventroposterior nuclei ot the thalamus (both pars lateralis and medialis). Comparable relay nuclei in the visceral sensory system are the nucleus of the solitary tract in the medulla, parabrachial nucleus of the pons, and ventroposterior nucleus of the thalamus (parvicellularis). Similar cell groups in the visual system are in the superior colliculus of the midbrain and the pregeniculate nucleus of the thalamus. A role for dysbindin-1 in visual system function is suggested by the finding that the amplitude of the P1 visual evoked potential, reflecting information processing in visual association cortex (Di Russo et al., 2001), is significantly reduced in schizophrenia cases carrying a DTNBP1 risk haplotype for that disorder compared with the P1 amplitude in such cases not carrying the haplotype (Donohoe et al., 2008).

In most of the sensory relay nuclei named, dysbindin-1 immunoreactivity largely coincides with known terminal fields of sensory or sensory relay neurons innervating those nuclei (see Parent, 1996).

The concentration of such neuropil protein is often modest compared with that found in the striatum and hippocampal formation (see below), but is marked in the somatosensory and visceral sensory system in the lower medulla, especially in the gracile and solitary nuclei (e.g., *Figure 2.2-13e*). It should also be noted that a cell layer of the spinal cord influencing relay of pain signals, namely layer II, displays a very high level of dysbindin-1 in its neurons and neuropil. The functional significance of that finding is discussed in *Section 2.2.6.5.8*.

6.3.2.3.2 Cerebellum and Inferior Olive

These two structures are reciprocally connected (see Parent, 1996, pp. 583–629). The cerebellum consists of a cortex with three layers (i.e., the molecular, Purkinje cell, and granule cell layers) and several nuclei beneath the cortex simply referred to as deep nuclei. Moderate to high levels of dysbindin-1 occur in Purkinje cells, known to be GABAergic, and in large neurons of deep cerebellar nuclei, known to be glutamatergic. Dysbindin-1 often extends far out into the dendritic tree of the Purkinje cells. The neuropil of the molecular layer and the cell layer of the deep nuclei is filled with generally moderate levels of the protein (*Figure 2.2-13e*). Only low levels are usually found in the granule cells. Using the Oxford dysbindin-1 antibody m10CT-FP, Benson et al. (2001) and Sillitoe et al. (2003) reported immunoreactivity in large axon terminals of mossy fibers innervating the granule cell layer of the mouse. We have also seen that with the same antibody in the mouse, but never in humans or in either species with other dysbindin-1 antibodies. It is probably due to cross reaction of the antibody with a protein other than dysbindin-1. That could explain why Sillitoe et al. (2003) found markedly elevated mossy fiber immunoreactivity in the mdx mouse, an animal model of Duchenne muscular dystrophy, but were unable to detect a significant change in the appropriate band of dysbindin-1 immunoblots of the cerebellum. If there were a change in cerebellar levels of dysbindin-1 in mdx mice, it should show up even in whole brain assays given that the cerebellum is a major brain structure. Yet Nazarian et al. (2006) found no change in whole brain dysbindin-1 levels in the mdx brain.

Moderate to high levels of dysbindin-1 neuropil delineate the cell layer of all the nuclei composing the inferior olivary complex at the base of the medulla (not clearly seen in *Figure 2.2-13e*). The relatively small glutamatergic neurons that comprise these nuclei have high concentrations of dysbindin-1. The protein is clearly present in their axons, which are a major source of the climbing fibers innervating cerebellar Purkinje cells. The only other cells from which we have been able to detect axons containing dysbindin-1 at the light microscopic level are motor neurons in the cerebral cortex, cranial motor nuclei, and spinal motor neurons.

6.3.2.3.3 Medial Habenula and Interpeduncular Nucleus

At least a subset of the small neurons that comprise the thalamic medial habenular nucleus expresses dysbindin-1 at moderate to high levels. Since the neurons are densely packed, it is difficult to see if the protein is present in the neuropil around them (*Figure 2.2-13c*). The immunoreactive neurons are probably cholinergic, because they are mainly in the ventral two thirds of the medial habenula, which is composed principally of cholinergic neurons (Contestabile et al., 1987). As demonstrated by the study just cited, those neurons are the source of the strong cholinergic innervation of the interpeduncular nucleus, principally its core. Since the moderately dense dysbindin-1 neuropil is also restricted to the core of the interpeduncular nucleus, the protein is probably present in cholinergic habenulo-interpeduncular efferents.

While there is no indication that dysbindin-1 is present in other cholinergic terminals in the CNS (e.g., in the striatum or hippocampal formation), it might exist in neuromuscular terminals judging from the protein's presence in large-caliber axons leaving cranial and spinal motor neurons in the brainstem and spinal cord. At the postsynaptic surface of neuromuscular junctions, however, there is no enrichment of dysbindin-1 as noted earlier (Benson et al., 2001).

6.3.2.3.4 Basal Ganglia and Substantia Nigra-Ventral Tegmental Area

These are intimately interconnected structures (see Parent, 1996, pp. 795–863). The larger of the two, the basal ganglia, consists of the striatum (caudate nucleus, putamen, and nucleus accumbens) and the globus pallidus. Most, if not all striatal neurons express dysbindin-1, including sparse giant aspiny cells and

numerous medium spiny cells. Basic features of these cell types must be known to understand the functional implications of dysbindin-1 in striatal structures. The giant aspiny cells are cholinergic neurons innervating only striatal tissue and are hence local circuit cells (i.e., interneurons). Like other striatal interneurons, nearly all giant aspiny cells express dopamine (D) receptors, specifically excitatory D1b (also known as D5) and inhibitory D2 receptors (Yan et al. 1997; cf. also Bergson et al., 1995, Aosaki et al., 1998, Pisani et al., 2000, and Centonze et al., 2003).

The dysbindin-1 medium spiny cells are not a homogeneous population. There are three groups of such cells, all of which are GABAergic (Parent, 1996, pp. 803, 822–828; Surmeier et al., 1996, 2007). One consists of striatonigral neurons innervating the substantia nigra, pars reticulata; its neurons are characterized by high expression of enkephalin, substance P, and excitatory D1a (= D1) dopamine receptors. Another group consists of striatopallidal neurons innervating the external globus pallidus; its neurons are characterized by high expression of enkephalin and inhibitory D2 dopamine receptors. The third group consists of striatopallidal neurons innervating the internal globus pallidus; its neurons are characterized by high expression of enkephalin, substance P, and excitatory D1 dopamine receptors.

The strong dysbindin-1 neuropil filling the striatum (*Figure 2.2-13b*) appears to derive largely from dendrites of its medium spiny cells, especially their densely packed dendritic spines. Our immunoEM work on macaques indicates that the dysbindin-1 neuropil is limited mainly to dendrites, particularly their spines, which in the striatum are nearly all on medium spiny cells. The dysbindin-1 containing spines are targets of axon terminals with which they form asymmetric synapses (i.e., so named because the postsynaptic membrane is clearly thicker than the presynaptic membrane). These synapses fit published descriptions of medium spiny targets of cerebrocortical and, to a lesser extent, thalamic axon terminals (Kötter, 1994; Smith et al., 1994). The axon terminals found on the dysbindin-1 containing spines lack dysbindin-1. The few axon terminals which do contain dysbindin-1 in the striatum form asymmetric synapses with unlabeled dendrites of unknown origin. Our view that dysbindin-1 neuropil in the striatum is mainly of local dendritic origin offers a simple explanation for the finding that such neuropil in humans is much denser in the putamen than in the caudate nucleus, because the degree of cellular dysbindin-1 gene expression in the human striatum is also much greater in the putamen than in the caudate nucleus (Weickert et al., 2004: see *Figure 2.2-10c* and *f*).

Striatal dysbindin-1 neuropil is thus postsynaptic to what are likely to be glutamatergic corticostriatal and thalamostriatal axons terminating on spines of medium spiny cells. These spines or the dendritic shafts from which they arise are also innervated by dopaminergic input from the substantia nigra and/or the ventral tegmental area (Freund et al., 1984; Kötter, 1994; Smith et al., 1994). The dopaminergic innervation modulates cortical input via activation of D1 dopamine receptors on striatonigral medium spiny cells and via either D1 or D2 dopamine receptors on striatopallidal medium spiny cells (Ferré et al., 2007; Surmeier et al., 2007). The deduced proximity of dysbindin-1 and dopamine at or near cortical synapses on medium spiny cells finds support in the very similar distribution of the two proteins in striatal neuropil, which is punctuated by patches of diffuse enkephalin and substance P immunoreactivity called striosomes. Mirroring the distribution of dopamine fibers mapped using antibodies to tyrosine hydroxylase (Prensa et al., 1999), dysbindin-1 fills the matrix neuropil *outside* the striasomes throughout the striatum but becomes concentrated *inside* the striasomes in the caudal half of the striatum. There is thus reason to believe that dysbindin-1 is enriched in areas near dopaminergic synapses.

Striatonigral medium spiny cells innervate the substantia nigra, pars reticulata, which has a moderately dense dysbindin-1 neuropil (*Figure 2.2-13d*; see also Benson et al., 2001, *Figure 2.2-9j*). This suggests that the protein is present in the GABAergic striatonigral pathway given that all medium spiny cells in the striatum appear to contain dysbindin-1 as noted above and given our immunoEM finding that axon terminals forming symmetric synapses in the substantia nigra, pars reticulata also contain the protein. The postsynaptic portion of these synapses does not contain dysbindin-1. The neurons on which the labeled axon terminals are seen may nevertheless be the dysbindin-1 containing cells in pars reticulata and/or pars compacta of the substantia nigra. Occasionally, some of the protein in those cells is found postsynaptically, but not opposite labeled terminals.

Neurons in the substantia nigra, pars compacta and the adjacent ventral tegmental area are rich in dysbindin-1, which is expected given the high level of dysbindin-1 gene expression in such cells

(Figure 2.2-10g, Weickert et al., 2004). Dopamine synthesis in these structures is probably not regulated by dysbindin-1, because loss of dysbindin-1 in mice with a deletion mutation in the Dtnbp1 gene does not alter midbrain levels of the rate limiting synthetic enzyme of dopamine, tyrosine hydroxylase (Murotani et al., 2007). Axons of the substantia nigra, pars compacta cells innervating the striatum appear to lack dysbindin-1 since axon terminals in the striatum are rarely immunoreactive for the protein. Those rare striatal axon terminals which do display dysbindin-1 immunoreactivity do not form the symmetric synapses common to dopaminergic terminals. Thus, unlike the striatonigral pathway, the reciprocal nigrostriatal pathway contains little, if any, dysbindin-1. We cannot say if the protein is present in other pathways originating in the substantia nigra, but there is suggestive evidence that dysbindin-1 may be present in dopaminergic axons of the ventral tegmental area that innervate the amygdala and hippocampal formation (Murotani et al., 2007; see Section 2.2.6.4.2.7). These axons form part of a mesolimbic pathway to several limbic structures in addition to the amygdala and hippocampal formation (i.e., habenula, bed nuclei of the stria terminalis, septal area, and entorhinal cortex (Beckstead et al., 1979) .

As noted above, the globus pallidus is another target of striatal medium spiny cells. One population of such cells innervates the external globus pallidum while another innervates the internal globus pallidus, which in rodents is known as the entopeduncular nucleus. These striatal outputs appear to contain little, if any dysbindin-1, because immunoreactivity for that protein is low in the neuropil of both pallidal segments in rodents and in the internal pallidum of primates. The external pallidum of primates, however, differs from the same structure in rodents by having large cells immunoreactive for dysbindin-1 scattered in a moderately dense immunoreactive neuropil. These cells resemble the larger-size calretinin-positive interneurons in the primate globus pallidus (see Parent et al., 1996). But denser dysbindin-1 neuropil in the external than internal pallidum in primates cannot be due to the presence of the protein in these calretinin cells, because they are common in both pallidal segments and because calretinin neuropil seems no denser in the external than in the internal pallidum (see Parent et al., 1996). The source of the denser dysbindin-1 neuropil in the external pallidum of primates thus remains unknown.

6.3.2.3.5 Hippocampal Formation

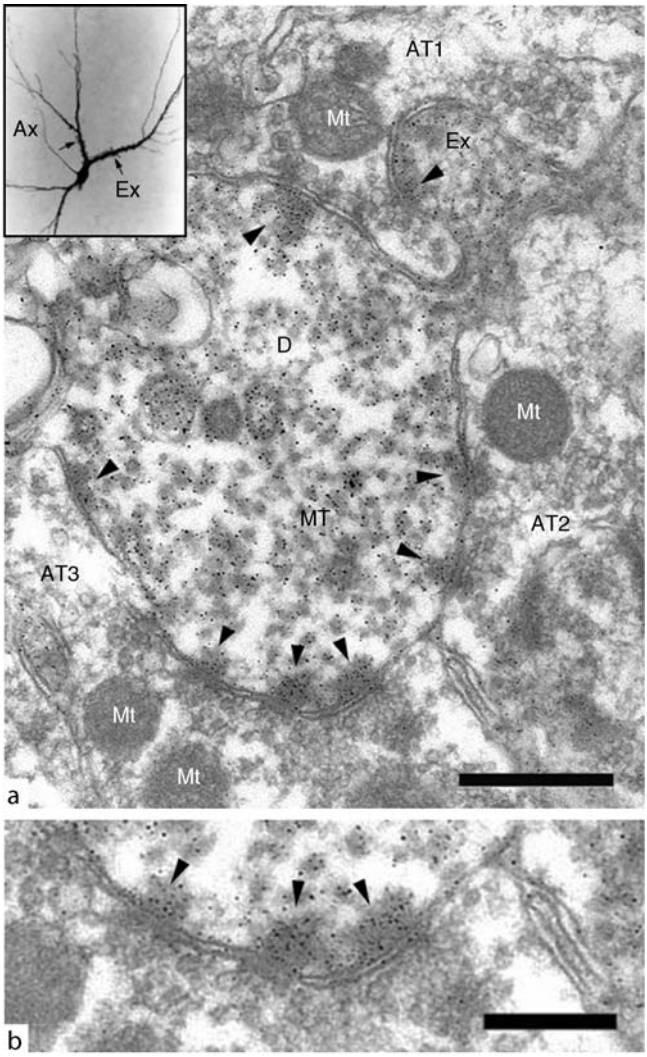
Unusually high levels of dysbindin-1 are found in neuronal and neuropil components of this brain area, which consists of the dentate gyrus (DG), hippocampus proper (i.e., cornu ammonis [CA] fields 1–3]), and the subiculum (Figures 2.2-13c–d and 2.2-14b). As in the case of the basal ganglia, an anatomical introduction clarifies the functional implications of dysbindin-1 found in the hippocampal formation (Figures 2.2-13–2.2-18). For a detailed review of its anatomy, see Amaral and Lavenex (2007).

The DG is composed of three layers. The outermost is the molecular layer, a cell-poor layer carrying afferents from the entorhinal cortex and adjoining cerebrocortical areas. Those fibers terminate on dendrites of granule cells whose cell bodies are densely packed in the second layer of the DG (i.e., the granule cell layer, DGg). Granule cell axons innervate the third layer of the DG (i.e., the polymorph layer) also known as the dentate gyrus hilus (DGh: see Figure 2.2-14b). This has scattered, large neurons of diverse shapes. Among the hilar neurons are mossy cells, so named because of their large, irregularly shaped dendritic spines (see inset in Figure 2.2-16). They have axons which synapse on granule cell dendrites in the deep (i.e., inner) portion of the molecular layer (Figures 2.2-14b and 2.2-18a). These axons are very long and each innervates much of the DG. They collectively comprise the intrinsic connections of the gyrus. Those innervating the molecular layer of the DG on the same side of the brain are called associational projections, while those innervating the molecular layer of the DG on the opposite side of the brain are called commissural projections. Granule cell axons divide into collateral branches some of which innervate hilar cells, while others innervate pyramidal cells in the CA3 sector of the hippocampus. These branched axons are called mossy fibers due to the large, irregular forms of their terminals. They should not be confused with the axons of hilar mossy neurons.

Dysbindin-1 is present in the associational/commissural projections of the DG (Figures 2.2-13c,d and 2.2-14; see also Talbot et al., 2004, 2006). This is deduced from several findings taken together. As was noted above, the terminal field of the associational/commissural projections in the DG is the inner molecular layer of the gyrus (DGiml). All input to that layer derives from the DG mossy cells (see Amaral and Lavenex, 2007, p. 63), which display the highest cellular levels of dysbindin-1 in the CNS. The DG

Figure 2.2-16

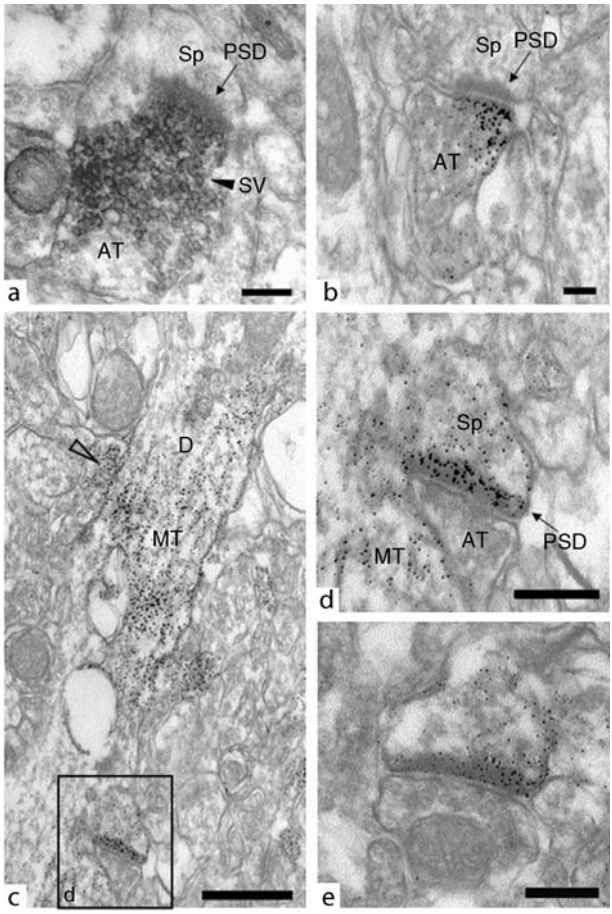
Postsynaptic localization of dysbindin-1 in a dentate gyrus mossy neuron of the DBA/2J mouse. Inset shows a dentate mossy neuron in a Golgi preparation; note thorny excrescences (Ex) on dendritic stalks, which are targets of dentate granule cell axons, also known as mossy fibers. In (a), a cross section of a mossy cell dendrite is shown in an electron micrograph. Dysbindin-1 was immunolabeled using PA3111 visualized with silver-gold treatment of the DAB reaction product, seen as dense, black grains. Note labeling of microtubules (MT) and *arrowed* PSDs, including those on the dendritic shaft and at least one in a thorny excrescence postsynaptic to a large, unlabeled mossy terminal (AT1). AT1 and AT2 may be a single terminal. In (b), immunolabeling of PSDs at bottom of *A* are shown at higher magnification. Scale bars in *A* and *B* are 500 nm and 250 nm, respectively. *Mt*, mitochondrion. Reprinted from Talbot et al. (2006)



associational/commissural projections innervate the entire width of DGiml, which is likewise filled with dysbindin-1 neuropil (e.g., *Figure 2.2-14f*). The projections are glutamatergic (Wenzel et al., 1997), a marker of which (vesicular glutamate transporter-1 [VGluT-1]) was found to be coextensive with dysbindin-1 neuropil in DGiml (Talbot et al., 2004). ImmunoEM shows that this dysbindin-1 neuropil is virtually

Figure 2.2-17

Pre- and post-synaptic dysbindin-1 in CA1 stratum radiatum of macaques (a) and DBA/2J mice (b–e). Immunolabeling with PA3111 was visualized with DAB reaction product treated with nickel in (a) and with silver-gold in (b–e), where dysbindin-1 labeling is seen as dense, black grains. In (a–b), labeling is seen in on clusters of synaptic vesicle (SV) in axon terminals (ATs) ending on spines (SPs) with unlabeled PSDs. Prominent labeling of microtubules (MT) in an apical dendrite (D) of a pyramidal neuron is seen in (c), the boxed area of which contains a labeled spine shown at higher magnification in (d). The hollow arrowhead in (c) points to a possible GABAergic AT forming a symmetric synapse on the shaft of a pyramidal cell apical dendrite. Another spine with labeled PSD is seen in (e). Scale bars in (a), (b), (d), and (e) = 100 nm; that in (c) = 500 nm. Reprinted from Talbot et al. (2006)

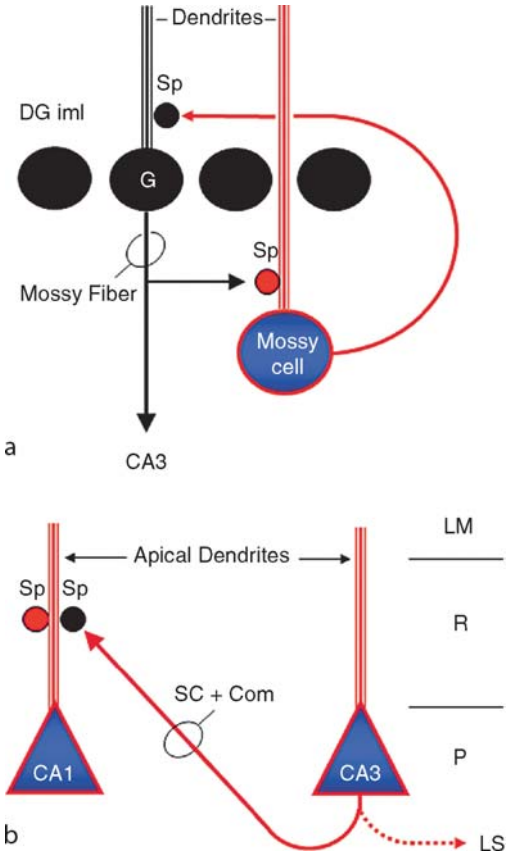


restricted to axon terminals forming asymmetric synapses on dendritic spines (*Figure 2.2-15*; Talbot et al., 2006), which fits published descriptions of associational/commissural synapses on granule cells (Buckmaster et al., 1996; Wenzel et al., 1997). Most dysbindin-1 in those terminals is associated closely with synaptic vesicles (*Figure 2.2-15*).

DGh mossy cells innervate not only granule cell dendrites in the DGiml, but also the dendritic shafts of GABAergic neurons in the DGh (Buckmaster et al., 1996; Wenzel et al., 1997). The latter are themselves

Figure 2.2-18

Likely localization of dysbindin-1 in two intrinsic circuits of the HF based on immunoEM findings. *Red* indicates tissue elements containing (or likely to contain) dysbindin-1; *black* indicates those lacking that protein. (a) A feedback circuit from dentate mossy cells to dentate granule cells. The primary target of mossy cell axons are granule cell spines (Sp) within the dentate gyrus inner molecular layer (DGiml). Since dysbindin-1 is highly expressed in mossy cells and in numerous axospinous terminals in DGiml, the protein is likely to be in the mossy cell terminals in DGiml, (b) a feed-forward circuit from CA3 to CA1. CA3 pyramidal cell axons collateralize to innervate CA1, CA3, and the lateral septum (LS) bilaterally. The collaterals innervating the ipsilateral CA1 are known as Schaffer collaterals (SC); those innervating the contralateral hippocampus are commissural collaterals (Com). Since dysbindin-1 is highly expressed in CA3 pyramidal cells and is present in axospinous terminals of CA1, the protein is likely to be in axon collaterals of CA3 pyramidal cells, perhaps including those to the LS. The location of the pyramidal, radiate, and lacunosum molecular layers of CA1 are indicated by the letters P, R, and LM, respectively. Reprinted from Talbot et al. (2006)



likely to express dysbindin-1 since all dentate hilar neurons appear to do so. This may explain our finding of rare DGh synapses in which dysbindin-1 is found both pre and postsynaptically (Talbot et al., 2006). Those synapses may be formed by axon terminals of glutamatergic mossy cells on GABAergic interneurons in the DGh. They are not expected to be synapses between one mossy cell and another, because such synapses occur on spines, not dendritic shafts (Wenzel et al., 1997).

DGh mossy cells are themselves innervated at postsynaptic sites that contain dysbindin-1 (*Figure 2.2-16*; Talbot et al., 2006). These sites are opposite axon terminals which are not immunoreactive for the protein, including the large mossy fiber terminals of DG granule cells (*Figure 2.2-16*). Dysbindin-1 thus plays not only a presynaptic role in DG associational/commissural projections, but a postsynaptic role in the granule cell mossy fiber projection as diagrammed in *Figure 2.2-18a*.

Elsewhere in the DG, dysbindin-1 is either absent or present at only low levels. Little more than background immunoreactivity occurs in the outer portion of the molecular layer (*Figure 2.2-14a*), which suggests that entorhinal afferents lack the protein. Consistent with DTNBP1 gene expression in the densely packed granule cell layer (Talbot et al., 2004; Weickert et al., 2008), granule cell bodies express at least low levels of dysbindin-1 protein. Granule cell axons (i.e., mossy fibers), however, are very unlikely to transport the protein to their terminals. It is true that Benson et al. (2001) reported that such terminals, like those of mossy fibers in the cerebellum, are immunoreactive for dysbindin-1 using the Oxford antibody m10CT-FP. With that antibody, we made the same observation in mice but not in humans. Nonetheless, we have never replicated that finding in mice or humans with other dysbindin-1 antibodies, including PA3111, which is particularly sensitive to synaptic dysbindin-1 (Talbot et al., 2006). ImmunoEM work shows an absence of PA3111 label in terminals of granule cell mossy fibers on the mossy cells of the DGh (*Figure 2.2-16*; Talbot et al., 2006). Light microscopy likewise shows no sign of immunoreactivity with PA3111 in stratum lucidum of CA3 where mossy fiber terminals are highly concentrated (*Figure 2.2-14c*).

The hippocampus is composed of four tissue layers: (1) a molecular layer carrying afferents from the same cerebrocortical areas innervating the DG, (2) a synaptically dense layer sparsely populated by several types of interneurons and penetrated by radiating dendrites of pyramidal cells deeper in the hippocampus, hence called stratum radiatum, (3) the pyramidal cell layer (stratum pyramidal) including nearly all hippocampal output neurons, and (4) a synaptically dense stratum oriens sparsely populated by many types of interneurons. The adjacent subiculum is composed of a molecular layer carrying cerebrocortical afferents and a pyramidal cell layer less dense than that in the hippocampus and filled with a synaptically dense neuropil. As in the DG, there are intrinsic projections from hippocampal neurons traveling long distances to innervate much of that structure on the same side of the brain (i.e., associational projections) and the opposite sides of the brain (i.e., commissural projections). These intrinsic projections arise from CA3 pyramidal cells and terminate in strata oriens and radiatum (see *Figure 2.2-14b*).

Consistent with their DTNBP1 gene expression levels (Talbot et al., 2004; Weickert et al., 2008), hippocampal pyramidal cells have a distinctly higher level of dysbindin-1 protein in CA2 and CA3 than in CA1 (Talbot et al., 2004, 2006). The protein in the CA2 and CA3 pyramidal cells fills the cell body, including its nucleus, and spreads far into the apical dendrites (*Figure 2.2-14c*). In pyramidal cells of CA1 and the subiculum, dysbindin-1 is less prominent in the cell body than in the apical dendrites extending far into stratum radiatum (Talbot et al., 2006). The protein in such dendrites is located along microtubules and in at least a subset of the dendritic spines, where the protein is concentrated in PSDs opposite axon terminals lacking dysbindin-1 and in what appear to be cytoskeletal elements such as microtubules and/or actin fibrils (*Figure 2.2-17*; Talbot et al., 2006).

In addition to pyramidal cells, a population of smaller neurons scattered in strata oriens and radiatum of the hippocampus are found to contain dysbindin-1 in truly optimal immunohistochemical preparations. They remain uncharacterized, but their distribution suggests that they include the subset of GABAergic hippocampal interneurons expressing the calcium-binding protein calbindin mapped by Freund and Buzsaki (1996).

Dysbindin-1 is present in the glutamatergic associational/commissural projections of CA3, just as it is in the glutamatergic associational/commissural projections of the DG (see *Figures 2.2-14b* and *2.2-18*). This conclusion is based on the following findings. A relatively dense dysbindin-1 neuropil fills strata oriens and radiatum throughout the hippocampus, particularly in CA3 (*Figures 2.2-13c,d* and *2.2-14*). No inputs from other brain areas can account for such complete filling of these layers. But associational and commissural inputs from CA3 pyramidal cells can. Such inputs are known to innervate the full extent of strata oriens and radiatum in all hippocampal sectors (CA1-CA3: see Amaral and Lavenex, 2007). They rarely extend beyond CA1 into the adjacent subiculum (Ishizuka et al., 1990), where there is a distinct drop in the level of dysbindin-1 neuropil (e.g., *Figure 2.2-14e*). The associational/commissural

projections of CA3 pyramidal cells derive from their abundant axon collaterals, many of which remain in CA3 and may explain why the dysbindin-1 neuropil there is especially dense (see *Figures 2.2-13c* and *2.2-14a,e*). Other CA3 collaterals known as Schaffer collaterals extend into CA1 on the same side of the brain. These collaterals and their terminals on spines of CA1 dendritic spines have been shown to contain dysbindin-1. In those terminals, the protein is closely associated with synaptic vesicles (*Figures 2.2-17a,b* and *2.2-18b*; Talbot et al. 2006). The spines they synapse upon do not contain the protein, however. Other CA3 pyramidal axon collaterals are commissural projections of the hippocampus. They reach their synaptic targets on the opposite side of the brain via the dorsal hippocampal commissure, which also contains dysbindin-1 (*Figure 2.2-13d*). Another set of CA3 collaterals innervate a structure outside the hippocampus, namely the lateral septum, where a moderately dense dysbindin-1 neuropil is found. Given the evidence that the protein is transported to terminals of other CA3 collaterals, the same may well be true for those collaterals ending in the lateral septal area (Talbot et al., 2006).

Apart from the lateral septum, there is no indication that dysbindin-1 is enriched in hippocampal or subicular output to extrinsic structures. Nor is there any evidence that the protein is enriched in extrinsic input to the hippocampal formation, except perhaps for minor dopaminergic input from the mesolimbic pathway originating in the ventral tegmental area of the midbrain (Murotani et al., 2007; see *Section 2.2.6.4.2.7*). Otherwise, dysbindin-1 neuropil in the hippocampal formation appears to be selectively concentrated in the intrinsic associational and commissural projections of that structure.

6.3.2.3.6 Neocortex

Although nearly all neocortical neurons express dysbindin-1, the level of expression is highly variable. Granule cells in layers II and IV, like those in the dentate gyrus and cerebellar cortex, express little if any dysbindin-1. In the small neurons of layer III and V, possibly aspiny GABAergic interneurons, the protein level is low to moderate. In medium-size pyramidal cells of layer V and fusiform neurons of layer VI, the protein level is higher, but still only moderate. In clear contrast, relatively high levels of dysbindin-1 are seen in both medium- and large-size pyramidal cells of layer III and in large pyramidal cells of layer V, the last seen mainly in somatosensory and motor areas. In these layer III and V cells, the protein extends far into the apical dendrites, previously mistaken for axons by Benson et al. (2001).

The relatively high levels of dysbindin-1 in layer III pyramidal cells are of special interest. These cells are glutamatergic and/or aspartamatergic and are the major source of long cortical associational and commissural projections of the neocortex (Parent, 1996, pp. 867–873; cf. also Barbas, 1986 and Zilles, 1990, pp. 795–796). It seems likely that dysbindin-1 is present in the axon terminals of these projections, which innervate primarily deep layer III and layer IV (Parent, 1996, p. 868 and Zilles, 1990, p. 796). Along with layer I, these are the cortical layers with the densest dysbindin-1 neuropil, although the density is clearly less than in the hippocampal formation or the striatum. Our immunoEM work indicates that most dysbindin-1 in the neuropil of the neocortex occurs in axon terminals. The immunoreactive terminals form the asymmetric junctions characteristic of excitatory synapses. The protein in these terminals, as in others containing the protein, is associated closely with synaptic vesicles. The postsynaptic targets of these dysbindin-1 containing terminals are dendritic spines of pyramidal cells and small diameter dendrites of apparent interneurons. Dysbindin-1 is also found in at least a small number of neocortical dendritic spines, but not in the axon terminals which synapse on those spines.

As in the hippocampus, then, dysbindin-1 in the neocortex is markedly enriched in the parent neurons of long intrinsic projections (i.e., in associational and commissural connections) and is likely to be in the axon terminals of those projections. In comparison, there is little indication that the protein is present in extrinsic afferents or efferents of the neocortex. While it is possible that some dysbindin-1 neuropil seen in upper layer IV of the sensory cortices derives from thalamic relay nuclei, there is little evidence that axons containing the protein cross the white matter separating the thalamus from the neocortex. That also argues against the presence of dysbindin-1 in neocortical output. Consistent with that view, we cannot detect dysbindin-1 in axon terminals of a major neocortical output target, namely the striatum. The dysbindin-1 in axons of motor neurons may be limited to recurrent collaterals remaining in the cortex. We thus consider dysbindin-1 in the neocortex to be locally derived and locally targeted.

6.4 The Sandy (Sdy) Mouse: A Dysbindin-1 Mutant

At the Jackson Laboratory in 1983, an autosomal recessive coat color mutation occurred spontaneously in the DBA/2J mouse strain. The sandy coat color of the homozygous animals gives the strain its name (*Figure 2.2-9b*). These animals are fully viable, but can be difficult to breed, perhaps due to impaired spermatogenesis as explained in *Section 2.2.6.5.1*). In 1991, Swank et al., reported that sdy mice have features of a platelet storage pool deficiency disorder called Hermansky-Pudlak Syndrome (HPS). This is a genetically heterogeneous, autosomal recessive disorder characterized by ocular and cutaneous pigment dilution (albinism), prolonged bleeding times after injury, and in some cases pulmonary fibrosis and deposits of a lipid pigment known as ceroid (Huizing et al., 2000; Wei 2006). The core symptoms of HPS are due to defects in lysosome-related organelles (LROs), namely melanosomes, platelet dense granules, and kidney secretory lysosomes (see *Section 2.2.6.4.2.3–2.2.6.4.2.5*).

Sdy mice share one or more HPS features with fourteen other mouse strains defined by their coat color mutations (cf. Swank et al., 1998; Nguyen and Wei, 2004; Wei, 2006). The mutation in each strain occurs in a gene encoding a subunit in one of the five protein assemblies important in normal LRO biogenesis, namely AP-3, BLOC-1, BLOC-2, BLOC-3, or HOPS (= the homotypic fusion and vacuole protein sorting complex) (cf. Di Pietro and Dell'Angelica, 2005; Wei, 2006; Li et al., 2007). The sdy mouse is one of the mouse strains with coat color mutations in genes encoding BLOC-1 subunits. The other strains are also known by names describing their fur color: cappuccino (Gwynn et al., 2000; Ciciotte et al., 2003), muted (Swank et al., 1991a; Zhang et al., 2002), pallid (Novak et al., 1984), and reduced pigmentation (Gwyn et al., 2004). The coat color mutations in these strains are in genes encoding the proteins cappuccino, muted, pallidin, and BLOS3 (BLOC-1 subunit 3), respectively. Of these, muted and pallidin are direct dysbindin-1 binding partners (Starcevic and Dell'Angelica, 2004; Nazarian et al., 2006). As it implies, the sdy mouse is not only a model of dysbindin-1 functions, but also of BLOC-1 functions. It should be recognized, however, that it is not a perfect model of the latter, because dysbindin-1 binds more than just BLOC-1 proteins (see introduction to *Section 2.2.6.5*).

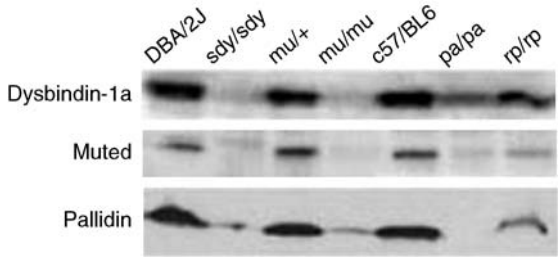
6.4.1 Mutations in Sdy Mice and the HPS-7 Case

Although, it was known in 1991 that the coat color mutation in sdy mice occurs on chromosome 13 in the vicinity of cytogenic band A5, it was not until 2003 that its specific locus was published. In that year, Wei Lei and his colleagues working in Swank's laboratory reported that sdy mice have a deletion mutation in Dtnbp1 and that transgenic addition of the normal gene largely rescues normal DBA/2J coat color (i.e., brown) and corrects other phenotypic abnormalities of sdy mice (Li et al., 2003). The same report identified a nonsense mutation in the human DTNBP1 gene associated with a novel form of HPS, which the authors named HPS type 7 (HPS-7; Li et al., 2003). As explained below, both the mouse and human mutations can reduce expression of normal dysbindin-1.

The coat-color mutation in sdy mice (*Dtnbp1*^{sdy}) is an in-frame deletion of 38,129 nucleotides from nucleotide number 3,701 in intron 5 to nucleotide 12,377 in intron 7 that excises exons 6 and 7 encoding a 52 aa segment of the CCD in dysbindin-1(i.e., amino acids 120–171: see *Figure 2.2-9c* and *e*). The mutant protein is predicted by PCoils to have a CCD between aa 81–123, which is 58 aa shorter than the wild type CCD. The truncated CCD lacks the last two amino acids of the LZ motif and is not divided into two helixes like the wild type protein (see *Section 2.2.3.2*), which may hinder flexibility of the CCD. Given the importance of the CCD in interactions of dysbindin-1 with its binding partners (see *Section 2.2.3.2*), it is not surprising that such interactions with the truncated protein in homozygous mice are eliminated in the case of β-dystrobrevin and greatly reduced in the case of muted and pallidin (Li et al., 2003).

In all tissues tested (brain, heart, kidney, liver, and skeletal muscle), Li et al. (2003) found that dysbindin-1A protein was undetectable in homozygous sdy mice. It was detected in heterozygous mice in the kidney, but at lower levels than in DBA/2J control animals. Our own work on whole brains from sdy animals with a DBA/2J or a C57BL/6J genetic background shows that dysbindin-1A and -1C are low in heterozygotes and absent in homozygotes (*Figures 2.2-9f*, *2.2-12b*). The reduction or loss of

Figure 2.2-19
Loss of BLOC-1 proteins following loss of individual BLOC-1 components as shown by Li et al. (2003) in kidney extracts. The loss of dysbindin-1A in homozygous sdy (sdy/sdy) mice is accompanied by loss of muted and a major reduction in pallidin. Loss of muted in mice homozygous for a deletion mutation in Muted (mu/mu) is similarly accompanied by loss of dysbindin-1A and a major reduction in pallidin. Likewise, loss of pallidin in mice homozygous for a deletion mutation in Pallid (pa/pa) causes loss of muted and reduction in dysbindin-1. To a lesser extent, the same phenomenon occurs in mice homozygous for a deletion mutation in another BLOC-1 gene called reduced pigmentation (rp/rp)



dysbindin-1 is not attributable to a loss of gene expression. Transcription of the mutated gene still occurs, but the transcripts are shorter (*Figure 2.2-9d*, Li et al., 2003). While the transcripts may simply be unstable and quickly degraded, the most likely explanation for the loss of dysbindin-1 in homozygotes is an inability of the mutant protein to form a stable complex with other BLOC-1 proteins. The truncated protein predicted by the smaller transcripts is seriously impaired in binding other BLOC-1 proteins as already described. That prevents proper assembly of BLOC-1, which leads to the degradation of its components. It is based on the finding that homozygous sdy mice display is not only a loss of full-length dysbindin-1A and -1C, but also reductions in several other members of BLOC-1, namely muted, pallidin, and the reduced pigmentation protein BLOS3 (*Figure 2.2-19*, Li et al., 2003), which is an acronym for BLOC-1 subunit 3 (Starcevic and Dell'Angelica, 2004). Loss of dysbindin-1A alone may trigger this phenomenon, because there is reason to believe that dysbindin-1C is not part of BLOC-1 (see introduction to *Section 2.2.6.5*).

The human DTNBP1 mutation found in the single known HPS-7 case is a nonsense mutation due to a cytosine to thymine (C –> T) substitution that causes a CAG sequence in the DNA, which is then transcribed in the mRNA as the stop codon UAG. Given its location in exon 5, the mutation causes translation to stop just after the glutamine (Q) at aa 103, which explains why the HPS-7 mutation is called Q103X. The resulting truncated dysbindin-1 protein would be less than a third of its normal size and lack all but a quarter of its CCD, lacking more than half of the LZ motif likely to be important in binding BLOC-1 and other proteins (see *Section 2.2.3.2*).

6.4.2 Abnormalities in Sdy Mice

The sdy mouse is a model system for studying the functions of dysbindin-1 due to its mutation in Dtnbp1 leading to reduction in (or loss of) the protein. Clues to these functions are found in phenotypic abnormalities of sdy mice. Such abnormalities are described in this chapter, starting with those in the DBA/2J genetic background, because they indicate confounding variables that must be taken into account in assessing all other abnormalities found in sdy mice derived from DBA/2J animals (i.e., sdy/DBA mice).

6.4.2.1 Genetic Background As noted above, the original sdy mice were Dtnbp1 mutants of the DBA/2J strain. This strain, unlike C57BL/6 mice, is homozygous for several mutations related to neurological, melanogenic, and/or inflammatory disorders. In particular, DBA/2J mice are homozygous for at least six pathologic alleles: (1) cadherin 23^{ahl} (Cdh23^{ahl} = Cdh^{753A}) associated with an age-related hearing loss (see [http://jaxmice.jax.org/strain/000671.html]; cf. also Johnson et al., 2000 and 2006), (2) a CD94 exon 6

deletion (cf. Wilhelm et al., 2003 and Vance et al., 2002) associated with altered immune responses to infection (Borrego et al., 2006), (3 and 4) glycoprotein (transmembrane) nmb^{R150X} ($Gpnmb^{R150X}$) and tyrosinase-related protein 1^{isa} ($Tyrp1^{isa}$) both associated with pigmentary glaucoma (Howell et al., 2007), (5) hemolytic $complement^{0}$ (Hc^{0}) associated with loss of immune complement component 5 (C5: Wetsel et al., 1990), and hence, with impaired inflammatory responses to infection (Allegretti et al., 2005), as well as impaired neuronal and astrocytic responses to excitotoxicity (Pasinetti et al., 1996), and (6) myosin $5a^{d}$ ($Myo5a^{d}$), the dilute pigment allele linked to an ecotropic murine leukemia [MuLV] virus DNA sequence (Jenkins et al., 1981) associated with disrupted trafficking of melanosomes and vesicles (Nguyen and Wei, 2004; Desnos et al., 2007) and possibly disrupted myelination (Sloane and Vartanian, 2007).

These six mutations must be present in sdy mice derived from DBA/2J animals, though not all sdy mice are necessarily homozygous for them as they have wild type Gpnmb (Anderson et al., 2002). Behavioral phenotypes resulting from these mutations in DBA/2J and sdy/DBA mice are described in *Section 2.2.6.4.2.9*.

6.4.2.2 Adrenal Chromaffin Granules Chromaffin cells in the medulla of the adrenal gland contain secretory granules characterized by electron-dense cores (Díaz-Flores et al., 2008). They are accordingly known as dense core vesicles (DCV), which are found in both the major and the minor population of chromaffin cells. DCVs in the major population are on average larger and are thus known as large DCVs (LDCVs). They are of two types: smaller LDCVs storing epinephrine in 80–85% of the chromaffin cells and larger LDCVs storing norepinephrine in the other chromaffin cells (Díaz-Flores et al., 2008). Splanchnic nerve activation of the adrenal medulla causes LDCVs to exocytose their catecholamine content, which is then taken up by blood vessels (Rosé et al., 2002; Díaz-Flores et al., 2008). Studies using physiological conditions show that weak to moderate stimuli cause a slow, incomplete catecholamine release of the kiss-and-run type from the LDCVs via small (about 4 nm) fusion pores formed by transient merger of vesicle and cell membranes, whereas strong stimuli due to acute stress causes a rapid release in which the fusion pore expands to the point where the vesicle collapses to merge completely with the cell membranes and releases all LDCV contents, catecholamines, and neuropeptides (Fulop et al., 2005; Elhamdani et al., 2006; Díaz-Flores et al., 2008).

Amperometry is a major means of investigating events controlling the release of catecholamines from LDCVs, the bulk of which is epinephrine. It uses a carbon fiber microelectrode 5–7 μm in diameter placed on the external surface of a secretory cell and set at a potential (650–780 mV) readily oxidizing secretory molecules (Mosharov and Sulzer, 2005). It is employed mainly in studies of cells secreting monoamines, because these are easily oxidized, unlike acetylcholine, GABA, and glutamate (see Westerink, 2004). Exocytosed catecholamines are oxidized on the surface of the carbon fiber microelectrode, where the transfer of electrons to the catecholamine molecules causes a current proportional to the number of oxidized molecules. Given the high temporal resolution and great sensitivity of amperometry, the fluctuations in current are generally registered as discrete spikes, each of which represents the exocytosis of one secretory vesicle such as an LDCV (Mosharov and Sulzer, 2005). The first noticeable event seen in many such spikes is a small, slowly rising "foot" current due to the opening of a fusion pore allowing slow exocytosis of molecules from the vesicle (see Amatore et al., 2005). This is followed by the spike proper, a sharply rising current due to accelerated exocytosis as the fusion pore expands (see Burgoyne and Barclay, 2002 and Amatore et al., 2005). The speed of fusion pore expansion is indicated by the rise time of the spike current. After reaching its peak amplitude, the spike current declines as the exocytosis ends due to closure of the fusion pore in a kiss-and-run event or due to its collapse with complete merger of the vesicle and cell membranes (see Burgoyne and Barclay, 2002 and Amatore et al., 2005). The speed of these events is indicated by the decay time of the current decline. The total amount of released catecholamines is indicated by the total charge transfer represented by the area under the recorded spike, including its foot.

Chen et al. (2008) demonstrated abnormalities in the size, content, and exocytosis of adrenal chromaffin LDCVs in 6–10 week old homozygous sdy/DBA mice compared with wild-type DBA/2J controls. They used EM to study the size and distribution of LDCVs and amperometry to study the electrochemical properties of single-LDCV exocytosis triggered by 80 mM KCl. This is a strong depolarizing stimulus, which limited the findings to conditions mimicking those during acute stress (see Fulop et al., 2005). The electrophysiological results reported do not cover data on the foot of the amperometric spikes and hence

do not indicate whether the kinetics of fusion poor opening were altered in the sdy mice. The rise time of the spikes was normal in these animals, indicating that the kinetics of fusion pore expansion were normal. But the decay time of the amperometric spikes was increased by 35%, indicating slower kinetics of fusion pore closure or collapse. Given the normal fusion pore expansion, the increased decay time appeared to reflect an increase in the amount of catecholamine molecules released per LDCV (i.e., quantal size per vesicle) since the 35% increase in release time was nearly matched by a 30% increase in the charge transfer of each spike. Consistent with increased quantal size, there was a 10% increase in LDCV diameter in the sdy mice. The observed increases in release duration and quantal release size were reversible upon dysbindin-1 dialysis for 5–10 min, an effect not exerted when that protein was denatured. Further amperometric tests on whole, patch-clamped chromaffin cells subjected to a 2-s depolarization pulse from −70 mV to 0 mV showed that the total number of amperometric spikes and their combined charge (i.e., total number of LDCV fusion events and catecholamine released) were both 30% less in sdy than in wild type mice. The decreased frequency of fusion events indicates a decreased probability of release, consistent with a 25% decrease in the number of LDCVs per μm^2 in the chromaffin cells. These findings show that in the sdy/DBA mice an increase in quantal size of LDCVs was more than offset by a decreased release probability.

The reasons for these abnormalities in chromaffin LDCVs are suggested by other findings of Chen et al. (2008). While they found no associated changes in adrenal levels of proteins involved in exocytosis (complexin 1 or 2, munc18–1, SNAP25, synaptotagmin 1, synaptotagmin 1, syntaxin 1, or VAMP2), they did find that the LDCVs in sdy/DBA were 10% larger in diameter and 25% lower in number per μm^2. Yet electrophysiological tests of Chen et al. (2008) demonstrated that sdy mice had normal rates of LDCV endocytosis and refilling. It thus seems likely that the observed abnormalities in the sdy LDCVs were at least partially due to defective biogenesis resulting from loss of BLOC-1. As argued later (see *Section 2.2.6.5.5*), BLOC-1 may well play a role in biogenesis of synaptic vesicles in the reserve pool of axon terminals. From that pool, synaptic vesicles are recruited into the readily releasable pool during strong stimulation as used in the study under discussion (see Akbergenova and Bykhovskaia, 2007, and supplementary Figure 1 in Elhamdani et al., 2006). While Chen et al. (2008) did not selectively study the reserve pool in adrenal chromaffin cells, they did find that the reserve pool in the hippocampus of sdy mice was reduced by ∼22%. Such a phenomenon in the adrenal chromaffin cells would help explain their finding of a 52% decrease in the pool of readily releasable LDCVs. Abnormalities in the size, number, catecholamine load, and fusion competence of these vesicles may thus reflect the loss of BLOC-1 effects on biogenesis of reserve pool vesicles from which many readily releasable LDCVs may ultimately derive. Fewer readily releasable LDCVs would naturally contribute to the noted decline in catecholamine release probability, which Chen et al. (2008) found was not due to decreased number of docked LDCVs or to reduced Ca^{2+} influx.

It is also possible, however, that the decreased probability of adrenal vesicle release in sdy mice reflects loss of interaction between dysbindin-1 and its binding partner snapin, which normally boosts the number of LDCVs kept in a readily releasable state (Tian et al., 2005) and enhances efficient, synchronous release of synaptic vesicles (Pan et al., 2009).

6.4.2.3 Kidney Secretory Lysosomes These organelles secrete hydrolases, among them β-glucuronidase and β-galactosidase, into proximal renal tubules. Such secretion is disrupted in homozygous sdy/DBA mice as demonstrated by the fact that their kidney concentrations of β-glucuronidase and β-galactosidase are 43–46% higher than in heterozygous animals, while their kidney secretion of those enzymes is reduced by about 48–53% compared with heterozygous animals (Swank et al., 1991b). Essentially the same phenomenon is observed in kidney secretory lysosomes of mice with mutations in other genes encoding BLOC-1 proteins (i.e., muted and pallid mice: see Swank et al., 1998). In contrast, levels of β-glucuronidase and β-galactosidase in the non-secretory lysosomes of the liver are normal in the sdy/DBA animals (Swank et al., 1991b). Since secretory (Blott and Griffiths, 2002; Holt et al., 2005) and non-secretory (Jaiswal et al., 2002; Arantes and Andrews, 2006) lysosomes can fuse with cell membranes, these findings suggest a selective effect of BLOC-1 on LRO secretion. It remains unknown whether the effect is related to accumulation of ceroid-like pigments, a common feature in HPS, in the renal proximal tubules of homozygous sdy/DBA mice (Swank et al., 1991b).

6.4.2.4 Melanosomes The most visible feature of homozygous sdy mice on a DBA or C57BL/6 background, is the lack of eye pigmentation (observed as red eyes) and very light pigmentation of the coat fur (Swank et al. 1991b). Vertebrate pigment cells, known as melanocytes, derive from neural crest melanoblasts that migrate to target locations in the epidermis, hair follicles, eye, cochlea, and the leptomeninges (Tolleson, 2005; Goding, 2007). Melanocytes both synthesize and store melanin pigment in LROs called melanosomes (Raposo and Marks, 2007). In cutaneous tissue, but not elsewhere, melanin is transferred to keratincoytes, probably by exocytosis followed by phagocytotic uptake (Van Den Bossche et al., 2006). Hair shafts comprising of mammalian fur are accumulations of keratinocytes pigmented by hair follicle melanocytes (Botchkarev and Paus, 2003; Slominski et al., 2005). In most mice with or without coat color mutations, the dominant hair pigments are brown to black eumelanins (Jackson, 1994) that are synthesized in elliptically-shaped melanosomes with a fibrillar matrix (Slominski et al., 2005).

As studied in various tissues, melanosomes derive from endosomes and go through four stages of development (Raposo and Marks, 2002; Raposo et al., 2007). The stage 1 premelanosomes resemble late endosomes or multivesicular bodies (MVBs) that are characterized by intraluminal vesicles and the absence of fibrillary structures or melanin. Stage 2 premelanosomes still lack melanin but do contain intraluminal fibers that are generally oriented parallel to the major axis of the organelle giving the interior of the premelanosomes a striated pattern. Stage 3 melanosomes have a better developed striated scaffold of intraluminal fibers upon which melanins are beginning to be deposited. Stage 4 melanosomes have a higher melanin content that commonly masks the fibrillar scaffold. Melanosome length normally grows from stage 1 to stages 2/3 and especially from stages 2/3 to stage 4 (cf. Nguyen et al., 2002; Nguyen and Wei, 2004).

Like nearly all mouse models of HPS, sdy/DBA mice display evidence of defective ocular and cutaneous melanogenesis (Li et al., 2003; Nguyen and Wei, 2004). Indeed, the defects are among the most prominently observed in the fifteen HPS mouse strains (cf. Nguyen et al., 2002 and Nguyen and Wei, 2004). This is not entirely due to the Dtnbp1 mutation since even wild type DBA/2J mice have a higher percentage of morphologically aberrant melanosomes than other wild type mouse strains and lack normally striated stage 2 and 3 melanosomes (Nguyen and Wei, 2004). But even compared with DBA/2J mice, homozygous sdy/DBA mice have a high percentage of morphologically aberrant melanosomes (52 vs. 23% in DBA/2J animals: Nguyen and Wei, 2004). This reflects early disruption in the biogenesis of cutaneous melanosomes since the normally significant increase in length of those organelles from stages 2/3 to stage 4 does not occur in sdy/DBA mice. The result is fewer fully pigmented (i.e., stage 4) melanosomes (i.e., 44% vs. 72% in DBA/2J and 76% in C57BL/6 mice: Nguyen and Wei, 2004). This can explain the very light coat color of homozygous sdy/DBA mice, because both the mature and immature melanosomes can be transferred to keratinocytes largely responsible for coat color (Nguyen and Wei, 2004). Without data on the rate or degree of transfer, however, it is possible that the light coat color may be due not only to fewer fully pigmented melanosomes, but also to decreased secretion of melanosomes to keratinocytes.

Melanosomal abnormalities similar to those found in sdy/DBA mice are found in cappuccino, muted, and pallid mice, which all have coat color mutations in genes that encode proteins integral to BLOC-1 (Nguyen et al., 2002; Nguyen and Wei, 2004). These and related findings have led to the proposal that BLOC-1 plays an important role in an early stage of LRO biogenesis (Wei, 2006; Li et al., 2007; Raposo et al., 2007).

6.4.2.5 Platelet Dense Granules After a tail snip, homozygous sdy/DBA mice bleed for a long time (>15 min.) compared with heterozygous sdy/DBA mice (2.0 $\pm$ 0.22 min.: Swank et al., 1991b) or C57BL/6 mice (3.8 $\pm$ 0.3 min.: Novak et al., 1984). As explained subsequently, this is attributable to abnormal biogenesis of platelet dense granules as explained below.

Platelets are non-nuclear, disc-shaped cytoplasmic bodies derived from mammalian bone marrow megakaryocytes that circulate in the blood as sensors of vascular integrity (Lasne et al., 2006; Ruggeri and Mendoliccino, 2007). When the endothelium forming the inner lining of blood vessels is breached, platelets play a critical role in arresting bleeding (hemostasis) by adhering to extracellular matrix components exposed beneath the endothelium and by aggregating with other adherent platelets to form clots (i.e., thrombi: Gibbins, 2004; Lasne et al., 2006). These events result from interactions between platelets and connective tissue (collagen) in the extracellular matrix. Especially under high shear rate in arteries, platelets first become tethered to collagen by binding von Willebrand factor and then are more securely attached by

direct binding to collagen (Gibbins, 2004; Ruggeri and Mendolicchio, 2007). Tethering and attachment, as well as the presence of plasma thrombin, activate the platelets (Ruggeri, 2002; Gibbins, 2004; Lasne et al., 2006). This triggers release of factors from their delta (δ) or dense granules that promote platelet aggregation. Platelet dense granules are LROs with a large, electron-opaque core rich in amines (serotonin and histamine), cations (Ca^{2+} and Mg^{2+} pyrophosphate), and nucleotides (ADP, ATP, GDP, GTP: McNicol and Isreals, 1999; Rendu and Brohard-Bohn, 2001). Upon platelet activation, virtually all the contents of their dense granules are released, in most cases causing further activation and/or aggregation of platelets (cf. Lages et al., 1983 and McNicol and Israels, 1999). Glutamate may also be released at the same time given that platelets are found to express VGluT-1 and VGluT-2 alongside vesicles resembling dense core granules and release glutamate upon collagen-induced aggregation (Tremolizzo et al., 2006).

Swank et al. (1991b) demonstrated that platelet dense granules are highly abnormal in homozygous sdy/DBA mice. While the numbers of such granules per platelet is normal as visualized with the fluorescent dye mepacrine (= quinacrine), few if any, can be visualized in standard EM preparations of unfixed, air-dried platelets. In other words, the core of the granules is no longer electron opaque in most instances. That probably reflects depletion of the core contents, which normally includes high concentrated serotonin (65 mM: Rendu and Brohard-Bohn, 2001). Indeed, platelet serotonin content of homozygous sdy/DBA mice is just 7% that of the heterozygous animals. Loss of dense granule contents explains the name "platelet storage pool deficiency" to describe a core feature of HPS, one which is shared by mice with mutations in genes encoding the proteins in BLOC-1.

In addition to content depletion, loading of platelet dense granules may be abnormal. The granules in homozygous sdy/DBA mice absorb just 67% of the mepacrine dye taken up by heterozygous sdy/DBA mice. Since mepacrine is a basic dye thought to accumulate in dense granules due to acidic entities in the granules (Picotti et al., 1976), lesser dye uptake in homozygous mice suggests an increase in pH of the granule lumen due to decreased activity of the proton-pumping ATPase in the granule membrane. Another membrane abnormality is indicated by the markedly reduced number of ultraviolet (UV) -induced flashes emitted by mepacrine-labeled dense granules. UV exposure is thought to cause normal mepacrine release into the less acidic cytosol, where it is less quenched and thus flashes briefly (Reddington et al., 1987). The number of flashes in the homozygous sdy/DBA mice is ten-fold less than in the heterozygous animals, suggesting decreased mepacrine release, and hence, an impairment in membrane dynamics. The impairment in granule release extends to collagen-induced ATP secretion and thrombin-induced serotonin secretion in homozygous compared with heterozygous sdy/DBA mice. The absence of dense granule ATP secretion upon exposure of platelets to low levels of collagen (i.e., 1 ug/ul) is correlated with a marked decrease in platelet aggregation, accounting for the long bleeding times in homozygous sdy/DBA mice.

The abnormalities just described in platelet dense granules of sdy/DBA mice are found in many other mouse models of HPS, including muted and pallid mice, all of which, as noted earlier, have coat color mutations in genes encoding proteins integral to BLOC-1. That complex is thus thought to be involved in the biogenesis of platelet dense granules.

6.4.2.6 Platelet Lysosomal Granules Like secretory lysosomes of the kidney, these LROs release hydrolases, including β-glucuronidase and β-galactosidase, into extracellular space, in this case blood plasma (Rendu and Brohard-Bohn, 2001). While levels of the two hydrolases named are the same in heterozygous and homozygous sdy/DBA mice, the latter animals secrete less of both enzymes in response to thrombin activation of platelets (Swank et al., 1991b). A similar phenomenon is observed in lysosomal granules of mice with mutations in other genes encoding BLOC-1 proteins, namely muted and pallid mice (see Swank et al., 1998). Since the membranes of platelet lysosomal granules are more fragile in homozygous than heterozygous sdy/DBA mice (Swank et al., 1991b), BLOC-1 may play a role in the biogenesis of those organelles as it does in other LROs.

6.4.2.7 Neurotransmitters Swank et al. (1991b) found that serotonin in platelet dense granules of homozygous sdy/DBA mice is only 7% of that in heterozygous mice and is not significantly secreted upon exposure to the platelet activators collagen or thrombin. No explanation has been given for these abnormalities, but they may reflect defective biogenesis of dense granule membranes that results in

impaired uptake and retention of serotonin. In homozygous versus heterozygous sdy/DBA mice, platelet dense granules initially take up less [^{3}H] serotonin (7,130 vs. 10,000 cpm per 10^8 platelets) and retain far less [^{3}H] serotonin as indicated by the level of radiolabel in culture supernatants 3 min later (40.0 vs. 5.3 cpm per 10^8 platelets) (see Swank et al., 1991b). It has not been determined if impaired serotonin uptake results from decreased levels of the amine or reduced trafficking of vesicular monoamine transporter 2 (VMAT2: Zucker et al., 2001) to the dense granules. Decreased retention could result from increased cytosolic pH, because incubation with weak bases causes leakage of serotonin from platelet dense granules (van Oost et al., 1985).

While there are no reports yet on serotonin in the nervous system of sdy mice, Murotani et al. (2007) and Hattori et al. (2008) have discovered abnormal dopamine levels and turnover in the brains of 2–3 month old male homozygous sdy/DBA mice compared with control DBA2J mice. In particular, they discovered that dopamine concentrations were significantly reduced in the hypothalamus, hippocampus, and cerebral cortex samples with the amygdala attached. Normal dopamine concentrations were found in the other brain areas (i.e., olfactory bulb, frontal cerebral cortex, striatum, thalamus, midbrain, cerebellum, and lower brain stem). In contrast, all brain areas studied in sdy mice had normal concentrations of glutamate. The normal dopamine concentration in the striatum is consistent with our immunoEM evidence that dysbindin-1 is absent in axon terminals of the nigrostriatal pathway (see *Section 2.2.6.3.2.3.4*). Reduced dopamine in the tissue containing the amygdala and the hippocampus suggests that dysbindin-1 may exist in axon terminals of the mesolimbic pathway, which originates in the ventral tegmental area of the midbrain. The dopamine reductions were not attributable to decreased levels of the rate limiting synthetic enzyme of dopamine (i.e., tyrosine hydroxylase), because midbrain levels of that enzyme were found normal in sdy mice (Murotani et al., 2007). A more likely explanation for the reductions in limbic dopamine was evident in an elevated ratio of dopamine to one of its metabolites, homovanillic acid, in tissue containing the amygdala and the hippocampus. This indicated an elevated dopamine turnover in those tissues, which suggests increased dopaminergic transmission (i.e., synaptic release with subsequent degradation). Elevated dopaminergic transmission in limbic structures of sdy mice is consistent with the observed increase in dopamine release by PC-12 cells transfected with Dtnbp1 siRNA to reduce dysbindin-1 levels, mimicking the loss of dysbindin-1 in sdy mice resulting from a deletion mutation in Dtnbp1 (Kumamoto et al., 2006: see *Section 2.2.6.5.6*).

6.4.2.8 Neurophysiology Although, glutamate levels are normal in all tested brain areas of homozygous sdy/DBA mice (Hattori et al., 2008), glutamatergic as well as GABAergic transmission are abnormal in such mice according to several electrophysiological studies conducted on the prelimbic cortex (the putative mouse homolog of the primate dorsolateral prefrontal cortex) and on the hippocampal formation.

In deep layers of the prelimbic cortex, Jentsch et al. (2009) studied basal and evoked transmitter release by pyramidal cells in homozygous sdy/DBA mice and wild type controls. Such cells are glutamatergic neurons in which dysbindin-1 loss should affect both basal and evoked transmitter release, because both types of release are reduced in cerebrocortical neuronal cultures transfected with Dtnbp1 siRNA to knockdown dysbindin-1 (Numakawa et al., 2004). Jentsch et al. (2009) investigated basal release by recording excitatory postsynaptic currents (EPSCs) with and without nerve conduction blockade. With such blockade, only miniature EPSCs (mEPSCs) are possible. Without such blockade, even in the absence of extrinsic stimulation, it is possible to record both mEPSCs and spontaneous EPSPs resulting from intrinsically generated action potentials; these miniature and spontaneous events are collectively called spontaneous EPSCs (sEPSCs). Jentsch et al. (2009) found a reduction in the amplitude of mEPSCs and sEPSCs, as well as a reduction in the frequency of sEPSCs in the prelimbic neurons of the sdy/DBA mice compared with wild type mice. Indicative of impaired evoked responses to stimulation, they found that only 50% of the prelimbic neurons in the sdy/DBA mice showed normal augmentation of response to the second of two electrical stimuli delivered after an interval greater than 20 ms (i.e., many of the cells failed to show paired-pulse facilitation). Loss of dysbindin-1 thus seems to impair synaptic release of glutamate by deep pyramidal cells in the prelimbic cortex.

The presynaptic anomalies in transmitter release just described are accompanied by postsynaptic anomalies in glutamatergic receptor responses. A study by Andrews et al. (2007) used whole-cell patch

clamp recordings to study ion channels of pyramidal cells deep in the prelimbic cortex. They found that in homozygous sdy/DBA versus wild type mice, glutamatergic current mediated by NMDA receptors (NMDARs) was reduced compared with that mediated by AMPA receptors (AMPARs), even though the neurons were normal in spike threshold and amplitude. The ratio of AMPAR current to total excitatory current remained normal. Since strong NMDAR currents are needed for induction of long-term potentiation (LTP, Citri and Malenka, 2008), this abnormality may contribute to the impaired spatial working memory displayed by such mice on a delayed non-match to position task known to be affected by prelimbic cortex lesions (Jentsch et al., 2009).

Further work on the prelimbic cortex of sdy/DBA mice has shown that the abnormalities in deep pyramidal cells extend to the fast-spiking interneurons innervating them. These are parvalbumin-containing cells (Kawaguchi and Kubota, 1997) which are driven via NMDAR-mediated input (Homayoun and Moghaddam (2007) and which in turn drive gamma frequency oscillations enhancing cognitive processes (Sohal et al., 2009; Cardin et al., 2009). Using whole-cell patch clamp recordings, Trantham-Davidson et al. (2008) found that fast-spiking interneurons in homozygous sdy/DBA mice showed a decreased frequency of sEPSC with reduced NMDAR-mediated currents, consistent with NMDAR hypofunction in these interneurons. This may explain why Ji et al. (2008) found decreased excitability of fast-spiking interneurons in deep layers of the prelimbic cortex in homozygous sdy/DBA mice. This may in turn explain why Trantham-Davison et al. (2008) also found decreased GABA-mediated inhibitory postsynaptic currents on pyramidal neurons of such mice. These findings are of special interest given that NMDAR hypofunction of fast-spiking interneurons (Homayoun and Moghaddam, 2007; Kehrer et al., 2008) and deficits in gamma oscillation generation (Lee et al., 2003; Light et al., 2006) are prominent biological features of schizophrenia.

In the hippocampal field CA1, Chen et al. (2008) studied miniature and evoked EPSCs in homozygous sdy/DBA mice and wild type controls. Given that glutamate is the dominant excitatory transmitter in the hippocampus (Kullman, 2007), the findings may be assumed to reflect glutamatergic events. Compared with wild type controls, the sdy mice showed a 45% decline in mEPSC frequency, which suggests a decrease in release probability. This may have been caused by decreased hippocampal levels of snapin in homozygous sdy/DBA mice reported elsewhere by the same investigators (Feng et al., 2008). As noted above, snapin promotes efficient, synchronous release of synaptic vesicles (Pan et al., 2009). Many other presynaptic proteins were eliminated as factors in the decreased vesicular release probability, because Chen et al. (2008) found that sdy mice had apparently normal levels of proteins involved in vesicular transmitter uptake (VGluT1), exocytosis (complexin 1 or 2, munc18–1, SNAP25, synaptotagmin 1, syntaxin 1, or VAMP2), or endocytosis (dynamin 1). Feng et al. (2008) showed that, unlike snapin, none of these are direct binding partners of dysbindin-1.

It is difficult to interpret two other presynaptic features reported by Chen et al. (2008), namely increases in decay time and charge transfer of the mEPSCs. The authors claim that these features indicate slower vesicular release kinetics and larger quantal size of the sdy synaptic vesicles, the latter consistent with their finding that the vesicles were on average 10% larger in diameter). Such changes were the case in the authors' amperometric study on catecholamine release from sdy adrenal chromaffin cells presented in the same report (see *Section 2.2.6.4.2.2*). But further study is needed to determine if such conclusions about glutamatergic vesicles in CA1 are valid, because – unlike amperometric data – mEPSCs themselves provide only indirect data on vesicular exocytosis and do so accurately only in the absence of postsynaptic changes. Chen et al. (2008) claim that the normal amplitude of mEPSCs in sdy mice is evidence that those mice were normal in the number and responsiveness of postsynaptic receptors. That is not supported, however, by results of the only direct tests of postsynaptic responsiveness given, specifically tests establishing the ratio of AMPAR to NMDAR currents evoked by Schaffer collateral input to CA1. While there is no reason to doubt that the ratio itself was unaltered, the recordings illustrating the point (Chen et al., 2008, suppl. Figure S2) show that both AMPAR and NMDAR currents were clearly reduced. Since strong NMDAR currents are needed for induction of long-term potentiation (LTP, Citri and Malenka, 2008), this abnormality may contribute to the impaired LTP displayed in CA1 of homozygous sdy mice on DBA/2J (Glen et al., 2008) or C57BL/6 (G. Carlson, in preparation) backgrounds.

Less problematic is the further finding of Chen et al. (2008) that the amplitudes of EPSCs evoked by Schaffer collateral inputs to CA1 were decreased by about 29%. This is consistent with the decreased

vesicular release probability evident in the lower frequency of mEPSCs found by the same authors and in results of paired-pulse tests of Jentsch et al. (2009) in the prefrontal cortex of sdy mice. EM work in CA1 showed that the decreased probability of release is probably not due to impairment in docking of vesicles with the presynaptic membrane, but could be due in part to an observed 22% reduction in the reserve pool of synaptic vesicles from which readily releasable vesicles may ultimately derive (see *Sections 2.2.6.5.5* and *2.2.6.5.6*). It could also be due, at least in part, to loss of interactions with snapin, which as mentioned earlier normally boosts the number of LDCVs kept in a readily releasable state (Tian et al., 2005) and enhances efficient, synchronous release of synaptic vesicles for (Pan et al., 2009). Other morphological changes at glutamatergic synapses in CA1 of sdy mice noted by Chen et al. (2008) (i.e., increased vesicular size, decreased synaptic cleft widths, and increased PSD thickness) may also contribute to the electrophysiological changes in that area. How they might do so is unknown.

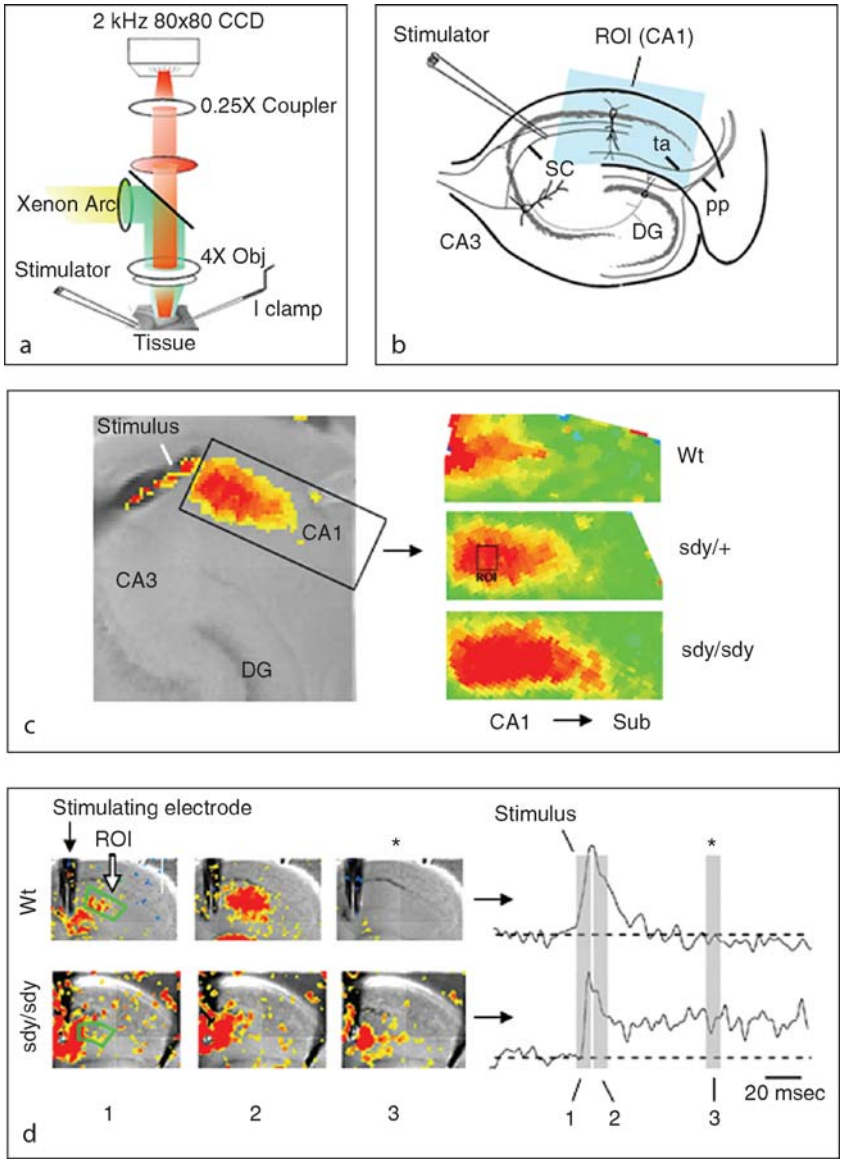
Our electrophysiological studies on the hippocampus of 2–3 month old sdy mice also find abnormalities compared with wild type animals. These abnormalities are shared by sdy mice on DBA and on C57BL/6 backgrounds (the latter is called sdy/BL6 mice). They were discovered by Gregory Carlson while studying CA1 neuron responses to input from CA3 Schaffer collaterals. The work was done on horizontal slices of the hippocampus in or near the ventral half of that structure (in *Figure 2.2-20a*). Voltage-sensitive dye recordings were used to capture the temporal and spatial properties of the CA1 responses to stimulation. Employing a camera with a 2 kHz frame rate, the method allows us to visualize postsynaptic potential kinetics and, unlike field recordings, enables direct assay of membrane voltage (Contreras and Llinas, 2001; Ang et al., 2005; Carlson and Coulter, 2008).

Stimulation of Schaffer collaterals (*Figure 2.2-20b*) evokes strong excitatory postsynaptic potentials (EPSPs) in CA1 that spread farther in the sdy mice, especially in homozygous mice (*Figure 2.2-20c*). The responses of CA1 cells to glutamatergic input from Schaffer collaterals (Arrigoni and Green, 2004) are thus greater in sdy mice. Stimulation of these collaterals normally evokes EPSPs followed by longer inhibitory postsynaptic potentials (IPSP: *Figure 2.2-20d*, upper panels), as seen previously in rats (Davies and Collingridge, 1989; Ang et al., 2005). The EPSP–IPSP sequence reflects initial excitation of CA1 pyramidal cells followed by feedback inhibition of those cells via local GABAergic neurons (Buhl and Whittington, 2007). In stark contrast, homozygous sdy mice display virtually no IPSPs following the EPSPs (*Figure 2.2-20d*, lower panels, Arnold et al., 2005). Since EPSP amplitudes in sdy mice appeared normal, the simplest explanation for this phenomenon is loss of synaptic strength at synapses of CA1 pyramidal cells on interneurons and/or at synapses of those GABAergic interneurons feeding back on the pyramidal cells. An effect at synapses of CA1 pyramidal cells on interneurons is consistent with evidence cited above for decreased synaptic glutamate release and/or postsynaptic effects in the prefrontal cortex and CA1 of sdy/DBA mice (Andrews et al., 2007; Jentsch et al. (2009); Chen et al., 2008). An effect at synapses of interneurons on CA1 pyramidal cells is more speculative, but is consistent with the presence of dysbindin-1 in interneurons in CA1 (see *Section 2.2.6.3.2.3.5*). Loss of the protein in those interneurons should reduce their capacity for GABA release, because dysbindin-1 knockdown lowers levels of synapsin 1 (Numakawa et al., 2004), which is known to reduce the pool of readily releasable synaptic vesicles in inhibitory hippocampal neurons (Baldelli et al., 2007).

6.4.2.9 Behavior and Cognition A number of behavioral studies have been conducted on sdy mice with DBA/2J or C57BL/6 backgrounds. Before discussing them, it should be recalled that DBA/2J mice are homozygous for at least six mutations, of which four are associated with neural impairments (see *Section 2.2.6.4.2.1*). These appear to account for a number of developmental abnormalities compared with C57BL/6 mice. DBA/2J mice develop a high frequency hearing loss between 3–4 weeks of age. By 5 weeks, hearing loss is detected at all frequencies tested and persists through old age (Erway et al., 1993; Zheng et al., 1999). Between 3–4 months, at least some DBA/2J mice begin showing symptoms of glaucoma with 56% of the females and 15% of the males showing loss of the iris pigment epithelium and transillumination of the peripheral iris (John et al., 1998). As early as 6–7 months of age, DBA/2J mice have abnormal irises, and by 9 months they show increased intraocular pressure (John et al., 1998). Between 10–19 months, there are fewer retinal ganglion cells (John et al., 1998; Moon et al., 2005) and fewer cholinergic and GABAergic amacrine cells than in C57BL/6 mice of the same age (Moon et al., 2005).

Figure 2.2-20

Loss of dysbindin-1 in homozygous sandy mice causes longer excitation and loss of inhibitory responses to Schaffer collateral input in CA1 hippocampal neurons. (a) shows the set-up for voltage-sensitive dye imaging. 350 μM horizontal slices of the hippocampus were exposed to the voltage-sensitive dye RH-795, placed in an interface chamber, and imaged via epifluorescence through a 4X objective. Epifluorescent changes were recorded with a CCD-SM camera at 2000 Hz before, during, and after stimulation of the Schaffer collaterals (see Carlson and Coulter, 2008). (b) shows the stimulation and recording set-up. (c) shows increased spread of excitatory postsynaptic currents into the recording area in CA1 of sdy mice. (d) shows loss of normal inhibition of CA1 neurons in homozygous sdy mice following initial excitation via Schaffer collateral input. See text *Section 2.2.6.4.2.8* **for a discussion of this phenomenon**



Since sdy/DBA mice are necessarily homozygous for most alleles causing these conditions (see *Section 2.2.6.4.2.1*), they must share most of the auditory and visual deficits of DBA/2J animals. They are also expected to share the enhanced responses to stress, decreased responses to dopamine agonists, and cognitive deficits on auditory, olfactory, and visual tasks shown by DBA/2J mice compared with C57BL/6 animals (cf. Rossi-Arnaud and Ammassari-Teule, 1998, Balogh et al., 2002, Cabib et al., 2002, and Restivo et al., 2006). Adult sdy/DBA animals are therefore not optimal for detecting deficits resulting from dysbindin-1 loss. Even with the most appropriate controls (i.e., littermate wild-type DBA/2J mice), it will be more difficult to detect impaired behavioral capacities on auditory, olfactory, or visual tasks in sdy/DBA than sdy/BL6 mice. Since most rodent behavioral tests are dependent on auditory, olfactory, and/or visual perception, caution is advised in inferring behavioral dysbindin-1 functions from tests on sdy/DBA mice.

A number of behavioral studies have nevertheless been conducted on sdy/DBA mice. The first such study was that of Hattori et al. (2008), who studied 6–9 weeks old male homozygous sdy/DBA animals versus wild-type DBA/2J mice not noted to be littermates. The sdy animals were found to be normal in many respects: body weight, hair coat quality and density, the number and length of whiskers, sensorimotor reflexes (i.e., eye blink, ear twitch, whisker response to touch, and righting reaction when turned over), and neuromuscular strength, as also reported by Takao et al. (2008) for such animals. Even though the sdy mice showed an exaggerated startled response to a loud (120 dB) sound or pulse, the response was inhibited as in wild-type animals by a weaker sound (74–82 dB) or prepulse presented shortly before the loud one. In other words, there was normal prepulse inhibition, as previously noted by Li et al. (2003). In other respects, however, the sdy mice were abnormal. They were hypoactive in the open field test and appeared to be anxious as suggested by their avoidance of the central space in the open field and of the open arms in an elevated maze. The sdy mice were also less sociable, spending less time in social contacts with wild-type or other sdy mice.

The findings of Hattori et al. (2008) are problematic for several reasons. At the ages studied, DBA/2J mice have clear auditory problems as noted above. It is premature, then, to conclude that prepulse inhibition is normal in sdy/DBA mice. In homozygous sdy/BL6 mice, which lack the auditory deficits of DBA/2J mice, prepulse inhibition is significantly reduced (Halene et al., 2009). Moreover, no abnormal locomotor activity in the open field was found in sdy/DBA mice by Bhardwaj et al. (2009) or by Feng et al. (2008), who also found no evidence of elevated anxiety in such animals.

In other respects, sdy/DBA mice display many abnormal behaviors, though not always in heterozygotes. Bhardwaj et al. (2009) found (1) decreased responsiveness to painful stimuli in heterozygotes and homozygotes, (2) loss of locomotor habituation to the open field in homozygotes, (3) impaired novel object recognition memory in heterozygotes and homozygotes, (4) contextual fear conditioning deficits in homozygotes, and (5) decreased locomotor responsiveness to a single amphetamine dose (2.5 mg/kg ip) in heterozygotes and homozygotes but sensitization of homozygotes to this amphetamine dose administered once a day for five days. Studying homozygotes versus wild type controls, Feng et al. (2008) likewise found sdy deficits in novel object recognition memory, as well as decreased time spent in social interactions. Also studying homozygotes versus controls, Takao et al. (2008) reported sdy deficits in tests of long-term spatial memory (Barnes circular maze test) and spatial working memory (T-maze forced alternation task).

Sdy/BL6 mice are also normal in body weight, physical appearance (apart from pigmentation), sensorimotor reflexes, and neuromuscular strength. Cox et al. (2009) demonstrated this in 3–4 months old littermate wild-type, heterozygote, and homozygous mice of both sexes. They found no differences among animal groups of either sex in body weight, number and length of whiskers, hair coat condition, grooming behavior, or sensorimotor reflexes. Motor coordination was not impaired, as reflected in ability to stay atop a rod revolving at an accelerating rate. The sdy mice also displayed no evidence of increased anxiety (i.e., they showed no preference for the edge of the open field or for the closed arms of an elevated maze). But the homozygous animals did display several behavioral abnormalities, all of which are consistent with dysfunction of the hippocampal formation. Unlike the findings made in sdy/DBA animals, homozygous sdy/BL6 mice of both the sexes were hyperactive in the open field, more so in the second test session, suggesting impaired habituation to the novel environment. Altered hippocampal neurotransmission in sdy mice noted above (see *Section 2.2.6.4.2.8*) may account for these abnormalities, given that hyperactivity and impaired habituation are common features of altered hippocampal function in rodents (cf. Galani et al., 1998; Wright et al., 2004; Sloan et al., 2006).

The most striking behavioral feature of sdy/BL6 mice currently known is a severe deficit in spatial learning and memory ability seen in homozygous but not in heterozygous animals (Cox et al., 2009). This was discovered using the Morris water maze (D'Hooge and De Deyn, 2001; Morris, 2007, pp. 629–634), in which rodents are tested for their ability to use spatial context cues above water to find a submerged platform from different starting locations along the perimeter of a circular pool. This is preceded by visible platform trials in which the location of the platform is cued (i.e., marked) by an attached flag. By the end of such trials, wild-type, heterozygous sdy, and homozygous sdy mice were not significantly different in time to reach the platform (i.e., escape latency), indicating equivalent levels of motivation, visual acuity, and final visual association learning. The next phase of testing consists of hidden platform trials in which the flag is removed so that the platform location must be learned with respect to spatial cues around the pool. In these trials, homozygous sdy mice improved much more slowly than did the heterozygous sdy or wild-type littermates and had significantly longer escape latencies than those mice. In the subsequent probe trial in which the platform had been removed from the pool, the homozygous sdy displayed no preference for the quadrant where the hidden platform had been, which reflects impaired spatial memory. Homozygous sdy/DBA mice also show such a deficit on the probe trial in the Morris water maze (Jentsch et al. (2007).

Spatial leaning and memory deficits without loss of visual association ability in homozygous sdy mice mirrors the water maze deficits of rodents with hippocampal lesions (see D'Hooge & De Deyn, 2001, Sloan et al., 2006, and Morris, 2007). Such deficits may be caused by the decreased responsiveness of hippocampal CA1 pyramidal neurons to excitatory input from CA3 found in sdy mice by Chen et al. (2008, see *Section 2.2.6.4.2.8*). Normal responsiveness to such input is important for spatial, but not visual association memory as shown by the finding that CA1-specific knockout of NMDA glutamate receptors impairs location of the hidden, but not the visible, platform in the water maze (Tsien et al., 1996).

A final behavioral abnormality reported in sdy mice is a deficit in short-term working memory revealed in performance on a delayed non-matching-to-position task by homozygous sdy/DBA (Jentsch et al., 2009). In this task, a food-deprived animal faces a food dispenser flanked by two retracted levers. It is then trained to release a food pellet into the dispenser by pressing the lever extended into the cage. That lever is then retracted and, after a delay of 0–24 s, both levers are extended. The animal must now learn to press the lever that was previously extended (i.e., a delayed matching-to-position task) or the lever that was not previously extended (i.e., a delayed non-matching-to-position task). Correct delayed responses on spatial tasks tax spatial working memory and thus provide measures of that cognitive capacity. Delayed matching- and non-matching-to-position tasks using the relatively short delays tested to date are insensitive to lesions of the hippocampal formation (Sloan et al., 2006). The deficits in delayed non-matching-to-position found in sdy/DBA mice thus appear to reflect short-term spatial working memory abnormalities outside the hippocampal formation. Of the brain areas mediating spatial working memory (see Curtis, 2006 and van Asselen et al., 2006), the prefrontal cortex is the most likely candidate, though it must be recognized that the literature is divided on its contribution to the delayed non-matching-to-position task (see Sloan et al., 2006).

Further evidence that dysbindin-1 plays a role in cognition is presented in *Section 2.2.6.5.10*.

6.4.3 Does the Sdy Mouse Model Aspects of Schizophrenia?

Since dysbindin-1 is reduced in the brains of sdy mice (see *Section 2.2.6.4.1*) and of schizophrenia cases (see *Section 2.2.6.6.2*), it must be asked whether these mice display phenotypical features of that disorder. That appears to be true for homozygous, but not necessarily heterozygous, sdy mice given the behavioral abnormalities described above (see *Section 2.2.6.4.2.9*). The case for the homozygote animals can be summarized as follows. Their hyperactivity and reduced auditory prepulse inhibition of the sdy/BL6 mice (Cox et al., 2009; Halene et al., 2009) is shared by many mouse models of schizophrenia (Powell and Miyakawa, 2006; see also Mohn et al., 1999, Hikidia et al., 2007, Powell et al., 2007). Their hyperactivity may have the same proximal causes as the psychomotor agitation estimated to occur in 21% of acute schizophrenia cases admitted for emergency psychiatric care in the U.S. (Marco and Vaughan, 2005). Impaired habituation to the open field shown by homozygous sdy/DBA and sdy/BL6 mice (Bhardwadj et al., 2009; Cox et al., 2009) mice resembles the decreased habituation to diverse stimuli reported in schizophrenia

(Taiminen et al., 2000; Meincke et al., 2004; Holt et al., 2006). The decreased social interactions of homozygous sdy/DBA mice (Feng et al., 2008; Hattori et al., 2008) is consistent with a negative symptom (i.e., social withdrawal) and perhaps with impaired social cognition in schizophrenia (see Couture et al., 2006 and Yager and Ehmann, 2006). Finally, the spatial memory deficits displayed by homozygous sdy mice in the Morris water maze (Cox et al., 2009) and the T-maze (Takao et al., 2008) resemble the deficits displayed by humans with schizophrenia in a virtual Morris water maze task (Hanlon et al., 2006) and in other tests of spatial working memory (Glahn et al., 2003). Homozygous sdy mice thus appear to model diverse aspects of schizophrenia.

Drosophila with a piggyBac insertion mutation ($Ddys^{e01028}$) may also model aspects of schizophrenia. Shao et al. (2008) have presented evidence that such fruit flies, which have reduced levels of dysbindin-1, are similar to sandy mice in several respects relevant to schizophrenia. These flies are hyperactive and show learning and memory deficits in an olfactory T-maze task. Also like sandy mice, the $Ddyse^{01028}$ flies show impaired glutamatergic transmission at the neuromuscular junction (not cholinergic in *Drosophila*) due to reduced levels of presynaptic dysbindin-1 and heightened dopaminergic transmission reflected in markedly elevated, reserpine-sensitive same-sex courtship among male flies. $Ddyse^{01028}$ flies are consequently a second dysbindin-1 deficient species available for studies on functions of that protein.

6.5 Binding Partners and Potential Functions

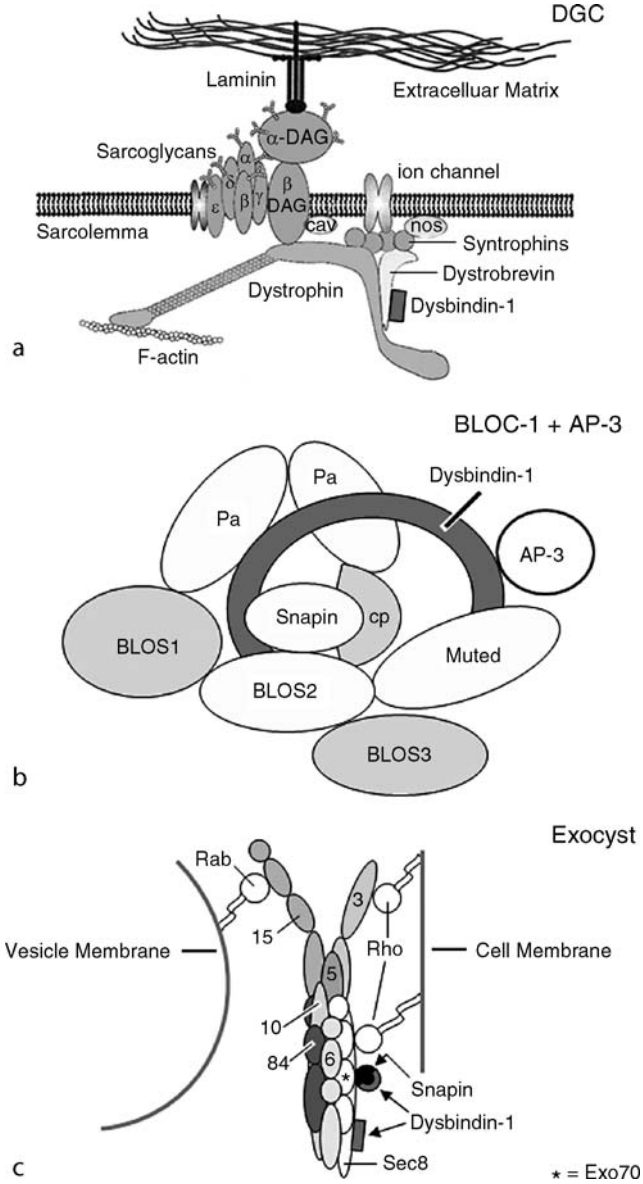
Dysbindin-1 has a large number of binding partners, which collectively comprise the dysbindin-1 interactome. Those known at present are listed in *Table 2.2-6*. Nearly half are members of BLOC-1 (muted, pallidin, and snapin). Another, mysospryn, binds a protein (desmin) that interacts with BLOC-1 in muscle (Kouloumenta et al., 2007). Two other members of the interactome, α- and β-dystrobrevin, are part of the dystrophin glycoprotein complex (in *Figure 2.2-21a*), but it has been questioned whether or not these are physiological binding partners of dysbindin-1. There is no doubt that dysbindin-1 *can* bind dystrobrevins, as demonstrated by yeast two-hybrid (Y2H) screens and in vitro tests with the isolated CCDs of the two proteins (Benson et al., 2001; Nazarian et al., 2006). The results of in vivo tests in the two reports just cited are nevertheless inconsistent. Nazarian et al. (2006) found no evidence that isolated CCDs of dysbindin-1 and dystrobrevins bind in extracts of HeLa cells or mouse tissues. As touched upon earlier (see *Section 2.2.3.2*), Benson et al. (2001) argue that it is not the CCD, but rather the CTR, of β-dystrobrevin, which binds dysbindin-1. They also demonstrated co-immunoprecipitation of the two full-length proteins in tissue from the brain and muscle of rats. Nazarian et al. (2006) were nevertheless unable to demonstrate such co-immunoprecipitation in brain or muscle of the mouse or rat even though they could co-immunoprecipitate dysbindin-1 and the BLOC-1 protein pallidin in the same experiments. Nazarian et al. (2006) imply that higher affinity interaction of dysbindin-1 with BLOC-1 in vivo may interfere with an interaction of dysbindin-1 with dystrobrevins. Such interference would be widespread in the body given the ubiquitous expression of BLOC-1 proteins. At present, it is questionable whether dystrobrevins are physiological binding partners of dysbindin-1.

The dysbindin-1 interactome may include many more members than currently reported. In addition to the known members of the interactome, there are many candidate members as listed in *Table 2.2-7*. These have been identified in Y2H screens, but remain to be tested in co-immunoprecipitation experiments on cultured cells or animal tissues. Most were discovered by Camargo et al. (2007) in Y2H screens using dysbindin-1 (amino acids 1–189) as the bait and fetal (i.e., 20 week old) human brain proteins derived from a cDNA library as prey. Of the 97 bait-prey interactions identified, 17 were judged high-confidence interactions and are accordingly listed in *Table 2.2-7*, except for the 3 whose identity is uncertain. The candidate binding partners could interact with all dysbindin-1 isoforms (as is true for the established binding partners), since the bait included the CCD (amino acids 81–181) shared by all those isoforms. But the candidates are not likely to interact with dysbindin-2 or -3, since the bait excluded the portion of dysbindin-1 with significant sequence homology to dysbindin-2 and -3 (see *Section 2.2.3.3.1*).

The spectrum of potential dysbindin-1 functions is indicated by the types of proteins in its interactome. If we include both the known and candidate binding partners, the interactome includes ten protein groups

Figure 2.2-21

Three of the protein complexes with which dysbindin-1 interacts. (a) Shows the components of the dystrophin glycoprotein complex (DGC), which spans the muscle membrane and connects the cytoskeleton to the extracellular matrix (adapted with permission from Biggar et al., 2002). All shaded molecules belong to the DGC, (b) shows the eight components of BLOC-1 (plus AP-3) and their binding partners within the complex. Those components which can bind dysbindin-1 are shown in direct contact. The diagram is not meant to be physical model of BLOC-1, because it is unlikely that any one dysbindin-1 molecule can bind more than three BLOC-1 members (possibly just one) at any one time (see introduction to *Section 2.2.6.5*) *Cp* cappuccino, (c) shows the exocyst complex tethering a secretory vesicle to the cell membrane (adapted with permission from Munson and Novick, 2006). The complex consists of temperature-sensitive secretory (sec) proteins 3, 5, 6, 8, 10, and 15, as well as exocyst (Exo) proteins 70 and 84. Dysbindin-1 can bind sec6 directly and Exo70 via snapin



(1) endosomal trafficking proteins that belong to BLOC-1 (muted, pallidin, snapin, BLOS1, and BLOS2), (2) other endosomal trafficking proteins (AP-3μA, neurobeachin, rab11A, and rabenosyn-5), (3) cytoskeleton-associated proteins (dystonin-1, MACF-1, sec8, and syntabulin), (4) exocyst and related proteins (sec8 and snapin), (5) muscle proteins (α- and β-dystrobrevin and myospryn), (6) nuclear proteins (cyclin A2, PA28γ, and TFIIIB), (7) nuclear envelope proteins (AKAP6, nesprin-1β, and RanBP5), (8) an E3 ubiquitin ligase (TRIM32), (9) spermatogenesis proteins (RNF151), and (10) proteins of unknown function (zinc finger protein 490). This suggests that dysbindin-1 serves diverse cellular functions mediated by different binding partners.

It is tempting to use computer-based models of protein-protein interactions to gain insight into the full range of dysbindin-1 functions as recently attempted by Guo et al. (2009). Their analysis is based on the assumption that dysbindin-1 has 31 direct binding partners. The results are misleading not only because they include as binding partners several proteins for which there is currently no evidence of direct interaction (i.e., gene products of BLOCS3, CCND3, CNO, AB13, ARFIP2, and DGCR6L), but also because they make no distinction between known and candidate binding partners. Of the 25 remaining proteins for which we have at least Y2H evidence of ability to bind dysbindindin-1 by Guo et al. (2009), only 7 are established (see *Table 2.2-6*). Of those 7, two (α- and β-dystrobrevin) may not be physiological-binding partners as explained above. It is thus premature to use computer modeling to gain insight into the functions of an extensive dysbindin-1 interactome.

It must be also appreciated that there are spatial and temporal constraints on dysbindin-1 interactions and hence on its physiological functions. This was touched upon previously while discussing whether the protein interacts with dystrobrevins in vivo. Most dysbindin-1 interactions probably entail supercoil formation between its CCD and that of another protein (see *Section 2.2.3.2*). But its CCD is short (101 aa), limiting the number of partners any given dysbindin-1 molecule can stably bind at one time. Its Interactions are further limited by access to, and affinity for, potential-binding partners in different cell types and cell compartments under different developmental and physiological conditions. Such constraints must be considered when evaluating hypotheses about possible dysbindin-1 functions and the binding partners which mediate them.

While key dysbindin-1 functions do appear to be mediated by BLOC-1 (see Ghiani et al., 2009 and *Section 2.2.6.5.5*), it is likely that others are mediated by interactions with proteins outside that complex. This is based on three considerations. First, only one dysbindin-1 isoform may be a BLOC-1 member given our finding that dysbindin-1A, but not -1C is reduced in muted mice, which display BLOC-1 loss (*Figure 2.2-19*, Li et al., 2003). Knockdown of muted in SH-SY5Y neuroblastoma cells also causes marked reduction in dysbindin-1A, but not in any other dysbindin-1 isoforms (see supplemental Figure 1a in Iizuka et al., 2007). Second, only 4 of the 24 known or candidate-binding partners of dysbindin-1 are identified as members of BLOC-1 (muted, pallidin, snapin, and BLOS 2). Third, dysbindin-1 is found in nuclei, where three of its candidate-binding partners are located (see *Section 2.2.4.3*), but where no other BLOC-1 members have been found.

We consider here the many potential functions of dysbindin-1 suggested by its binding partners, tissue localization, in vitro effects in cell cultures, and the abnormalities in sdy mice and in humans with certain SNPs in DTNBP1. Few of the many potential functions have been tested experimentally, however.

6.5.1 Spermatogenesis

Dysbindin-1 is a known binding partner of RNF151 (RING finger protein 151: see *Table 2.2-6*). As shown by Nian et al. (2007), gene expression of RNF151 occurs only in the testes and begins with onset of spermatogenesis in mice on postnatal day 21, increases until postnatal day 35, and remains high until at least postnatal day 70 (the last day tested). Spermatogenesis, which occurs in the seminiferous tubules of the testes, produces round spermatids which mature into elongated spermatids. In situ hybridization revealed that RNF151 mRNA is located in round spermatids of stages VII-VIII seminiferous tubules. Immunohistochemistry shows that RNF151 protein is subsequently located in elongated spermatids of stage VIII-IX seminiferous tubules. These findings suggest that RNF151 plays a role in spermatogenesis. Nian et al. (2007)

speculate that interaction of RNF151 and dysbindin-1 may initiate acrosome formation beginning in round spermatids. The acrosome is an organelle spread over the head of sperm that contains digestive enzymes enabling sperm to fuse with an egg. Acrosomes are LROs (Moreno and Alvarado, 2006) and hence organelles of the type whose biogenesis is facilitated by BLOC-1 (Dell'Angelica, 2004, Di Pietro and Dell'Angelica, 2005; Wei, 2006, Li et al., 2007). Reduced interaction between RNF151 and dysbindin-1 in sdy mice may explain why breeding of these mice can be difficult.

6.5.2 Cell Growth and Proliferation

A role for dysbindin-1 in cell growth and proliferation has not been tested, but is expected for four reasons. First, the first putative promoter of DTNBP1 contains binding sites for three transcription factors (E2F1, NF-1, and Sp1) regulating cell growth and proliferation (see *Section 2.2.6.1.3*). Second, dysbindin-1 is known to promote activation of Akt (Numakawa et al., 2004), which is a major trigger for growth and proliferation of cells (Manning and Cantley, 2007), including neuronal progenitor cells of the hippocampal formation (Peltier et al., 2007). Such an effect on Akt signaling may explain why overexpression of DTNBP1 is a feature of so many cancer tissues (see *Section 2.2.6.2.2* and *Table 2.2-13*). Third, the peak period for gene expression of DTNBP1 in prenatal development largely coincides with the peak in proliferation of neuronal precursors (see *Section 2.2.6.2.1*). Fourth, unlike other BLOC-1 proteins, dysbindin-1 is enriched in neuronal nuclei (see *Section 2.2.4.3*), and has candidate-binding partners that which are known to regulate cell growth and proliferation (i.e., cyclin A2, PA28γ, and transcription factor IIIB, isoform 3: see *Table 2.2-7*).

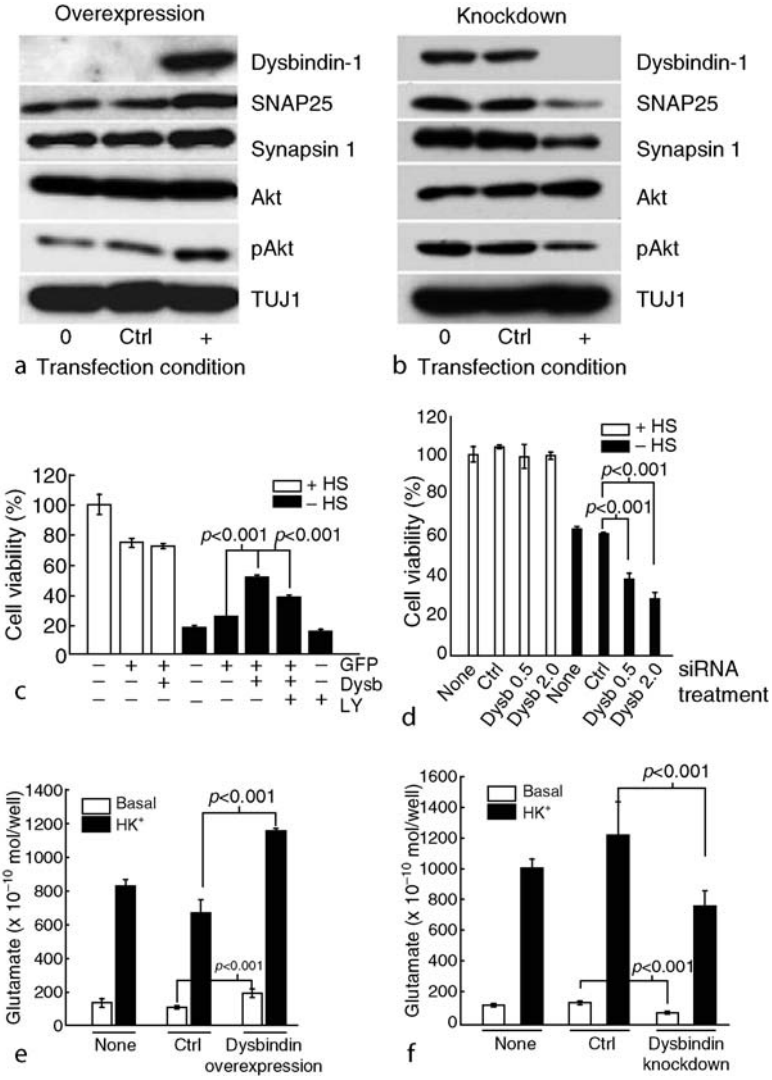
The candidate nuclear binding partners are, in fact, critical for cell growth and proliferation. Cyclin A2 is critical in promoting the G1/S and G2/M transitions in the cell cycle of proliferating cells (Murphy et al., 1997). PA28γ also promotes the G1/S and G2/M transitions in proliferating cells (Murata et al., 1999; Barton et al., 2004; Rechsteiner and Hill, 2005). Transcription initiation factor IIIB, isoform 3 is part of a protein complex allowing RNA polymerase III to start transcription of genes encoding structural RNAs, including (1) transfer RNAs and the 5S subunit of ribosomal RNA required for protein synthesis, (2) the U6 small nuclear RNA involved in posttranscriptional processing, and (3) the 7SL ribosomal RNA used in translocation of new proteins from ribosomes to the ER (Geiduschek and Kassavetis, 2001). Transcriptional control of RNA polymerase III, accordingly regulates the rapid increase in new proteins needed for the cell growth on which cell proliferation is dependent. Transcriptional regulation of RNA polymerase III proves to be tightly coupled with rate of cell growth and proliferation (Mauger and Scott, 2004; Goodfellow and White, 2007).

6.5.3 Anti-Apoptosis in Neurons

Apoptosis, which is the most common form of programmed cell death, can occur in the CNS at all ages, but is a conspicuous feature of late the embryonic and fetal brain development (Blaschke et al., 1996; Kuan et al., 2000; Rakic and Zecevic, 2000). As noted earlier (see *Section 2.2.6.2.1*), the peak period for dysbindin-1 gene expression in neuronal proliferative zones of the embryonic mouse brain (G12.5 – 16.5: Kumamoto et al., 2007) falls within the peak period for programmed cell death in those germinal zones (i.e., G12–18: Blaschke et al., 1996). Dysbindin-1 may play an anti-apoptotic role during this period in prenatal development for two reasons. First, the DTNBP1 promoter preceding its first exon contains a binding site for the Sp1 transcription factor (Liao and Chen, 2004), which is induced in immature cerebrocortical neurons by apoptotic stimuli and decreases occurrence of apoptotic reactions in those cells (Ryu et al., 2003; Lee et al., 2006). Second, the cell viability of immature cerebrocortical neurons following serum deprivation, measured by mitochondrial conversion of a dimethylthiazol tetrazolium bromide (MTT), is increased (*Figure 2.2-22c*) when the cells were transfected to overexpress dysbindin-1 and decreased (*Figure 2.2-22d*) when they were transfected with DTNBP1 siRNA to knockdown dysbindin-1 (Numakawa et al., 2004). Where such positive neuroprotective results are obtained, the MTT method is effective in measuring the apoptotic effects of serum deprivation (Lobner, 2000).

Figure 2.2-22

Effect of dysbindin-1 overexpression or knockdown on neuronal proteins (a and b), cell viability (c and d), and glutamate release (e and f) in primary cultures of rat cerebrocortical neurons. Overexpression increases Akt phosphorylation and levels of SNAP-25 and synapsin 1, whereas knockdown has the opposite effect (see text *Sections 2.2.6.5.3* and *2.2.6.5.6* for discussion). The symbols 0 and + in (a) and (b) refer to wild type and expression-altered conditions. *GFP* green fluorescent protein; *HK*$^+$ high KCL condition; *HS* horse serum; *LY* the PI3K inhibitor LY294002 Adapted with from Numakawa et al. (2004)



The anti-apoptotic effect of dysbindin-1 is due in part to increased Akt signaling, which is well known for its ability to suppress apoptosis (see Manning and Cantley, 2007). As shown by Numakawa et al. (2004), overexpression of dysbindin-1 in immature cerebrocortical neurons increases phosphorylation of Akt (*Figure 2.2-22a*), whereas dysbindin-1 knockdown had the opposite effect (*Figure 2.2-22b*).

The anti-apoptotic effect was significantly reduced when the neurons were treated with a PI3K inhibitor (LY294002) able to decrease Akt phosphorylation (Numakawa et al., 2004). It is unclear how dysbindin-1 is able to activate Akt, though there is a predicted Akt binding site in the NTR of dysbindin-1 enabling a direct feedback for activated Akt (see *Figure 2.2-2* and *Table 2.2-5*).

It is also unclear what role the predicted DNA-PK site in dysbindin-1 may have in enabling or mediating its anti-apoptotic effect. As explained earlier (see *Section 2.2.3.3.1*), DNA-PK, which phosphorylates dysbindin-1 (Oyama et al., 2009), helps repair double-strand DNA breaks and suppresses apoptosis in developing and adult neurons (cf., Chechlacz et al., 2001, Culmsee et al., 2001, Vermuri et al., 2001, and Neema et al., 2005). Anti-apoptotic effects of DNA-PK on prenatal neurons (Vermuri et al., 2001) presumably have a major impact on brain development, especially given that the highest levels of DNA-PK are found in fetal tissue (Oka et al., 2000). It will thus be important to test if DNA-PK phosphorylation of nuclear dysbindin-1 in developing neurons contributes to the survival of those cells.

6.5.4 Growth of Axons, Dendrites, and Dendritic Spines

Studies on cultured SH-SY5Y neuroblastoma cells and embryonic hippocampal neurons find that dysbindin-1 is present in neurites (developing dendrites and axons) and in axonal growth cones (Kubota et al., 2009; Taneichi-Kuroda et al., 2009). Kubota et al. (2009) showed that siRNA knockdown of dysbindin-1 in the SH-SY5Y cells disrupts the organization of the actin cytoskeleton at the tips of neurites, which was associated with stunted growth. They also found cytoskeletal anomalies in growth cone layers of embryonic hippocampal neurons from homozygous sdy/DBA mice, namely the loss of actin filaments in one layer and a decrease in microtubules in another. Kubota et al. (2009) argued that this may have been due to the lower than normal levels of activated c-Jun N-terminal kinase (JNK) they discovered in sdy/DBA brains, because there is evidence that activated JNK is critical for axon formation (Oliva et al., 2006) and because siRNA knockdown of dysbindin-1 in SH-SY5Y cells can by itself reduce levels of activated JNK.

At least four known or candidate dysbindin-1 binding partners may regulate (1) growth of neuronal processes during development and (2) growth of dendritic spines during and after development. These four binding partners are pallidin, snapin, rab11A, and sec8 (see *Tables 2.2-6* and *2.2-7*).

Neurite growth can be influenced directly or indirectly by pallidin, snapin, and sec8. Pallidin-deficient fibroblasts are deficient in VAMP7 (= tetanus neurotoxin-insensitive vesicle associated membrane protein [TI-VAMP]) (Salazar et al., 2006), a vesicular (v) SNARE (*s*oluble *N*-ethylmaleimide-sensitive factor *a*ttachment protein *re*ceptor; see Kao, this volume). VAMP7 regulates growth of dendrites and axons by promoting vesicle fusion with growth cone membranes (Martinez-Arca et al., 2001; Alberts et al., 2003, 2006). Snapin can also regulate dendritic growth, a role less well known than its ability to regulate transmitter release (e.g., Thacker et al., 2004 and Tian et al., 2005; Pan et al., 2009). It reduces the number of primary dendrites grown by embryonic hippocampal neurons in culture by competing with tubulin for binding of the novel protein cypin (Chen et al., 2005), which increases the number of primary dendrites and promotes microtubule assembly in the same cells (Akum et al., 2004). Cypin also regulates interaction between postsynaptic density protein-95 (PSD-95) and sec8 (Riefler et al., 2003), which itself regulates dendritic branching and microtubule assembly (Liebl et al., 2005). Dysbindin-1 is closely associated with microtubules in neurons expressing the protein (e.g., *Figure 2.2-17c*, Talbot et al., 2006).

Dendritic spine growth can be influenced directly or indirectly by pallidin and rab11A. One of pallidin's binding partners is syntaxin-13 (Huang et al., 1999; Moriyama and Bonifacino, 2002), which is a target (t) SNARE involved in the transport of membrane cargos from early to recycling endosomes (Prekeris et al., 1998). Subsequent transport of membrane cargos from recycling endosomes to the cell membrane requires the small GTPase rab11A or rab11B (Ullrich et al., 1996; Ren et al., 1998; Lapierre et al., 2001). Both syntaxin 13 and rab11A are components of the recycling endosomes required for growth and maintenance of dendritic spines that appear under conditions promoting long-term potentiation (e.g., activation of NMDA receptors: Park et al., 2006). As noted earlier (see introduction to *Section 2.2.6.3.2.3*), there is reason to believe that dysbindin-1 may be associated with recycling endosomes in dendritic spines on

hippocampal cells. Park et al. (2006) showed that transfection of hippocampal neurons with wild-type syntaxin 13 or rab11A increases spine density, whereas transfection with inactive forms of these proteins reduces spine density markedly.

6.5.5 AP-3 Cargo Transport to LROs and Synaptic Vesicles

Key functions of dysbindin-1 may be mediated by BLOC-1, a protein assembly discovered independently by Falcón-Pérez et al. (2002) and Moriyama and Bonifacino (2002). It was the first known of at least three metazoan assemblies (see Dell'Angelica, 2004 and Di Pietro and Dell, Angelica, 2005) that contribute to the biogenesis of LROs (lysosome-related organelles) (De Pietro et al., 2006; Salazar et al., 2006; Setty et al., 2007). BLOC-1 is composed of at least seven proteins in addition to dysbindin-1: cappuccino, muted, pallidin, snapin, and the BLOC-1 subunits 1–3 (BLOS1–3) (Starcevic and Dell'Angelica, 2004). Dysbindin-1 can bind four of these proteins (muted, pallidin, snapin, and BLOS 2: see *Tables 2.2-6* and *2.2-7* and *Figure 2.2-21b*). This is striking, considering that dysbindin-1 has only one binding partner in all the other protein complexes with which it is likely to interact. Loss of dysbindin-1 in sdy mice is accompanied by loss of all BLOC-1 members studied to date (see *Section 2.2.6.4.1*). Consequently, many abnormalities in sdy mice (see *Section 2.2.6.4.2*) may result from loss of BLOC-1 functions. We consider here what those functions are.

BLOC-1 contributes to the biogenesis of LROs by promoting delivery of proteins they require for functional maturation (cf. Di Pietro et al., 2006; Salazar et al., 2006; Setty et al., 2007). The complex is found in cytosolic and membrane tissue fractions (Falcón-Pérez et al., 2002; Moriyama and Bonifacino, 2002). On the latter, BLOC-1 can bind two other protein assemblies important in trafficking proteins to LROs, specifically BLOC-2 and AP-3 (Di Pietro et al., 2006). BLOC-2, which contains proteins of genes causally associated with HPS-3, -5, and -6 (Di Pietro et al., 2004), facilitates BLOC-1 trafficking of at least one protein (tyrosinase-related protein 1 [Tryp1]) (Setty et al., 2007). But its direct interaction with BLOC-1 would be limited in vivo, because BLOC-1 and BLOC-2 facilitate trafficking of Tryp1 to LROs in melanocytes from intracellular sites that are largely separate (Setty et al., 2007). In contrast, BLOC-1 and AP-3 appear to be localized to the same sites in melanocytes (cf. Theos et al., 2005 and Di Pietro et al., 2006). Their proximity is established in neuroendocrine and neuronal cells, where BLOC-1 and AP-3 have been found on the same population of synaptophysin-containing vesicles (Salazar et al., 2005b, 2006). Synaptophysin is an integral membrane protein of SVLMs in PC-12 cells and of synaptic vesicles derived either from presynaptic membranes by AP-2 or from endosomes by AP-3 (cf. Salazar et al., 2004b, 2005b and Takamori et al., 2006).

AP-3 is one of four adaptor protein complexes, each of which forms a coat on the cytosolic surface of certain intracellular membranes for the purpose of forming a transport vesicle with selected proteins needed elsewhere in the cell (see Bonifacino and Glick, 2004, Robinson, 2004, and Newell-Litwa et al., 2007). AP complexes recognize proteins with certain sorting signals, which are tyrosine- or dileucine-based in the case of AP-3 (Bonifacino and Traub, 2003). One of those sorting signals is the motif YXXΦ found at the start of the dysbindin domain as a YLQI sequence in vertebrate orthologs of dysbindin-1 as noted earlier. Upon being recruited to what is called a donor membrane, AP complexes bind proteins with the appropriate sorting signals, collectively concentrating them within the coated area and assisting in budding the coated membrane. Now enriched in the newly selected (sorted) proteins, the bud is pinched off from the donor membrane as a transport vesicle. Upon contact with an acceptor membrane, the transport vesicle fuses and thereby delivers its cargo to a target structure such as an LRO (e.g., a melanosome) or the cell membrane (see Bonifacino and Glick, 2004). AP-3, like other AP complexes, is involved in protein trafficking along particular intracellular pathways. It specifically binds TGN and early endosomal membrane proteins that are targeted to late endosomes and/or lysosomes (Ihrke et al., 2004; Peden et al., 2004; Theos et al., 2005). There is evidence that AP-3 trafficking of proteins to lysosomes occurs in both neuronal and non-neuronal cells (see Newell-Litwa et al., 2007).

With few exceptions, the functions of BLOC-1 are inextricably linked to those of AP-3, which greatly facilitates recruitment of BLOC-1 to membranes (Di Pietro et al., 2006; Salazar et al., 2006). Studies on

fibroblasts and melanocytes show that both BLOC-1 and AP-3 facilitate trafficking of the same proteins from early endosomes to their normal targets in LROs or late endosomes (cf. Di Pietro et al., 2006, Salazar et al., 2006, and Setty et al., 2007). This has been shown for trafficking of Tryp1 and to a lesser extent tyrosinase to LROs in melanocytes (Di Pietro et al., 2006; Setty et al., 2007) and for trafficking of phosphatidylinositol-4-kinase type IIα (PI4K IIα) to late endosomes in fibroblasts (Salazar et al., 2006). In cells lacking BLOC-1 or AP-3, these proteins are redirected (i.e., mistargeted) to other intracellular compartments (e.g., lysosomes) and/or to the cell surface (Di Pietro et al., 2006; Salazar et al., 2006; Setty et al., 2007). Redirection to the cell surface in the absence of BLOC-1 or AP-3 is a common phenomenon, which has been demonstrated for LAMP-1, LAMP-3 (CD63), and Tyrp1 (Di Pietro et al., 2006, Salazar et al., 2006, and Setty et al., 2007). Apart from syntaxins 7 and 8, all proteins known to be mistargeted in BLOC-1 deficient cells (i.e., LAMP-1, -2, -3, PI4K IIα, tyrosinase, and Tryp1) have AP-3 sorting signals (cf. Bonifacino and Traub, 2003 and Craige et al., 2008).

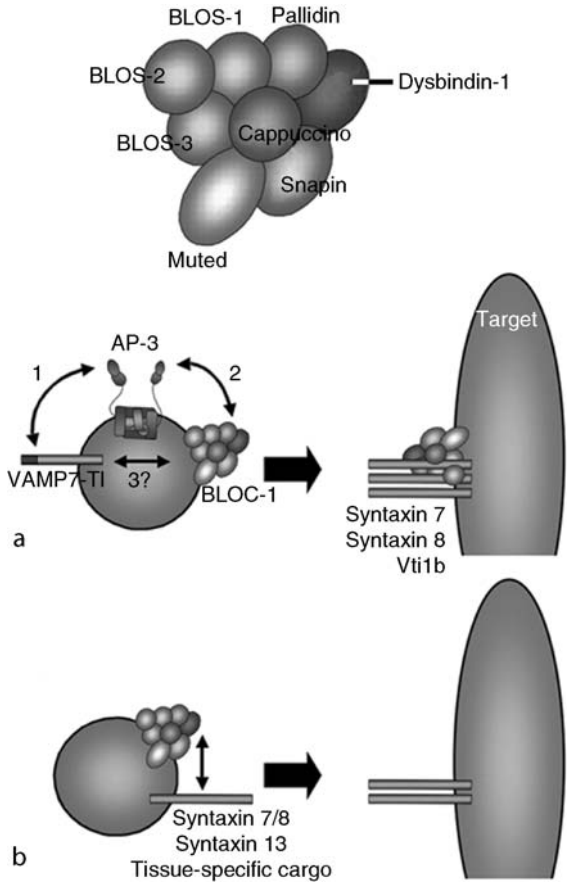
These observations indicate that BLOC-1 regulates targeting of AP-3 cargoes, as proposed by Salazar et al. (2006). The basis for such a regulatory effect is suggested by their discovery that loss of BLOC-1 or AP-3 in fibroblasts markedly reduces fibroblast levels of the vesicular v-SNARE VAMP7, which is another AP-3 cargo exported from early endosomes (Martinez-Arca et al., 2003). BLOC-1 may thus facilitate AP-3 recognition of specific cargoes like VAMP7 (*Figure 2.2-23a*), as proposed by Newell-Litwa et al. (2007). This would increase chances of the cargo being incorporated into transport vesicles and would thereby decrease chances of rapid degradation. Reduced VAMP7 in those vesicles would impair delivery of their cargoes since delivery depends on vesicle fusion with target structures via interaction of a vesicular SNARE and its cognate target t-SNARE on the acceptor membrane. Three molecules collectively serve as the cognate t-SNARE of VAMP7 in endosomes: Vti1b, syntaxin 7, and syntaxin 8 (Bogdanovic et al., 2002; Pryor et al., 2004). Co-localization of syntaxin 7 and 8 is largely lost in fibroblasts lacking BLOC-1, but not in those lacking only AP-3 (Salazar et al., 2006). Consequently, BLOC-1 can also affect protein trafficking independent of AP-3 (*Figure 2.2-23b*) by regulating the sorting of syntaxin 7 and/or 8 into transport vesicles and/or by maintaining close proximity of those syntaxins on target membranes (Salazar et al., 2006; Newell-Litwa et al., 2007).

As in peripheral tissues, BLOC-1 may regulate the recognition of AP-3 cargoes in the brain. As noted earlier (see *Section 2.2.2.2.1*), AP-3 derived synaptic vesicles are candidate LROs. These are synaptic vesicles generated by budding of early endosomes in axon terminals (Danglot and Galli, 2007 and Voglmaier and Edwards, 2007). Like AP-3 derived SVLMs in PC-12 cells, they have all the key features of LROs: an early endosomal origin, secretory capacity, and at least one lysosomal membrane protein (i.e., LAMP-1: cf. Salazar et al., 2004a, 2005b). Synaptic vesicles in the brain derived from early endosomes via AP-3 are well established (cf. Nakatsu et al., 2004 and Salazar et al., 2004a, b, 2005, 2006). As mentioned earlier, at least four BLOC-1 members (i.e., dysbindin-1, muted, pallidin, and snapin) are closely associated with AP-3 derived synaptic vesicles and SVLMs (Salazar et al., 2005b, 2006). In AP-3 deficient mice, such vesicles have reduced levels of LAMP-1, PI4K IIα, VAMP7, vesicle chloride channel 3, VGluT-1, and zinc transporter 3 (cf. Salazar et al., 2004a, b, 2005a, b, 2006). They might also have lower levels of other synaptic vesicle components since dileucine sorting signals recognized by AP-3 (as well as by AP-1 and AP-2) are found on VAMP4 and on the vesicular transporters for acetylcholine, GABA, and monoamines (Bonifacino and Traub, 2003). Consequently, BLOC-1 may regulate incorporation of many AP-3 cargoes into endosomally-derived synaptic vesicles. Considering the specific proteins involved, BLOC-1 is likely to affect the amount of transmitter stored in such vesicles and their ability to fuse with the presynaptic membrane.

Dysbindin-1 may play both peripheral and central roles in the interaction of BLOC-1 with AP-3. The peripheral role would be stabilization of BLOC-1 (see *Section 2.2.6.4.1* and *Figure 2.2-19*). The central role would be enabling the two complexes to bind. As illustrated in *Figure 2.2-6*, dysbindin-1's CCD can bind multiple BLOC-1 components (Nazarian et al., 2006), while the YXXΦ motif in its DD can bind the μ subunit of AP-3 (Taneichi-Kuroda et al., 2009), specifically the μA isoform found in both neuronal and non-neuronal cells. The binding of AP-3μ subunits to cargo with the YXXΦ motif is well known (Bonifacino and Traub, 2003). There is thus reason to suspect that dysbindin-1 acts as an adaptor for interactions of BLOC-1 with AP-3 and in that manner influence intracellular transport.

Figure 2.2-23

BLOC-1 roles in transporting cargo proteins as proposed by Newell-Litwa et al. (2007). Dysbindin-1 as part of BLOC-1 could participate in mechanisms transferring proteins from transport vesicles to target membranes. In one such mechanism, (a), BLOC-1 facilitates recognition of AP-3 cargoes such as VAMP7 either by stabilizing AP-3 interactions with the cargoes (1 and 2) or by direct interactions with the cargo (2 and 3). BLOC-1 could also affect transport at the target membrane by stabilizing a t-SNARE complex composed of syntaxin 7, syntaxin 8, and Vti1b. In a mechanism entirely separate from AP-3, (b), BLOC-1 could regulate cargo transport independently of AP-3 by selectively sorting the SNAREs syntaxin 7, 8, and/or 13 into transport vesicles. The evidence for such roles of BLOC-1 is covered in *Section 2.2.6.5.5*. Adapted from Newell-Litwa et al. (2007)



6.5.6 Synaptic Vesicle Release

Dysbindin-1 is enriched in presynaptic fields at diverse locations in the CNS as described earlier (see *Section 2.2.6.3.2.3*). Where investigated with immunoEM, the protein in those fields is localized primarily to synaptic vesicles (e.g., *Figures 2.2-16–2.2-18*, Talbot et al., 2006). That may be explained by the finding discussed above that dysbindin-1 and at least three other BLOC-1 members are peripheral membrane components of synaptic vesicles and PC-12 cell SVLMs derived from endosomes by an AP-3 mediated process (cf. Salazar et al., 2005b, 2006). These probably constitute the reserve pool of synaptic vesicles (Voglmaier and Edwards, 2007), which is estimated to include 80–90% of all synaptic vesicles in an axon terminal (Rizzoli and Betz, 2005). Loss of dysbindin-1 in homozygous sdy mice leads to a significant loss in the size of the reserve pool (Chen et al., 2008). A preferential localization of BLOC-1 in the reserve pool may

explain why it is not uncommon to find dysbindin-1 immunoreactivity in axon terminals confined largely to clusters of synaptic vesicles situated far from the presynaptic membrane (Talbot et al., in preparation). In other terminals, however, synaptic vesicles immunoreactive for the protein are located both near and far away from the presynaptic membrane (*Figures 2.2-16a*, *19a,b*). This may reflect different stimulation states of synaptic terminals at the time of death.

AP-3 derived synaptic vesicles have two v-SNAREs: VAMP2 and to a lesser degree VAMP7 (cf. Salazar et al., 2004b, 2006). VAMP2 (synaptobrevin 2) is the v-SNARE critical for synaptic vesicle fusion with the presynaptic membrane and hence for transmitter release (Lang and Jahn, 2008). VAMP7 may serve the same purpose because, in addition to forming SNARE complexes with t-SNAREs common on endosomes (i.e., Vti1, syntaxin 7 and 8), it also forms such SNARE complexes with the t-SNAREs required for fusion of synaptic vesicles with the presynaptic membrane (i.e., syntaxin 1 and SNAP25: Yang et al., 1999; Alberts et al., 2003).

A study by Numakawa et al. (2004) on cultured cerebrocortical neurons showed that dysbindin-1 can affect neurotransmitter release. Of special interest was the effect on release of glutamate evoked by 50 mM KCl depolarization for 1–3 min, which is the range of exposure times in works to which the authors refer readers for such data. Compared with control cultures, those transfected with Dtnbp1 constructs to overexpress dysbindin-1 released more glutamate, while those transfected with Dtnbp1 siRNA to knock-down dysbindin-1 released less glutamate (*Figure 2.2-22e,f*). This is probably due in part to an effect of dysbindin-1 on the reserve pool of synaptic vesicles, which are released only by stimulation strong enough to deplete recycling synaptic vesicles (Rizzoli and Betz, 2005). The stimulus used by Numakawa et al. (2004) should have had that effect given the finding of Leenders et al. (2002) that exposure of brain synaptosomes to 50 mM KCl for 15 s or 3 min depletes synaptic vesicles docked at the presynaptic membrane and induces generation of endosome-like organelles indicative of the bulk endocytosis from which synaptic vesicles of the reserve pool are produced (see Rizzoli et al., 2003).

The effect of dysbindin-1 on glutamate release from what appear to be synaptic vesicles of the reserve pool might be explained by an effect of the protein on synapsin 1. Numakawa et al. (2004) found that overexpression and knockdown of dysbindin-1 in their cerebrocortical neuron cultures respectively caused increases and decreases in the level of synapsin 1 (*Figure 2.2-22a,b*), which may interact directly with the dysbindin-1 binding partner snapin (Krapivinsky et al. 2006). Synapsin 1 and 2 control the availability of synaptic vesicles in the reserve pool for release at the active zone of the presynaptic membrane (Chi et al., 2003). Under resting conditions, synapsins bind the reserve vesicles and tether them to actin filaments in a cluster kept away from the active zone and thereby prevent them from docking with the active zone. With strong stimulation, however, synapsins dissociate from the reserve vesicles and instead promote their export (mobilization) to the readily releasable pool and thereby facilitate release of their transmitter at the active zone (Akbergenova and Bykhovskaia, 2007). As in cortical neurons transfected to knockdown dysbindin-1 (Numakawa et al., 2004), synaptosomes from mice with double knockouts of synapsin 1 and 2 show decreased glutamate release upon stimulation with a concentration of KCl (25 mM for 30 s: Lonart and Simsek-Duran, 2006).

An additional effect of dysbindin-1 on the readily releasable pool may explain why Numakawa et al (2004) found that even basal glutamate release by cerebrocortical neuronal cultures was increased when they were transfected to overexpress dysbindin-1 and decreased when transfected to knockdown the protein (*Figure 2.2-22e,f*). This is not attributable to the changes in synapsin 1 levels noted above, because synapsins do not affect either basal levels of transmitter release (see Akbergenova and Bykhovskaia, 2007) or such release in a manner opposite to that seen when dysbindin-1 is down regulated (see Lonart and Simsek-Duran, 2006). Consequently, the increased basal glutamate release observed in cerebrocortical cells overexpressing dysbindin-1 may be due to more direct effects of the protein on synaptic vesicle release, specifically on processes controlling vesicle fusion with the presynaptic membrane. Here too, Numakawa et al. (2004) provided a possible answer. They discovered that overexpression and knockdown of dysbindin-1 caused respective increases and decreases in SNAP-25 (*Figure 2.2-22a,b*), which is a critical t-SNARE for synaptic vesicle fusion with the presynaptic membrane (Lang and Jahn, 2008).

Dysbdindin-1 may also affect synaptic vesicle fusion via interaction with its known or candidate binding partners sec8, snapin, and syntabulin. Sec8, like dysbindin-1A and -1B, is associated with presynaptic membranes (Hsu et al., 1996; Vik-mo et al., 2003). It is a member of the exocyst complex

(*Figure 2.2-21c*), an assembly of eight proteins that is vital in docking (tethering) of a vesicle to the cell membrane. Dysbindin-1 can bind the exocyst direct via sec8 and indirectly via snapin, which binds the Exo70 component of the exocyst (Bao et al., 2008). Since there is some evidence that dysbindin-1 is not involved in docking (Chen et al., 2008), however, its ability to influence vesicle release via interaction with the exocyst must be regarded with caution. Docking leads to priming of the vesicle for fusion, a process enabling formation of a SNARE complex via interactions of the v-SNARE on the vesicle membrane with the t-SNAREs (i.e., SNAP-25 and syntaxin-1) on the presynaptic membrane (Becherer and Rettig, 2006). There are at least two ways dysbindin-1 could affect priming. One is via its interaction with syntabulin, which is known to be important in transporting syntaxin-1 cargo vesicles down axonal processes for transfer to the neuronal membrane (Su et al., 2004a). The other is via its interaction with snapin. During the fusion clamp stage in priming, snapin stabilizes coupling of synaptotagmin-1 to the SNARE complex via SNAP-25. This has the effect of maximizing the size of the readily releasable pool of vesicles before Ca^{2+} influx, which in turn maximizes the number of synchronously released vesicles upon Ca^{2+} influx (Pan et al., 2009).

A study by Kumamoto et al. (2006) suggests that dysbindin-1 also affects dopamine release. But unlike the case with glutamate release, which is facilitated by dysbindin-1 as described above, the effect on dopamine release was found to be inhibitory. Kumamoto et al. (2006) specifically studied PC-12 cells, which are derived from a chromaffin cell tumor of the adrenal medulla. These cells do not store glutamate for release (see Salazar et al., 2005a), but they do secrete other molecules used as neurotransmitters in the nervous system, especially dopamine (see Fornai et al., 2007). As noted earlier, dysbindin-1 and several other BLOC-1 proteins are closely associated with AP-3 derived SVLMs in PC-12 cells (Salazar et al., 2005b, 2006). As with AP-3 derived synaptic vesicles, this class of SVLMs may be a reserve pool released upon strong stimulation. Such a reserve pool has not been reported in PC-12 cells, but there is evidence for it in their cells of origin (i.e., chromaffin cells: see Rose et al., 2002). Kumamoto et al. (2006) found no change in basal dopamine release in PC-12 cultures transfected to overexpress or knockdown dysbindin-1 compared with untransfected PC-12 cultures. But after strong depolarization with 50 mM KCl, they found that PC-12 cultures transfected to knockdown dysbindin-1 released more dopamine than controls. Dysbindin-1 thus appears to suppress dopamine secretion under normal circumstances. This may explain why the dysbindin-1 loss in sdy mice is associated with increased dopamine turnover suggestive of increased dopamine release in targets of the midbrain dopamine neurons, namely the amygdala and hippocampal formation (Murotani et al., 2007). That nevertheless remains a speculative hypothesis, given that dopamine synthesis, storage, and release in PC-12 cells is quite different in dopamine-rich brain tissue (Fornai et al., 2007). Amperometric tests on PC-12 cells and dopaminergic brain tissue similar to those done on the adrenal medulla of sdy mice (Chen et al., 2008) are needed to identify the specific effects of dysbindin-1 loss on exocytosis of dopamine.

There is a potentially simple reason why dysbindin-1 exerts an inhibitory effect on dopamine release by PC-12 cells, but an excitatory effect on glutamate release by cerebrocortical neurons. Knockdown of dysbindin-1 in these two cell types leads to opposite effects on SNAP-25, which is a critical t-SNARE as noted above. Knockdown of dysbindin-1 raises SNAP-25 in PC-12 cells (Kumamoto et al., 2006), but lowers SNAP-25 in cerebrocortical neurons (Numakawa et al., 2004).

An additional factor may contribute to reduced dopamine release by PC-12 cells after dysbindin-1 knockdown. Such knockdown in other cells increases cell surface expression of dopamine type 2 receptors (D2Rs: Iizuka et al., 2007; see *Section 2.2.6.5.7*). Activation of D2Rs on PC-12 cells reduces dopamine release from such cells (Courtney et al., 1991; Pothos et al., 1998), just as activation of presynaptic D2Rs on dopaminergic axons in the striatum (Benoit-Marchand et al., 2001; Kita et al., 2007), and the neocortex (Fedele et al., 1999; Löffler et al., 2006) generally reduces dopamine release by those terminals. If the density of D2Rs on PC-12 cells does increase following dysbindin-1 knockdown as in other cells, it would potentiate an inhibitory feedback circuit activated by dopamine release from the cells. Since, D2Rs are located not only postsynaptically, but also presynaptically in certain tissues (Sesack et al., 1994; Delle Donne et al., 1997; Negyessy et al. 2005; Paspalas et al., 2006), it can also affect dopaminergic transmission in another way, by limiting the duration and spatial extent of transmission by recruiting to the plasma membrane, the dopamine transporter (Bolan et al., 2007; Lee et al., 2007), which removes dopamine from the synaptic cleft (Williams and Galli, 2006). This would counteract the effects of increased cell surface expression of D2Rs. It remains to be seen, then, the net effect of such increased expression is on dopaminergic transmission.

As detailed earlier (see Section 2.2.6.4.2.2), Chen et al. (2008) have recently shown with amperometry that loss of dysbindin-1 in sdy/DBA mice results in increased quantal size of vesicular epinephrine and/or norepinephrine in adrenal chromaffin cells, but that the total catecholamine amount released per stimulus is smaller due to decreased release probability. This suggests that dysbindin-1 normally facilitates adrenal epinephrine and/or norepinephrine release.

The studies described indicate that dysbindin-1 can affect the release of diverse neurotransmitters and neurohormones. It facilitates release of synaptic glutamate and adrenal epinephrine and/or norepinephrine, but inhibits PC-12 dopamine release. The effect dysbindin-1 has on release of other neurotransmitters or neurohormones is unknown. It should be noted, however, that one of the candidate binding partners of dysbindin-1, neurobeachin, has been found essential in evoked acetylcholine release at the neuromuscular junction (Su et al., 2004b). Dysbindin-1 is probably present in terminals of motor neurons since it is present in their cell bodies and axons (see Section 2.2.6.3.2.3.3).

6.5.7 Postsynaptic Receptor Trafficking

While axon terminals containing dysbindin-1 have rarely been seen to synapse at postsynaptic sites, which that also possess that protein, there are other synapses where the reverse is true. In all brain structures examined (i.e., hippocampal formation, neocortex, and the striatum), dysbindin-1 has been found at postsynaptic sites opposite axon terminals lacking the protein (see Section 2.2.6.3.2.3.4–2.2.6.3.2.3.6). These sites are sometimes dendritic shafts (e.g., Figure 2.2-16), but most are dendritic spines (e.g., Figure 2.2-17c–e). In such spines, dysbindin-1 is found mainly in the PSD, to a lesser degree along underlying cytoskeletal elements, and sometimes along the inner surface of the plasma membrane (e.g., Figure 2.2-17c–e). The PSDs enriched in the protein are relatively thick, suggesting that they are targets of glutamatergic terminals (Okabe, 2007). That is consistent with evidence that dendritic spines are the primary targets of glutamatergic axons in the neocortex, hippocampal formation, and striatum (cf. Smith et al., 1994 and Okabe, 2007). A subset of the dendritic spines in neocortex (Negyessy et al. 2005; Paspalas et al., 2006) and striatum (Freund et al., 1984; Smith et al., 1994) are also targets of dopaminergic axons. Whether this is also true in the hippocampal formation is unknown. We consider here evidence for postsynaptic actions of dysbindin-1 at dopaminergic and glutamatergic synapses.

Iizuka et al. (2007) discovered that dysbindin-1 affects cell membrane recycling of D2R, but not that of the dopamine D1 receptor (D1R). Upon binding to agonists at the cell surface, D1R and D2R are rapidly endocytosed in about 10 min and are then quickly returned to the cell membrane within about 15–30 min or slowly trafficked to lysosomes, where they are degraded in about 14 h (Vickery and von Zastrow, 1999). In their cell culture experiments, Iizuka et al. (2007) found that SH-SY5Y neuroblastoma cells and cerebrocortical neurons transfected with Dtnbp1 siRNA to knockdown dysbindin-1 (mainly dysbindin-1A) caused an increase in cell surface levels of D2R, but not of D1R. In SH-SY5Y cells transfected with Muted siRNA, they found the same effects on dysbindin-1A and cell surface D2R. These findings implicate BLOC-1 in regulation of D2R cell surface expression since dysbindin-1 and muted are both BLOC-1 members. The increase in cell surface D2R may reflect reduced internalization and/or increased recycling of the receptor from early (i.e., sorting) endosomes. Iizuka et al. (2007) imply that reduced internalization is more likely, because dysbindin-1 knockdown in SH-SY5Y cells appeared to block dopamine-induced internalization of D2R. But such blockage is uncertain since cell surface D2R was measured at a time point (>30 min after dopamine treatment) when the bulk of the labeled D2R would have been returned to the cell membrane. It is thus possible that reduced dysbindin-1 does not block internalization of D2R, but simply facilitates recycling of that receptor to the cell membrane. Such an effect would be another example of a BLOC-1 loss accelerating trafficking of proteins from early endosomes to the cell membrane (cf. Di Pietro et al., 2006, Salazar et al., 2006, and Setty et al., 2007). This occurs when further downstream trafficking is prevented. If that is the case, the normal role of dysbindin-1 in D2R trafficking would be a typical BLOC-1 function, namely regulating transport of a cargo (in this case D2R) from early endosomes toward lysosomes for degradation (see Section 2.2.6.5.5).

It seems probable that dysbindin-1 only affects trafficking of those D2Rs that are constitutively internalized, which refers to endocytosis in the absence of an agonist. This would explain why Iizuka

et al. (2007) found that cell surface D2R was increased after knockdown of dysbindin-1 in the absence of dopamine and that the increase was unaffected by 10 μM dopamine, which would activate both high and low affinity D2Rs (Seeman et al., 2003). It would also explain why they found no effect of dysbindin-1 on cell surface D1R. There is no appreciable constitutive internalization of D1R, yet such endocytosis is a prominent feature of D2R (Vickery and von Zastrow, 1999). Constitutive internalization of D2R relies on a process separate from the dopamine-induced internalization of D1R or D2R. It is not dependent on dynamin, which has an important role in clathrin-mediated endocytosis (Kabbani et al., 2004). This suggests separate trafficking pathways for constitutive and agonist-induced internalized of D2R. Only the latter pathway would be shared by D1R since its internalization is effectively agonist-dependent. The existence of such separate trafficking pathways probably explains why only about 11% of labeled D2R sites on dendrites in the prefrontal cortex are clathrin-coated pits (Paspalas et al., 2006) and why in the presence of dopamine there is still only limited overlap in the cell surface vessels labeled with D1R and D2R in neuroblastoma cells (Vickery and von Zastrow, 1999). The role of dysbindin-1 in trafficking of DR2 may thus be limited to constitutively internalized receptors not dependent on interaction at the cell surface with clathrin associated AP-2. That is consistent with our knowledge of BLOC-1, which only appears to interact at the surface of endosomally derived membranes with cargo-associated AP-3 (see *Section 2.2.6.5.5*). It remains to be determined whether D2R has an AP-3 sorting motif.

D2R is found on dendritic shafts, dendritic spines, and axon terminals in the prefrontal cortex (Negyessy et al., 2005; Paspalas et al., 2006) and striatum (Sesack et al., 1994; Delle Donne et al., 1997). These receptors are often close to glutamatergic synapses and help mediate dopamine modulation of glutamate transmission at those synapses (cf. Bamford et al., 2004, Tseng and O'Donnell, 2004, and Ferré et al., 2007). Dysbindin-1 may contribute to such modulation not only by regulating cell surface levels and D2R levels as described above, but also by affecting postsynaptic glutamate receptors. As noted earlier (see *Section 2.2.6.4.2.8*), pyramidal cells deep in the prefrontal cortex display reduced NMDA currents in homozygous sdy/DBA mice (Andrews et al., 2007). In such cells, dysbindin-1 is concentrated in the PSD of asymmetric synapses (Talbot et al., in preparation). NMDA receptors are embedded in the PSDs of such synapses, as is a candidate-binding partner of dysbindin-1, sec8 (Riefler et al., 2003; Sans et al., 2003). As mentioned previously (see *Section 2.2.6.5.6*), sec8 is part of the exocyst complex involved in docking of vesicles (including those carrying cargo proteins) with the cell membrane. It forms a complex with other PSD proteins and is important in delivery of newly synthesized NMDARs to cell membranes, including those of dendritic spines (Sans et al., 2003; see also Lau and Zukin, 2007). Sec8 also plays a major role in delivery of AMPA receptors (AMPARs) from dendritic-stalks into dendritic spines, where another member of the exocyst, Exo70 (a binding partner of snapin: Bao et al., 2008), is critical for insertion of the receptor into peripheral portions of the PSD (Gerges et al., 2006). Unlike NMDAR, however, AMPAR currents are not affected in the only brain area of sdy mice adequately studied to date (i.e., prefrontal cortex: Andrews et al., 2007).

Sec8 involvement in delivery of NMDARs to dendritic spines might be explained by the role of the exocyst in vesicle transfer from microtubules to the actin network beneath the cell membrane, a role applicable in both dendrites and axons (see Wang and Hsu, 2006). With that in mind, dendritic transport vesicles carrying NMDARs may be tethered by the exocyst to microtubules and thereby carried to the actin network at the base of dendritic spines. Upon reaching that location, the exocyst may regulate vesicle transfer to actin fibers for delivery of the NMDAR cargo to the PSD. Dybindin-1 might assist in that transfer given its presence along microtubules (e.g., *Figure 2.2-17c*; Talbot et al., 2006) and its potential interaction with the candidate-binding partners sec8 and MACF1 (= ACF7). The latter protein, like sec8 (Vik-Mo et al., 2003), is highly expressed in brain (Bernier et al., 2000) and is important in transient tethering of microtubules to the actin network near the cell membrane (Kodama et al., 2003). A role for dysbindin-1 in NMDAR delivery to the PSD has clear functional implications, because long-term potentiation and long-term depression are dependent on strong and modest NMDAR-mediated calcium influx (Citri and Malenka, 2008), respectively.

Postsynaptic localization of dysbindin-1 at GABAergic synapses has not been investigated to our knowledge. This might be expected to occur given the finding that elements of the dystrophin glycoprotein complex (i.e., dystrophin, β-dystroglycan, and syntrophin) are associated with $GABA_A$ receptors (Knuesel et al., 1999; Brünig et al., 2002). But dystrobrevins, which connect dysbindin-1 to the dystrophin complex, have only been co-localized with postsynaptic targets of GABAergic terminals in the cerebellum (Grady et al., 2006). Such co-localization is not evident in the cerebral cortex and hippocampus (Grady et al., 2006). We should also keep

in mind, as explained earlier (see introduction to *Section 2.2.6.5*), that it is unclear if dystrobrevins are physiological-binding partners of dysbindin-1 (Nazarian et al., 2006). Thus, whatever postsynaptic dysbindin-1 there may be in GABAergic synapses may not interact with the dystrophin glycoprotein complex.

6.5.8 Relay and Gating of Spinal Pain Transmission

A step-wise decrease in responsiveness to thermally induced tail pain occurs from wild type to heterozygote to homozygote sdy/DBA mice (Bhardwaj et al., 2009). Our Immunohistochemical mapping of dysbindin-1 in the CNS suggests an anatomical basis for this phenomenon. We find that dysbindin-1 is enriched in neurons and axon terminal fields along relay pathways of several sensory systems, especially the somatosensory and visceral sensory systems (see *Section 2.2.6.3.2.3.1*). In the former system, very high concentrations of neuronal and neuropil dysbindin-1 are found in the substantia gelatinosa of the spinal cord and in its extension into the spinal trigeminal nucleus (*Figure 2.2-13e*; see also Figure 9i in Benson et al., 2001). The substantia gelatinosa forms layer II of the spinal cord gray matter. It is composed of small neurons (68% are glutamatergic and 31% are GABAergic: see Santos et al. 2007) receiving nociceptive information from finely myelinated (Aδ) and unmyelinated (C) somatosensory afferents (Yasaka et al., 2007). These fibers cannot account for the unusually high level of dysbindin-1 neuropil in layer II, because a distinctly lower neuropil level is seen in layer I even though it also receives Aδ and C fiber input. Some of the dysbindin-1 neuropil in layer II may derive instead from neurons in the same layer. Most of the axons or axon collaterals of those neurons remain in layer II (Dubner and Bennett, 1983, pp. 396–397; Santos et al., 2007).

Most interactions among substantia gelatinosa neurons are excitatory, as are the limited outputs of the substantia gelatinosa to neurons in layer I of the spinal cord (Santos et al., 2007). That layer relays nociceptive-specific signals to the thalamus and thereby the cerebral cortex (Ralston, 2005). The relay can be inhibited by a pathway that descends from neurons near the magnocellular raphe nucleus that innervate the dorsal horn (Dubner and Bennett, 1983). The neurons responsible are GABAergic and/or glycinergic cells in the gigantocelluar portion of the medullary reticular formation that directly inhibit neurons in the substantia gelatinosa (Kato et al., 2006). Since gigantocellular neurons have high levels of dysbindin-1 as noted above, the presence of the protein in their terminals may contribute to the strong dysbindin-1 neuropil in the substantia gelatinosa. It is possible, then, that dysbindin-1 plays a role in both transmission of somatic pain signals and the gating of those signals. Since the sdy mice showed increased, not decreased pain thresholds, it should be investigated whether dysbindin-1 reductions have a greater impact on spinal relay of pain signals than on their gating by brainstem mechanisms.

6.5.9 Motor Functions?

Dysbindin-1 is enriched in neurons and axons of upper motor neurons in the cerebral cortex and lower motor neurons of the brainstem and spinal cord (see *Section 2.2.6.3.2.3*). Since lower motor neurons innervate skeletal muscle, there may be presynaptic dysbindin-1 at the neuromuscular junction. The protein is also found near the sarcolemma of skeletal muscles, though it is diminished at the neuromuscular junction (Benson et al., 2001). Skeletal muscle expresses many known or candidate-binding partners of dysbindin-1 (e.g., AKAP6, dystrobrevins, dystonin-1, myospryn, pallidin, and snapin: cf. Dalpé et al., 1999, Benson et al., 2001, 2004, McConnachie et al., 2006, Nazarian et al., 2006, and Kouloumenta et al., 2007). A function for dysbindin-1 in muscle is nevertheless unclear. It is not enriched at postsynaptic sites of neuromuscular junctions (see *Section 2.2.6.3.2.2*) and hence is unlikely to be involved in roles of its binding partner α-dystrobrevin at such junctions (e.g., controlling the density and stability of acetylcholinesterase there: Martinez-Pena y Valenzuela and Akaaboune, 2007). Nor can we deduce a motor function from the increase of sarcolemmal dysbindin-1 seen in dystrophic mice (Benson et al., 2002; Nazarian et al., 2006). The only dysbindin-1 binding partners known to be increased in muscles of such mice are those belonging to BLOC-1, loss of which does not induce muscle pathology or muscle strength (Nazarian et al., 2006). That is consistent with the absence of any indications of behavioral motor deficits in sdy mice lacking dysbindin-1 (Hattori et al., 2008; Takao et al., 2008; Cox et al., 2009).

6.5.10 Cognition

As noted in the introduction, dysbindin-1 was discovered in the course of seeking protein interactions whose disruption might help explain the cognitive deficits in patients with certain forms of muscular dystrophy. The success of that effort is indicated by the recent evidence that sdy mice lacking dysbindin-1 display deficits in learning and memory. As discussed earlier (see *Section 2.2.6.4.2.9*), homozygous sdy mice display deficits in spatial learning and memory (Jentsch et al., 2009; Takao et al., 2008; Cox et al., 2009), novel object recognition (Feng et al., 2008; Bhardwaj et al., 2009), and contextual fear conditioning (Bhardwaj et al., 2009). A role for dysbindin-1 in human cognition is suggested by an increasing number of studies finding that genetic variation in DTNBP1 among normal individuals affects diverse cognitive process, including (1) selective attention as described below, (2) general cognitive ability (Burdick et al., 2006), (3) general, verbal, and sometimes visual memory measured on the revised Wechsler Memory Scales (Hashimoto et al., 2009a, 2009b), (4) working memory, verbal declarative memory, mental processing speed, and executive function measured with multiple tests (Luciano et al., 2009), and (5) semantic verbal fluency reflected in activation levels of the anterior cingulate and superior temporal cortices in the right hemisphere (Markov et al., 2009).

Evidence for a role of dysbindin-1 in selective attention comes from a study by Fallgatter et al. (2006) on nonpsychiatric subjects carrying DTNBP1 SNPs previously associated in the same ethnic population with an increased risk of schizophrenia (Schwab et al., 2003). While connected to 21 electrodes in an array over the entire scalp, the subjects watched a computer screen and were instructed to press a key whenever the letter "O" was followed by the letter "X" (= Go stimulus) but not if it was followed by any other letter (= NoGo stimulus). This is a cued version of the continuous performance task originally used to assess selective attention, one which permits testing attentional control of motor function (Fallgatter et al., 1997; Fallgatter and Strik, 1999). Evoked response potentials (ERPs) recorded from electrodes along the midline between the hemispheres were analyzed at 284–440 ms after the Go and NoGo stimuli. In normal subjects, ERPs to NoGo stimuli are centered at more anterior levels of the brain than are ERPs to Go stimuli, a phenomenon known as NoGo-anteriorization (Fallgater et al., 1997; Fallgatter and Strik, 1999). As found in chronic schizophrenia cases (Fallgatter and Müller, 2001; Fallgatter et al., 2003), NoGo- anteriorization was found to be reduced in the nonpsychiatric subjects homozygous for either of two SNPs in DTNBP1 (P1765 and P1320) associated with schizophrenia. This was attributable to a change in location of ERPs to Go stimuli, which were larger over the prefrontal/premotor cortex in homozygotes and were clearly centered in those individuals at more anterior brain levels than in nonhomozygotes. Homozygous and nonhomozygous individuals also differed in the source location of ERPs to the Go stimuli as determined using low resolution electromagnetic tomography (LORETA). These differences occurred in the right hemisphere, specifically in the premotor cortex (i.e., Brodman area [BA] 8), primary somatosensory cortex (BA 3), fusiform gyrus (BA 37), lingual gyrus (BA 19), and hippocampus. Differences of this type indicate altered information processing in several telencephalic areas. Since ERPs to NoGo stimuli were normal in homozygous carriers of the DTNBP1 SNPs, these carriers were specifically impaired in attentional response control of prepared movements to Go stimuli, not in the inhibition of those movements. It has been found, in fact, that one of the DTNBP1 SNPs associated with this phenomenon (P1320 = rs760761) is negatively correlated with performance on another sustained attention task in a large cohort of young male adults (Stefanis et al., 2007).

Several features of dysbindin-1 already discussed may explain its ability to influence cognition. One is its enrichment in the source (and probably the axon terminals of) associational and commissural pathways in both the neocortex and hippocampal formation (see *Section 2.2.6.3.2.3.5* and *2.2.6.3.2.3.6*). These structures are important in diverse learning and memory processes (cf. Miller, 2000, Constantinidis and Procyk, 2004; Sweatt, 2004; Morris, 2007). Integrated activity of widely separated neuronal pools in the neocortex (Goldman-Rakic, 1988; Miller, 2000) and in the hippocampal formation (Lisman, 1999; Small, 2002) necessarily depends on the long axons of the associational and commissural pathways. Dysbindin-1 could affect the synaptic efficacy of these pathways, which are glutamatergic and/or aspartamatergic (see Parent, 1996, p. 873), given the evidence that it can affect (1) membrane composition of AP-3 derived synaptic vesicles in reserve pools, (2) cerebrocortical glutamate release, and (3) NMDAR currents (see *Sections 2.2.6.5.5–2.2.6.5.6*).

Dysbindin-1 may also affect cognition by regulating dopaminergic effects on glutamatergic synapses in the prefrontal cortex, hippocampal formation, and striatum. These three interconnected structures work together to mediate various cognitive functions (Atallah et al., 2004; see also Chudasama and Robbins, 2006). The excitatory effect of glutamate release in the prefrontal cortex, hippocampus, and striatum is modulated by inhibitory dopaminergic input (cf. Hsu, 1996, Sesack et al., 2003, Bamford et al., 2004, Tseng and O'Donnell, 2004, and Ferré et al., 2007). This input derives from the substantia nigra or the adjacent ventral tegmental area. Dysbindin-1 could regulate such modulation postsynaptically by increasing surface levels of D2R (Iizuka et al., 2007) and enhancing NMDAR currents (Andrews et al., 2007; see *Section 2.2.6.4.2.8* and *2.2.6.5.7*).

Dopaminergic modulation by dysbindin-1 could also be exerted via presynaptic D2R autoreceptors, because dopamine release by cells having such autoreceptors (i.e., PC-12 cells) is altered when their dysbindin-1 stores are reduced (Kumamoto et al., 2006; see *Section 2.2.6.5.6*). We do not expect such a presynaptic effect in the dopaminergic axons of the substantia nigra innervating the striatum, because we cannot detect dysbindin-1 in striatal axon terminals possessing the morphology of dopaminergic synapses. But dysbindin-1 may be present in dopaminergic axons of the ventral tegmental area, which innervates the prefrontal cortex and limbic structures (e.g., the amygdala, hippocampus, and nucleus accumbens: see *Section 2.2.6.3.2.3.4*).

We should note finally that dysbindin-1 may affect cognition indirectly via effects on neuronal development, especially axonal and dendritic development (see *Section 2.2.6.5.4*). Even in adulthood, however, it is possible that the protein affects dendritic spine growth associated with long-term potentiation via its affect on NMDAR (Andrews et al., 2007; see *Sections 2.2.6.4.2.8* and *2.2.6.5.7*).

6.6 Involvement in Psychiatric Disorders

Many studies have found that genetic variation in DTNBP1 is associated with psychiatric conditions. This was first reported for schizophrenia as discussed below. Support for association with bipolar disorder (see Serretti and Mandelli, 2008) comes from four reports (Fallin et al., 2005; Breen et al., 2006; Joo et al., 2007; Pae et al., 2007b), though they are not supported by two others (Raybould et al., 2005; Perlis et al., 2008). While no association with major depression was found by Zil et al. (2004), this has been found by Kim et al. (2008), and there is some evidence of an association between genetic variation in DTNBP1 and the antidepressant effectiveness of selective serotonin reuptake inhibitors (Pae et al., 2007a). In the one brain area studied (i.e., DGiml), we found a trend toward lower than normal dysbindin-1 in major depression ($p = 0.08$, Talbot et al., 2004). The same study also found dysbindin-1 levels in that brain area are normal in bipolar disorder, but highly abnormal in schizophrenia (see *Section 2.2.6.6.2*).

A clinical feature sometimes shared by schizophrenia, bipolar disorder, and major depression is psychosis (i.e., a mental state characterized by loss of contact with reality). It is possible that the genetic variation of DTNBP1 reportedly associated with those disorders is associated with psychosis independent of the disorder in which it appears (i.e., psychosis defined as a distinct neurobiological syndrome: see Fujii and Ahmed, 2004). This proposal is consistent with the evidence that genetic variation in DTNBP1 is associated with psychosis in general (Kohn et al., 2004), with prolonged psychosis after methamphetamine abuse (Kishimoto et al., 2008), with bipolar 1 cases in which mood disturbance often has psychotic features (Raybould et al., 2005), and with measures of premorbid adjustment in cases of childhood-onset psychosis not meeting the full criteria for schizophrenia (Gornick et al., 2005). Further investigation is needed, however, to establish a specific connection between dysbindin-1 and psychosis in phenotypes other than schizophrenia.

We focus only on schizophrenia below, because it is the only psychiatric disorder in which dysbindin-1 has been studied to date at many levels of analysis (i.e., genetics, mRNA and protein expression, clinical symptoms, and cognition).

6.6.1 Association of DTNBP1 Variants with Schizophrenia

In order to facilitate detection of genetic factors linked to schizophrenia, Kenneth Kendler and Dermot Walsh recruited members of families in Ireland with a history of the disorder (Kendler et al., 1996). The field

work on this project, known as the Irish Study of High-Density Schizophrenia Families was completed by 1992. Of the 1,770 individuals in the study, 1,480 in 265 families served as a linkage sample, so called because its members supplied both the DNA and adequate clinical records needed for genetic linkage analyses. Such analyses by Straub et al. (1995) soon showed linkage of chromosome region 6p24–22 with schizophrenia. Fine mapping of this linkage region in the Irish sample by Straub et al. (2002) yielded strong, family based evidence for association of multiple SNPs in (and three-SNP haplotypes of) DTNBP1 with schizophrenia. Subsequent analysis of the same genotyped data set led to the report of a single risk haplotype consisting of eight SNPs in a 30.1 kb block of DTNBP1 covering introns 1–4 (van den Oord et al., 2003).

By mid 2009, there were 31 published studies testing association of DTNBP1 variants with schizophrenia in samples on four continents (*Table 2.2-14*). Eighteen of those studies reported significant association in one or more sample. They found relatively common SNPs and/or combinations of them (haplotypes) in DTNBP1 significantly associated with schizophrenia. Most of these variants were reported to confer increased risk of schizophrenia, but five studies also reported haplotypes conferring protection against (i.e., reduced risk for) the disorder (Kirov et al., 2004; Williams et al., 2004; Vilella et al., 2008; Hashimoto et al., 2009a; Pae et al., 2009). Except for two SNPs in the first promoter region (Numakawa et al., 2004; Williams et al., 2004), all SNPs in DTNBP1 thus far associated with schizophrenia are in introns (cf. Straub et al., 2002, Riley and Kendler, 2006, and Duan et al., 2007). No schizophrenia cases have been found to harbor deletion or insertion mutations in promoters or exons of DTNBP1 (Liao and Chen, 2004; Williams et al., 2004; Duan et al., 2007).

Despite the many positive association studies, doubts have been raised by some groups about DTNBP1 as a susceptibility gene for schizophrenia. There are three major reasons given for these doubts. First, the positive studies often differ in the specific SNPs or haplotypes found to be associated with the disorder (see *Table 2.2-14*). Second, there have been 12 follow-up studies that failed to find an association of any SNPs in DTNBP1 with schizophrenia (see *Table 2.2-14*). The largest of these negative studies are those of Sanders et al. (2008) on 1870 Caucasian cases of European ancestry and Sullivan et al. (2008) on 738 cases of European, African, or other ancestry. These studies found no association between schizophrenia and any of the 15 DTNBP1 SNPs associated with the disorder in one or more of the earlier studies. Third, no association has been found between a wide variety of SNPs in DTNBP1 and premorbid adjustment in adult-onset schizophrenia (Schirmbeck et al., 2008).

In our opinion, the controversy raised by these negative studies is more apparent than real. It is clear that schizophrenia is the product of multiple genetic and environmental factors, as well as of interactions among and between those factors (see Tsuang et al., 2001, Sullivan et al., 2003, Riley and Kendler, 2006). Judging from studies done to date, any one gene probably makes only a small contribution to risk of schizophrenia at the population level and perhaps even in relatively small sets of collected families (see Craddock et al., 2008). Moreover, the actual size of the contribution by a single gene probably varies across individuals and families due to differing genetic backgrounds, epigenetics, and environmental factors. In the light of such obstacles in detecting a single-gene association signal, it seems highly improbable that false positive results alone account for the 18 studies reporting significant association between DTNBP1 variants and schizophrenia. It is entirely possible that those studies failing to find such an association did so because their samples included a higher proportion of cases in which the genetic background, epigenetic factors, and/or environmental influences masked the contribution of DTNBP1 to risk of schizophrenia.

The negative studies noted in *Table 2.2-14* are thus insufficient to challenge a growing consensus that DTNBP1 is a susceptibility gene for schizophrenia in at least some families in some populations. This consensus is supported by recent survey analyses of the case-control studies. It is true that the first meta-analysis on the topic (Li et al., 2007) did reach a negative conclusion, but it is of limited use since it excluded some of the most positive studies. A more inclusive survey of a different kind found that out of the 75 top candidates among susceptibility genes for schizophrenia, DTNBP1 had a combined odds ratio (1.36) of association with the disorder across studies, achieving the highest significance level of all the candidates genes (i.e., $p = 3.8 \times 10^{-10}$: Sun et al. 2008). A more narrowly focused meta-analysis discovered that one SNP in intron 4 of DTNBP1 (rs1011313 = P1325) was not only significantly associated with schizophrenia in Caucasians ($p < 0.003$), but was one of only four SNPs in the human genome showing a strong degree of

Table 2.2-14

DTNBP1 genetic variants associated with schizophrenia across studies

Sample Studied	Association Found?	SNPs Studied[a, b] (5′ → 3′ End of DTNBP1)																			
		rs2619538 (A/T)	rs909706 (A/G)	rs1474605 (A/G)	rs1018381 (C/T)	rs2619522 (T/G)	rs760761 (C/T)	rs2005976 (G/A)	rs2619528 (G/A)	rs1011313 (G/A)	rs3213207 (A/G)	rs2619539 (C/G)	rs760666 (C/T)	rs742105 (G/A)	rs7758659 (G/A)	rs6926401 (A/C)	rs1040410 (C/T)	rs875462 (A/G)	rs742106 (C/T)	rs1047631 (A/G)
		SNP A	P1583	P1792	P1578	P1763	P1320	P1757	P1765	P1325	P1635	P1655	P1287	P1333					P1328	P3230
Asia																				
(1) 233 Han Chinese trios (Tang et al., 2003)	Yes		A		C	T						G							T	
(2) 638 Han Chinese nuclear families (Li et al., 2005)	Yes		–			–	–	G	**G**	–	**A**	–								
(3) 693 Taiwanese nuclear families with at least wo affected siblings (Liu et al., 2007)	Yes		–		–	–		G	–	**A**		–							–	
(4) 670 Japanese cases vs. 588 controls (Numakawa et al., 2004)	Yes	**A**				**G**	**T**			G	**G**	–								
(5) 314 Japanese cases vs. 314 controls (Tochigi et al., 2006)	Yes		G		C	T	–			–	–	C		A						
(6) 194 Korean cases vs. 351 controls (Joo et al., 2006)	No	–				–	–				–	–								
(7) 908 Korean cases vs. 601 controls (Pae et al., 2009)	Yes					A/C	C/T			C	A									
Australia																				
(8) 336 cases vs. 172 controls[c] (Peters et al., 2008)	No	–	–		–	–			–	–	–	–					–	–	–	–
Ireland + UK																				
(9) 270 Irish high-density pedigrees (Straub et al., 2002)	Yes		–		–	**G**	**T**	**A**	**A**	**A**	**G**	**C**	–	**A**					–	
(10) 268 Irish high-density pedigrees (van den Oord et al., 2003)	Yes		–	**G**	C	**G**	**T**	**A**	**A**	G	**G**	–	–	–					–	
(11) 219 Irish cases vs. 231 controls (Morris et al., 2003)	No[d]						–	–			–		–							–

continued

■ Table 2.2-14 (continued)

Sample Studied	Association Found?	SNPs Studied[a, b] (5′ → 3′ End of DTNBP1)																			
		rs2619538 (A/T)	rs909706 (A/G)	rs1474605 (A/G)	rs1018381 (C/T)	rs2619522 (T/G)	rs760761 (C/T)	rs2005976 (G/A)	rs2619528 (G/A)	rs1011313 (G/A)	rs3213207 (A/G)	rs2619539 (C/G)	rs760666 (C/T)	rs742105 (G/A)	rs7758659 (G/A)	rs6926401 (A/C)	rs1040410 (C/T)	rs875462 (A/G)	rs742106 (C/T)	rs1047631 (A/G)
		SNP A	P1583	P1792	P1578	P1763	P1320	P1757	P1765	P1325	P1635	P1655	P1287	P1333					P1328	P3230
(12) 708 Irish and UK cases vs. 711 controls (Williams et al., 2004)	Yes	T/T						–		–	A/G	C/G								–
(13) 219 Irish cases vs. 231 controls (Williams et al., 2004)	Yes	T/T						–		–	A/G	C/G								–
(14) 580 Scottish cases vs. 620 controls (Li et al., 2005)	Yes	–	–		–	–	C	A	–	–	–	–	–							
(15) 450 Irish and UK cases vs. 450 controls (Datta et al., 2007)	No	–				–	–	–		–	–	–								
Other European Countries																				
(16) 488 Bulgarian trios (Kirov et al. 2004)	Yes	–	–					**G**/A		G	**A**/G	G								
(17) 273 Dutch cases vs. 580 controls (Bakker et al. 2007)	No	–							–		–	–								
(18) 441 Finnish nuclear families (Turunen et al., 2007)	No		–			–			–	–	–	–	–	–			–	–	–	–
(19) 125 German triads and 78 sibling pairs (Schwab et al., 2003)	Yes				–	**T**	**C**		**G**	**G**	**A**									
(20) 418 German cases vs. 285 controls (Van den Bogaert et al., 2003)	No				–		–	–		–	–									
(21) 294 Polish cases vs. 113 controls (Van den Bogaert et al., 2003)	No				–		–	–		–	–									
(22) 589 Spanish case vs. 405 controls (Vilella et al., 2008)	Yes					–					**A**	–	–			A/A	**T/C**	A/G		
(23) 142 Swedish cases vs. 272 controls (Van den Bogaert et al., 2003)	Yes				**T**	–	T	**A**		G	A									

North America																								
(24) 215 African-American cases vs. 74 controls; 51 Hispanic cases vs. 32 controls; 258 Caucasian cases vs. 467 controls (Funke et al., 2004)	Yes		G			**T**		**G**		T			**A**		G									
(25) 210 U.S. triads and 233 Afrikaner families (Hall et al., 2004)	No							–					–				–					–		
(26) 274 Ashkenazi Jewish trios from 263 families (Fallin et al., 2005)	Yes	*				*		*		*			*		*		*		*			*		*
(27) 136 Caucasian and African-Amer. nuclear families containing 273 schizophrenia patients (Duan et al., 2007)	Yes	–	–					–						–	A	–			**G**				–	
(28) 311 Caucasians with schizophrenia vs. 291 controls (Wood et al., 2007)	No		–			–				–			–	–	–	–								
(29) 117 Canadian nuclear families (106 of Caucasian ancestry) (De Luca et al. (2005)	No		–																				–	–
(30) 738 U.S. cases of African, European, or other ancestry and 733 controls (Sullivan et al., 2008)	No	–	–	–		–		–		–			–	–	–	–			–	–	–	–	–	–
(31) 1870 Caucasians of European ancestry vs. 2002 controls (Sanders et al., 2008)	No	–	–	–		–		–		–			–	–	–	–			–	–	–	–	–	–

[a]rs ID numbers are from the NCBI SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). Letters in parentheses specify major/minor alleles on the minus strand. A = adenine, C = cytosine, G = guanine, and T = thymine. The alias ID listed below some of the rs IDs are those of Straub et al. (2002) or Williams et al. (2004). SNPs are listed from left to right in their order from the 5′ to 3′ end of DTNBP1. Only SNPs genotyped by two independent studies or shown to be associated with schizophrenia in at least one study are included in this table.

[b]The findings summarized in this table are listed using the following code:

A, C, T, or G = SNP by itself significantly associated with schizophrenia

A, C, T, or G = SNP by itself not signifcantly associated with schizophrenia, but is part of a risk haplotype for the disorder

A, C, T, or G = SNP signficiantly associated with schizophrenia by itself and as part of a risk haplotype for the disorder

A, C, T, or G = SNP significantly associated with schizophrenia as a part of a protective haplotype

– = SNP was genotyped but was not found significantly associated with schizophrenia by itself or as part of a haplotype

* = SNP was genotyped but no allele or haplotype information is given

[c]The cases and controls in Peters et al. (2008) were of Anglo-Irish descent.

[d]While the findings were negative, the same population yielded positive results when more SNPs were tested (see Williams et al., 2004).

epidemiological credibility (Allen et al., 2008). Though these analyses were conducted before publication of the latest negative studies, their inclusion still results in a significant ($p < 0.05$) association of rs1011313 with the disorder (see http://www.schizophreniaforum.org/res/sczgene/meta.asp?geneID = 1). The updated effect size is smaller (odds ratio = 1.12) but is comparable to that of nearly all known SNPs associated with schizophrenia (see website just cited) and with other diseases such as type 2 diabetes (see Craddock et al., 2008).

Two other findings also support the view that the DTNBP1 gene is involved in schizophrenia. One is the observation of Zuo et al. (2009) that the effectiveness of typical and atypical antipsychotics in schizophrenia patients is related to their specific DTNBP1 genotypes. The other finding of note here is the discovery of Morris et al. (2008) that risk for the disorder is increased by an epistatic interaction of DTNBP1 with another gene independently shown to be a risk factor for schizophrenia, namely MUTED (Straub et al., 2005), which is the gene encoding the dysbindin-1 binding partner muted (see *Table 2.2-6*). This raises the possibility that the contribution of DTNBP1 variants to schizophrenia risk may depend on the existence of interacting variants of other susceptibility genes, the frequency of which may vary across populations and across samples from the same population. The importance of genetic interactions affecting risk posed by DTNBP1 variants is further indicated by the previously mentioned finding of Bray et al. (2008) that DTNBP1 expression is regulated by trans-acting control sequences located in 8p22–12. This locus contains several other genes associated with schizophrenia, among them neuregulin (Bray et al., 2008), which is itself a major susceptibility gene for the disorder (Lawrie et al., 2008; see also Chen, this volume).

While differences in epistatic interactions with other genes (and with environmental variables) may help explain why some studies are positive while others are negative, the question remains why the positive studies find different risk haplotypes. The most basic answer lies in the extreme difficulty of replicating any study in psychiatric genetics due to often uncontrolled (and uncontrollable) confounding variables such as unknown genetic differences across samples, diagnostic criteria used in selecting subjects (i.e., ascertainment criteria), allelic and phenotypic heterogeneity of the subjects selected, genotyping error, and recruitment of sufficient subjects to detect small magnitude associations (see Schork et al., 2007 and Craddock et al., 2008). We will focus on the related issues of population differences and allelic heterogeneity since an understanding of those issues clarifies the lack of consistency in studies on the association of DTNBP1 with schizophrenia.

Genetic differences across populations present obstacles in replicating any association study in psychiatric genetics. In an attempt to observe such differences in evaluating evidence for association of DTNBP1 variants with schizophrenia, Mutsuddi et al. (2006) built a composite haplotype map for Utah residents with northern or western European ancestry (i.e., the CEU/CEPH sample of the International HapMap Project) using HapMap genotyping data on all SNPs in DTNBP1 that had been associated with the disorder. They found that the six haplotypes in the 5' region of DTNBP1 reported by studies on European samples (see *Table 2.2-15*) were present in the CEU sample at roughly the same frequencies. They thus proposed that the European-derived samples used in the positive association studies were unlikely to produce different risk haplotypes (i.e., that the samples were genetically similar enough that population differences were unlikely to cause "conflicting results"). Upon closer inspection of the data used by Mutsuddi et al. (2006), however, it is not clear that such strong similarity across samples can be inferred from haplotype similarity based on common SNPs. Although DTNBP1 haplotype structures among both cases and controls are fairly similar among the European (and even non-European) based samples, it is evident that the frequencies of the different haplotypes in those populations are often quite different (see *Table 2.2-15*). Lesser, but still notable differences are seen even across populations with European ancestry (e.g., Irish vs. the CEU population from Utah: see *Table 2.2-15*). Consequently, genetic differences across samples cannot be excluded as a source of differences in risk haplotypes across studies. The very fact that different DTNBP1 risk haplotypes have been reported in different samples is evidence that those samples (but perhaps not their source populations) are genetically different with respect to their most detectable risk haplotypes.

We are thus led to consider allelic heterogeneity, the existence of different susceptibility variants in the same gene. This is a primary reason for what are often termed "inconsistent" findings in psychiatric

Table 2.2-15

Haplotpes in 5′ region of DTNBP1 across populations[a]

	Haplotype frequencies[c]				SNPs in haplotypes[d]							
					rs1474605	rs1018381	rs2619522	rs760761	rs2005976	rs2619528	rs1011313	rs3213207
Haplotype[b]	Ireland[e] (%)	Utah[f] (%)	Nigeria[f] (%)	Asia[f] (%)	P1792	P1578	P1763	P1320	P1757	P1765	P1325	P1635
1	73.00	75.00	63.33	70.55	A	C	T	C	G	G	G	A
2	5.80	10.83	5.83	0.56	G	C	G	T	A	A	G	G
3	7.10	5.83	–	21.11	A	C	T	C	G	G	A	A
4	1.00	–	–	–	A	C	T	T	G	G	G	A
5	6.00	7.50	30.83	7.2	G	T	G	T	A	A	G	A
6	1.50	0.83	–	–	G	C	T	T	G	G	A	G
Other haplotypes	5.30	0.01	0.01	0.58								

[a]Genotype data were obtained from http://www.hapmap.org/. The haplotypes were constructed using the Haploview program

[b]Haplotypes numbered as identified in Irish families with a high density of schizophrenia cases (van den Oord et al., 2003; Riley and Kendler, 2006). Haplotype 1 may represent a schizophrenia risk haplotype identified by Schwab et al. (2003), Tang et al. (2003), Williams et al. (2004), Li et al. (2005) and Tochigi et al. (2006). Haplotype 2 may represent a schizophrenia haplotype identified by van den Oord et al. (2003), Kirov et al. (2004), Numakawa et al. (2004), and Li et al. (2005). Finally, haplotype 5 may represent a schizophrenia risk haplotype identified by Van Den Bogaert et al. (2003) and Funke et al. (2004)

[c]Defined as the percentage of all individuals (normal and psychiatric) carrying a specific DTNBP1 haplotype in a given population

[d]SNPs are identified by their NCBI rs numbers and by their aliases (e.g., P1792) given by Straub et al. (2002)

[e]Frequencies are those reported by van den Oord et al. (2003)

[f]Frequencies listed are based on data of the International Hapmap Project (http://www.hapmap.org/index.html.en) for Utah residents of northern and western European ancestry (i.e., the CEU population), Yorubans of Ibadan, Nigeria (the YRI population), and the combined Asian groups of Han Chinese in Beijing (the CHB population) and Japanese in Tokyo (the JPT population). Dashes indicate apparent absence of a specific haplotype in a given population

genetics. Like most, if not all, susceptibility genes, there may well be multiple undetected susceptibility variants in DTNBP1 that are tagged in variable ways across studies. We should expect, then, that studies may differ in the sets of variants they detect depending on the nature of the sample studied, which depends on which population is sampled and the ascertainment method used to select cases (Riley et al., 2006). The actual susceptibility variants in DTNBP1 may not even be the SNPs which are currently associated with schizophrenia individually or as part of a haplotype (see Williams et al., 2005). Those SNPs may simply tag the susceptibility variants. Among the unidentified susceptibility variants may be SNPs in cis-acting control sequences in or near the gene that regulate its transcription (Bray et al., 2008; see *Section 2.2.6.1.3*). Indeed, Bray et al. (2005) discovered that a DTNBP1 haplotype conferring increased risk of schizophrenia tags one or more cis-acting sequences reducing DTNBP1 gene expression in the dorsolateral prefrontal cortex of the human brain (Bray et al., 2005). Altered levels of dysbindin-1 gene and protein expression have been found in that brain area of schizophrenia cases (Weickert et al., 2004; Tang et al., 2007) as addressed next.

6.6.2 Gene and Protein Expression of Dysbindin-1 in Schizophrenia

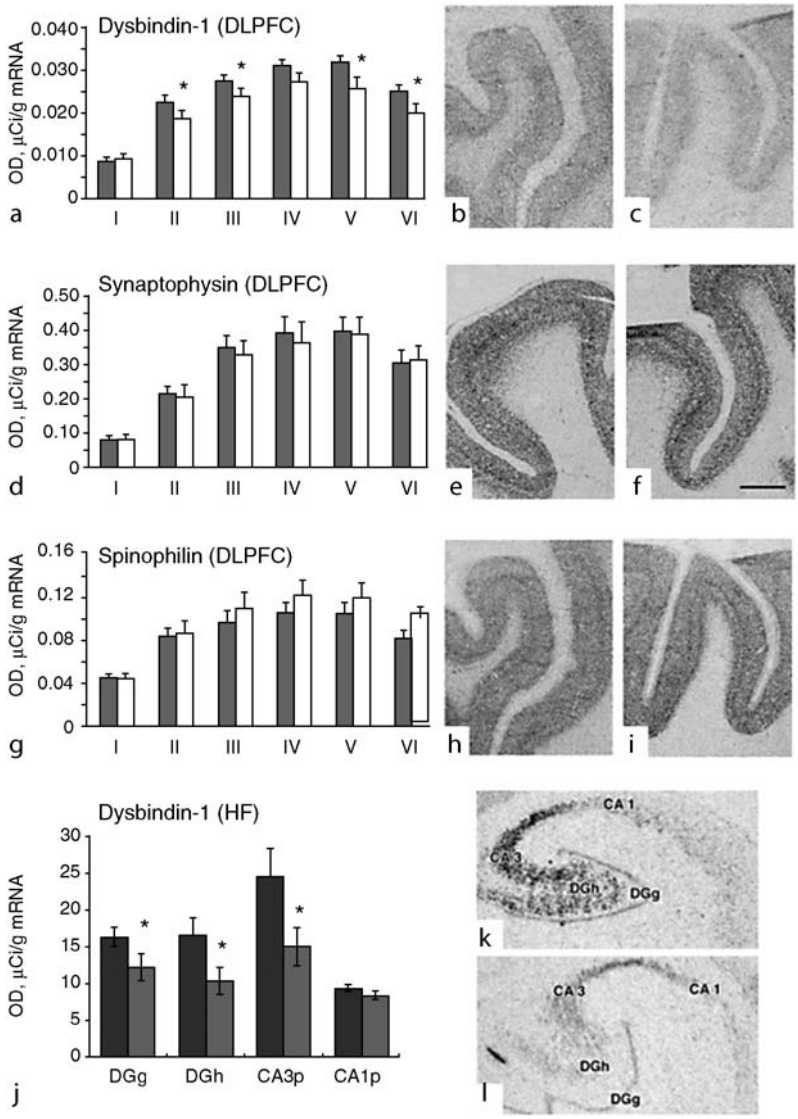
While schizophrenia-associated SNPs in DTNBP1 have not been demonstrated to affect transcription of the gene, it has been reported that altered DTNBP1 gene expression does occur in schizophrenia, specifically in immortalized lymphocytes, the dorsolateral prefrontal cortex (DLPFC), and the hippocampal formation. In immortalized lymphocytes of schizophrenia cases, Chagnon et al. (2008) found a mean 28% reduction in DTNBP1 transcript *a* not significantly affected by the atypical neuroleptic olanzapine. Similar changes in the DLPFC and the hippocampal formation attracted special interest, because those brain areas are among the most commonly affected in schizophrenia (Harrison and Lewis, 2003; cf. also Harrison, 2004, and Lewis and González-Burgos, 2008). Using in situ hybridization with a riboprobe recognizing the *a*, *b*, and *c* transcripts (see *Section 2.2.6.2.1*), Weickert and her colleagues found that DTNBP1 gene expression in schizophrenia cases was 15–20% lower in all DLPFC cell layers except layer IV (*Figure 2.2-24a–c*, Weickert et al., 2004) and 20–40% lower in all the hippocampal formation cell layers except the pyramidal layer of CA1 (*Figure 2.2-24j–l*, Weickert et al., 2008).

In a larger set of schizophrenia cases and matched controls, however, we could not confirm the findings of Weickert et al. (2004) using quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) with primer sets permitting quantification of the three major DTNBP1 transcripts (Tang et al., 2009). Levels of dysbindin-1A and -1B mRNA were actually elevated in the schizophrenia cases, while levels of dysbindin-1C mRNA were normal. It is unclear if the discrepant findings between the two studies are due to the different methods used and/or the different genetic background of the samples studied (i.e., primarily African-American in Weickert et al., 2004 and entirely American Caucasian in Tang et al., 2009).

More consistent results have been found with respect to dysbindin-1 protein expression in schizophrenia, which is significantly reduced in the DLPFC (Straub et al., 2004; Tang et al., 2009), auditory cortices (Talbot et al., submitted), and hippocampal formation (Talbot et al., 2004). This does not reflect global down-regulation of the protein, because normal levels are maintained in the anterior cingulate cortex (Talbot et al., 2004). As assessed with immunoblotting to date, the dysbindin-1 reductions in schizophrenia are isoform specific. In whole tissue lysates of the DLPFC, 71% of the case-control pairs showed reductions limited to dysbindin-1C with a mean decrease of 60% (Tang et al., 2009). In synaptosomal fractions of auditory cortices, 92% of our case control pairs showed reductions limited to dysbindin-1A with a mean decrease of 48% (Talbot et al., submitted). In synaptosomal fractions of the hippocampal formation, 67% and 80% of our case-control pairs showed reductions limited to dysbindin-1B and -1C, respectively, with mean decreases of 33% in the former isoform and 35% in the latter (Talbot et al., submitted). Neuroleptic medication taken by schizophrenia cases is unlikely to account for these reductions, because chronic haldoperidol administration in rodents does not affect dysbindin-1 immunoreactivity in such brain areas (Straub et al., 2004; Talbot et al., 2004). Where tested to date (i.e., the DLPFC), the dysbindin-1 reductions are not due to decreased DTNBP1 gene expression (Tang et al., 2009) and are thus likely to reflect posttranslational modifications promoting degradation (e.g., ubiquitination by TRIM32).

Figure 2.2-24

Reduced dysbindin-1 gene expression in dorsolateral prefrontal cortex (DLPFC) and hippocampal formation (HF) of schizophrenia cases studied with in situ hybridization. Asterisks in histograms indicate a significant difference between controls (darker bars) and schizophrenia cases (lighter bars). DLPFC reductions in dysbindin-1 (a) were found in all cell layers in the absence of any changes in the presynaptic marker synaptophysin (d) or the postsynaptic marker spinophilin A (g). The reductions found in the HF occurred in all areas except CA1. Graphs are accompanied by representative sections from normal (b, e, h, and k) and schizophrenia (c, f, i, and l) cases. Adapted from Weickert et al. (2004) for the DLPFC and Weickert et al. (2008) for the HF



As noted earlier (see *Section 2.2.6.3.1*), dysbindin-1B in human synapses is almost exclusively associated with synaptic vesicles. Consequently, our recent finding that dysbindin-1B is reduced in the hippocampal formation of schizophrenia cases confirms our previous quantitative immunohistochemical finding that reduced dysbindin-1 occurs in glutamatergic presynaptic fields of associational and

commissural pathways in that brain area of two schizophrenia cohorts (Talbot et al., 2004; see *Figures 2.2-25a* and *2.2-25b*). We specified earlier the origin and termination of these pathways and summarized the evidence for dysbindin-1 in their axon terminals (see *Section 2.2.6.3.2.3.5* and *Figures 2.2-14b*, *2.2-18*, and *2.2-25g*). Compared with matched controls, 73–93% of the schizophrenia cases in the immunohistochemical study showed 18–42% reductions in presynaptic dysbindin-1 depending on the case population studied and the hippocampal formation area examined. The reductions were largest and most consistent in the DGiml and in strata oriens and radiatum of CA3. Smaller reductions occurred in the neuropil of CA1 and the subiculum. These reductions may be due in part to decreased DTNBP1 gene expression since schizophrenia cases show such decrements in hippocampal formation cell layers that innervate DGiml and the neuropil of CA1–3 and the subiculum (Weickert et al., 2008).

The apparent reduction in presynaptic dysbindin-1 in the DLPFC and hippocampal formation of schizophrenia cases occurs without evidence of any synaptic degeneration. The reductions were not accompanied by decreased expression of the presynaptic markers synaptophysin and synapsin 1 or of the postsynaptic marker spinophilin (*Figure 2.2-24d–i*). Where tested, the reductions are accompanied instead by altered levels of VGluT-1. Talbot et al. (2004) reported that decreased dysbindin-1 was correlated with increased VGluT-1 in DGiml of schizophrenia cases. Subsequent reports on other schizophrenia populations, however, found that VGluT-1 gene expression in neurons innervating the DGiml was reduced (Eastwood et al., 2005) and that VGluT-1 immunoreactivity was reduced in DGiml when another antibody was used (Sawada et al., 2005). It is unclear why there are discordant VGluT-1 results across cohorts.

The cause of altered dysbindin-1 gene and protein expression found in schizophrenia is probably not limited to the DTNBP1 haplotypes reported to be associated with the disorder. This is indicated by the fact that abnormalities in gene and protein expression were detected even in relatively small samples of schizophrenia cases (N = 15–38 cases). Those cases were not chosen for the presence of any genetic risk factors and are unlikely to include more than a few individuals with high risk DTNBP1 haplotypes, which are usually found in no more than 18% of schizophrenia cases (e.g., cf. Straub et al., 2002, van den Oord et al., 2003, Van Den Bogaert et al., 2003, Funke et al., 2004, Kirov et al., 2004, and Tosato et al., 2007). Indeed, only two of the schizophrenia cases in the study by Talbot et al. (2004) were later found to be carriers of a high-risk DTNBP1 haplotype. Other genetic, epigenetic, and/or nongenetic factors must accordingly account for the dysbindin-1 gene and protein expression changes in schizophrenia.

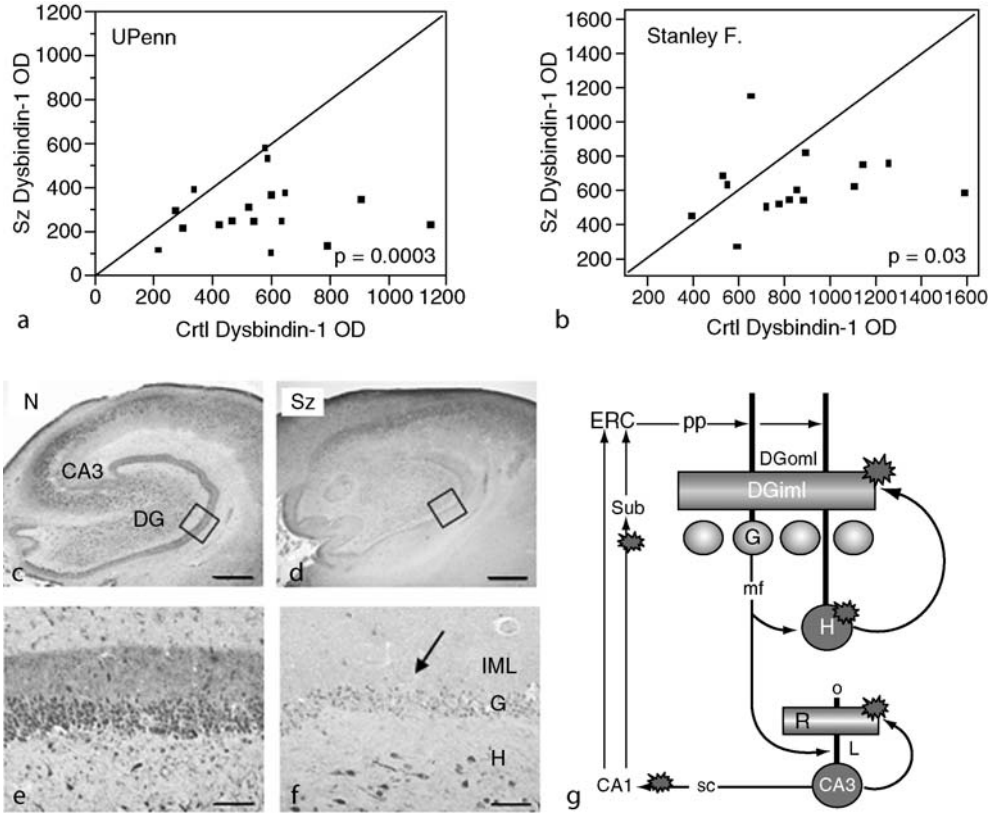
6.6.3 Potential Contributions of Altered Dysbindin-1 Expression to Neurobiological and Clinical Features of Schizophrenia

Dysbindin-1 reductions in the DLPC and hippocampal formation of schizophrenia cases could contribute to many of their neurobiological and clinical abnormalities. We consider here what those contributions may be during and after development using what is known about core abnormalities in schizophrenia, clinical correlates of DTNBP1 risk haplotypes, and normal functions of dysbindin-1.

6.6.3.1 Abnormal Brain Development Schizophrenia results from interactions among genetic and environmental factors that are believed to cause aberrant brain development pre and postnatally (cf. Maynard et al., 2001, Lewis and Levitt, 2002, and Rapoport et al., 2005). There is compelling evidence that the disorder is the behavioral outcome of abnormal neurodevelopmental processes starting long before onset of clinical symptoms (see Rapoport et al., 2005). Peak DTNBP1 gene expression occurs prenatally (Kumamoto et al., 2007), as does dysbindin-1A protein expression (Ghiani et al., 2009). The prenatal peak occurs during a period of maximum cell proliferation and the largely overlapping period of programmed cell death (see *Section 2.2.6.2.1*). Dysbindin-1 may well play a role in these prenatal events, because there is reason to believe it promotes cell growth and proliferation (see *Section 2.2.6.5.2*) and has an anti-apoptotic effect on immature neurons (Numakawa et al., 2004; see *Section 2.2.6.5.3*). The latter effect might regulate the proportion of neurons and their precursors escaping programmed cell death before and after birth. Disruption of this effect in carriers of a DTNBP1 risk SNP for schizophrenia (P1578 = rs1018381) might help explain why such individuals are found to have smaller brain volumes

Figure 2.2-25

Reduced dysbindin-1 protein in certain intrinsic, glutamatergic pathways of the hippocampal formation in schizophrenia. This is especially notable in the inner molecular layer (IML) of the dentate gyrus (DGiml). Relative levels of dysbindin-1 were assessed by measuring the optical density (OD) of dysbindin-1 immunoreactivity to antibody PA3111. (a) and (b) are bivariate graphs in which each data point gives the OD of dysbindin-1 immunoreactivity in the DGiml of a schizophrenia case (y-axis) versus that in its matched control (x-axis). OD values represent shades of gray on a scale of 0–4095. Points below the diagonal line represent matched pairs in which immunoreactivity was lower in the schizophrenia case than in its matched control. (a) Plots data from University of Pennsylvania (UPenn) cases, while (b) plots data from Stanley Foundation. The reduction of dysbindin-1 immunoreactivity seen in the hippocampal formation of a schizophrenia (Sz) case compared with its matched control (N) is shown at low magnification in (c) and (d) (scale bars = 1 mm) and at higher magnification of the boxed areas through DGiml in (e) and (f) (scale bars = 50 μm). The pathways in which dysbindin-1 reduction probably occur in schizophrenia are indicated by starbursts in (g). The major input to the hippocampal formation is that from the entorhinal cortex (ERC), which travels via the perforant path (pp) to terminate (in part) on dendrites of granule cells (G) in the outer molecular layer of the dentate gyrus (DGoml). The granule cell axons innervate hilar cells (H) in the polymorph layer of the dentate gyrus and dendrites of CA3 pyramidal cells. The axons of CA3 cells divide into collaterals, some which terminate locally while others terminate in CA1. CA1 pyramidal cells in turn innervate the subiculum (Sub) and the entorhinal cortex. In schizophrenia, dysbindin-1 is reduced in hilar cells and their terminal field in DGiml, the local and distal terminal fields of CA3, and the terminal field of CA1 in the subiculum. For other abbreviations, see List of Anatomical Abbreviations (pp. 109–110). Adapted from Talbot et al. (2004)



than noncarriers (Narr et al., 2009). The protective effect might also limit apoptosis-mediated delayed cell death after hypoxic conditions induced by obstetric complications, some of which are risk factors for schizophrenia (Cannon et al., 2002; Bryne et al., 2007). This may help explain why it has been found that a SNP in DTNBP1 is over-represented in schizophrenia cases born with a serious obstetric complication (Nicodemus et al., 2008).

As discussed earlier (see *Sections 2.2.6.5.5–2.2.6.5.6*), dysbindin-1 can affect the release of dopamine and glutamate, as well as the behavior of D2Rs and NMDARs. Alterations in these two transmitter systems during brain development have diverse consequences. These can begin early in the prenatal period. Gene expression and agonist binding of D2Rs begins with neuronal generation as early as gestational days 12–14 in rodents (cf. Jung and Bennett, 1996 and Araki et al., 2007). Gene expression of NMDARs likewise starts as early as gestational day 14 in rodents (Babb et al., 2005), although these receptors are not functionally mature until 3 weeks after birth (see Haberny et al., 2002). D2R promotes the G1/S cell cycle transition (Ohtani et al., 2003) and cell proliferation (Popolo et al., 2004) in germinal zones of the cerebral cortex (i.e., the subventricular zone) and the striatum (i.e., the lateral ganglionic eminence), as well as neurite outgrowth in the late fetal cerebral cortex (Reinsoso et al., 1996) and substantia nigra-ventral tegmental area (Kim et al., 2006). NMDAR in the late fetal period also stimulates cell proliferation of striatal and cerebrocortical neuronal progenitors (cf. Luk et al., 2003 and Luk and Sadikot, 2004). In the pre-adolescent period, NMDAR has been found to regulate the density of synapses in the hippocampus (Lüthi et al., 2001) and the dendritic spines in the cerebral cortex (Ultanir et al., 2007). In the same period, D2R inhibits migration of GABA neurons into the cerebral cortex (Crandall et al., 2007), whereas NMDAR stimulates the migration and functional maturation of glutamate neurons in the cerebral cortex (Reiprich et al., 2005). Via these effects on dopaminergic and glutamatergic actions mediated by D2R and NMDAR, reductions of dysbindin-1 early in brain development could affect cell growth and proliferation of neuronal precursors, as well as migration and maturation of cerebrocortical, hippocampal, striatal, and SN-VTA neurons.

Via several of its known or candidate-binding partners (pallidin, snapin, sec8, and rab 11A), dysbindin-1 could also affect growth of axons, dendrites, and dendritic spines as described earlier (see *Section 2.2.6.5.4*). Reduced expression of dysbindin-1 in development may thus contribute to the decreased neuronal size, dendritic length, and densities of dendritic spines and axon terminals found in the DLPFC, hippocampal formation, and other brain areas in schizophrenia (Harrison and Lewis, 2003; Lewis and Gonzalez-Burgos, 2008). This raises the possibility that decreased dysbindin-1 during development might alter synaptic architecture, which is of special interest in view of evidence that synaptic dysfunction is a central feature of schizophrenia (cf. Mirnics et al., 2001, Honer and Young, 2004, McCullumsmith et al., 2004, Lewis and Moghaddam, 2006, and Hashimoto et al., 2007).

6.6.3.2 Dopaminergic and Glutamatergic Dysregulation While abnormal brain development appears to be a distal or root cause of schizophrenia, its proximal or immediate cause appears to be abnormal synaptic functions (cf. Mirnics et al., 2001, McCullumsmith et al., 2004, MacDonald and Chafee, 2006, and Lewis and Moghaddam, 2006). Among the most frequently noted synaptic abnormalities in schizophrenia are those occurring in two interacting transmitter systems, namely dopaminergic (Laruelle, 2003; Winterer and Weinberger, 2004; Toda and Abi-Dargham, 2007) and glutamatergic (Coyle, 2006; MacDonald and Chafee, 2006) systems.

In its current form, the dopamine hypothesis of schizophrenia posits an imbalance in the activity of two different dopaminergic pathways originating in the mesencephalon (see Abi-Dargham, this volume). The hypothesis asserts that (1) the mesocortical dopamine pathway is *hypo*active, resulting in hypostimulation of target D1Rs (including those in the DLPFC) causing negative clinical symptoms (e.g., flattened affect, poverty of speech, lack of initiative, and social withdrawal: see Carpenter, this volume) and that (2) the mesolimbic pathway is *hyper*active, resulting in *hyper*stimulation of D2Rs (including those in the ventral striatum, hippocampus, and amygdala) causing positive clinical symptoms (e.g., delusions, hallucinations, and thought disorders) (Laruelle, 2003). Mesolimbic hyperactivity is evident in abnormally high amphetamine-induced striatal dopamine release, increased occupancy of D2Rs by dopamine in the striatum, and at least a small increase in the density of striatal D2Rs (Laruelle, 2003; Toda and Abi-Dargham, 2007).

The dopaminergic hyperactivity in the mesolimbic pathway could be caused in part by a reduction in dysbindin-1 for several reasons. Dysbindin-1 is highly expressed in the source neurons of the mesolimbic pathway (i.e., VTA neurons of the mesencephalon) and in their target neurons in the striatum (i.e., medium spiny cells: see *Section 2.2.6.3.2.3.4*). Moreover, sdy mice lacking dysbindin-1 show evidence of increased dopamine release in targets of the mesolimbic pathway (Murotani et al., 2007: see *Section 2.2.6.5.6*). That is consistent with the finding that knockdown of dysbindin-1 in PC-12 cells causes an elevation in stimulus-induced dopamine release (Kumamoto et al., 2006). Dysbindin-1 knockdown in neuroblastoma cells and cerebrocortical neurons also increases cell surface expression of D2Rs (Iizuka et al., 2007; see *Section 2.2.6.5.7*). If that occurs in target neurons of the mesocortical pathway in schizophrenia, it would contribute to the dopaminergic hyperactivity in that pathway.

Glutamatergic abnormalities are also a major feature of schizophrenia. They may actually cause the dopaminergic abnormalities in that disorder and then be exacerbated by them (Laruelle et al., 2005). The glutamate hypothesis simply states that NMDAR hypofunction contributes to the pathophysiology of schizophrenia (Coyle, 2006; Lindsley et al., 2006). A variant of this is the NMDA synaptic deficit hypothesis of MacDonald and Chafee (2006), which posits that each risk factor for schizophrenia impacts the NMDA glutamate synapse such that the cumulative effect drives NMDA synaptic function below a threshold for normal function. Supporting either form of the glutamate hypothesis is evidence that (1) many candidate susceptibility genes for schizophrenia encode proteins directly or indirectly affecting glutamatergic transmission (i.e., DAAO, DTNBP1, G72, GRM3, NRG1, and RGS: see Shi, this volume), (2) NMDAR blockers like phencyclidine (PCP) and ketamine induce clinical symptoms, whereas NMDAR potentiators like D-serine and sarcosine reduce those symptoms (see Javitt, this volume), and (3) NMDAR hypofunction may be responsible for the reduced GABAergic function often observed in schizophrenia (cf. McCullumsmith et al., 2004, Coyle, 2006, Lindsley et al., 2006, and MacDonald and Chafee, 2006).

Dysbindin-1 is expressed by glutamatergic neurons and by their targets cells in brain areas affected in schizophrenia, including the DLPFC, hippocampal formation, and striatum (see *Sections 2.2.6.3.2.3.4–2.2.6.3.2.3.6*). Reduced dysbindin-1 levels in such cells and their targets is likely to contribute to glutamatergic dysfunction in schizophrenia, because dysbindin-1 knockdown in cerebrocortical cells reduces stimulus-induced release of glutamate (Numakawa et al., 2004) and because dysbindin-1 loss in sdy mice leads to reduced NMDA-mediated currents in the rodent homolog of the primate DLPFC (Andrew et al., 2007), inducing NMDAR hypofunction in its neurons, including fast-spiking parvalbumin interneurons with consequent reductions in their GABAergic output to pyramidal cells (Trantham-Davidson et al., 2008). As noted earlier (see *Section 2.2.6.4.2.8*), gamma frequency oscillations are driven by this class of interneurons (Sohal et al., 2009; Cardin et al., 2009). Dysbindin-1 reductions in fast-spiking interneurons may thus contribute not only to NMDAR hypofunction in schizophrenia (Homayoun and Moghaddam, 2007; Kehrer et al, 2008), but also to the marked deficits in generation of gamma frequency oscillations seen in that disorder (Lee et al., 2003; Light et al., 2006).

6.6.3.3 Dysconnection in Cerebral Cortex and Hippocampal Formation Mounting evidence indicates that schizophrenia is characterized by reduced functional connectivity among cerebrocortical areas (Friston, 1998; Stephan et al., 2006). This appears to be the basis for reduced beta and especially gamma band synchrony induced during sensory processing (Uhlhaas and Singer, 2006; cf. also Symond et al., 2005 and Spencer et al., 2003). Since associational and commissural pathways mediating connections among cerebrocortical areas are glutamatergic and/or aspartamatergic (Parent, 1996, pp. 867–873), the functional dysconnections among those areas in schizophrenia are probably due at least in part to the NMDA hypofunction evident in that disorder (see above and Stephan et al., 2006). As described previously (see *Sections 2.2.6.3.2.3.5–2.2.6.3.2.3.6*), associational and commissural pathways in both the cerebral cortex and the hippocampal formation are highly enriched in dysbindin-1. Consequently, its reduction in those pathways may contribute to the functional dysconnection among areas of both the cerebral cortex and hippocampal formation, especially since – as mentioned above – loss of the protein diminishes stimulus-induced glutamate release (Numakawa et al., 2004) and reduces NMDA-mediated currents (Andrew et al., 2007).

A dysconnection effect related to dysbindin-1 may help explain why the P1 visual evoked potential is smaller in those schizophrenia cases carrying a DTNBP1 risk haplotype than in those not carrying that haplotype (Donohoe et al., 2008). The P1 visual evoked potential originates in extrastriate cortex (i.e., visual association cortex) (Di Russo et al., 2001), which receives part of its visual input from striate cortex (i.e., primary visual cortex) via an associational pathway (see Parent, 1996, p. 905). Dysbindin-1 is concentrated in the neurons giving rise to the association pathways of the striate cortex. If, then, dysbindin-1 levels in that pathway are reduced in carriers of the DTNBP1 risk haplotypes, the effect of the protein on glutamate release in vitro (Numakawa et al., 2004) lead us to expect reduced synaptic strength of visual cortex output to extrastriate cortex. Reduced input to the latter cortex would contribute to abnormally small P1 visual evoked potentials in those carrying the DTNBP1 risk haplotype.

6.6.3.4 Positive and Negative Symptoms Apart from social/occupational dysfunction, the diagnosis of schizophrenia is based on expression and duration of positive symptoms (e.g., delusions, hallucinations, and thought disorders) and negative symptoms (e.g., flattened affect, poverty of speech, lack of initiative, and social withdrawal) (see Eaton et al., 1995). It is unknown if decreased gene or protein expression of dysbindin-1 is related to expression of these symptoms. But relationships have been found between symptom severity and DTNBP1 haplotypes associated with risk for the disorder. The relationships found are not entirely consistent across studies, perhaps because different haplotypes were examined. Compared with schizophrenia cases not carrying high risk DTNBP1 haplotypes in the Irish Study of High Density Schizophrenia Families (van den Oord et al., 2003) and in a U.S. cohort (Funke et al., 2004), those which do carry such haplotypes have higher ratings on negative (but not positive symptom) factors (Fanous et al., 2005; De Rosse et al., 2006). In the more detailed study on the U.S. cohort, it was found that the carriers specifically had higher ratings on measures of avolition, alogia, and flattened affect (De Rosse et al., 2006). In Irish schizophrenia and schizoaffective cases not included in the Irish High Density Schizophrenia Families, Corvin et al. (2008) found no differences on a negative symptom factor between carriers and noncarriers of yet another DTNBP1 risk haplotype, but did find that the carriers had lower ratings on a positive symptom factor for hostility/excitability, which represents correlated measures of hostility, excitability, grandiosity, impulsivity, and uncooperativeness. Their risk haplotype appears to protect against manic-type symptoms in the cases studied. In Korean schizophrenia cases, Pae et al. (2008) found both protective and exacerbating DTNBP1 haplotypes associated with positive symptom severity assessed upon hospitalization for psychosis. The SNPs in these haplotypes were among the most strongly associated with schizophrenia in the initial study on the Irish High Density Schizophrenia Families (Straub et al., 2002). Several DTNBP1 SNPs associated with schizophrenia in that study have since been found significantly correlated with schizotypical personality factors, especially paranoia, in a large cohort of normal males 18–24 years old (Stefanis et al., 2007), a peak period for onset of schizophrenia (see Häfner et al., 1998).

6.6.3.5 Cognitive Impairment While not a diagnostic feature of the disorder, cognitive impairment is nevertheless a core feature of schizophrenia (Elvevåg and Goldberg, 2000; Barch, 2005). There is direct and indirect evidence for a role of dysbindin-1 in normal learning and memory processes (see *Section 2.2.6.5.10*). As argued above, decreased dysbindin-1 in schizophrenia may contribute to several features of that disorder: abnormal brain development, dopamine hyperactivity, NMDAR hypofunction, reduced generation of gamma frequency oscillations, and functional disconnection within the cerebral cortex and hippocampal formation. All these changes could affect learning and memory processes (cf. McCullumsmith et al., 2004, Stephan et al., 2006, Tanaka, 2006, and Dumonthiel et al., 2008; Sohal et al., 2009).

The cognitive status of schizophrenia cases is often reported to be worse in those carrying SNPs in DTNBP1 individually or collectively associated with the disorder (Straub et al., 2003; Burdick et al., 2006, 2007; Donohoe et al., 2007; Strohmaier et al., 2007; Zinstock et al., 2007). Although there is one study to the contrary (Peters et al., 2008), such SNPs in schizophrenia have been associated with (1) greater decline in general cognitive ability from a premorbid to a clinical state (Burdick et al., 2007), (2) lower general cognitive ability in the clinical state (Burdick et al., 2006), (3) lower scores on verbal, performance, and full-scale IQ tests measured on the Wechsler Adult Intelligence Scales (WAIS) (Straub et al., 2003; Zinstock

et al., 2007), (4) deficits on a spatial working memory task from the Cambridge Automated Test Battery and on a Go NoGo attentional response task similar to that described in *Section 2.2.6.5.10* (Donohoe et al., 2007), and finally (5) poorer performance on Trail-Making Tests A and B (= TMT-A and TMT-B) (Strohmaier et al., 2007). Performance on TMT-A measures visual search skills plus motor speed, while performance on TMT-B measures visual search skills plus the ability to alternate between cognitive categories (Crowe, 1998). Results of TMT-B testing also yield indirect measures of attention and working memory capacities (Crowe 1998).

The tasks on which schizophrenia cases with high-risk DTNBP1 SNPs perform poorly compared with cases without those SNPs are all known to activate lateral prefrontal cortex with or without activation of other brain areas, mainly the hippocampus and association areas of parietal and temporal cortex (cf. Laurens et al., 2005, Zakzanis et al., 2005, Colom et al., 2006, and Ricciardi et al., 2006). These SNPs in DTNBP1 may thus contribute to dysfunction in lateral prefrontal cortex in schizophrenia. This possibility is consistent with decreased efficiency of DLPFC information processing in schizophrenia cases with high-risk DTNBP1 SNPs during WAIS-R testing deduced by Straub et al. (2003). That may help explain the finding that schizophrenia cases carrying a high-risk DTNBP1 haplotype are more likely to display high levels of negative symptoms defined in part by cognitive impairments such as incoherence and thought disorders (Fanous et al., 2005).

Given the number of studies reporting associations between cognitive measures and SNPs in DTNBP1, interest has grown in determining how such SNPs may affect transcription of the gene and its potential epistatic effects on other genes. This is one of the most challenging areas of research on dysbindin-1, because the DTNBP1 SNPs in question are intronic and not predicted to affect splicing. It is especially challenging since the actual susceptibility variants in DTNBP1 have yet to be identified. Until they are, it may be more profitable to focus research efforts on establishing the relationship between cognition and the expression of dysbindin-1 proteins.

We began this review with the question that led to the discovery of dysbindin-1, namely what disrupted protein interactions of dystrobrevin may help account for the cognitive deficits in Duchenne and Becker muscular dystrophy? The discovery of dysbindin-1 has not led to an answer to that question, but has led to the discovery that abnormalities in the DTNBP1 gene or in levels of its protein product do affect cognition in normal individuals and in schizophrenia cases. Establishing the mechanisms responsible for that effect is perhaps the most important topic of future basic and translational research on dysbindin-1.

7 Synopsis

The dysbindin protein family consists of proteins sharing significant aa sequence homology with the first discovered member of the family (i.e., dysbindin-1A). The homologous aa sequence in each family member of a given species is called the dysbindin domain (DD), a 41–54 aa segment of the C-terminus region containing a predicted site for DNA protein kinase phosphorylation site. The dysbindin family contains three paralogs, dysbindin-1, -2, and -3, each of which is encoded by a different gene. Each paralog can exist in more than one isoform, currently defined by the mRNA encoding it. In humans, the family has at least eight members (dysbindin-1A, -1B, -1C, dysbindin-2A, -2B, -2C, dysbindin-3A and -3B). While also expressed in the frog, dysbindin-1B is not expressed in the mouse, which has only four known family members (dysbindin-1A, -1C, -2B, and -3B). Except for dysbindin-2C, all members of the family have many predicted phosphorylation sites (mainly serine or threonine residues) for diverse kinases.

With few exceptions, dysbindin family members are predicted to be both cytosolic and nuclear proteins. As that implies, none are likely to be integral membrane proteins or linked to the outer surface of the cell membrane. None has motifs indicative of binding to actin, DNA, RNA, or ribosomes. Apart from dysbindin-2A, none are probable secretory proteins. If dysbindin-1 is an accurate guide, family members in the cytosol are instead likely to be associated with the periphery of intracellular membranes and with cytoskeletal binding proteins.

Further discussion of the dysbindin family is limited to dysbindin-1 since little is known about other family members apart from the fact that dysbindin-2B is a casein kinase 1 binding partner and a stem cell factor apoptosis response protein. In contrast, much has been learned about dysbindin-1 since its discovery was reported by Benson et al. (2001). It is the only member of the dysbindin family known to exist in invertebrates, specifically the fruit fly, and may thus date back 600 million years. Unlike all other dysbindin paralogs, it contains a coiled coil domain (CCD) allowing extensive interactions with other proteins. A leucine zipper motif in the CCD changed in the course of evolution in a manner permitting more durable interactions with binding partners.

Dysbindin-1 is encoded by the dystrobrevin binding protein 1 (DTNBP1) gene at 6p22.3 in humans and in 13A5 in mice. There is evidence of 13 exons in humans and 10 exons in mice. Of the many deduced transcripts, the best established are those for the three dysbindin-1 isoforms recognized in this chapter. The transcript for dysbindin-1A is clearly the most widely and abundantly expressed, but all three transcripts are expressed in the human brain. These appear to encode proteins at ~50, 37, and 33 kDa in western blots, which we accordingly designate as dysbindin-1A, -1B, and -1C proteins. Transcriptional regulation of DTNBP1 is still poorly understood, but evidence has been presented for cis- and trans-acting control sequences and for consensus binding sites for nuclear factor 1 (NF-1) and specificity protein 1 (SP1) in the first of four possible promoters in DTNBP1.

Gene expression of dysbindin-1 is ubiquitous in the body, including the brain and is often elevated in cancerous tissue. In the developing brain, there is peak expression during neurogenesis and during adolescence. In the adult brain, the highest gene expression found to date is in the temporal neocortex, hippocampal formation, and dopamine-rich areas of the brain, specifically the substantia nigra-ventral tegmental area and the areas it innervates (i.e., the amygdala, dorsolateral prefrontal cortex, rostral entorhinal cortex, nucleus accumbens, and putamen.

Protein expression of dysbindin-1 is also ubiquitous in the body and is detectable in cell bodies of virtually all neuronal populations. Levels of somatal protein are variable, however, with the highest levels found in areas listed above where gene expression is highest. High levels of dysbindin-1 protein expression are also seen in certain synaptic fields. Where these have been examined with immunoEM, dysbindin-1 has been found mainly along microtubules of dendrites and axons, in PSDs of dendritic spines, and around synaptic vesicles. Tissue fractionation of whole brain tissue reveals that dysbindin-1A is most highly concentrated in PSD fractions, dysbindin -1B in synaptic vesicle fractions, and dysbindin-1C in both PSD and synaptic vesicle fractions.

The synaptic fields enriched in dysbindin-1 are most often dopaminergic, glutamatergic, and/or GABAergic. While dysbindin-1 appears to be absent in axons of the nigrostriatal pathway, it is present in their postsynaptic targets. In the dopaminergic mesolimbic pathway to the amygdala and the hippocampal formation, however, dysbindin-1 may be localized both pre and postsynaptically. In the glutamatergic (and/or aspartamatergic) associational and commissural pathways of the cerebral cortex and the hippocampal formation, dysbindin-1 has been found both pre and postsynaptically, though not in the same synapse. Subcellular localization of dysbindin-1 in GABAergic synaptic fields has not been studied.

An animal model of dysbindin-1 functions is available in the sandy (sdy) mouse, which has a naturally occurring deletion mutation of exons 6 and 7 in the gene (Dtnbp1) encoding of the mouse protein. The mutation results in loss of dysbindin-1 in homozygous animals, as well as loss or reduction in other BLOC-1 proteins (i.e., muted, pallidin, and BLOS3). Homozygous sdy mice thus model both dysbindin-1 and BLOC-1 functions. In addition to abnormalities typical of BLOC-1 dysfunctions in peripheral tissues, such mice show evidence of increased dopamine transmission in limbic tissue, decreased catecholamine release from the adrenal chromaffin cells, reduced glutamate release in the prefrontal cortex and hippocampal formation, and loss of evoked inhibitory responses in the latter area. The homozygous sdy mice show impaired auditory prepulse inhibition, are less responsive to painful stimuli, spend less time in social interactions, habituate less to the open field, and display prominent deficits in contextual fear conditioning, novel object memory, and spatial memory. They thus share many features of mice considered animal models of schizophrenia.

As indicated by its many known and candidate binding partners listed in *Tables 2.2-6* and *2.2-7*, dysbindin-1 may have diverse functions not limited to those of BLOC-1. We specify what those functions may be based on the nature of the protein's binding partners, its tissue localization, results of in vitro experiments, and the abnormalities in sdy mice and humans with certain SNPs in DTNBP1. The potential functions proposed are: (1) spermatogenesis, (2) promotion of cell growth and proliferation, (3) neuroprotection against apoptosis, (4) promotion of axon, dendrite, and dendritic spine growth, (5) regulation of AP-3 cargo transport to lysosome-related organelles (including synaptic vesicles in reserve pools), (6) facilitation of glutamate and adrenal catecholamine release, but inhibition of dopamine release, (7) regulation of D2R and NMDAR trafficking, (8) relay and gating of spinal pain transmission, and (9) enhancement of cognition. A role in motor functions is considered and found to be unlikely.

Genetic variation in DTNBP1 has often been associated with psychiatric conditions in which psychosis may be a present, specifically bipolar disorder, major depression, and schizophrenia. Fifteen studies have found a significant association between schizophrenia and one or more SNP in DTNBP1. One of those SNPs (rs1011313 = P1325) has recently been identified as among only four SNPs in the human genome showing a strong degree of epidemiological credibility. These findings have become controversial in the last 2 years, because the DTNBP1 SNPs associated with schizophrenia often vary among populations and because there are now 11 negative studies. In our view, however, the negative studies are insufficient to discount the possibility that DTNBP1 is a susceptibility gene for schizophrenia for reasons detailed in *Section 2.2.6.6.1*. Indeed, the combined odds ratio of association between genetic variation in DTNBP1 and schizophrenia across studies achieves a significance level ($p = 3.8 \times 10^{-10}$) higher than for variation in any of the other top candidate genes for that disorder (Sun et al., 2008). Independent o the genetic controversy, there is evidence of an involvement of dysbindin-1 protein expression in two brain areas commonly affected in the disorder (i.e., the dorsolateral prefrontal cortex and hippocampal formation). Given the potential functions of dysbindin-1, such changes may add to the pathophysiology of the disorder by altering brain development, dopaminergic and glutamatergic transmission, associational and commissural connections in the cerebral cortex and hippocampal formation, gamma oscillations in those structures, and cognitive processes in general.

This chapter highlights both what is known about the dysbindin protein family, especially dysbindin-1, and what remains to be learned. The incentive for further work on dysbindin-1 lies in increasing evidence for its diverse neuronal functions, including cognition, and for the consequences of its disruption on the pathophysiology of schizophrenia. Used as intended, this chapter serves as a source of background information in the context needed to generate plausible hypotheses for further study and to design the experiments testing them.

8 Note on Authorship and Contact Information

This review was conceived and written by the corresponding author, Konrad Talbot (talbotk2@mail.med.upenn.edu), with assistance from Derek J. Blake on dysbindin protein family members and binding partners of dysbindin -1, Gregory C. Carlson on neurophysiological studies on the sandy mouse, Natalia Louneva on dysbindin-1 isoforms in tissue fractions of the mouse and human brain, Wei-Yi Ong on the distribution of dysbindin-1 in the brain of macaques at the electron microscopic level, and Junxia Tang on the transcripts of DTNBP1 and the association of genetic variants in DTNBP1 with schizophrenia. Steven E. Arnold provided feedback on the review as a whole.

Acknowledgments

This work was supported by grants to S.E.A. from the National Institute of Mental Health (R01 MH072880 and P50 MH064045) and from the National Alliance for Research on Schizophrenia and Depression (NARSAD).

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2.3 Neuregulin 1 and Schizophrenia

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_2.3,

Abstract: Signaling pathways regulated by the interaction of neuregulin1 (*NRG1*) and ErbB family members are essential for the formation and proper functioning of virtually all organ systems. Dysregulation of NRG1/ErbB signaling can severely compromise health, contributing to cancer, neurodegenerative and neuropsychiatric disorders, cardiac failure, and compromised reproductive capacity. Moreover, genetic studies of human cohorts from Iceland to East Asia have identified and confirmed that polymorphisms within the Nrg1 gene are associated with susceptibility to schizophrenia.

Numerous genetic modeling studies in mice demonstrate that *NRG1* signaling is an important player in the development of normal and pathognomonic features of neuronal connectivity. Recent studies have identified novel signaling mechanisms and revealed unexpected roles of NRG1 isoforms in both the developing and adult nervous system. Of specific pertinence to this review are recent findings linking deficits in neuronal NRG1 expression with perturbations of synaptic transmission, myelination, and the survival of particular sets of neurons and glia. Despite the recent deluge of reports linking the NRG1/ErbB interaction to fundamental aspects of neuronal function, the molecular mediators of this bidirectional signaling pathway remain unknown.

In the following pages, we will first review the evidence linking *NRG1* with schizophrenia, and provide a general review of the neuregulin gene family. Then we will discuss more specifically the emerging role for neuregulins, particularly the Type III isoforms, in the formation and maintenance of peripheral and central synapses. We hope that this will provide a framework for probing how alterations in the normal function of NRG1/ErbB signaling contribute to the etiology of schizophrenia.

NRG nomenclature used for this chapter:

Human gene name: *NRG1* (*italics*)

Mouse or other nonhuman gene name: *Nrg1* (*italics*)

Protein (all species): NRG1 (not in italics)

List of Abbreviations: COS, childhood-onset schizophrenia; EGF, epidermal growth factor; MGE, medial ganglionic eminence; *NRG1*, neuregulin1; SNPs, single-nucleotide polymorphisms

1 Neuregulin 1 Gene as a Schizophrenia Susceptibility Gene

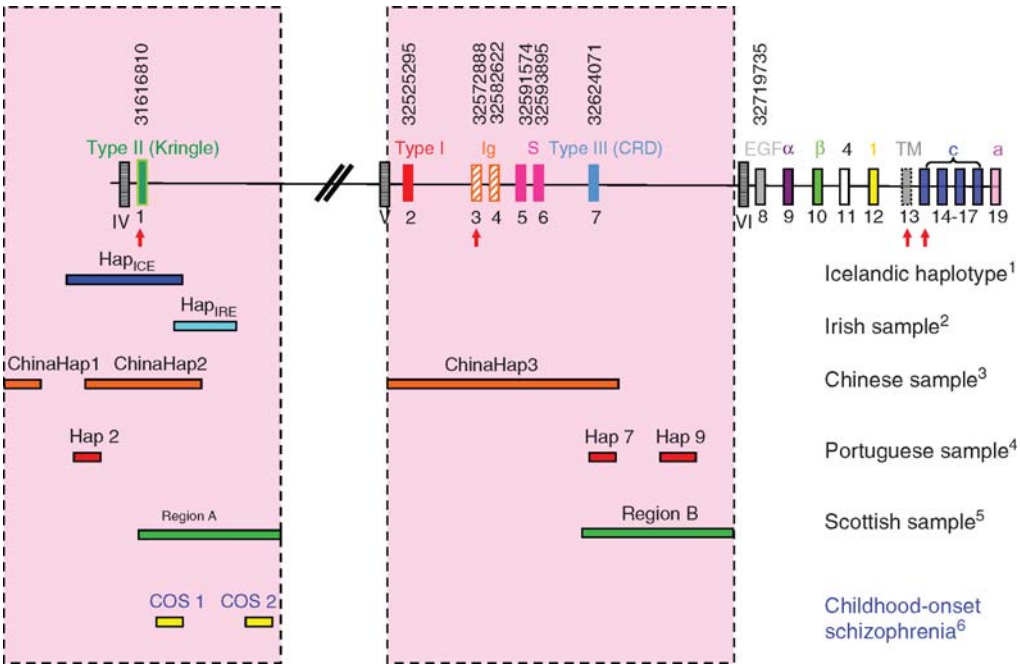
1.1 Human Genetic Studies on Neuregulin 1 Gene

Since the first paper identifying neuregulin 1 (*NRG1*) gene as one of the schizophrenia susceptibility genes (Stefansson et al., 2002), there have been over two dozen published studies attempting to replicate this association. Our review on human genetic studies is not intended to provide meta-analyses (readers who are interested in results of meta-analysis should refer to (Li et al., 2006; Munafo et al., 2006; Tosato et al., 2005), rather we would like to provide a summary of all the published results up to August 2007. A comprehensive up-to-date overview of published schizophrenia association studies for *NRG1* can be found at the Schizophrenia Research Forum website: (http://www.schizophreniaforum.org/res/sczgene/geneoverview.asp?geneid=311).

Multiple genome linkage studies identified regions on chromosome 8p, which harbors the *NRG1* gene, as the locations of plausible schizophrenia susceptibility genes (Blouin et al., 1998; Gurling et al., 2001; Hovatta et al., 1998; Kendler et al., 1999; Kendler et al., 1996; Pulver et al., 1995; Stefansson et al., 2002; Suarez et al., 2006). Fine mapping and association studies using single-nucleotide polymorphisms (SNPs) and microsatellite markers first identified a region near the 5′ end of the *NRG1* gene that was associated with schizophrenia in an Icelandic population (Stefansson et al., 2002). A core haplotype (Hap_{ICE}), which consists of 5 SNPs and two microsatellite markers (*Figure 2.3-1*), was found to be overrepresented in affected individuals (Stefansson et al., 2002). Subsequent replication with this core haplotype and other markers near or within *NRG1* gene demonstrated a role of *NRG1* in schizophrenia susceptibility from Scottish, Irish, British, Portuguese, South African, Japanese, and Han Chinese samples (Corvin et al., 2004; Fukui et al., 2006; Hall et al., 2004; Hong et al., 2004; Li et al., 2004; Petryshen et al., 2005; Stefansson et al.,

Figure 2.3-1

Neuregulin gene structure. The organization of the *NRG1* gene is shown at the top (numbers refer to nucleotide numbers of human chromosome 8). Exons are shown as vertical boxes (drawn approximately to scale) and connected by a horizontal line indicating introns (not drawn to scale). The six major families of isoforms are designated I–VI based on their first coding exon. Other functionally significant exons are noted. Below the gene structure are horizontal bars that indicate the regions encompassing multiple marker haplotypes that are associated with risk of developing schizophrenia (described in detail in text). Two major regions of overlapping risk haplotypes from multiple studies are shaded



2003a; Stefansson et al., 2003b; Tang et al., 2004; Thomson et al., 2007; Yang et al., 2003; Zhao et al., 2004). Other studies did not detect association between those SNPs or the core haplotype in the *NRG1* gene and schizophrenia (Hong et al., 2004; Ingason et al., 2006; Iwata et al., 2004; Thiselton et al., 2004).

Additional SNPs and haplotypes were identified throughout the entire *NRG1* gene that facilitate systematic association studies between *NRG1* and schizophrenia (Corvin et al., 2004; Li et al., 2004; Petryshen et al., 2005; Thomson et al., 2007). In particular, linkage disequilibrium (LD)-based studies have helped identify haplotype blocks in the *NRG1* gene that bear a strong association with schizophrenia. Among these haplotype blocks, Hap_{IRE}, ChinaHap2, Hap2, and region A (*Figure 2.3-1*) are located at the 5′ region of the *NRG1* gene overlapping with the Icelandic haplotype (Hap_{ICE}) and include promoter regions, exon 1 and intron 1 of the *NRG1* gene (Corvin et al., 2004; Li et al., 2004; Petryshen et al., 2005; Thomson et al., 2007). Hap 7 and Hap 9 are located in more 3′ regions of the *NRG1* gene spanning intron 6–7 and overlap with ChinaHap3 and region B (*Figure 2.3-1*) (Li et al., 2004; Petryshen et al., 2005; Thomson et al., 2007). It appears that there are at least two haplotype regions, one at the 5′ end of the locus spans the transcriptional start sites of the Type IV and Type II isoforms, and the other that spans the 5′ ends of Type I and Type III isoforms. Most critical is to identify the DNA sequence variations that directly contribute to the development of schizophrenia.

Childhood-onset schizophrenia (COS) represents a rare and severe form of the disorder with a much earlier age of onset of psychosis (prior to age 12), as opposed to the typical age of onset at 18–20 for men and 25 for womens (Rapoport and Inoff-Germain, 2000). Clinically and neurobiologically, COS is considered to be continuous with the adult-onset schizophrenia; yet the higher rate of familial illness/endophenotypes in the COS group than that in the families of adult-onset patients point to a stronger genetic basis for COS (Addington et al., 2007; Keller et al., 2003). Genetic association studies in COS patients have identified two risk haplotypes (COS1 and COS2 in *Figure 2.3-1*) in the 5′ end of the *NRG1* gene. The haplotype COS1 overlaps with the 3′ end of the Icelandic haplotype Hap_{ICE}, whereas COS2 is located 238 kb downstream of COS1. Using one of the markers from COS1, 420M9-1395, which by itself provides strong association with COS, Addington et al. (2007) demonstrated that within the COS group, the risk-allele carriers have bigger gray and white matter volumes than the no-risk allele carriers at the age of 8. Furthermore, the changes (from the age of 8 to 20) of grey and white matter volumes within the COS group are also different between carriers and noncarriers. Over a period of 12 years, the difference between carriers and noncarriers gradually decreases and becomes insignificant by the end of the study – the risk-allele carriers have accelerated gray matter loss over time, while the no-risk allele carriers have slower gray matter loss. The white matter volume is decreased in the carriers while it is increased substantially in the noncarriers. There are similar trends in the gray matter volume difference and the changes of the gray matter volume among the risk-allele carriers and noncarriers in healthy controls (Addington et al., 2007). These findings are consistent with the established role of NRG1 during neural development (see later) and provide the first study that correlates a risk allele and disorder-specific changes in brain development.

Neuregulin 1 protein signals through the interaction with ErbB receptors. Two studies also have reported genetic association studies with markers in the ErbB4 gene in schizophrenia (Norton et al., 2006; Silberberg et al., 2006). In one study, 15 SNPs were identified in the ErbB4 gene. None of the identified SNPs change amino acid sequence, nor do they have significant association with schizophrenia (Norton et al., 2006). However, there was genetic epistasis between markers on ErbB4 and the *NRG1* risk haplotype Hap_{ICE}. Genetic interaction between markers at *NRG1* and at ErbB4 results in effect sizes that are greater than that of the *NRG1* or the ErbB4 markers alone (Norton et al., 2006). Another LD-based study, in contrast, demonstrates highly significant association between three SNPs and a haplotype of the ErbB4 gene with schizophrenia (Silberberg et al., 2006). Together these demonstrate that NRG1/ErbB signaling pathway may be involved in the pathophysiology of schizophrenia.

1.2 Correlation of the Expression of Neuregulin 1 and the Disease State of Schizophrenia

Despite of the extensive DNA sequencing within the *NRG1* gene, only 4 (SNP8NRG433E1006, rs3924999, rs10503929, and a novel SNP yet to be named) out of more than 80 SNPs identified so far are nonsynonymous (i.e., that causes amino acid sequence change) (Hong et al., 2004; Stefansson et al., 2002; Walss-Bass et al., 2006a; Yang et al., 2003)). SNP8NRG433E1006 is one of the five SNPs of the Hap_{ICE}; it alters an amino acid residue from Arg to Gly in the exon 1 of the *NRG1* gene. This SNP itself is not significantly associated with schizophrenia and indeed the allele found in the risk haplotype is the more common allele in the general population (Petryshen et al., 2005; Stefansson et al., 2003a; Stefansson et al., 2004). SNP rs3924999 changes an amino acid residue from Arg to Gln in the exon 3 of the *NRG1* gene, and is significantly associated with schizophrenia in one, but not the other, Han Chinese samples (Hong et al., 2004; Yang et al., 2003). SNP rs10503929 causes an amino acid residue change from Met to Thr in the exon 14. There has yet to be an association study to determine its role in schizophrenia susceptibility. The yet-to-be named novel SNP identified using subjects with history of psychosis from the Central Valley of Costa Rica is very intriguing (Walss-Bass et al., 2006a; Walss-Bass et al., 2006b). It is significantly associated with schizophrenia and with psychosis from the same population, and it changes an evolutionally conserved (and thus potentially functionally significant) amino acid residue from Val to Leu in the exon 13 of the *NRG1* gene. Changes between Val and Leu in the Presenillin-1 or Amyloid Precursor Protein (APP) have been associated with Alzheimer's disease, suggesting the significance of stereochemistry of the aliphatic

residues of Leu or Val in proper protein functions (Furuya et al., 2003; Jia et al., 2005; Murrell et al., 2000; Raux et al., 2000). Thus, this particular SNP deserves further analyses from more independent samples.

The lack of reproducible association of individual nonsynonymous SNPs of the *NRG1* gene with schizophrenia, and the abundant associated SNPs residing within the noncoding regions of the *NRG1* gene have led to the hypothesis that altered expression (as opposed to changes in amino acid sequences) of *NRG1* accounts for the contribution of NRG1 to increased schizophrenia susceptibility (Harrison and Weinberger 2005; Stefansson et al., 2002; Stefansson et al., 2004). Several studies have attempted to test this hypothesis using either postmortem tissues or peripheral leukocytes from schizophrenics (Hahn et al., 2006b; Hashimoto et al., 2004; Law et al., 2006; Petryshen et al., 2005). There are small but significant increases of Type I NRG1 (*Figure 2.3-1* and see later for isoform definitions) transcripts in the dorsolateral prefrontal cortex and the hippocampus and of Type IV transcripts in the hippocampus (Harrison and Law, 2006; Hashimoto et al., 2004; Law et al., 2006). The risk allele SNP*8NRG122132 was associated with decreased Type I NRG1 mRNA in controls but with increased Type I mRNA in schizophrenics. The underlying molecular basis for this complex interaction between SNP*NRG122132 and Type I NRG1 expression is unknown. Changes in Type IV NRG1 mRNA levels are also associated with the genotype at a specific risk SNP, SNP*NRG243177. In this case there is a gene dosage affect with the risk allele being associated with increased Type IV mRNA levels, an association that is more pronounced in schizophrenics than in controls, and that is associated with increased activity of reporter constructs in transfected kidney cells.

Despite these intriguing findings at the mRNA level, no changes in the overall NRG1 mRNA and protein levels have been documented (Hahn et al., 2006a; Hashimoto et al., 2004). ErbB4 hyperresponsiveness of brain slices from the schizophrenics to recombinant NRG1 was reported and the phenomena was linked to more pronounced suppression of NMDA receptor activation (Hahn et al., 2006a). It remains to be determined whether increased Type I or Type IV NRG1 expression or hyperresponsive ErbB4 signaling contributes to the development of schizophrenia, or are secondary to disease pathology (or both).

1.3 Neuregulin 1 Gene and Psychoses

In addition to the reported association between some SNPs in the *NRG1* gene and schizophrenia, several studies have reported associations of SNPs in the *NRG1* gene with bipolar disorder, psychoses, as well as late onset Alzheimer's disease with psychoses (Go et al., 2005; Green et al., 2005; Hall et al., 2006; Meeks et al., 2006; Thomson et al., 2007; Walss-Bass et al., 2006a). Green et al. reported that the strength of the association with the *NRG1* Hap_{ICE} haplotype was increased if only considering schizophrenia samples who had experienced mania. Similarly, when a subset of bipolar disorder cases with predominantly mood incongruent psychotic features was examined against the *NRG1* haplotype, the association was greater compared to that with all the bipolar disorder cases (Green et al., 2005). In their efforts of identifying markers across the *NRG1* gene that are associated with schizophrenia and bipolar disorder, Thomson et al. (2007) found that both region A and B within the *NRG1* gene (*Figure 2.3-1*) harbor risk alleles for both schizophrenia and bipolar disorder. With a group of young people at high risk of developing schizophrenia, Hall et al. (2006) found that SNPNRG243177 of Hap_{ICE} was associated with increased development of psychotic symptoms. They did not find association with other SNPs from the Hap_{ICE}; the association of Hap_{ICE} in general is lower than to SNPNRG243177 specifically. Alzheimer's disease is the major cause of dementia and is characterized by the presence of amyloid plaques and neurofibrillary tangles in the cortical areas and the medial temporal lobe of the brain (Blennow et al., 2006). Although cognitive deficits are hallmarks of Alzheimer's disease, there is increasing evidence that Alzheimer's disease is associated with increased psychoses, depression, and sleep disturbance (Meeks et al., 2006). The schizophrenia at-risk polymorphisms on the *NRG1* gene also are found to be associated with the increased psychosis of Alzheimer's disease (Go et al., 2005; Meeks et al., 2006). Together, these studies point to partial overlap in the genetic structure of schizophrenia and bipolar disorder, and further identify *NRG1* as a susceptibility gene for the development of psychoses (Craddock et al., 2005, 2006; Murray et al., 2004).

2 Neuregulin 1 and ErbB Genes

2.1 Neuregulin–ErbB Signaling: Meet the Extended Family

Products of the neuregulin 1 gene belong to a signaling network composed of four receptor tyrosine kinases and ~14–20 ligands. The core component of the system, found in organisms from worms to humans, is a ligand-dependent receptor tyrosine kinase and a ligand containing a core EGF (epidermal growth factor)-like domain. In *Caenorhabditis elegans*, this function is served by a single ligand receptor pair, encoded by lin-3 and let-23; *Drosophila melanogaster* also has a single receptor (DEGFR) but uses one of four ligands to activate it, spitz, gurken, keren, and vein (the latter being most closely related to the vertebrate neuregulins). An additional feature of this network in *Drosphila* is the presence of ErbB specific inhibitors, argos and kekkon. Argos is a secreted antagonistic ligand that inhibits ErbB signaling in a paracrine manner, whereas kekkon/LRIG-1 are transmembrane proteins that interact with, and inhibit ErbB receptors in a cell autonomous manner.

As we turn to vertebrates, the complexity grows to include four receptors: ErbB1, ErbB2, ErbB3, and ErbB4 (ErbB1 = EGFR; ErbB2 was originally identified as Neu, and in humans the alternative nomenclature is HER1–HER4). The ErbB receptors function as homodimers (ErbB1 and ErbB4) and as heterodimers (in particular ErbB1:ErbB2, ErbB2:ErbB3, and ErbB2:ErbB4). Signaling involves ligand-dependent activation of tyrosine kinase activity intrinsic to the receptors. Within the ErbB family, ErbB1, ErbB3, and ErbB4 bind ligands, whereas ErbB2 functions as a general coreceptor. Once homo- or heterodimers are activated, the intracellular kinase domains autophosphorylate and activate numerous signaling pathways. One persistent question is how, given the multitude of possible receptor–ligand combinations, signaling specificity is conferred. Both ErbB2 and ErbB3 must form heterodimers to function because ErbB2 is unable to bind ligand and ErbB3 lacks kinase activity.

Vertebrate evolution expanded not only the ErbB family, but the wealth of ligands as well: 14 genes are currently known to encode ErbB ligands. These can be divided into four groups based on their receptor binding specificity. EGF, transforming growth factor-α (TGF-α), amphiregulin, and epigen bind to ErbB1 only; heparin binding EGF (HB-EGF), epiregulin, and betacellulin bind to both ErbB1 and ErbB4, neuregulin 1 and 2 bind to ErbB3 and ErbB4, and neuregulin 3 and 4 appear to bind only to ErbB4.

All of the ErbB ligands are initially synthesized as single pass transmembrane proteins, with the exception of a subfamily of neuregulin 1 splice variants (discussed in detail later). Activation of ErbB receptors typically requires proteolytic cleavage of the precursor form of the ligand and release of the extracellular portion containing the EGF-domain. In vertebrates cell surface-associated ADAMS family (including ADAM 17 and 19) metalloproteases and the β-secretase enzyme (BACE-1) cleave in the extracellular juxtamembrane domain of neuregulin 1.

2.2 The Neuregulin Subfamily

The neuregulins are encoded by four distinct genes; *Nrg1*, *Nrg2*, *Nrg3*, and *Nrg4*. Within the neuregulin subfamily, *Nrg1* and *Nrg2* are most closely related, *Nrg3* is a bit more distant, and *Nrg4* is very distant. *Nrg1* and *Nrg2* share a number of features that are absent from *Nrg3* and *Nrg4*. Both *Nrg1* and *Nrg2* undergo complex alternative splicing resulting in the synthesis of multiple isoforms; in contrast, two isoforms of NRG3 and one NRG4 isoform have been described. NRG1 and NRG2 also bind to both ErbB3 and ErbB4 in contrast to NRG3 and NRG4, which only bind ErbB4. *Nrg1* and *Nrg2* are expressed in a variety of tissues, although the major site of expression for both is the nervous system. *Nrg3* expression is limited to the nervous system and testis, whereas *Nrg4* is expressed predominantly in the pancreas.

Nrg1 and *Nrg2* share the same basic exon/intron organization giving rise to similar functional domains in the protein products (Busfield et al., 1997; Carraway et al., 1997; Chang et al., 1997; Falls, 2003; Higashiyama et al., 1997; Ring et al., 1999; Yamada et al., 2000). *Nrg1* and *Nrg2* undergo alternative splicing resulting in expression of 15+ reported Nrg1 isoforms and 6–8 Nrg2 isoforms.

Type I, II, IV, and V isoforms of NRG1 and all NRG2 isoforms contain immunoglobulin (Ig)-like domains near their N-termini (~35% identity). The EGF-like domain is followed by hypervariable sequences, a transmembrane domain (90% identical) and a relatively long intracellular domain (overall about 35% identity). As a result of alternative splicing in the hypervariable region that follows the highly conserved EGF-like domain, both *Nrg1* and *Nrg2* products include membrane-anchored and secreted proteins. There are three regions in the intracellular domains of unknown function that share high levels of sequence identity as well (50–85%).

2.3 Neuregulin 1: Isoform Diversity

The neuregulin 1 (*NRG1*) gene spans about 1.5 million base pairs of human chromosome 8p (Falls, 2003; Stefansson et al., 2002; Stefansson et al., 2004). The general genomic organization of the neuregulin 1 gene is shown in *Figure 2.3-1* (note that the exons are drawn approximately to scale, the introns are not). In the human genome, the neuregulin 1 gene is located on chromosome 8 in the p12–p21 region. Linkage analysis and mapping experiments implicate this region in two conditions of great import for human health, genetic susceptibility to development of schizophrenia and cancer. Birnbaum and colleagues have identified breast and pancreatic tumor associated breakpoints within the *NRG1* locus and propose that the *NRG1* gene lies within a fragile site on chromosome 8.

Complex differential splicing coupled to utilization of multiple promoters generates more than 15 mRNAs encoding proteins with both shared and distinct signaling properties (discussed in depth later). Current nomenclature distinguishes isoforms based on the N-terminal domain (Types I–VI), one of two classes of EGF-like domains (alpha vs. beta), the highly variable juxtamembrane domain (1, 2, 3, or 4) and the length of the intracellular domain (the "c" form is the shortest, the "a" form the longest). The relative abundance of the multiple possible splicing isoforms in different cell types in vivo is not known.

The six major classes of NRG1 isoforms (Types I–III found in all vertebrates and IV–VI that appear to be primate-specific) are defined based on unique 5′ coding exons. It is generally assumed that these major isoforms are transcribed from distinct promoters. The activity of these promoters is believed to be differentially regulated, resulting in distinct temporal and spatial patterns of expression of Type I, II, III, and IV NRG1 (no information is available on expression patterns of Types V and VI) (Buonanno and Fischbach, 2001; Falls, 2003; Meyer and Birchmeier, 1994, 1995; Meyer et al., 1997; Shinoda et al., 1997; Wolpowitz et al., 2000; Yang et al., 1998). In general, Type I isoforms are most widely expressed, and play key roles in embryonic and postnatal development. Type II isoform expression is more restricted, being limited to both neuronal and nonneuronal cells of the nervous system. Expression of the Type III isoforms is restricted to subsets of peripheral and central neurons under normal conditions in vivo (Meyer et al., 1997; Wolpowitz et al., 2000; Yang et al., 1998). What combinations of cis-acting elements and transcription factors establish and maintain these expression patterns is not known. Neurotrohins, including BDNF and NT-3 induce increased NRG1 mRNA levels and NRG1 protein release from ventral spinal neurons in vitro (Loeb and Fischbach, 1997). BDNF specifically increased expression of Ig-containing isoforms, whereas NT-3 affected non-Ig containing isoforms. It is not clear whether the differential effect of BDNF and NT-3 on NRG1 isoform expression results from differential stimulation of distinct neuronal populations or divergence of signaling between activated TrkB versus TrkC and the *Nrg1* promoters.

This largely underexplored area of transcriptional regulation of neuregulin 1 expression is likely to be of importance for several reasons. First, changes in NRG1 expression occur in pathological conditions, including injury to the nervous system. Injury-associated changes in NRG1 expression include increased expression of Type I NRG1 isoforms in astrocytes after ischemia and decreased Type III NRG1 isoforms after nerve transection (Kerber et al., 2003; Lindholm et al., 2002; Parker et al., 2002; Tokita et al., 2001). In addition, as noted earlier, although Type III NRG1 expression in vivo is restricted to subsets of peripheral and central neurons this isoform is expressed in neoplastic Schwann cells (Stonecypher et al., 2005).

Second, although several haplotypes spanning the neuregulin 1 gene (*Figure 2.3-1*) are believed to confer a degree of susceptibility for developing schizophrenia, few changes that would alter protein function have been detected. A plausible explanation for how these noncoding polymorphisms contribute to disease

is that they alter the level of expression, the pattern of expression, or the relative expression of different isoforms of neuregulin 1, and that the resulting changes in NRG1 protein levels contribute to the pathology of this disease. Data consistent with this hypothesis include the finding of modest changes in the levels of Type I and Type IV NRG1 mRNAs in postmortem brains from schizophrenics compared with controls (discussed earlier). The additional demonstration that reduced expression of subsets of NRG1 isoforms in mice result in a number of profound phenotypes (see *Section 2.3.3*) underscores the point that altering the expression of NRG1 can contribute in a profound manner to pathology.

Classification of NRG1 isoforms does not simply reflect differential expression patterns; the distinct N-terminal domains impart functional differences as well (Buonanno and Fischbach, 2001; Falls, 2003; Han and Fischbach, 1999; Liu et al., 1998b; Liu et al., 2001; Loeb et al., 1998; Lu et al., 1995; Montero et al., 2000; Schroering and Carey, 1998; Wang et al., 2001). All functional NRG1 isoforms contain an EGF-like domain. The N-terminal portion of the EGF-like domain is identical for all isoforms, whereas there is extensive variability in the C-terminal region. The full significance of this hypervariability is not known, although there are clear differences in receptor binding affinity between the alpha and beta groups of isoforms (Beerli et al., 1995; Karunagaran et al., 1996; Ni et al., 2001; Pinkas-Kramarski et al., 1996; Pinkas-Kramarski et al., 1998).

The different N-terminal domains impart distinct properties that influence the manner in which each set of NRG1 isoforms signals to, or interacts with ErbB-expressing target cells. Types I, IV, and V NRG1 contain an Ig-like domain, Type II NRG1s contain both an Ig-domain and a so-called Kringle domain, and Type III NRG1s contain a cysteine-rich domain (Buonanno and Fischbach, 2001; Falls, 2003). Type I and IV NRG1s are expressed on the cell surface, where they undergo both basal and inducible proteolysis. Type II NRG1 contains a classic signal peptide, and many Type II mRNAs (e.g., glia growth factor 2, GGF2) terminate before the transmembrane domain. This Type II NRG1 undergoes constitutive secretion. Once released by either route, these Ig-containing NRG1 isoforms could, in principle, diffuse over some distance to interact with ErbB-expressing target cells.

Because the N-terminal cysteine-rich domain of Type III NRG1 contains a unique transmembrane domain, these isoforms remain membrane-tethered (Michailov et al., 2004; Schroering and Carey, 1998; Wang et al., 2001). Indeed, when Wang et al. quantified surface NRG1 levels in cells expressing comparable amounts of total Type I NRG1 or Type III NRG1, they found that there was at least 25 times more Type III NRG1 on the surface of cells than Type I NRG1 (Wang et al., 2001). Thus, NRG1 signals as soluble growth factors (Types I, II, IV, and presumably V and VI) and membrane-tethered ligands (Type III).

2.4 Neuregulin 1: Functional Diversity

Neuregulin was isolated initially as the putative ligand for the ErbB2/HER2 human oncogene (Corfas and Fischbach, 1993; Falls et al., 1993; Goodearl et al., 1993; Holmes et al., 1992; Peles et al., 1992; Wen et al., 1992). Almost simultaneously, NRG1 was discovered by several independent groups each searching for a molecule with a distinct biological activity; for example, as the agent responsible for inducing nicotinic acetylcholine receptor expression at the neuromuscular junction and at nerve–nerve synapses, and as a growth and survival factor for glial cells (Buonanno and Fischbach, 2001; Corfas et al., 1993; Falls, 2003; Yang et al., 1998). All of these biological functions require NRG1 binding to, and activating ErbB receptor tyrosine kinases. NRG1 binding to ErbB receptors activates numerous intracellular signaling pathways that ultimately regulate gene expression, cell proliferation, cell survival and differentiation, and cell motility (Agus et al., 2002; Alroy et al., 1999; Chausovsky et al., 2000; Esparis-Ogando et al., 2002; Flores et al., 2000; Fu et al., 2001; Goodearl et al., 1993; Guerra-Vladusic et al., 2001; Hellyer et al., 2001; Hijazi et al., 2000; Hobbs et al., 2002; Li et al., 2001; Marchionni et al., 1993; Pinkas-Kramarski et al., 1998; Riese et al., 1995; Rio et al., 1997; Russell et al., 1999; Si et al., 1999; Vartanian et al., 1997; Won et al., 1999). We refer to these "classical" responses in ErbB-expressing cells, as forward signaling responses.

2.5 Neuregulin 1: Novel Signaling Mechanism

The NRG1 C-terminal intracellular domain (NRG1-ICD) is required for membrane insertion, intracellular trafficking, and surface expression for Type I NRG1 isoforms. In addition, the NRG1-ICD mediates novel signal transduction events, at least for the Type III isoforms. The importance of the NRG1-ICD has been substantiated from several lines of direct and indirect evidence.

The NRG1-ICD is essential for cell survival. Mice in which a stop codon was introduced immediately following the transmembrane domain die during mid-gestation with a phenotype indistinguishable from that of mice in which all NRG1 isoform expression has been disrupted (the EGF-like domain, or pan-Nrg knockouts) (Liu et al., 1998a).

The NRG1-ICD is essential for surface expression of Type I Nrg 1(Liu et al., 1998a). In vitro experiments demonstrate that Type I isoforms of NRG1 lacking an intact intracellular domain fail to be expressed at the cell surface, and therefore fail to release soluble growth factor. Because the Type I NRG1 isoform is required for cardiac development, the so-called "transmembrane mutant" animals, the pan-knockout animals, and the Ig-mutant animals (in which the Ig-like domain common to all Type I and Type II isoforms is disrupted) all show the same embryonic lethal phenotype.

We recently generated a line of mice unable to express Type III NRG1 (Type 1 and Type II NRG1 expression was unaffected). Homozygous mutant animals die at birth because they are unable to breathe, secondary to death of the phrenic nerve (Wolpowitz et al., 2000). A detailed analysis of the birth, early differentiation, and death of motor and sensory neurons in these embryos demonstrated that there was a general, but not universal, progressive loss of specific pools of motor and sensory neurons that would normally express Type III NRG1. We proposed that during normal development, axonal Type III NRG1 interactions with ErbB receptors in the target fields (for example, ErbBs expressed on differentiating myotubes or on neurons in the dorsal horn of the spinal cord) elicits a signal that is required for stable neuron–target interactions (and synaptic maintenance) and for the survival of the Type III NRG1-expressing neurons.

Based on these observations, we have demonstrated that Type III NRG1 isoforms are in fact bidirectional signaling molecules that serve as both ligands for ErbB3 and ErbB4 RTKs and as receptors (Bao et al., 2004; Bao et al., 2003). Activation of Type III NRG1 as a receptor results in intramembranous proteolysis and release of the NRG1-ICD, which then translocates to the nucleus and alters gene expression. Thus Type III NRG1 activates ErbB receptor tyrosine kinases on target cells, and receives and transduces a "survival signal."

In sum, different isoforms of NRG1 are generated via distinct transcriptional regulatory mechanisms, and complex patterns of alternate splicing. The resultant family of signaling proteins utilize distinct albeit overlapping strategies for directing local cellular responses. As yet we do not have a clear picture of which aspects of NRG1 signaling are inappropriate in the development of neuropsychiatric disorders, such as schizophrenia, but postmortem studies point toward increased levels of specific isoforms, perhaps resulting in a combination of excess ErbB4 activation and an imbalance between paracrine and juxtacrine signaling. Possible sites for therapeutic intervention include differential manipulation of isoforms specific transcription and targeting one or both of the two proteolytic processing steps that are key for eliciting the full NRG1 signaling repertoire. Indeed, targeting the proteases (ADAMS family members) responsible for constitutive and controlled release of other ErbB ligands and of the proteases responsible for intramembranous processing of transcriptional regulators (e.g., the presenilins/γ-secretases) is being actively pursued.

3 Neuregulin 1 and Schizophrenia: Lessons from In Vitro and In Vivo Animal Studies

3.1 Mouse Models for Schizophrenia?

Genetically modified mouse models of human disease have proven invaluable in deepening our understanding of the molecular, cellular, and physiological events that underlie human diseases. Developing

adequate animal models for neuropsychiatric disorders, especially schizophrenia, has proven more intractable for several reasons. At the molecular and cellular level the "cause" of schizophrenia is believed to be the confluence of developmental, environmental, and genetic events. The underlying genetic contribution to this disease is strong, but is the result of the action (interaction) of multiple genes. In other words, no one gene by itself has a major impact on development of schizophrenia. Most DNA sequence changes associated with disease are not rare mutations. Instead, most represent polymorphisms in noncoding sequences, and as such are relatively common. The effects of these sequence changes remain largely unknown but are likely to alter in subtle ways the expression levels, expression patterns, and splicing patterns of the associated gene products, rather than grossly affecting protein function. Perhaps, more salient is the essential humanness of schizophrenia. Schizophrenia is a disease (or spectrum of disorders) that cannot be diagnosed by a quantifiable measure. Diagnosis relies on the observation and communication of a constellation of abnormal behaviors that range from difficult to impossible to model in other organisms. Despite these reservations and limitations, mouse models have proven effective at giving insight on the potential links between altered NRG1/ErbB signaling and schizophrenia. In particular, these mouse models are exceedingly useful for addressing a number of key questions including probing the function of these proteins in the development and function of key neural circuits and neurotransmitter systems that function abnormally in individuals with schizophrenia. (Arguello and Gogos, 2006; Braff et al., 2001; Braff and Light, 2005; Cannon, 2005; Castner et al., 2004; Chen et al., 2006; El-Ghundi et al., 2007; Geyer et al., 2001; Goldman-Rakic, 1999; Gottesman and Gould, 2003; Harrison and Weinberger, 2005; Kennedy et al., 2003; Lipska and Weinberger, 2002; O'Donovan et al., 2003; O'Tuathaigh et al., 2007; Owen et al., 2004; Rapoport et al., 2005; Swerdlow et al., 2000).

In this section, we will review first the literature dealing with how alterations in NRG1/ErbB signaling affect neurotransmitter systems and synapses that are hypothesized to underlie aspects of schizophrenia. Then we will discuss the results of behavioral analyses of NRG1 and ErbB mutant mice, focusing on behavioral tests designed to probe circuits associated with endophenotypes of the disease.

3.2 Neuregulin 1/ErbB Signaling and Synaptic Maturation

The pathology of schizophrenia has been associated with underlying deficits in the function of oligodendrocytes, in dopamine signaling (see Abi-Dargham, this volume), in the function of specific populations of cortical GABAergic interneurons (see Kristiansen, this volume), and in NMDA-mediated glutamatergic transmission (see Javitt, this volume) (Coyle and Tsai, 2004; Harrison and Weinberger, 2005; Hof et al., 2002; Lewis, 2000). Evidence linking NRG1–ErbB signaling to each of these targets comes from both in vivo studies in rodents and in vitro studies using tissue from a variety of species including human postmortem samples.

Myelination defects and schizophrenia. Microarray expression profiling of postmortem tissues revealed decreased expression of a number of genes whose products are essential for myelination and/or oligodendrocyte function including the NRG1 receptor, ErbB3 (Chambers and Perrone-Bizzozero, 2004; Dwork et al., 2007; Flynn et al., 2003; Hakak et al., 2001). Additional support for white matter abnormalities (although not necessarily myelination defects) comes from imaging studies (see Kubicki et al. (2007) for recent review). In the special case of COS, patients who carry *NRG1* at-risk haplotypes show different trajectories of white matter changes during adolescence than either controls or patients without *NRG1* risk alleles (Addington et al., 2007). NRG1–ErbB signaling is a critical regulator of oligodendrocyte proliferation, survival, and differentiation, both in vitro and in vivo. In mice, altering the *Nrg1* gene dosage affects the extent of myelination of peripheral nerves (Michailov et al., 2004; Taveggia et al., 2005) and disruption of oligodendroglial ErbB signaling alters myelination of central axons (Roy et al., 2007b). Based on these findings one might predict that decreased NRG1–ErbB3 signaling contributes to white matter changes that are associated with disease. In mouse peripheral nerves, regulation of myelination requires axonally presented, Type III NRG1 (Michailov et al., 2004; Taveggia et al., 2005). It is not clear whether this isoform specificity holds for proper myelination of central tracts as well, but if so (or at least if the requirement for axonally presented NRG1 holds), then the increased levels of soluble NRG1 (Types I and IV) predicted

from postmortem mRNA analyses (Hashimoto et al., 2004; Law et al., 2006) might interfere with oligodendrocytes–axon signaling.

Neuregulin 1 and dopamine. There is no data linking altered NRG1–ErbB signaling with the changes in dopaminergic transmission that characterize schizophrenia. However, several recent studies in rodents raise this as a potentially fruitful area of investigation. First, dopaminergic neurons in the ventral tegmental area and the substantia nigra express ErbB receptors and respond to acute application of NRG1 in vivo by releasing dopamine (Steiner et al., 1999; Yurek et al., 2004). Second, Roy et al. (2007a) have demonstrated that expressing a dominant negative mutant of ErbB4 in mouse oligodendrocytes not only affects myelination, but results in global upregulation of dopamine receptors (D_1 and D_2) and dopamine transporter.

Neuregulin 1 and GABAergic systems. There is a wealth of converging evidence for disfunction of cortical GABAerbic signaling in schizophrenia. Postmortem analyses have demonstrated reductions in the levels of expression of the 67 kD isoform of glutamic acid decarboxylase (GAD67), parvalbumin, the GABA membrane transporter, GAT-1, and specialized axon–axonal synapses between chandelier interneurons and layer 3 pyramidal neurons (Hashimoto et al., 2008; Hashimoto et al., 2003; Lewis, 2000; Lewis et al., 2005; Lewis et al., 2004; Volk and Lewis, 2002). The chandelier class of interneurons strongly inhibits pyramidal neuron firing and these interneurons have been postulated to impose synchrony on excitatory output from the cortex, and deficits in chandelier neuronal function is believed to contribute to impairments in cognitive tasks mediated by the dorsal lateral prefrontal cortex.

There are several levels at which NRG1–ErbB signaling could influence this population of cortical interneurons. Cortical interneurons are derived from progenitor populations in the medial ganglionic eminence (MGE). The immature neurons migrate tangentially from the MGE into the neocortex and then radially to occupy appropriate cortical layers. The tangential migration of these interneurons and the targeting of dorsal thalamic axons into the neocortex require NRG1–ErbB4 signaling (Flames et al., 2004; Flames and Marin, 2005; Lopez-Bendito et al., 2006). Adult mice mutant for ErbB4 or heterozygous for disruption of Type III NRG1 have decreased numbers of cortical interneurons (Flames et al., 2004; Johnson, 2007). Although it is logical that these decreases could represent migration failure during development, they might also represent loss of expression of the marker proteins used to identify them (including parvalbumin). What affect these decreases (in number, function, or both) has on circuit function and behavior is not clear. Second, acute treatment of brain slices with recombinant NRG1 peptides alters transcription of $GABA_A$ receptors, although the magnitude and direction of the response depends on the type of neuron studied (Okada and Corfas, 2004; Rieff et al., 1999). Third, cortical GABAergic axon terminals contain ErbB4 and activation of ErbB4 modulates the probability of GABA release (Woo et al., 2007). Thus, presynaptic NRG1–ErbB4 signaling can modulate the strength of GABAergic transmission.

Neuregulin and glutamate. Functional imaging, pharmacological, and postmortem studies all point to aberrant cortical glutamatergic transmission in schizophrenics (Coyle, 2004; Coyle and Tsai, 2004; Harrison et al., 2003; Harrison and Weinberger, 2005; Laruelle et al., 2003). NRG1–ErbB signaling affects glutamatergic transmission in a number of complex ways. As noted earlier, NRG1–ErbB signaling during early development is required for targeting of glutamatergic projections from the dorsal thalamus to the neocortex (Lopez-Bendito et al., 2006). In addition, NRG1–ErbB signaling is important for the radial migration of differentiating pyramidal neurons (Anton et al., 1997; Lopez-Bendito et al., 2006). How or whether the apparently subtle changes in NRG1–ErbB signaling that contribute to schizophrenia affect these early developmental events is not known.

NRG1–ErbB signaling contributes to activity-dependent synaptic plasticity in hippocampal and cortical slice cultures by regulating NMDA receptor levels and phosphorylation (Bjarnadottir et al., 2007a; Gu et al., 2005; Hahn et al., 2006a; Ozaki et al., 1997; Stefansson et al., 2002) and by regulating AMPA receptor trafficking (Kwon et al., 2005; Li et al., 2007). These studies report complex, and seemingly contradictory effects of NRG1–ErbB signaling at glutamatergic synapses. Several early reports found that acute exposure of hippocampal or cortical slices to recombinant NRG1 peptides resulted in decreased glutamatergic transmission (Gu et al., 2005; Huang et al., 2000; Kwon et al., 2005). In contrast, a more recent study of hippocampal CA3 to CA1 synapses found that decreasing either presynaptic CA3 NRG1 levels or postsynaptic CA1 ErbB4 levels prevented activity-dependent potentiation of glutamatergic transmission (Li et al., 2007). Another recent study provides a possible resolution to these contradictory results. Bjarnadottir et al.

compared glutamate synapses in wild-type animals with those from animals heterozygous for NRG1 mutations, and further tested the responses of these synapses to various concentrations of acutely applied NRG1 peptide (Bjarnadottir et al., 2007a). As seen in previous studies of hippocampal slices from wild-type animals, exogenous NRG1 over a wide range of concentrations interfered with LTP. In slices from heterozygotes, the magnitude of theta burst induced LTP was reduced relative to wild-type slices. Adding low concentrations of exogenous NRG1 to slices from NRG1 heterozygotes enhanced LTP, whereas high concentrations inhibited LTP. Taken together, these studies point to the need for a fine balance of NRG1–ErbB signaling with both deficient and excessive signaling interfering with synaptic plasticity (Bjarnadottir et al., 2007b; Gu et al., 2005; Hahn et al., 2006a; Kwon et al., 2005; Li et al., 2007; Role and Talmage, 2007). It is likely that there is a similar requirement for balanced signaling at other synapses and at extrasynaptic sites. Given the apparent sensitivity of the NRG1–ErbB signaling network to perturbations, it is becoming clearer how subtle changes in the levels and types of NRG1–ErbB interactions could alter the ability of key brain circuits to withstand additional genetic and environmental insults and contribute to disease.

3.3 Using Mutant Mice for *Nrg1* to Model Disease Endophenotypes

The ideal approach to studying the biological changes that underlie psychiatric diseases would be to examine the effects of introducing disease-associated mutations into model organisms. Most DNA sequences linking *NRG1* with schizophrenia do not change protein-coding sequences and are instead believed to alter the levels, timing and/or pattern of *NRG1* expression. As such, genetic studies of NRG1 (and ErbB receptors) in mice have utilized standard gene disruption techniques. A variety of mouse lines have been generated that carry mutations in the *Nrg1* gene, as well as lines with mutations in the ErbB2, ErbB3, and ErbB4 genes (*Table 2.3-1*). Homozygous disruption of each of these genes results in embryonic or perinatal lethality limiting their usefulness to studies of early brain development. Mice that are heterozygotes for targeted disruption of *Nrg1* (or ErbB2–B4) are viable and fertile and provide a window of opportunity for dissecting the effects of reducing Neuregulin 1 expression by half on CNS circuits in adult animals. A wide variety of behavioral assays have been performed on *Nrg1* heterozygotes (and to a lesser extent on ErbB mutant animals), with a special focus on assays that are believed to probe the function of circuits that underlie endophenotypes that characterize schizophrenia. Neuregulin 1 mutations that have been examined include those that eliminate all Neuregulin 1 expression as well as ones that eliminate Ig-NRG1 expression (Types I and II) (Bjarnadottir et al., 2007b; Gerlai et al., 2000), Type III NRG1 expression, or all NRG1 isoforms that contain the common transmembrane domain (all Type I and subsets of Types II and III) (Bjarnadottir et al., 2007b; Boucher et al., 2007a; Karl et al., 2007; O'Tuathaigh et al., 2006; Stefansson et al., 2002).

Not surprisingly, the results are complicated. Heterozygous *Nrg1*, ErbB2, ErbB3, and ErbB4 mutant mice are quantifiably different from their wild-type littermates (*Tables 2.3-1* and *2.3-2*). Some behaviors are altered in all mutants examined, whereas others are only associated with the disruption of specific NRG1 isoforms. In general, *Nrg1* mutant mice show elevated levels of activity in a novel open environment (Gerlai et al., 2000; Karl et al., 2007; O'Tuathaigh et al., 2006; Stefansson et al., 2002), have deficits in pre-pulse inhibition of an acoustic startle reflex (a model of sensorimotor gating) (Chen et al., 2008; Stefansson et al., 2002), have altered anxiety related behaviors (Karl et al., 2007), altered social interactions (Karl et al., 2007), deficits in working/short term memory (Chen et al., 2008), and altered responses to drugs such as THC and nicotine (Boucher et al., 2007a, b; Chen et al., 2008). Similar results have been noted in mice bearing mutations in ErbB receptors (see later).

The study of endophenotypes that characterize human diseases has greatly assisted the development of quantitative studies in other organisms. In the case of neuropsychiatric disorders, there has been a great effort devoted to the development of behavioral assays that maintain some degree of cross species validity and thus allow probing disease-associated endophenotypes. In the specific example of *Nrg1* mouse models of schizophrenia-associated endophenotypes, three behaviors stand out; hyperactivity in a novel environment, prepulse inhibition (PPI), and working/short-term memory. The psychotic symptoms in patients

Table 2.3-1
Summary of neuregulin 1 phenotypic alleles

Allele	Allele name	Allele definition
$Nrg1^{tm1Cbm}$	Targeted mutation 1, Carmen Birchmeier	A neomycin resistance gene replaces exons 9, 10, and 11, which encode the carboxy-terminus of the EGF domain of all known Nrg1 variants
$Nrg1^{tm1Fej}$	Targeted mutation 1, Frank E Jones	The endogenous locus was modified to contain a stop codon in exon 9 and single loxP sites flanking exons 9, 10, and 11. The mutation was designed to specifically target the α-isoform, which encoded by exon 9. RT-PCR analysis showed reduced levels of mRNA, putatively due to nonsense mediated decay
$Nrg1^{tm1Gne}$	Targeted mutation 1, Genentech	Replacement of the exon encoding the N-terminal half of the EGF domain with a neomycin cassette. This null mutation results in disruption of all splice forms of Nrg1
$Nrg1^{tm1Leth}$	targeted mutation 1, Lars E Theill	Disruption was caused by insertion of vector Part of exon 3, encoding the Ig domain, was replaced with a PGK-neomycin cassette
$Nrg1^{tm1Lwr}$	targeted mutation 1, Lorna W Role	Disruption of the exon 7 by insertion of a nonsense mutation and an Xba1 site in the exon encoding the CRD domain. A lox-P flanked neo gene is also inserted into the intron following the CRD-encoding exon
$Nrg1^{tm1Zhou}$	Targeted mutation 1, Mingdong Zhou	Specific disruption of the intracellular domain of the Nrg1 gene. A stop codon and a poly(A) sequence was introduced immediately 3′ to the coding region for the first three amino acids of the intracellular domain, followed by a neomycin cassette
$Nrg1^{tm2Cbm}$	Targeted mutation 2, Carmen Birchmeier	Exon 8 is fused to a lacZ gene. Coding sequences downstream of the fusion point, including the N-terminal half of the EGF domain, are deleted
$Nrg1^{tm2Zhou}$	Targeted mutation 2, Mingdong Zhou	The majority of exon 13 and a portion of intron 13 were replaced with a neomycin selection cassette inserted by homologous recombination. The deleted region encoded the transmembrane domain
$Nrg1^{tm3Cbm}$	Targeted mutation 3, Carmen Birchmeier	A floxed allele was generated that contained loxP sites inserted into intron 7 and in intron 1

with schizophrenia are related to increased dopaminergic transmission, which usually manifests as hyperactivity. In addition patients with schizophrenia show deficits in PPI and in working memory tasks.

Two lines of *Nrg1* mutant mice (TM-$Nrg1^{+/-}$ and EGF-$Nrg1^{+/-}$) exhibit hyperactivity in an open field assay (Gerlai et al., 2000; Stefansson et al., 2002), and acute clozapine administration normalized (TM-$Nrg1^{+/-}$) locomotor activity. Both TM-$Nrg1^{+/-}$ and CRD-$Nrg1^{+/-}$ mice have PPI deficits that are exacerbated with age (Boucher et al., 2007b; Chen et al., 2008; Stefansson et al., 2002). Chronic nicotine administration to CRD-$Nrg1^{+/-}$ mice ameliorates their PPI deficits (Chen et al., 2008), reminiscent of reported effects of nicotine on the functional deficits of schizophrenia (George et al., 2006; Kumari and Postma, 2005; Postma et al., 2006) (see Freedman, this volume). Working memory deficits are core to the cognitive deficits in schizophrenia and have been proposed to be a useful intermediate phenotype to study the genetic predisposition toward schizophrenia development (Glahn et al., 2003; Goldberg et al., 2003; Winterer et al., 2004). CRD-$Nrg1^{+/-}$ mice have impaired performance in a short-term memory/working memory test (Chen et al., 2008). In addition, *Nrg1* mutant mice show enhanced behavioral responses to delta 9-tetrahydrocannabinol, a result that might provide insight into gene–environment interactions that underlie progression to disease (Boucher et al., 2007b).

Table 2.3-2
Comparison of phenotypes in *Nrg1*

Nrg1 heterozygous mice	Ig domain[a, b]	CRD domain (Type III)	TM domain[c–f]	EGF domain[f, g]
Behavioral analyses				
Prepulse inhibition (PPI)	ND	Strong deficits	Weak deficits	ND
Locomotor activity	No difference	No difference	Hyperactivity	Hyperactivity
Memory	ND	Impaired STM/WM	ND	ND
Response to acute stress	ND	Blunted CORT	ND	ND
Anxiety-related assays	ND	No difference	Less anxious	ND
Drug effects				
Clozapine	Decreased activity	ND	Decreased activity	ND
Nicotine	ND	Ameliorate PPI deficits	ND	ND
Morphological studies				
Lateral ventricle (LV)	ND	Enlarged LV	ND	ND
PFC interneurons	ND	↓ PV + cells	ND	ND
Dendritic spine density	ND	↓ in VSub	ND	ND
Myelination[b, h]	No change (PNS)	Hypomyelination (PNS)	ND	Hypomyelination (PNS)
Synaptic functions				
NMDA receptors	ND	ND	↓ Phosphorylated NR2B	↓ NMDA receptors
Nicotinic ACh receptors	ND	↓ α7 nAChR protein	ND	ND
Synaptic transmission	ND	↓ Glu transmission	ND	Normal basal transmission
Short term plasticity	ND	ND	ND	Enhanced PPF
Long term plasticity	ND	Impaired	ND	Impaired

Note: Ig, immunoglobulin; CRD, cysteine-rich domain; TM, transmembrane; EGF, epidermal growth factor-like; ND, not determined; STM/WM, short-term or working memory; CORT, corticosterone; PNS, peripheral nervous system; PV, Parvalbumin; VSub, ventral subiculum; PPF, paired-pulse facilitation

It is a bit surprising that there is a paucity of published information on morphological analyses of *Nrg1* mutant mice that might link the behavioral parallels between these mice and disease endophenotypes with postmortem findings. As described in the previous sections, there is a *Nrg1* gene dosage effect on myelination, specifically the Type III NRG1 isoform (Michailov et al., 2004; Taveggia et al., 2005) and interference with NRG1 processing or ErbB signaling impairs cerebral myelination (Hu et al., 2006; Roy et al., 2007a; Willem et al., 2006). We have begun a systematic analysis of morphological changes in CRD-$Nrg1^{+/-}$ mice relative to their wild-type littermates. To date, these studies reveal several schizophrenia-related abnormalities. Compared with wild-type mice, heterozygous mice exhibit enlarged lateral ventricles, decreased parvalbumin (PV)-positive interneurons in prefrontal cortical regions, and decreased dendritic spine density of pyramidal neurons in the ventral hippocampal subicular region (Chen et al., 2008). Interestingly, the phenotype of enlarged lateral ventricle of CRD-$Nrg1^{+/-}$ mice is exacerbated by age, a finding that is reminiscent to imaging studies in patients with schizophrenia over time (Lieberman et al., 2001;

Marsh et al., 1994; Steen et al., 2006). Given these intriguing parallels, it is likely that additional studies along these lines will add to our understanding of the roles of NRG1–ErbB signaling at the cellular, circuit, and behavioral levels and provide insight into how disruption of normal NRG1–ErbB signaling contributes to disease.

3.4 Phenotypes of Mutant Mice for ErbB Receptors

In contrast to *Nrg1* heterozygous mice, there are relatively fewer reports on the behavioral and morphological phenotypes for ErbB2, ErbB3, or ErbB4 heterozygous mice (*Tables 2.3-2* and *2.3-3*). Normal locomotor activity is reported for ErbB2$^{+/-}$ and ErbB3$^{+/-}$ mice while ErbB4$^{+/-}$ mice exhibit hyperactivity and slight PPI deficits. There is no difference in myelination between ErbB2$^{+/-}$ and ErbB3$^{+/-}$ mice and control mice (Atanasoski et al., 2006; Michailov et al., 2004). In contrast to the hyperactivity reported for ErbB4$^{+/-}$ mice, DN-ErbB4 mice exhibit hypoactivity in an open field test (Roy et al., 2007a). Interestingly, mice with a conditional mutation of ErbB4 (see *Tables 2.3-2* and *2.3-3* for details) also display hypoactivity and impaired spatial Morris Water Maze memory (Golub et al., 2004). In addition, mice lacking ErbB4 in all noncardiac muscle cells have reduced numbers of prefrontal interneurons (Flames et al., 2004), consistent with a role of NRG1/ErbB signaling in regulating tangential migration of interneurons, and is reminiscent to the phenotypes described in CRD-*Nrg1*$^{+/-}$ mice (decreased PV-positive interneurons).

3.5 Type III Neuregulin 1 Heterozygous Mutant Mice as a Model for Understanding Disease Conditions, and for Testing Therapeutics for Schizophrenia

The Neuregulin 1 gene is one of the leading candidate susceptibility genes for schizophrenia (Harrison and Law, 2006; Harrison and Weinberger, 2005). Human genetic studies continue to implicate the association between the *NRG1* gene and schizophrenia (as described earlier). The pleiotropic functions of the NRG1 protein also make it a plausible etiological and pathological risk factor for schizophrenia as delineated earlier (also see review from (Falls, 2003) and (Harrison and Law, 2006)). Mutant mice for *Nrg1* and ErbB have provided us insights about roles of NRG1/ErbB signaling in circuits underlying sensorimotor gating, memory performance, and in synaptic functions relating to dopamine, glutamate, and acetylcholine. We argue that through analyses of mutant mice for *Nrg1* and ErbB4, we will be better informed about the possible roles of the NRG1/ErbB signaling in the endophenotypes and phenotypes of schizophrenia. Further, we will be able to use these mice to study molecular mechanisms underlying these phenotypes and test potential therapeutics for schizophrenia and psychotic symptoms. Further studies on the effects of antipsychotic drugs (e.g., clozapine) and of other drugs that improve symptoms of schizophrenia (e.g., nicotine) in *Nrg1* heterozygous mice will help us determine whether (1) behavioral, morphological, and synaptic impairments that result from altered NRG1 expression levels can be ameliorated by antipsychotic medication; (2) the therapeutic effects of antipsychotic medications are mediated by modulating NRG1/ErbB signaling.

Type III *Nrg1* heterozygous mice display a wide array of behavioral deficits and morphological alterations that are reminiscent of schizophrenia. This indicates that decreased Type III *Nrg1* expression leads to impairments in neural circuits whose malfunctions are implicated in schizophrenia. Together with postmortem findings of increased Type I and Type IV NRG1 mRNA expression (Hashimoto et al., 2004; Law et al., 2006), and increased or altered ErbB4 signaling upon NRG1 stimulation in schizophrenia tissues (Hahn et al., 2006a; Law et al., 2007), we propose that in the disease condition, there is an increased paracrine signaling by soluble NRG1. Increased soluble NRG1 disrupts bidirectional Type III NRG1, juxtacrine signaling, and results in excessive responses in ErbB-expressing neurons or glia and deficient

Table 2.3-3
Comparison of phenotypes in ErbB mutant mice

ErbB mutant mice	ErbB2$^{+/-}$ or ErbB3$^{(+/-)b, g}$	ErbB4$^{(+/-)g}$	Conditional ErB4^{i-k}	DN-ErbB4$^{l, m}$
Behavioral analyses				
PPI	ND	No/weak deficit	ND	ND
Locomotor activity	No difference	Hyperactivity	Hypoactivity/no difference[n]	Hypoactivity
Memory	ND	ND	Impaired SM	ND
Morphological studies				
Gross morphology (brain)	ND	ND	↓ PFC interneurons[o]	ND
Myelination	No change (PNS)	ND	ND	Hypomyelination[p] (CNS and PNS)
Synaptic functions				
Dopamine receptors	ND	ND	ND	↑ D1 receptors/ transporters
Synaptic transmission	ND	ND	ND	Enhanced dopamine transmission

Note: SM, Spatial Morris Water Maze memory; CNS, central nervous system
[a]Rimer et al. (2005)
[b]Michailov et al. (2004)
[c]O'Tuathaigh et al. (2006)
[d]Stefansson et al. (2002)
[e]Karl et al. (2007)
[f]Bjarnadottir et al. (2007)
[g]Gerlai et al. (2000)
[h]Taveggia et al. (2005)
[i]Thuret et al. (2004)
[j]Golub et al. (2004)
[k]Flames et al. (2004)
[l]Chen et al. (2006)
[m]Roy et al. (2007)
[n]Conditional ErbB4 mutant mice are generated with Nestin-cre X Floxed ErbB4 (Thuret et al., 2004; Golub et al., 2004). Thus these animals do not have ErbB4 expression in all the neurons. Heterozygous ErbB4 conditional mutant mice exhibit similar activity as the wild-type controls. However, homozygous ErbB4 conditional mutant mice exhibit hypoactivity compared with both wild-type and heterozygous mutant mice (Golub et al., 2004). In another paper, homozygous ErbB4 conditional mutant mice display normal locomotor activity and have grossly normal morphology in the substantial nigra pars comparcta (Thuret et al., 2004)
[o]Conditioanl ErbB4 mutants are generated by crossing Cardiac-HER4 × ErbB4$^{-/-}$ (Flames et al., 2004). Mice in this condition do not have functional ErbB4 signaling except for the cardiac cells
[p]DN-ErbB4 mutant mice are generated by placing a dominant negative ErbB4 under the CNP (2′, 3′-cyclic nucleotide 3′-phophodiesterase) promoter. This leads to disruption of the functions of ErbB2, 3, and 4 in the oligodendrocyte and myelinating Schwann cell lineages. In CNS, both optic nerve and corpus callosum are affected (Roy et al., 2007); in PNS, sciatic nerves of CNP-DN-ErbB4 mice are thinner compared with wild-type controls (Chen et al., 2006)

responses in Type III NRG1-expressing neurons. If our hypothesis is correct, then compounds that augment Type III NRG1 signaling might be useful in treatment. In summary, we suggest in-depth physiological studies on NRG1/ErbB signaling through mice with mutations in NRG1 and ErbB4 will help us gain significant understanding about the link between *NRG1* and schizophrenia.

Acknowledgments

Work cited from the authors' laboratories was supported by grants from the NIH (NS29071 and NS22061), NARSAD and a McKnight Brain Disorders Award.

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2.4 Synaptic Vesicle Associated Proteins and Schizophrenia

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_2.4,

Abstract: There is increasing evidence implicating proteins associated with the synaptic vesicle, a presynaptic organelle that is essential for neurotransmission and synaptic connectivity, to schizophrenia. This chapter reviews the postmortem, genetic, and behavioral data, examines the trends and their relevance.

List of Abbreviations: GABA, gamma aminobutyric acid; MAPK, mitogen-activated protein kinase; SNARE, soluble *N*-ethylmaleimide-sensitive fusion attachment protein receptor; SNP, single nucleotide polymorphism

1 Introduction

Schizophrenia is a severe, chronic disorder, in which affected individuals experience hallucinations, delusions, disorganized thinking, and a decline in social functioning. Although the etiology is unknown, schizophrenia is currently hypothesized to be a neurodevelopmental disorder, as well as a disease of aberrant synaptic connectivity. Heredity is known to play a strong role in schizophrenia, and multiple genes of small effect interacting with environmental factors are believed to contribute to this illness. This is consistent with the finding that the clinical presentation and outcomes are heterogeneous, and may relate to the various etiological factors leading to schizophrenia. In this regard, it seems likely that schizophrenia is not a unique disease, but rather, a collection of conditions that share a common pathophysiology.

In support of the neurodevelopmental basis of schizophrenia, several studies have found an increased incidence of schizophrenia with obstetric complications, malnutrition, infection during pregnancy, association with congenital abnormalities, and association with soft neurological signs during development (reviewed by Lewis and Levitt, 2002). On the other hand, numerous reports are consistent with alterations in neurotransmission and synaptic connectivity (Selemon and Goldman-Rakic, 1999; Lewis and Gonzalez-Burgos, 2000). For example, postmortem studies have documented cellular changes in the hippocampal formation in the absence of neurodegenerative changes (Harrison, 2004), decreased neuronal size (Selemon and Goldman-Rakic, 1999), and changes in the level or posttranslational processing of presynaptic and dendritic markers. These findings, in addition to DNA microarray studies (Mirnics et al., 2000; Hemby et al., 2002), have led many investigators to conclude that schizophrenia is a "disease of the synapse" (Andreasen et al., 1997; Harrison, 1999; Mirnics et al., 2001; Frankle et al., 2003; Eastwood, 2004). Thus, convergent findings from a variety of different research areas have suggested that alterations of neurotransmission and synaptic connectivity may be a fundamental feature of schizophrenia, which could lead to the symptoms of psychosis and cognitive deficiency that are characteristic of this disorder.

The molecular substrates for perturbed neurotransmission and synaptic connectivity in schizophrenia or other severe mental disorders remain unclear. These functions require appropriate information transfer between neurons, and in order to accomplish this task, both the presynaptic and postsynaptic compartments of the synapse must be functioning normally. Within the presynaptic terminal, the *synaptic vesicle* is central to the molecular machinery required for proper information transfer between neurons. The synaptic vesicle is a specialized organelle that stores neurotransmitter, is concentrated at presynaptic nerve terminals, and is essential for neurotransmission in both the peripheral and central nervous system. Due to its small, uniform size, only a limited number of proteins are associated with the synaptic vesicle membrane (Südhof, 2004; Coughenour et al., 2005), but many other proteins not bound to this organelle are also involved in regulating its function. Several studies have implicated specific synaptic vesicle proteins in schizophrenia, supporting the notion that regulation of this organelle could contribute to the synaptic misconnectivity believed to underlie this disorder.

It is noteworthy that some attempts have been made to visualize synaptic vesicles at an ultrastructural level in the brains of individuals with schizophrenia. In one study, tissue obtained from a temporal lobe biopsy of an individual with schizophrenia (Ong and Garey, 1993) showed many unusual asymmetric synapses, including clumped but numerous, synaptic vesicles located at short synaptic active zones. In another study, electron microscopic examination of the postmortem caudate nucleus revealed swelling of some axon terminals, shrinkage of some axon boutons and fewer synaptic vesicles (Uranova et al., 1996). These observations are similar to a series of prior postmortem electron microscopic studies, where

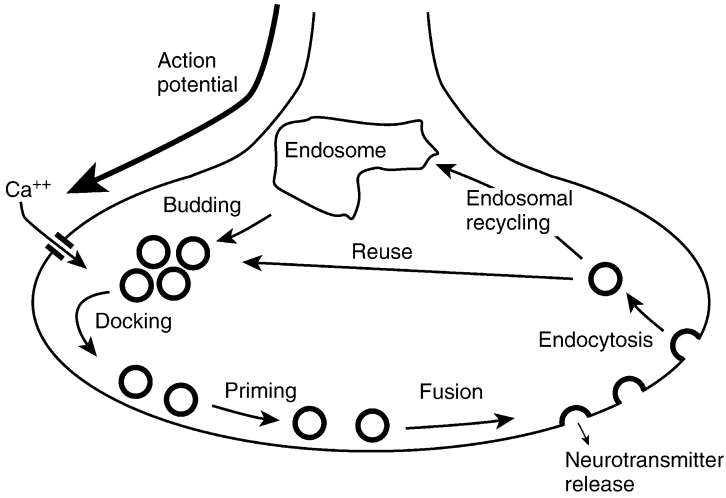
aggregation of synaptic vesicles were reported in the cortex of individuals with schizophrenia (Soustek, 1989; Soustek, 1990).

In this chapter, we will review the evidence suggesting a role for specific synaptic vesicle-associated proteins in schizophrenia. First, we present a brief overview of the synaptic vesicle cycle in the broader context of synaptic neurotransmission at chemical synapses. We then describe the experimental evidence linking specific molecular components of the synaptic vesicle to schizophrenia. Since not all synaptic vesicle proteins have been studied in relationship to schizophrenia, this review focuses only on those proteins for which such an effort was made. Finally, we describe the potential roles these proteins could play in the context of current etiological theories of schizophrenia, and discuss the relevance of the experimental findings in the context of this enigmatic disorder.

2 Neurotransmission at Chemical Synapses

Neurotransmission at chemical synapses is carried out by a complex sequence of events. Initially, an action potential arrives at the presynaptic terminal, inducing a rapid, transient influx of Ca^{2+} locally (*Figure 2.4-1*).

Figure 2.4-1
Neurotransmission and the synaptic vescile cycle



The elevation of presynaptic Ca^{2+} causes synaptic vesicles to fuse with the plasma membrane and release neurotransmitter into the synaptic cleft. Neurotransmitters then diffuse across the synaptic cleft and bind to specific receptors on the postsynaptic membrane. This can result in the opening or closing of ion channels, thus changing the probability that the postsynaptic neuron will fire an action potential, or the triggering of biochemical cascades within the postsynaptic neuron (collectively termed signal transduction). In this manner, neuronal communication occurs as information is transmitted from the presynaptic membrane to the postsynaptic membrane, and thus from one neuron to another.

Synaptic vesicles, once formed and transported to the presynaptic terminal, are not destroyed after each use, but are "recycled." A series of sequential steps has been determined whereby vesicles are used for neurotransmitter release, and then reassembled so that they can be used again – the so-called synaptic vesicle cycle (Südhof, 2004) (*Figure 2.4-1*). In the first step of the synaptic vesicle cycle, neurotransmitters

are actively transported into synaptic vesicles, which then accumulate in the active zone. Synaptic vesicles "dock" at the active zone and are "primed" to enable calcium-dependent fusion and pore opening, or exocytosis. After exocytosis, the vesicle is reformed by endocytosis, and neurotransmitter is again transported back into the vesicle. Any step within the synaptic vesicle cycle can potentially regulate neurotransmitter release.

Neurotransmitter release can also be regulated outside of the synaptic vesicle cycle, by the rate of synaptic vesicle formation and transport to the presynaptic terminal, or their spatial distribution. Within the synaptic vesicle cycle, steps such as docking, fusion, and endocytosis are regulated by a variety of proteins. Since many of these steps require energy, they can also be indirectly regulated by the local energy supply at the presynaptic terminal.

3 Molecular Components of Synaptic Vesicles Implicated in Schizophrenia

3.1 Synapsins

Synapsins were the first family of proteins discovered to be associated with synaptic vesicles (reviewed by Greengard et al., 1993), and have been shown to play dual roles in synaptic transmission and neurodevelopment, processes believed to be aberrant in schizophrenia. Synapsins are also substrates for a large number of protein kinases, which regulate the activity of many proteins via covalent transfer of a phosphate group from ATP. Unlike many proteins intimately linked to the synaptic vesicle cycle, synapsins can either bind or dissociate from synaptic vesicles depending on their phosphorylation state (Hosaka et al., 1999). The current hypothesis regarding the actions of synapsins is that these proteins regulate the size of the reserve pool of synaptic vesicles (Greengard et al., 1993; Hilfiker et al., 1999). Synapsins have also been implicated in the regulation of a variety of neurodevelopmental processes, including axon formation (Han et al., 1991; Ferreira et al., 1994; Chin et al., 1995; Ferreira et al., 2000), axon elongation (Ferreira et al., 1994; Chin et al., 1995), and synapse formation (Chin et al., 1995; Ferreira et al., 1995).

All synapsins have been implicated in the neurobiology of schizophrenia, but the current evidence is strongest for synapsins II and III. Initial postmortem studies suggested an involvement of synapsin I (Browning et al., 1993) and synapsin II (Grebb and Greengard, 1990) with schizophrenia. However, recent postmortem studies in the hippocampus (Vawter et al., 2002) or frontal cortex (Albert et al., 2002) have failed to demonstrate changes in synapsin I levels in schizophrenia. On the other hand, gene expression profiling using DNA microarrays has demonstrated that, of 9,846 genes analyzed, synapsin II was one of two genes (*N*-ethylmaleimide-sensitive factor (NSF), described later, is the other) that were the most specifically downregulated in the prefrontal cortex of individuals with schizophrenia (Mirnics et al., 2000). The same study showed no significant changes in 98% of the gene groups analyzed, and the synapsin II finding has been replicated in another gene-profiling study (Hof et al., 2002). These observations were also consistent with studies indicating that brain synapsin II levels were increased by chronic treatment with haloperidol, a conventional antipsychotic used to treat schizophrenia (Chong et al., 2002). None of the gene profiling studies examined levels of synapsins I or III. However, when proteins were quantitated, levels of synapsins II and III were found to be decreased by more than 50% in the postmortem hippocampi of individuals with schizophrenia (Vawter et al., 2002), and levels of synapsin III were also found to be decreased in the dorsolateral prefrontal cortex of individuals with schizophrenia (Porton and Wetsel, 2007).

Genetic studies have also provided evidence implicating synapsins II and III in schizophrenia. Both synapsins II and III are located in schizophrenia susceptibility loci, on chromosome 3p24 (Li et al., 1995; Pulver et al., 1995), and chromosome 22q12.3 (Kao et al., 1998; Gill et al., 1998), respectively. By contrast, synapsin I, which resides on the X chromosome, is not located on a schizophrenia susceptibility locus (Craddock et al., 2005). Both case-control and family-based association analyses support a role for synapsin II gene haplotypes in schizophrenia (Chen et al., 2004a; Chen et al., 2004b). Preliminary studies also suggest a similar association between single nucleotide polymorphisms (SNPs) residing in the synapsin III gene and schizophrenia (Chen et al., 2005). In addition, a missense polymorphism, S470N, which was identified in the synapsin III gene, was found to occur more frequently in individuals with

schizophrenia than in controls (Porton et al., 2004; Lachman et al., 2005). Other studies on synapsin III have described noncoding polymorphisms in this gene that are not associated with schizophrenia (Ohmori et al., 2000; Ohtsuki et al., 2000; Tsai et al., 2002). However, S470N was not detected in these reports, and factors such as genetic and racial background, diagnoses, and region of the gene analyzed, could account for the findings. S470N is of significance, because it prevents phosphorylation at Ser-470, a substrate for mitogen-activated protein kinase (MAPK), which is a downstream effector of neurotrophin action (Porton et al., 2004). Neurotrophins are known to regulate both neurodevelopment and neurotransmission through MAPK phosphorylation of neural substrates. Moreover, it was recently found that S470N affects neurotrophin-mediated neurotransmission, indicating that the polymorphism is indeed functional and may have relevance to schizophrenia (Cheng et al., in preparation).

Animal behavioral studies using mice with a deletion in the synapsin III gene (synapsin III KO) are also consistent with a role for this gene in schizophrenia (William Wetsel, personal communication 2008). These mice do not harbor obvious anatomical deficits or neurological disorders. However, synapsin III KO mice are hyperactive in the open field. Hyperlocomotion in rodents may be relevant to schizophrenia because it can be related to vulnerability to stress or sensitivity to neurotransmitters in this disorder (Lipska and Weinberger, 2000). Another neurophysiological parameter, sensorimotor gating, is deficient in schizophrenia (Braff et al., 2001), and can sometimes be reversed by administration of antipsychotics (Kumari and Sharma, 2002). Synapsin III KO mice are deficient in a test for sensorimotor gating, prepulse inhibition, and this deficit was restored by clozapine or raclopride, but not by haloperidol. This finding suggests that synapsin III may be related to a specific subtype of the disorder. In addition, synapsin III KO mice also exhibit abnormalities in latent inhibition (a test of attention), learning and memory, and contextual and cued fear conditioning (a test of emotional processing). Thus, synapsin III KO mice exhibit abnormalities in a battery of behavioral paradigms that test neural circuits, cognition, and emotional processing, that may have relevance to schizophrenia.

Moreover, a neurodevelopmental role for the synapsin III gene has clearly been established, and as discussed earlier, schizophrenia is believed to have neurodevelopmental origins. Synapsin III is expressed during early development (Porton et al., 1999; Pieribone et al., 2002; Porton et al., 2004), is important for axonogenesis, and its absence leads to abnormal axon growth and hypertrophied growth cones (Ferreira et al., 2000; Feng et al., 2002). Synapsin III is enriched in young precursor cells of the hippocampal dentate gyrus (Pieribone et al., 2002), a region of the brain, where neurogenesis is known to exist well into adulthood (reviewed by Gage, 2000). Recent studies demonstrate that synapsin III KO mice display abnormal neurogenesis (Kao et al., 2008), and there is increasing evidence that neurogenesis in the adult is highly relevant to neuropsychiatric disease.

3.2 Synaptotagmins

Increases in intracellular calcium stimulate exocytosis at presynaptic terminals by triggering the fusion of synaptic vesicles with the plasma membrane. The synaptotagmins, a family of proteins initially identified because they bind to calcium, are believed to be the calcium sensors involved in this regulated form of exocytosis (Jahn et al., 2003). Synaptotagmins interact with the plasma membrane proteins neurexin and syntaxin, and display increased affinity for lipid membranes when intracellular calcium levels are increased. The synaptotagmin gene family is surprisingly large, with at least 15 genes identified so far in mammals (Südhof, 2002; Fukuda, 2003; Inoue and Oishi, 2005). Most of the biochemical, genetic, and physiological studies have focused on synaptotagmins I and II. Synaptotagmin I is essential for calcium-mediated neurotransmission in the central nervous system, and deletion of this gene in mice is lethal (Geppert et al., 1994). Synaptotagmin II forms dimers with synaptotagmin I, and probably subserves a similar function to synaptotagmin I (Osborne et al., 1999). Of all the isoforms, the greatest evidence linking this gene family to schizophrenia has been found with synaptotagmins IV and XI. Curiously, both these isoforms are the only synaptotagmins unable to bind to calcium (von Poser et al., 1997), and synaptotagmin IV is most homologous to synaptotagmin XI within the rat species (Ferguson et al., 2001).

The human synaptotagmin IV gene has been mapped to chromosome 18q12.3 (Ferguson et al., 2000b), a region that was recently identified as a potential susceptibility locus for both schizophrenia and bipolar disorder (Walss-Bass et al., 2005). Synaptotagmin IV mRNA is expressed in the brain and is not detectable in nonneuronal tissues. The highest levels of synaptotagmin IV are found in the hippocampus, and lower levels found in the cortex and cerebellum. Synaptotagmin IV is the predominant synaptotagmin isoform in the hippocampus and neocortex during the first week of postnatal development, while levels steadily decline into adulthood (Berton et al., 1997). In contrast, synaptotagmin I levels increase and stabilize by postnatal day 15–20. The spatial and temporal pattern of synaptotagmin IV expression suggests that this isoform may play a role in neurodevelopmental functions, which are believed to be perturbed in schizophrenia, such as synaptogenesis and axon connectivity (Ferguson et al., 2001).

Behavioral studies conducted in mice lacking the synaptotagmin IV gene have also provided evidence suggesting that depletion of this gene could result in psychiatric symptoms. Mice possessing a homozygous deletion of the synaptotagmin IV gene (synaptotagmin IV knockout) showed no gross structural brain abnormalities and were viable (Ferguson et al., 2000a), indicating that this gene is not essential for long-term survival. Synaptotagmin IV knockout mice displayed impaired motor coordination, abnormal contextual fear conditioning, and deficiencies in hippocampal-dependent learning and memory (Ferguson et al., 2000a). Synaptotagmin IV knockout mice also displayed increased locomotion in the open field and reduced anxiety-like and depression-like behavior (Ferguson et al., 2004).

The human synaptotagmin XI gene is located on chromosome 1q21-22, a region where a major susceptibility locus for schizophrenia has been reported (Brzustowicz et al., 2000; Brzustowicz et al., 2004). Expression of this gene is induced by the atypical antipsychotic drug clozapine, and decreased by treatment with MK-108, an NMDA receptor antagonist (Kontkanen et al., 2002; Paulson et al., 2003). This finding suggests that expression of synaptotagmin XI could be related to the mental status of individuals with schizophrenia, since clozapine ameliorates symptoms while MK-801 worsens them. In addition, a tandem repeat of a 33-bp region with promoter-like activity has been identified immediately upstream from the transcription start site of the synaptotagmin XI gene. In a small genetic study, 2 out of 60 individuals with schizophrenia had 3 instead of 2 tandem repeats, while none were found in normal controls (Yokota et al., 2003). This finding could have significance since promoter-like activity was correlated with an increased number of repeat units (Yokota et al., 2003).

3.3 Synaptophysin I and its Homologs

Synaptophysin was the first integral synaptic vesicle membrane protein to be isolated and cloned, and antibodies against this protein are the most widely used immunohistological marker for synapses, accounting for most of the 3,000 citations quoting this protein (Valtorta et al., 2004). Although synaptophysin has been studied intensively for over a decade, the actual function of this protein is still unclear. Synaptophysin is one of the most abundant synaptic vesicle proteins, accounting for 7% of the protein in this organelle. Synaptophysin forms multimeric complexes that have been reported to form channels in lipid bilayers, suggesting a role in fusion and exocytosis. Synaptophysin also interacts with synaptobrevin II, a v-SNARE protein that will be discussed later. Taken together, synaptophysin may play a role in regulating the rate of fusion between vesicles and the plasma membrane (Valtorta et al., 2004).

To date, more postmortem studies in schizophrenia have been performed using synaptophysin as a synaptic marker than any other protein. However, the range of techniques, brain regions, and materials assayed make it difficult to compare the studies meaningfully. More than half the studies that examined synaptophysin protein did not find a statistically significant change in schizophrenia (summarized in *Table 2.4-1*). However, about one third of the protein studies and more than half of the RNA studies found a decrease in synaptophysin levels, suggesting significant synaptic pathology in at least a subset of individuals with schizophrenia. Differences in synaptophysin expression also varied greatly depending on the brain region examined. In the prefrontal cortex, a region of the brain implicated in schizophrenia (Weinberger and Berman, 1996), seven out of eight studies found no change in synaptophysin levels while

Table 2.4-1
Synaptophysin postmortem studies in schizophrenia

References	Method	Brain region	Protein	RNA	No. of cases of schizophrenia	No. of cases of controls
Browning et al. (1993)	Western blot	Hippocampus	No change		7	7
Chambers et al. (2005)	Immunocytochemistry	Hippocampus: dentate gyrus	Decreased		14	14
Davidsson et al. (1999)	Western blot	Thalamus	Not done		15	21
		Cerebellum	No change		12	7
		Cingulate gyrus	Decreased		18	12
		Temporal cortex	No change		13	9
		Parietal cortex	No change		15	10
		Hippocampus	Decreased		13	10
		Frontal cortex	No change		5	6
Eastwood and Harrison (1995a)	Immunoautoradiography	Medial temporal lobe	Decreased		11	14
Eastwood et al. (1995b)	In situ hybridization	Medial temporal lobe	Decreased	Decreased	7	13
Eastwood and Harrison (1999)	In situ hybridization	Hippocampus		Decreased	11	11
Eastwood et al. (2000)	Immunoautoradiography	Prefrontal cortex	No change	No change	19	19
	Western blot	Anterior cingulate	No change	No change	19	19
	In situ hybridization	Temporal cortex	No change	Decreased	19	19
		Occipital cortex	Decreased	Decreased	19	19
Eastwood and Harrison (2001a)	Western blot	Ant cingulate cortex	No change		15	14
Eastwood et al. (2001b)	In situ hybridization	Cerebellum		Decreased	16	16
Gabriel et al. (1997)	Immunocytochemistry	Cingulate gyrus	Increased		19	16
		Temporal cortex	No change		19	16
		Frontal cortex	No change		19	16
		Parietal cortex	No change		19	16
Glantz et al. (2000)	In situ hybridization	Prefrontal cortex		No change	10	10
Halim et al. (2003)	Western blot	Prefrontal cortex	No change		18	23

continued

Table 2.4-1 (continued)

References	Method	Brain region	Protein	RNA	No. of cases of schizophrenia	No. of cases of controls
Hemby et al. (2002)	Gene array	Entorrhinal cortex		Decreased	8	9
Honer et al. (1995)	ELISA	Cingulate gyrus	No change		18	24
Honer et al. 1999	Western blot	Anterior frontal cortex	Decreased (natural causes)		13	10
Karson et al. (1999)	Western blot Northern blot	Prefrontal cortex	Decreased	No change	14	12
Landen et al. (1999)	Radioimmunoassay	Left thalamus	Decreased		9	9
Landen et al. (2002)	Western blot	Cingulate cortex	Decreased		11	13
Mukaetova-Ladinska et al. (2002)	ELISA (synaptosomes)	Cerebellum	No change		8	8
Perrone-Bizzozero et al. (1996)	Western blot	Visual cortex Frontal lobe Prefrontal cortex	Decreased Decreased Decreased		5–10 5–6	4–6 4–6
Rioux et al. (2005)	Immunocytochemistry	Olfactory bulb	No change		14	17
Scarr et al. (2006)	Western blot	Prefrontal cortex		No change	20	20
Sokolov et al. (2000)	RT-PCR	Superior temporal gyrus		Increased	14	9
Tcherepanov and Sokolov (1997)	RT-PCR	Left middle temporal gyrus Left superior temporal gyrus		Increased Increased	7 5	4 4
Vawter et al. (1999)	Western blot	Hippocampus	No change		16	13
Vawter et al. (2002)	Western blot	Hippocampus	No change		16	13
Weickert et al. (2004)	In situ hybridization	Prefrontal cortex	No change		14	15
Young et al. (1998)	Immunocytochemistry	Hippocampus	Increased		13	13

only one study found a decrease. In the hippocampus, another brain region implicated in schizophrenia, three out of six studies found a decrease in synaptophysin levels. Most of the postmortem studies used synaptophysin as a marker for synapses, with changes in its expression interpreted as a sign of synaptic pathology. There have been no genetic studies of the synaptophysin gene in humans reported to date. The human synaptophysin gene is located on human chromosome Xp11.23-p11.22, a region of the genome, which has not been implicated in schizophrenia (Craddock et al., 2005). Thus, despite the many postmortem studies performed, it has not been established whether synaptophysin itself actually plays a role in schizophrenia.

Surprisingly, mice lacking the synaptophysin gene do not display any apparent electrophysiological abnormality (McMahon et al., 1996). Nor do these mice appear to have neurological abnormalities (McMahon et al., 1996), although formal behavioral testing has not been reported. The surprising lack of a phenotype in the synaptophysin knockout mice suggested the possibility of redundant genes compensating for the loss of synaptophysin, and led many investigators to seek homologs for synaptophysin. Out of this extensive search, several homologs were identified, including synaptoporin (also known as synaptophysin II), synaptogyrin, and several nonneuronal members of the synaptophysin family. It is believed that these synaptophysin homologs may play a similar role to synaptophysin, both in neurons as well as nonneuronal tissues.

Of the synaptophysin homologs, only synaptogyrin I has been investigated as a candidate susceptibility gene for schizophrenia. The synaptogyrin I gene is located on chromosome 22q13, a region implicated in schizophrenia by some studies (Gill et al., 1998), and is close to a region containing a CAG repeat expansion that is overrepresented in schizophrenia and bipolar disorder in the Indian population (Saleem et al., 2001). A recent screen for mutations revealed the presence of a nonsense mutation (Trp27Ter in exon 2) of the SYNGR1 gene in an Indian family; one unaffected and three siblings with paranoid schizophrenia possessed the mutation (Verma et al., 2004).

Like synaptophysin, the function of synaptogyrin I is still unclear. Synaptogyrin I knockout mice do not have an apparent phenotype. Both the synaptophysin I and synaptogyrin I genes have been knocked out in mice simultaneously (Janz et al., 1999). Electrophysiological studies showed that short-term and long-term plasticity were dramatically reduced in the synaptophysin/synaptogyrin double knockout mice. This study demonstrated that synaptophysin I and synaptogyrin I perform redundant functions in synaptic plasticity, and that more than one gene may need to be disrupted in order to reveal a phenotype.

3.4 SNARE Proteins and Their Regulators

Protein transport between intracellular compartments is mediated by a mechanism that is well-conserved among all eukaryotes, from yeast to man. The transport mechanism involves carrier vesicles that bud from one organelle and fuse selectively to another. Specialized proteins are required for vesicle transport, docking, and fusion, and they have been generically named SNAREs (an acronym for soluble *N*-ethylmaleimide-sensitive fusion attachment protein receptor). SNAREs have been divided into those associated with the vesicle (termed v-SNAREs), and those associated with the target (termed t-SNAREs). The key protein, which led to the discovery of SNAREs was NSF, an ATPase found ubiquitously in all cells, and involved in numerous intracellular transport events. The subsequent identification of soluble proteins stably bound to NSF, the so-called SNARE complex, led to the formulation of the SNARE hypothesis, which posits that all intracellular fusion events are mediated by SNAREs (Rothman, 2002).

In neurons, the SNARE complex consists of three main proteins: the v-SNARE synaptobrevin or VAMP (vesicle-associated membrane protein), and two t-SNAREs, syntaxin and SNAP-25 (synaptosomal associated protein of 25 kD). Synaptobrevins traverse the synaptic vesicle membrane in an asymmetric manner: a few amino acids are found inside the vesicle, but most of the molecule lies outside the vesicle, within the cytoplasm. Synaptobrevin makes contact with another protein anchored to the plasma membrane of the presynaptic neuron, syntaxin, which is associated with SNAP-25. Via these interactions, the SNARE proteins play a role in the docking and fusion of synaptic vesicles to the active zone.

Because of their critical role in neurotransmitter release, SNARE proteins represent a potential molecular substrate for synaptic dysfunction or misconnectivity. Accordingly, a number of postmortem studies have reported changes in the level of expression for SNARE proteins in schizophrenia.

Several studies have consistently implicated SNAP-25 in schizophrenia. Postmortem SNAP-25 protein was found to be decreased in the hippocampus (Young et al., 1998; Fatemi et al., 2001; Thompson et al., 2003a), prefrontal cortex (Thompson et al., 1998; Karson et al., 1999), temporal cortex (Thompson et al., 1998), anterior frontal cortex (Honer et al., 2002), and cerebellum (Mukaetova-Ladinska et al., 2002) of individuals with schizophrenia. Conversely, elevated SNAP-25 in cerebrospinal fluid of patients with schizophrenia was observed (Thompson et al., 1999; Thompson et al., 2003b). One study failed to find a change in SNAP-25 in the prefrontal cortex (Brodman's area 9) of individuals with schizophrenia, but did find an increase in individuals with bipolar disorder (Scarr et al., 2006).

Behavioral studies in mice lacking the SNAP-25 gene also suggest that a deficiency in this gene could give rise to psychiatric symptoms. These mice exhibit hyperactivity that can be reduced upon administration of amphetamines, a drug that acts presynaptically, suggesting that these mice could be a model for attention deficit disorder (Hess et al., 1996). It should be noted that individuals with schizophrenia also have deficits in attention, and there is high comorbidity of schizophrenia with attention deficit disorder (Stahlberg et al., 2004).

Postmortem studies have also implicated the other SNARE proteins, syntaxin and synaptobrevin, in schizophrenia. Syntaxin protein levels were found to be increased in the cingulate gyrus of individuals with schizophrenia (Gabriel et al., 1997; Honer et al., 1997), but normal in other regions of the cortex (Gabriel et al., 1997). Synaptobrevin protein levels were found to be decreased in the prefrontal cortex of individuals with schizophrenia, but other vesicular proteins such as SNAP-25, syntaxin, and synaptophysin were unchanged in the same study (Halim et al., 2003). In accord with these findings, the mRNAs of several presynaptic proteins, including synaptotagmin I, synaptobrevin I and II, syntaxin IA, and SNAP-25, were also found to be decreased in the temporal cortex of individuals with schizophrenia (Sokolov et al., 2000).

The changes in SNARE protein expression do not appear to be due to the effects of antipsychotic treatment. In rats, haloperidol and chlorpromazine increased SNAP-25 protein levels in the hippocampus (Barr et al., 2006), while in the postmortem brains of individuals with schizophrenia, SNAP-25 levels are lower in the hippocampus (Young et al., 1998; Fatemi et al., 2001; Thompson et al., 2003a). Likewise, no changes in the level of mRNA encoding SNAP-25, syntaxin or synaptobrevin were observed in the prefrontal cortex of rats chronically treated with haloperidol (Nakahara et al., 1998).

Some genetic studies have also implicated SNARE proteins in schizophrenia. Polymorphisms identified in the promoter region of SNAP-29, a member of the SNAP-25 gene family, have been associated with schizophrenia (Saito et al., 2001; Wonodi et al., 2005). SNAP-29 is located on chromosome 22q11, a region that also harbors the velocardiofacial microdeletion, a chromosomal abnormality strongly associated with schizophrenia (Saito et al., 2001). A polymorphism identified in the SNAP-25 gene, located on chromosome 20p12, did not show any association with schizophrenia (Tachikawa et al., 2001); however, some SNAP-25 polymorphisms may be associated with treatment response (Muller et al., 2005). Polymorphisms in the syntaxin IA gene have also been associated with schizophrenia (Wong et al., 2004). To date, no genetic studies have been performed on the synaptobrevin gene.

The SNARE complex is controlled by a multitude of other proteins, and the complex probably regulates other proteins as well. One protein critical for SNARE function is NSF, which was initially found along with synapsin II to be one of the most significantly downregulated genes in schizophrenia (Mirnics et al., 2001). However, two subsequent studies failed to replicate this original finding (Imai et al., 2001; Gray et al., 2006).

Another set of proteins that bind to the SNARE complex are complexins I and II. These small proteins (~15 kD) are highly enriched in brain, particularly at synapses. Complexins are implicated in calcium-mediated neurotransmitter release, where their role is thought to be in the stabilization of the SNARE complex (Marz and Hanson, 2002). Both complexins are also implicated in schizophrenia, as levels of complexin I and II mRNA are decreased in the temporal lobe and hippocampus (Eastwood and Harrison, 1998), while levels of complexin II mRNA are decreased in the cerebellum (Eastwood et al., 2001b). This is consistent with the finding that complexin I protein is decreased in the prefrontal cortex (Sawada et al., 2002), and both complexins I and II protein are decreased in the hippocampus (Sawada et al., 2005) of

affected individuals. Mice lacking either the complexin I (Glynn et al., 2005) or II (Glynn et al., 2003) gene possess severe neurological deficits, and these mice also exhibit behaviors suggesting that complexins could be involved in psychiatric symptoms. Among the behavioral abnormalities observed in complexin I knockout mice are deficits in grooming and rearing behavior, and reduced exploration behavior. Complexin II knockout mice display poor performance on hippocampal-dependent memory and learning tasks only when they are maternally deprived (Yamauchi et al., 2005). Genetic studies have not revealed evidence of association of complexins to schizophrenia (Kishi et al., 2006), but there is a report suggesting that if variants of both the complexin II and synapsin II genes are evaluated, a positive association with schizophrenia is observed (Lee et al., 2005).

4 Potential Roles of Synaptic Vesicle Associated Proteins in the Etiology of Schizophrenia

What potential roles could synaptic vesicle associated proteins have in the context of two major theories regarding the etiology of schizophrenia, namely the "synaptic" hypothesis and the "neurodevelopmental" hypothesis?

With respect to the "synaptic" hypothesis, several neurotransmitter systems have been implicated in schizophrenia, including dopamine, glutamate, and gamma aminobutyric acid (GABA). Dopamine is implicated because all conventional antipsychotics are antagonists which bind to the dopamine D2 receptor. Glutamate is also implicated because drugs that antagonize the NMDA receptor can mimic the symptoms of schizophrenia, but other agents that modulate this site can ameliorate symptoms (Javitt, 2004). GABA, the major inhibitory neurotransmitter in the brain, plays modulatory roles in regulating dopaminergic and glutamatergic neurotransmission. Some postmortem studies also suggest that GABAergic neurons may be affected in schizophrenia (Lewis et al., 1999).

Since the presynaptic machinery for all these neurotransmitter systems is similar, it would not be surprising that perturbation of specific synaptic vesicle proteins could disrupt some or all these systems. Indeed, there is evidence that specific synaptic vesicle associated proteins preferentially affect neurotransmitter systems, potentially creating an imbalance in circuits that have been hypothesized to be relevant for psychiatric disorders (Benes, 2000). For example, synapsin I preferentially regulates glutamatergic neurotransmission (Jovanovic et al., 2000), but if all synapsins are depleted in mice, the primary deficit is in GABAergic neurotransmission (Gitler et al., 2005). Pharmacological studies in synapsin III KO mice suggest that some of their behaviors are due, in part, to a hyperdopaminergic state (William Wetsel, personal communication 2008). SNAP25 deficiency leads to dysregulation of catecholamine regulation (Jones et al., 2001). In addition, postmortem studies have revealed distinct regional differences in levels of complexin I and II in the brains of individuals with schizophrenia. This is significant because complexin I is associated primarily with inhibitory synapses, while complexin II is associated with excitatory synapses (Eastwood and Harrison, 1998). Taken together, the findings raise the possibility that subtypes of schizophrenia might be explained by variable differences in synaptic vesicle associated proteins. This is of practical importance because schizophrenia is a heterogeneous disorder, and affected individuals respond differently to medication.

Schizophrenia is currently conceptualized as being a neurodevelopmental disorder. How do proteins associated with synaptic vesicle function fit into this paradigm? Although the vast majority of studies have focused on these proteins as regulators of neurotransmitter release, there is accumulating evidence that specific synaptic vesicle proteins are also involved in neurodevelopment. Synapsins are probably the best known example of a synaptic vesicle protein that plays dual roles in neurodevelopment as well as synaptic transmission. It is well-documented that these proteins regulate nerve terminal development (Han et al., 1991), axon formation (Ferreira et al., 2000), axon elongation (Chin et al., 1995), synapse development (Chin et al., 1995; Li et al., 1995), and synapse maintenance (Ferreira et al., 1995). Moreover, synapsins have also been implicated in neurogenesis (Pieribone et al., 2002; Kao et al., 2008), which is intriguing since growing evidence suggests a relationship between psychiatric disorders and neurogenesis. For example, studies have shown that neurogenesis is disrupted by depression and stress (McEwen, 2000; Duman et al., 2001),

but stimulated by antidepressants (Malberg et al., 2000), lithium (Chen et al., 2000) and atypical antipsychotics (Wakade et al., 2002). In addition, the SNARE protein, VAMP2, stimulates axon elongation, while SNAP-25A stimulated neurite sprouting (Shirasu et al., 2000; Kimura et al., 2003). Synaptotagmins have also been shown to stimulate process formation (Fukuda and Mikoshiba, 2000). Thus, synaptic vesicle-associated proteins can have dual roles in neurotransmission and neurodevelopment. The precise mechanism by which this occurs remains to be determined.

5 Discussion

The synaptic vesicle and proteins associated with this organelle are critical for neurotransmitter release and synaptic connectivity, and have been intensely investigated in schizophrenia. Although evidence for a link between synaptic vesicle proteins and schizophrenia has been gathered from different investigations, the great majority of studies involve postmortem brain analyses of RNA or protein. It is well-known that there are limitations to the quantification of protein or RNA in postmortem tissue. For example, the postmortem time interval, age, medical condition, and cause of death can all have significant effects on the quality of RNA or protein. Another concern is that schizophrenia is likely a heterogeneous disorder, and subtype differences would not be observed in a postmortem study using a small number of samples. Finally, individuals with schizophrenia have high comorbidity with substance abuse and medical illnesses, and have often received years of treatment with antipsychotics. All these factors could confound the final interpretation of these studies. Nonetheless, independent laboratories have reported consistent changes in the expression of certain vesicle-associated proteins in schizophrenia, including synaptophysin, synapsins, complexins, and SNARE proteins.

Genetic studies have also been employed to examine the role of genes encoding synaptic vesicle proteins, their polymorphisms, and their relationship to schizophrenia. To date, linkage analyses have not conclusively identified any one gene which confers susceptibility to schizophrenia, but has identified loci where putative candidate genes could reside (Craddock et al., 2005). Several genetic association studies have identified gene variations in synapsins, SNAP-25, syntaxin, and possibly complexin II, which may have relevance for schizophrenia. However, only a few potentially functional polymorphisms in candidate genes have been identified to date, and these findings need to be replicated in much larger populations. Clearly, more research needs to be conducted in this area. In order to achieve meaningful results, statistical analyses examining the effect of multiple gene variants (or SNPs) simultaneously may need to be performed. A recent example of this type of study demonstrated that variants of both the synapsin II and complexin II genes were associated with greater susceptibility to schizophrenia than either gene variants considered alone (Lee et al., 2005).

Behavioral studies of genetically engineered mice have also provided intriguing evidence that disruption of some synaptic vesicle genes could result in psychiatric disorders. For example, hyperactivity in mice may be similar to the agitation observed in some psychiatric disorders, and could be related to a hyperdopaminergic state. Accordingly, hyperactivity has been reported in mice bearing a deletion in the genes for synapsin III (William Wetsel, personal communication 2008), synaptotagmin IV (Ferguson et al., 2001), and SNAP-25 (Hess et al., 1996). In addition, deficits in emotional processing (as assessed by fear conditioning) and cognitive testing have been reported for mice lacking synaptotagmin IV (Ferguson et al., 2001).

It may be useful to consider the different types of evidence linking specific synaptic vesicle-associated proteins with schizophrenia (*Table 2.4-2*). The evidence implicating some proteins, such as synapsin III, SNAP-25, and complexins, in schizophrenia has been accumulated at postmortem, genetic, and behavioral levels.

Although we have discussed specific synaptic vesicle-associated proteins in this chapter, it is important to recognize that these proteins do not function alone. Extensive protein–protein interactions are required for the orchestrated release of neurotransmitter at the synaptic cleft. SNARE proteins form a complex that must interact with synaptotagmins, synaptophysin, and other molecules for fusion and exocytosis to occur. Synapsins bind to multiple signaling molecules as well as the synaptic vesicle and actin, in order to

Table 2.4-2
Evidence linking proteins associated with the synaptic vesicle to schizophrenia

Protein	Postmortem data	Human genetic data	Mouse behavioral data
Synapsin I	Yes	No support	Not done
Synapsin II	Yes	Yes	Not done
Synapsin III	Yes	Yes	Yes
Synaptotagmin IV	Not done	Not done	Yes
Synaptophysin	Yes	Not done	No support
Synaptogyrin I	Yes	Yes	No support
SNARE proteins and their regulators			
Synaptobrevin	Yes	Not done	Not done
N-ethylmaleimide-sensitive factor (NSF)	Little support	Not done	Not done
SNAP-25	Yes	Yes	Yes
Complexins	Yes	Yes	Yes

exert its action. In order to reveal phenotypes, more than one synaptic vesicle gene may need to be deleted in mice, as is the case for synaptophysin and synaptogyrin. This theme is reiterated in the notion that schizophrenia is a complex genetic disease involving multiple genes that exert small effects on overall susceptibility. Thus, if genes associated with synaptic vesicle function are involved in schizophrenia, it would not be difficult to appreciate that they must interact to produce the symptoms characteristic of the disorder.

In summary, a convergence of research from diverse areas of investigation – postmortem, genetic, and behavioral studies – has implicated proteins associated with the synaptic vesicle in the pathophysiology of schizophrenia. This general line of research has implications for dissecting the genetic complexity, understanding the pathogenesis, and devising treatment strategies for this complex illness.

Acknowledgments

Supported in part by NS 047209 (H. T. Kao) and MH070898 (B. Porton).

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2.5 Redox Dysregulation in Schizophrenia: Genetic Susceptibility and Pathophysiological Mechanisms

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_2.5,

Abstract: Schizophrenia is a severe, frequent, chronic and complex disease implying an interplay of genetic, environmental and developmental vulnerability factors. Its physiopathology involves an impairment of neural connectivity both at the functional and structural levels, and various transmitter systems. Increasing evidence points to the involvement in the disease of oxidative stress and redox imbalance, although their origin is still elusive. Glutathione plays a major role as a redox regulator and is required as antioxidant for cellular protection against oxidative damage. It is synthesized by the rate-limiting enzyme glutamate-cysteine ligase (GCL).

The evidence for its involvement in schizophrenia is summarized below.

- Glutathione synthesis is impaired in patients at the level of the GCL enzyme, as measured in fibroblasts. In addition, genetic analysis showed an association between schizophrenia and the two GCL subunits. These findings are consistent with low brain and cerebrospinal fluid (CSF) glutathione levels.
- It is proposed that a brain glutathione deficit leads to the depression of *N*-methyl-D-aspartate (NMDA) responses and affect synaptic contacts in catecholamine innervated regions.
- Experimental pharmacological in vitro models in which glutathione is decreased reveal a hypofunction of NMDA receptors. In vivo developmental models show a reduction of dendritic spine density and of γ-aminobutyric acid (GABA)-parvalbumin immunoreactive interneurones in prefrontal cortex, as well as memory and sensory integration impairments. These anomalies are similar to that observed in schizophrenia.
- In a clinical trial, oral administration of *N*-acetyl-cysteine, a glutathione precursor, improves clinical symptoms and mismatch negativity.
- The data are compatible with the dopamine, glutamate and neurodevelopmental hypotheses as well as with schizophrenia comorbidities, including cardiovascular diseases, diabetes, viral infections, neonatal complications and hyperhomocysteinemia.

A redox/antioxidant dysregulation due to an impairment of glutathione synthesis of genetic origin is a vulnerability factor for schizophrenia.

List of Abbreviations: BSO, Buthionine Sulfoximine; CAT, Catalase; CSF, Cerebrospinal Fluid; DA, Dopamine; GAD-67, Glutamate Decarboxylase 67; GCL, Glutamate-Cysteine Ligase; GCLC, Glutamate-Cysteine Ligase Catalytic Subunit; GCLM, Glutamate-Cysteine Ligase Modifier Subunit; GGT, γ-Glutamyl Transpeptidase; GPX, Glutathione Peroxidase; GSH, Glutathione; GSS, Glutathione Synthetase; GSSG, Oxidized Form of Glutathione; GSTM, Glutamyl-S-Transferase; LPS, Lipopolysaccharide; MMN, Mismatch Negativity; NAC, N-Acetyl Cysteine; NEG, Negative; ODS, "Osteogenic Disorder Shionogi"; Oligo, Oligodendrocytes; PFC, Prefrontal Cortex; POS, Positive; PV, Parvalbumin; RNS, Reactive Nitrogen Species; ROS, Reactive Oxygen Species; SOD, Superoxide Dismutase; t-BHQ, *tert*-Buthylhydroquinone; TNR, Trinucleotide Repeat

1 Schizophrenia, Disconnectivity Syndrome and Oxidative Stress

Schizophrenia remains a multifaceted disorder, with all evidence of its onset and etiology pointing to a complex interplay of genetic, environmental and developmental factors.

1.1 Etiopathogeny

Family, twin and adoption studies have demonstrated that the morbidity risk of schizophrenia in relatives correlates with the degree of shared genes. However, the mode of inheritance of the disease is not simple. Numerous studies have focused on identifying genetic vulnerability factors. Results from several genome-wide scans (Barr et al., 1994; Coon et al., 1994; Blouin et al., 1998; Brzustowicz et al., 1999; Brzustowicz et al., 2000) have identified chromosomal regions of interest, and cumulative evidence from replication efforts suggest that schizophrenia susceptibility genes may be found on chromosomes 1, 6, 8, 10, 13 and 22 (see review Pulver, 2000; Faraone et al., 2002; Lewis et al., 2003; Mcguffin et al., 2003; Carlson et al., 2004).

Recently, several putative susceptibility genes were identified: the most promising being neuregulin (see Chapter 2.3, this volume) (Stefansson et al., 2004), dysbindin or DTNBP1 (Benson et al., 2004), GCL catalytic subunit (Gysin et al., 2007), GCL modulatory subunit (Tosic et al., 2006), catechol-*o*-methyltransferase (COMT) (Egan et al., 2001; Shifman et al., 2002; Glatt et al., 2003); see also negative findings (Fan et al., 2005; Munafo et al., 2005; Williams et al., 2005), DISC1 (Millar et al., 2000), RGS4 (Williams et al., 2004), PRODH2 (Liu et al., 2002), calcineurin PP3CC (Gerber et al., 2003) and G72/G30 (Chumakov et al., 2002) (for review see (Chapter 2.1, this volume) (Owen et al., 2004; Harrison and Weinberger 2005; Riley and Kendler, 2006; Ross et al. 2006). However, understanding how genetic variation at each locus confers susceptibility and/or protection, or what is the contribution of each gene, their relationship with the phenotype and their interaction with environmental risk factors (Caspi et al., 2005; Nicodemus et al., 2005), will all need further investigations.

Environmental factors such as exposure to viral infections (Leweke et al., 2004); autoimmune, toxic or traumatic insults; and stress during gestation, birth or childhood (Marcelis et al., 1998; Cannon et al., 2000; Rosso et al., 2000) have been implied in the pathogenesis of schizophrenia. Lately, models based on epigenic factors and interaction between a susceptible genotype and the environmental factors have been proposed for this complex disease (Petronis, 2004).

Moreover, attempts to produce a unifying concept of the etiology of schizophrenia have posited that these biological mechanisms have their origins in developmental processes that emerge prior to the onset of clinical symptoms. Indeed, evidences for pre- and perinatal epidemiological risk factors of schizophrenia and for premorbid dysfunction during infancy and childhood have led to the formulation of the so-called neurodevelopmental hypothesis: schizophrenia is viewed as resulting from etiological events acting between conception and birth and interfering with normal maturational processes of the central nervous system (Murray and Lewis, 1987; Weinberger, 1987; Lewis and Levitt, 2002). Moreover, it is also hypothesized that the interaction of a genetic diathesis and early neurodevelopmental insults results in defective connectivity between a number of brain regions, including the midbrain, nucleus accumbens, thalamus as well as temporolimbic and prefrontal cortices (Parnas et al., 1996; Friston, 1998; Selemon and Goldman-Rakic, 1999; Andreasen, 2000). This defective neural circuitry is then vulnerable to dysfunction when unmasked by the developmental processes and events of adolescence (myelination, synaptic pruning and hormonal effects of puberty on central nervous system) (see Chapter 2.4, this volume) and exposure to stressors as the individual moves through the age of risk (Lewis and Lieberman, 2000; Raedler et al., 2000; Lewis and Levitt, 2002).

1.2 Pathophysiology

There are a number of theories that implicate aberrant neurotransmission systems in schizophrenia: in particular, aberrant dopaminergic, serotonergic, glutamatergic and γ-aminobutyric acid (GABA)-ergic systems implying dysfunction in presynaptic storage as well as vesicular transport, release, reuptake and metabolic mechanisms (see review Lewis and Lieberman, 2000; Ross et al., 2006). It is unclear, however, to what extent any neurochemical findings reflect primary rather than secondary pathology, compensatory mechanisms or environmental influences.

Evidence for a dopamine (DA) system dysfunction includes the psychosis-inducing effects of dopaminergic agonists and the antipsychotic potency of antagonists (Matthysse, 1973; Carlsson, 1988). There is emerging evidence (see Chapter 1.1, this volume) for a presynaptic dopaminergic abnormality in schizophrenia (Laruelle et al., 1999). Besides the initial hypothesis based on functional excess of subcortical DA neurotransmission (Carlsson, 2006), other lines of evidence indicate that working memory impairments in schizophrenia might be related to deficit in DA neurotransmission in dorsolateral prefrontal cortex. Both cortical hypodopaminergia and subcortical hyperdopaminergia have been put forward (Lewis and Gonzalez-Burgos, 2006) (See Abi-Dargham, this volume).

The glutamate hypofunction hypothesis relies on the fact that phencyclidine and ketamine, both potent noncompetitive *N*-methyl-D-aspartate (NMDA)-glutamate receptor antagonists, induce schizophrenia-like symptoms in healthy individuals and worsen some symptoms in schizophrenia patients

(Kim et al., 1980; Javitt and Zukin, 1991; Olney et al., 1999; Lahti et al., 2001). Recent postmortem and genetic findings support the hypothesis of dysregulation of NMDA receptor modulation in schizophrenia (for review, see Coyle et al., 2003; Moghaddam, 2003; Tamminga et al., 2003; Coyle, 2006) (see Chapter 1.3, this volume). Moreover, indirect pharmacological evidence also points to the implication of serotonergic (Lieberman et al., 1998), cholinergic (see Chapter 1.2, this volume) and GABA-ergic systems.

Recent postmortem studies provided consistent evidence of a defect in prefrontal GABAergic interneurons in schizophrenia (see Chapter 4.2, this volume), (Benes and Berretta, 2001; Volk et al., 2002; Beasley et al., 2002b; Hashimoto et al., 2003; Lewis et al., 2005). These cells provide both inhibitory and disinhibitory modulation in cortical and hippocampal circuits and contribute to the generation of oscillatory rhythms (Traub et al., 1996; Whittington et al., 2000), discriminative information processing gating of sensory information (Wilson et al., 1994; Rao et al., 2000) and shaping the temporal flow of information (Constantinidis et al., 2002). All these functions are abnormal in schizophrenia (Kwon et al., 1999; Spencer et al., 2003).

Multiple lines of evidence suggest that schizophrenia is associated with abnormalities in neural circuitry and impaired structural connectivity. Magnetic resonance spectroscopy (MRS) studies reveal alterations of membrane phospholipid metabolism and reduction in *N*-acetyl aspartate, a marker for neuronal integrity, in frontal and temporal lobes (Pettegrew et al., 1991; Horrobin, 1998; Keshavan et al., 2003). Postmortem histological studies (Harrison, 1999) have shown (a) a reduction in cortical thickness in prefrontal cortex (PFC); (b) an increase in cell density without change in total neuron number (Selemon et al., 1995; Selemon et al., 1998); (c) a decrease in the number of dendritic spines indicating a loss of synaptic connectivity in prefrontal cortex and superior temporal cortex (Garey et al., 1998; Glantz and Lewis, 2000; Rosoklija et al., 2000; Kolluri et al., 2005); (d) alteration of synaptic protein expression (Eastwood et al., 1995); (e) a decrease in GAD67 and parvalbumin expression in a subset of inhibitory GABA-parvalbumin interneurons of the dorsal PFC (Volk et al., 2000; Lewis et al., 2005); and (f) a decrease in the total number of neurons in the thalamic nuclei mediodorsal and anterior (Pakkenberg, 1990; Popken et al., 2000; Rose et al., 2006). Moreover, recent advances in diffusion tensor imaging allowed in vivo explorations of anatomical connectivity in human brain. It has pointed to connectivity abnormalities in frontoparietal and frontotemporal circuitry in schizophrenia (Burns et al., 2003), for review see Lim and Helpern (2002), Kanaan et al. (2005). It has been proposed that oligodendroglia, myelin and white matter abnormalities may be the underlying mechanisms (Davis et al., 2003; Davis and Haroutunian, 2003; Tkachev et al., 2003; Kubicki et al., 2005). Besides this anatomical evidence for a structural disconnectivity, anomalies in information integration across brain networks are accumulating.

Functional connectivity is based on the study of dynamic, context-dependent processes, which require the preferential recruitment of some networks over others. Methods for analysis of these processes are based on the premise that functionally interacting regions will show correlated patterns of activity (Engel et al., 1991; Singer and Gray, 1995; Tallon-Baudry and Bertrand, 1999). In schizophrenia, there is emerging evidence for a failure of the gamma band synchronization (40 Hz range) of anterior-posterior neural circuits in response to gestalt stimuli (Spencer et al., 2003) and for binding capacity (Uhlhaas and Singer, 2006). Such perturbation of brain connectivity might be the result of functional anomalies of various neurotransmission systems, including dopaminergic, serotonergic, glutamatergic and GABA-ergic systems (Shelley et al., 1991; Umbricht et al., 1998; Umbricht et al., 2000). The perceptive, cognitive and behavioral symptoms of schizophrenia are thus likely to be due to the above-summarized structural and functional disconnectivity.

In summary, the existing neuroanatomical, neurochemical, neurophysiological and psychopathological arguments converge to suggest that schizophrenia may be considered as a developmental disconnectivity syndrome having complex polygenic and environmental origins. In the following, we will review the role of redox impairment as underlying pathophysiological mechanisms.

1.3 Oxidative Stress in Schizophrenia

In general, oxidative and nitrosative stress result from an imbalance between an overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) on one side and a deficiency of enzymatic and

nonenzymatic antioxidants on the other, which can damage cellular lipids, proteins or DNA. The ROS include superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$) and peroxyl radical ($ROO^{\cdot}$), whereas RNS include nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^-$). The defense mechanisms against this free radical-induced oxidative and nitrosative stress consist of enzymatic systems, such as superoxide dismutase (SOD), glutathione peroxidases (GPX), catalase (CAT), and nonenzymatic antioxidants which include glutathione (GSH), ascorbic acid (vitamin C), α-tocopherol (vitamin E), carotenoids and flavonoids, among others.

The brain is particularly vulnerable to oxidative damage because of its high oxygen utilization, its high content of oxidizable polyunsaturated fatty acids and the presence of redox-active metals (Cu, Fe). There is increasing evidence for the involvement of oxidative stress-induced cellular damage in the pathogenesis of various neurodegenerative diseases (Andersen, 2004; Valko et al., 2007).

It has been proposed that schizophrenia is associated with ROS-mediated pathologies (Hoffer et al., 1954; Mahadik and Mukherjee, 1996; Horrobin, 1998). Indeed, there is increasing evidence of impaired antioxidant defense systems and increased lipid peroxidation in patients suffering from schizophrenia. Reduction of plasma antioxidants such as bilirubin and uric acid was reported both in first-episode (Reddy et al., 2003) and chronic patients (Yao et al., 2000). Abnormal antioxidant enzyme activities such as SOD, GPX and CAT were also observed in plasma and/or blood cells of patients (Mahadik and Mukherjee, 1996; Herken et al., 2001; Yao et al., 2001; Zhang et al., 2006). SOD converts superoxide radicals to hydrogen peroxide, which, in turn, is decomposed to water and oxygen by GPX and CAT, thereby preventing the formation of the toxic hydroxyl radicals.

Such imbalanced antioxidant systems in schizophrenia could lead to oxidative stress- and ROS-mediated injury as supported by increased lipid peroxidation products and reduced membrane polyunsaturated fatty acids (PUFAs). Decrease in membrane phospholipids in blood cells of psychotic patients (Keshavan et al., 1993; Reddy et al., 2004) and fibroblasts from drug-naïve patients (Mahadik et al., 1994) as well as in postmortem brains (Horrobin et al., 1991) have indeed been reported. It has also been suggested that peripheral membrane anomalies correlate with abnormal central phospholipid metabolism in first-episode and chronic schizophrenia patients (Pettegrew et al., 1991; Yao et al., 2002). Recently, a microarray and proteomic study on postmortem brain showed anomalies of mitochondrial function and oxidative stress pathways in schizophrenia (Prabakaran et al., 2004). Mitochondrial dysfunction in schizophrenia has also been observed by Ben-Shachar (2002) and Altar et al. (2005). As main ROS producers, mitochondria are particularly susceptible to oxidative damage. Thus, a deficit in glutathione (GSH) or immobilization stress induce greater increase in lipid peroxidation and protein oxidation in mitochondrial rather than in cytosolic fractions of cerebral cortex (Liu et al., 1996).

However, studies on the various antioxidant systems in the peripheral tissue of schizophrenia patients showed large discrepancies. In patients, when compared to control subjects, SOD activity was found to be increased (Reddy et al., 1991; Zhang et al., 2001), decreased (Herken et al., 2001; Zhang et al., 2006) or unchanged (Yao et al., 1998; Herken et al., 2001). Similarly, GPX activity was found to be elevated (Herken et al., 2001), diminished (Zhang et al., 2006) or unchanged (Reddy et al., 1991; Yao et al., 1999). Finally, the results concerning the CAT were also contradictory, with lower (Reddy et al., 1991), elevated (Herken et al., 2001) or normal (Yao et al., 1998; Zhang et al., 2006) activities in schizophrenia patients. These discrepancies might be due to differences in analytical methodologies, testing materials (blood cells vs. plasma or serum), exposure to medication (naïve vs. drug withdrawal vs. medicated), stages of disease (acute vs. chronic or active vs. remission phase), lifestyle or dietary pattern and origin of the patient populations. It is also important to remember that these antioxidant enzymes have different localizations. While SODs are present both in cytosol (copper-zinc type) and mitochondria (manganese type), the heme-containing metalloenzyme CAT is mostly localized in peroxisomes, lysosomes and mitochondria. Owing to its cytosolic localization, the selenium metalloenzyme GPX, which eliminates hydrogen and lipid peroxides by using glutathione, is an effective protection against cytosolic injuries.

Besides these inconsistent findings in peripheral tissue, analysis of patient's postmortem brain indicates lower GPX and glutathione reductase activities in striatum (Yao et al., 2006), elevated Mn-SOD activity (Loven et al., 1996) and elevated Mn-SOD protein levels in cortex (Michel et al., 2004). On the other hand, Cu- and Zn-SOD levels were reported not to be altered (Loven et al., 1996), whereas their enzymatic activity were

increased (Michel et al., 2004). Studies in drug-naïve, first-episode psychotic patients suggest that impairment of the antioxidant systems precedes the illness and that antipsychotic medication may differentially alter it.

To summarize, there is evidence that oxidative stress is associated with schizophrenia, at least in a subgroup of patients. It is, however, not known whether this oxidative stress is due to an excess of ROS, a deficit in antioxidant mechanisms or a combination of both. An excess of ROS could originate from an excess of endogenous products, toxic environmental compounds or pathological insults (such as infections or hypoxia/anoxia). A deficit in antioxidant mechanisms could be due to a genetic defect in one or some enzymes involved in the defense mechanisms such as SOD, CAT or those of the GSH metabolism (see below). Impairment of the antioxidant systems might render the organism particularly vulnerable during a temporary excess of ROS. We propose here that a genetic defect of the GSH system is one of the origins of the failure of antioxidant defenses in schizophrenia.

1.4 Glutathione (GSH) Function and Metabolism

The tripeptide GSH (γ-glutamyl-cysteine-glycine) is known as the major intracellular non-protein redox regulator and enzyme-catalyzed antioxidant in living cells. It is required for the maintenance of the thiol redox status, protection against oxidative damage, detoxification of endogenous and exogenous reactive electrophiles, storage and transport of cysteine, as well as protein and DNA synthesis (Orlowski and Karkowsky, 1976; Meister, 1981; Meister and Anderson, 1983; Dalton et al., 1999; Hammond et al., 2001). Brain cells utilize 20% of the oxygen consumed by the body but constitute only 2% of the body's weight (Sokoloff, 1999). This points to the potential generation of high amounts of ROS during oxidative phosphorylation in the brain, which has to be counterbalanced by an appropriate antioxidant defense mechanism. Thus the brain is particularly vulnerable to redox dysregulation, and GSH plays a key role in protecting nerve cells against oxidative stress and harmful xenobiotics (Ogita et al., 1995; Janaky et al., 1999; Dringen, 2000).

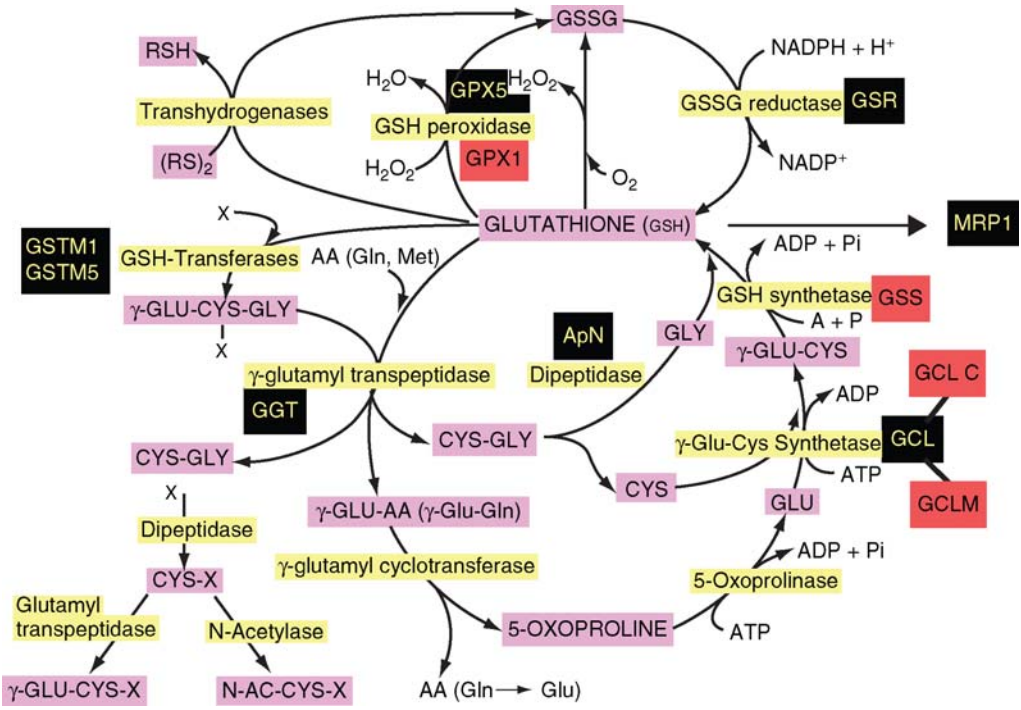
Evidence supports a link between cellular GSH status and apoptosis (Hall, 1999). Because oxidants (production of ROS) often elicit redox imbalance (as indicated by the ratio between GSH and the oxidized form GSSG), it is difficult to distinguish the direct effects of cellular redox imbalance from that of oxidants. Pias and Aw (2002) have shown that induction of an early and transient loss of cellular redox balance, independent of ROS production, initiated apoptosis in PC-12 cells through mitochondrial signaling and activation of caspase-3. The supply of cellular nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) was critical to the maintenance of the GSH/GSSG status through the pentose phosphate pathway. Indeed, decrease of glucose availability and/or inhibition of the pentose phosphate pathway led to dramatic loss of NADPH necessary for GSSG reduction, severe redox imbalance and accelerated cell apoptosis.

As a redox regulator, GSH is also involved during development in cell cycle regulation and cell differentiation (Shi et al., 2000). Intracellular redox state, controlled by GSH, appears to be a necessary and sufficient modulator of the balance between self-renewal and differentiation in dividing glial precursor cell (Smith et al., 2000): a more reducing environment (maintained by elevated GSH levels) stimulates cell proliferation, and a slight shift towards a mildly oxidizing environment initiates cell differentiation. A further shift towards a more oxidizing environment in the cell leads to apoptosis and necrosis. Moreover, oligodendrocytes precursors are more sensitive to the toxic effects of GSH depletion than mature oligodendrocytes (Back et al., 1998). GSH also plays an important role in the redox control of various signal transduction pathways and gene expression (Esposito et al., 2004; Wu et al., 2004). Besides its intracellular functions, GSH appears to have also essential extracellular functions (Dringen and Hirrlinger, 2003) as it is released into the extracellular space (Zangerle et al., 1992). Finally, GSH has been proposed to play a neuromodulator or neurotransmitter role (Janaky et al., 1999). GSH is known to potentiate the NMDA receptors' response to glutamate (Kohr et al., 1994) and to modulate other channels and receptors implicated in neurotransmission (Kohr et al., 1994; Janaky et al., 1999; Dringen et al., 2000).

The GSH metabolism is well established (Meister, 1981) (see *Figure 2.5-1*). GSH is synthesized intracellularly by the enzymes glutamate-cysteine ligase (GCL) and GSH synthetase (GSS). GCL, the first

Figure 2.5-1

Glutathione metabolic pathway: The genes for which expression was studied in fibroblast cultures from skin biopsies are shown in black box (no difference between controls and patients) or in dark grey box (lower expression in patents compared to controls). These genes code for the enzymes shown in unhighlighted text. Substrates and products are in light grey box. Abbreviations are as in the text



and rate-limiting synthesizing enzyme, forms γ-glutamylcysteine (γ-Glu-Cys) from glutamate (Glu) and cysteine (Cys). Glutathione synthetase (GSS), the second synthesizing enzyme, catalyzes the reaction between γ-Glu-Cys and glycine to produce GSH (Meister and Anderson, 1983). In contrast to GSH synthesis, which occurs intracellularly, GSH degradation occurs in the extracellular space and in cells expressing the membrane-bound enzyme γ-glutamyl transpeptidase (GGT). GGT removes the glutamyl moiety from GSH to give cysteinylglycine. Aminopeptidase N (ApN) is a dipeptase involved in degradation of cysteinylglycine, giving rise to cysteine (Dringen et al., 2001). The toxic ROS such as hydrogen peroxide that are formed from aerobic metabolism are reduced through the redox cycle and catalyzed by the enzymes GSH peroxidase (GPX) and GSH reductase (GSR). Electrophylic compounds, environmental toxins and reactive metabolites are detoxified via the formation GSH adducts by a reaction catalyzed by GSH-S-transferases (GSTM). GSH is also released from astroglial cells together with toxic compounds via the multidrug resistance protein MRP1 (Lorico et al., 1997; Minich et al., 2006). As GSH pathways and enzymes are reviewed in detail elsewhere (Sies, 1999; Dringen and Hirrlinger, 2003; Dalton et al., 2004; Wu et al., 2004), we will focus below on the key rate-limiting synthesizing enzyme GCL.

The GCL enzyme was first isolated and characterized in rat liver more than 25 years ago (Meister, 1981). Only a decade later, the human subunits were purified and enzymatically characterized (Misra and Griffith, 1998). GCL is a heterodimer composed of two subunits: glutamate-cysteine ligase modifier subunit (GCLM) (30 kDa, light subunit) (Huang et al., 1993a) and glutamate-cysteine ligase catalytic subunit (GCLC) (73 kDa, heavy subunit) (Huang et al., 1993b), encoded by two different genes (Tsuchiya et al., 1995). The chromosomal localization in humans is 6p12.1 for GCLC and 1p21.1 for GCLM (Tsuchiya

et al., 1995). GCL activity and *de novo* synthesis of GSH so far is known to be influenced mainly by the following factors: the total amount of GCLC and GCLM, the ratio of GCLM to GCLC, the intracellular GSH concentration via its feedback inhibition on GCL activity, the post-translational modifications of GCL, the availability of the cofactor ATP, and the substrate cysteine (Sun et al., 1996).

The GCLC subunit can function independently of GCLM, but GCLM enhances GCLC activity and reduces the feedback inhibition of GCL by GSH (Huang et al., 1993a). GCLC (−/−) mice studies showed that this gene is required for survival (Dalton et al., 2000). Only heterozygotes GCLC (+/−) are viable and fertile, with their protein level and GSH level reduced in the liver to 50% and 20%, respectively.

The GCLM subunit is responsible for the modulation of GCL activity (Yang et al., 2002). GCLM is not necessary for animal survival. Thus, GCLM (−/−) mice have normal survival in spite of the fact that their GSH levels are reduced to 9–40% (depending on the tissue) of the normal levels. In the brain, GSH levels are decreased by 60–70% in GCLM (−/−) mice compared to littermate controls (Chen et al., 2005), (unpublished observations). GCLM regulates the enzymatic activity of the GCL holoenzyme via several mechanisms. GCLM decreases the feedback inhibition of GCL activity by GSH and increases the affinity for glutamate, cysteine and the cofactor ATP on their respective binding sites on GCLC (Chen et al., 2005). In addition, an increased sensitivity to oxidative stress was reported in GCLM (−/−) mice (Yang et al., 2002). Finally, sequence comparisons, motif and 3D structure predictions revealed that GCLM belongs to the superfamily of aldo-keto reductases (AKR). AKR have a common requirement for NAD(P)H. $NADP^+$ and NADPH have been shown to influence GCL activity (Toroser et al., 2006). For these reasons it is conceivable that GCLM has activities independent of the modulation of GCL (Soltaninassab et al., 2000).

2 Impairment of GSH Metabolism in Patients

The observations reported below, made in five different cohorts with various methodologies, converge to suggest that anomalies of GSH metabolism represent a vulnerability factor in at least a subgroup of schizophrenia patients. They concern cerebrospinal fluid (CSF) and brain GSH levels, enzymatic activity, protein and gene expression in fibroblasts and allelic variants of the GCL genes (*Figure 2.5-2*).

2.1 Decrease of GSH levels

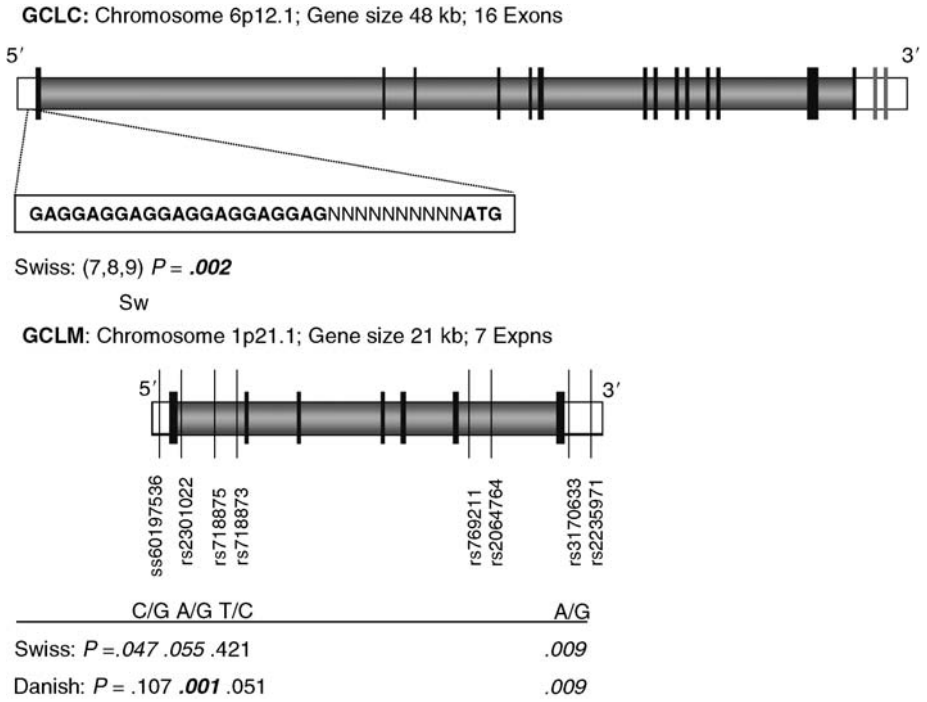
Levels of GSH (−27%) and of its direct metabolite γ-glutamylglutamine (−16%) have been shown to be decreased in the CSF of a group of drug-naïve/drug-free schizophrenic patients compared to control subjects (Do et al., 1995, 2000), suggesting that the deficit in GSH may underlie the pathophysiology of the disease and is not a consequence of treatment. A noninvasive in vivo proton MRS quantification method for GSH (Trabesinger et al., 1999) revealed that GSH levels in the medial prefrontal cortex were 52% lower in patients than in controls (Do et al., 2000). Recently, Yao and collaborators also reported a decrease of GSH levels in postmortem striatum of schizophrenic patients (Yao et al., 2006).

2.2 GCL Dysregulation Under Oxidative Stress Conditions

Searching for the cause(s) of such a GSH deficit, we investigated the activity of the GSH rate-limiting synthesizing enzyme GCL in fibroblasts derived from skin biopsies of schizophrenia patients and control subjects. We assumed that any limitation in GSH synthesis would be more pronounced under conditions of oxidative stress. In order to induce oxidative stress, fibroblasts were challenged with *tert*-buthylhydroquinone (*t*-BHQ), an electrophylic substance known to induce the expression of phase II genes such as GCLM and GCLC and raise GSH level through stimulation of GSH synthesis (Prestera et al., 1993; Soltaninassab et al., 2000). Following *t*-BHQ treatment, GCL activity was 26% lower in patients than in controls. *t*-BHQ treatment increased GCL activity by a factor of 3.45 in control subjects but only by a factor

Figure 2.5-2

Map of GCLC and GCLM genomic structures with the positions of analyzed polymorphisms. Tables show *P*-values for GAG distribution in GCLC gene and for key SNPs in GCLM gene in a Swiss population, and for GCLM also in a Danish population. Values in *italics* indicate a significantly different distribution between patients and controls. Values in *bold italics* indicate a significant difference after correction for multiple testing



of 2.95 in patients (Gysin et al., 2005, 2007). This indicates a significant deficit in the ability to react against oxidative stress in schizophrenia patients, and is consistent with the involvement of oxidative stress-induced impairment of neuronal processes and mitochondrial function reported in schizophrenia (Mahadik and Mukherjee, 1996; Herken et al., 2001; Yao et al., 2001; Prabakaran et al., 2004; Altar et al., 2005). The decrease of GSH levels in the CSF and prefrontal cortex of patients, as well as the dysregulation of GCL activity observed in patients' fibroblasts, could be due to polymorphisms in the GCLC and/or GCLM genes.

2.3 Decrease of GCLC Protein Expression

In parallel with the reduced GCL activity in patients, GCLC protein expression was decreased both under baseline conditions and under oxidative stress conditions by 22% and 29%, respectively. Treatment with *t*-BHQ increased GCLC expression in controls by a factor of 1.44 versus baseline, but the increase in patients (by a factor of 1.32) was significantly less. Thus, the induction of GCLC protein expression in response to *t*-BHQ treatment was lower in patients than in controls, leading to a more pronounced GCLC deficit under conditions of oxidative stress. In contrast to GCLC, GCLM protein expression was not decreased in schizophrenic patients. Moreover, an uncoupling in the regulation of GCLM and GCLC protein expression was revealed in patients. While GCLM and GCLC protein expression in control subjects was positively correlated under both baseline and oxidative stress conditions, no such relationship was present in patients (Gysin et al., 2005, 2007).

2.4 Decreased mRNA Levels of GSH-related Enzymes

Among various genes involved in GSH metabolism (*Figure 2.5-1*), the expression of the genes coding for the synthesis of GSH was significantly reduced in fibroblasts from patients: mRNA levels of GCLM, GCLC and GSS were decreased by 41%, 39% and 37%, respectively. In addition, mRNA levels of GPX1 was also lower by 29% (Gysin et al., 2005; Tosic et al., 2006, and unpublished results).

2.5 Genetic Association of GCL Subunits with Schizophrenia

Association with a GAG trinucleotide repeat (TNR) polymorphism in the GCLC gene:

The GCLC gene contains a TNR polymorphism 10 bp upstream of the start codon with 7–9 GAG repeats (*Figure 2.5-2*). Genetic analysis in two case–control studies (Swiss sample of 66 schizophrenia patients and 48 control subjects; Danish sample of 322 schizophrenia patients and 331 control subjects) showed a significant intergroup difference regarding the overall genotype distribution. In the Swiss sample, 50% of the controls had a genotype 7/7, 44% had 7/9, 4% had 7/8 and 2% had 8/9. Ninety-four percent of control subjects had no 8 TNR allele, and genotypes 8/8 and 9/9 were not present. In contrast, 36% of schizophrenia patients had a genotype with an 8 TNR allele, or had genotype 9/9. The Danish sample showed a very similar genotype distribution and revealed an overrepresentation of genotype 8/8 in patients (OR = 2.96, $P = 0.007$) and an over-representation of genotype 7/7 in controls (OR = 0.61, $P = 0.003$).

In addition, this polymorphism has functional consequences. As genotypes 7/7 and 7/9 (low risk) were overrepresented in control subjects and genotypes 7/8, 8/8, 8/9 and 9/9 (high risk) in patients, functional data of all Swiss subjects were classified according these two groups. Subjects with "high risk" genotypes had lower GCL activity, GCLC protein expression and GSH content than subjects with "low risk" genotypes (Gysin et al., 2007). Thus the "high risk genotype" is present in 36–40% of patients and is 3 times more frequent in patients, while the "low risk" one is predominant in healthy subjects. Thus this marker is of special interest, as it allows identification of an individual at risk.

Association with GCLM gene variants:

Analysis in the same two samples (Swiss sample: 40 patients, 31 controls; Danish sample: 349 patients, 348 controls) also showed an association between schizophrenia and allelic variants (SNP rs2301022 in intron 1 and rs3170633 at the 3′ end) of GCLM (*Figure 2.5-2*). Analysis in an independent family linkage study (262 subjects; proband-parent triads; NIMH Genetics Initiative for Schizophrenia) confirmed these findings. The family-based association statistic test (FBAT v1.7.2) revealed an association ($Z = 3.247$; $p = 0.0012$) in the presence of linkage between SNP marker rs2301022 and schizophrenia phenotype; allele G at the marker appeared as significantly over-transmitted to the affected offspring (Tosic et al., 2006). It is notable that the GCLM gene is localized on chromosome 1p21, the region shown by previous linkage studies to be one of the several regions critical for schizophrenia (Pulver, 2000; Arinami et al., 2005).

In conclusion, these genetic and functional results support the concept that a dysregulation of GSH metabolism, and in particular GSH synthesis, is one of the vulnerability factors that could contribute to the development of schizophrenia. These markers may contribute to obtain a complete picture of genetic risk factors of schizophrenia and may help to identify critical period and specific brain locations during development, where GSH deficits are important to the emergence of the disease.

3 Potential Contribution of GSH Deficit to Schizophrenia Pathophysiology

In the following section, we will present the effects of GSH deficit on brain morphology and physiology and on behavior that has been observed in rodents. We will discuss how these effects could relate to the pathophysiology of schizophrenia.

3.1 Acute GSH Deficit

3.1.1 Lipid Peroxidation and Impairment of Mitochondrial Function

GSH plays a major role in protecting cells against toxic effects of free radicals produced by the metabolism of catecholamines, particularly DA (Rabinovic et al., 2000). A GSH deficit leads to lipid peroxidation, impairment of mitochondrial function as well as dysregulation of glutamatergic and dopaminergic systems. Moreover, GSH deficit during postnatal development causes morphological abnormalities in the prefrontal cortex, with cognitive impairments becoming apparent mostly at adult age. Some of these adverse developmental effects appear, or are exacerbated, when an additional stress factor (i.e., excess of extracellular DA induced by a DA uptake inhibitor) is combined with the GSH deficit. A GSH deficit could thus lead to lipid membrane peroxidation (Hoyt et al., 1997) in the surroundings of dopaminergic terminals and during excessive oxidative stress. We observed a 20–30% increase in lipid peroxidation products levels in the diencephalon and pons/medulla of the mutant rats "osteogenic disorder shionogi" (ODS), in which a GSH deficit was induced by a specific inhibitor of GCL, buthionine sulfoximine (BSO) (Rougemont et al., 2003). These rats are particularly vulnerable to oxidative stress, as they are, like humans, unable to synthesize ascorbic acid and compensate for an impaired GSH synthesis.

Dopamine has been shown to form semiquinone/quinone and hydrogen peroxide (Hirrlinger et al., 2002a; Grima et al., 2003). In neuronal cultures, this leads to a 50% decrease in GSH levels through direct conjugation of GSH with dopamine semiquinone/quinone. When GSH levels are decreased by ethacrynic acid, DA further decreases the GSH content via activation of DA receptors and generation of ROS, leading to a reduction of the mitochondrial membrane potential ($\Delta\psi$) (Grima et al., 2003). This latter observation is consistent with the finding that depletion of brain GSH is accompanied by impaired mitochondrial function and decreased *N*-acetyl aspartate (Heales et al., 1995). The decreased GSH level observed by us in schizophrenia might therfore be related to the established *N*-acetyl aspartate reduction observed with MRS (Bertolino et al., 2000; Do et al., 2000).

These results suggest that a GSH deficit significantly contributes to the oxidative stress-induced impairment of membrane phospholipid and mitochondrial function proposed to be involved in the pathophysiology of schizophrenia (Mahadik and Mukherjee, 1996; Mahadik et al., 1998; Yao et al., 1999; Herken et al., 2001; Evans et al., 2003; Marchbanks et al., 2003; Prabakaran et al., 2004).

3.1.2 Synaptic Transmission, Plasticity and Neuromodulation

Many channels, receptors and other molecules involved in neurotransmission and modulation are redox-sensitive, implying that their function can be modulated by the redox state of the extra- and/or intracellular compartments. This includes NMDA-R (Kohr et al., 1994), $GABA_A$-R (Amato et al., 1999), calcium-activated K^+-channels (DiChiara and Reinhart, 1997), L-type calcium channels (Campbell et al., 1996), calcineurin, protein phosphatase 1 (Sommer et al., 2000, 2002; O'Loghlen et al., 2003) and ryanodine receptors (Bull et al., 2003; Hidalgo et al., 2004). An impairment of GSH synthesis might therefore alter many aspects of neurotransmission. In rat hippocampal slices, a BSO-induced GSH deficit impaired long-term potentiation and short-term plasticity, such as paired-pulse facilitation, and increased cell excitability (Steullet et al., 2006). Rats with diethylmaleate-induced GSH deficit displayed also a reduced long-term potentiation and paired-pulse facilitation in the dentate gyrus (Almaguer-Melian et al., 2000). Most interesting, however, are the effects of a GSH deficit on the function of NMDA receptors (NMDA-R) and on dopamine signaling.

3.1.2.1 Hypofunction of NMDA-R A hypofunction of the NMDA-R (see Javitt, this volume) has been implied in schizophrenia for the following reasons: Administration of phencyclidine or ketamine, both noncompetitive antagonists of the NMDA-R, induce schizophrenia-like symptoms in healthy individuals and worsen some symptoms in schizophrenia patients (Kim et al., 1980; Javitt and Zukin, 1991; Krystal et al., 1994; Olney et al., 1999), while add-on treatments with agonists of the glycine site of NMDA-R (i.e., glycine and D-serine) ameliorate negative symptoms (Heresco-Levy and Javitt, 2004). Furthermore, the mismatch negativity (MMN), a differential response to deviant stimuli compared to distracters presented in

an auditory oddball paradigm, was shown to be impaired in schizophrenia (Shelley et al., 1991; Javitt et al., 1993; Catts et al., 1995; Shutara et al., 1996; Javitt et al., 1998). This decrease in MMN amplitude can be induced by ketamine administration in normal subjects (Umbricht et al., 2000), suggesting that MMN reflects selective current flowing through open, unblocked NMDA channels. We can therefore ask the question whether a deficit in GSH will possibly affect the NMDA-R function. NMDA-Rs possess extracellular redox sites, which modulate the NMDA response (Sullivan et al., 1994).

When presented extracellularly, GSH or other reducing agents increase glutamate-induced depolarization via NMDA-R activation, while extracellular oxidizing conditions decrease this response (Kohr et al., 1994). Although most of GSH is contained within cells, GSH is released during cell depolarization (Zangerle et al., 1992) and also by astrocytes via the transport protein Mrp1 (Sagara et al., 1996; Hirrlinger et al., 2002b). The amount of GSH release is correlated with the intracellular GSH content (Sagara et al., 1996). Therefore, a deficit in intracellular GSH may lead to a concomitant decrease in its extracellular concentration, to an excessive oxidation of the extracellular redox sites of NMDA receptors and to a subsequent diminution of the function of these receptors. We observed that a BSO-induced GSH deficit led indeed to a decrease of NMDA-R-mediated field excitatory postsynaptic potentials (fEPSPs) in the CA1 region of the hippocampus (Steullet et al., 2006). Hypofunction of NMDA-R under such GSH deficit was also explained in part by an excessive oxidation of the extracellular redox-sensitive sites of the NMDA-R. These results suggest that GSH deficit could be one causal factor for the hypofunction of NMDA receptors in schizophrenia.

3.1.2.2 Dopamine Signaling The psychosis-inducing effects of dopaminergic agonists and the antipsychotic potency of D2R antagonists (Matthysse, 1973; Carlsson, 1988) indirectly speak for an alteration of the dopaminergic system in schizophrenia. More direct evidence for a dysfunction of dopaminergic systems in schizophrenia has, however, also emerged recently (Breier et al., 1997; Okubo et al., 1997; Laruelle et al., 1999; Bertolino et al., 2000; Albert et al. 2002; Suhara et al., 2002; Koh et al., 2003). Several molecules (i.e., calcineurin, protein phosphatase, adenylate cyclase) that are implicated in dopamine-mediated intracellular pathways are known to be redox-sensitive (Mukherjee and Lynn, 1979; Sommer et al., 2000, 2002). Therefore, we investigated whether low intracellular GSH levels could alter dopamine receptor-mediated signaling. The effects of GSH deficit on dopamine signaling were studied by focusing on the DA modulation of NMDA-mediated calcium responses in cultured neurons.

We found that a GSH deficit reversed the direction of DA modulation of NMDA responses (Steullet et al., 2008). In control neurons, DA enhanced NMDA responses. But in neurons with a BSO-induced GSH deficit, DA decreased NMDA responses via activation of D2-type receptors. This decrease disappeared when normal GSH levels were restored by GSH-ethyl ester, a membrane-permeable GSH analog. The difference in dopamine modulation of NMDA responses in control neurons and in neurons with a GSH deficit was mostly explained by a differential modulation of L-type calcium channels. DA enhanced the function of these channels in control neurons, but decreased it in BSO-treated neurons. The redox-sensitive ryanodine receptors (RyRs), which were enhanced in BSO-treated neurons, played an essential role in altering DA signaling in neurons with a GSH deficit. These data suggest that enhancement of the function of RyRs in neurons with low GSH levels favors D2-type receptor-mediated and calcium-dependent pathways, causing a change in DA modulation of L-type calcium channels and ultimately in DA modulation of NMDA responses.

It is tempting to speculate that these mechanisms may underlie some of the D2 receptor effects of antipsychotics. In addition, an acute GSH deficit in adult mice enhanced amphetamine-induced DA release in the nucleus accumbens (Jacobsen et al., 2005). Altogether, these results show that GSH deficit has profound effects on DA-mediated signaling and could contribute to the dysfunction of dopaminergic systems in schizophrenia.

3.2 GSH Deficit During Early Postnatal Development

Pre- and perinatal epidemiological risk factors of schizophrenia as well as premorbid dysfunction during infancy and childhood strongly suggest that etiological events during brain development can interfere with normal maturation processes and neuronal migration in the central nervous system leading to the

manifestation of the illness (Murray and Lewis, 1987; Weinberger, 1987; Lewis and Levitt, 2002). In this context, anomalies of GSH regulation, especially when combined with a brain-specific insult or stress, could play a crucial role in the development of abnormal nervous connections, leading to cognitive and behavioral impairments as observed in schizophrenia. Therefore, we investigated the effects of a transitory BSO-induced decrease in GSH levels during the rat development (postnatal day p5 until p16) on brain morphology (mostly prefrontal cortex) and cognitive functions. The combined effect of GSH deficit and a stress factor (i.e., excessive extracellular dopamine induced by GBR 12909, a blocker of DA uptake) was also evaluated.

3.2.1 Morphology of Neurons in the Prefrontal Cortex

3.2.1.1 Dendritic Spines A GSH deficit combined with hyperdopaminergy during development led to alteration of dendritic spines on pyramidal cells of the anterior cingulate. Golgi staining and quantitative morphometric analysis revealed an expansion in the size and number of apical dendrite branches, resulting in a wider lateral extension. In addition, the dendritic spines on both apical and basal dendrites of layer II/III showed a 20% reduced linear density and a lower proportion of spines with normal morphologies (Do et al., 2004, unpublished data). A similar effect was also observed in cultured cortical neurons: GSH deficit combined with DA application induced a decrease of the density of philopodia, neural processes somehow analogous to spines in mature neurons (Grima et al., 2003).

These results are consistent with the morphological findings reported in postmortem brains of patients with schizophrenia (i.e., decrease number of spines in prefrontal cortex (Garey et al., 1998; Glantz and Lewis, 2000; Rosoklija et al., 2000; Kolluri et al., 2005), and thus could be related to the deficit in synaptic connectivity assumed to underlie the disease (structural misconnectivity), leading to cognitive deficits.

3.2.1.2 Abnormal GABA Neurons A GSH deficit combined with hyperdopaminergy during development also caused a reduction in the immunoreactivity profiles of the GABAergic interneurons that express the protein parvalbumin. No significant effect was observed in the immunoreactivity profiles of the two other major calcium-binding protein markers – calbindin and calretinin (Cabungcal et al., 2006). In addition to the neuronal specificity, this decrease was specifically observed in the anterior cingulate cortex and not in somatosensory cortex. Interestingly, similar observations involving the same GABAergic (parvalbumin positive) interneurons have been reported in the same area of postmortem brains of schizophrenic patients (Beasley et al., 2002a,b; Hashimoto et al., 2003). This is of special interest since these fast-spiking, parvalbumin-expressing, soma-inhibiting interneurons are proposed to play a crucial role in gamma frequency oscillations which contribute to cognitive functions such as working memory, a core feature of schizophrenia (Lewis and Gonzalez-Burgos, 2006; Bartos et al., 2007). More recently, in a ketamine schizophrenia animal model, the loss of parvalbumin and GAD67 in cortical fast-spiking inhibitory interneurons was prevented by apocynin, an inhibitor of NADPH oxidase, the main generator of superoxide (Behrens et al., 2007). Thus oxidative stress appears to be the underlying mechanism of this hypoglutamatergic schizophrenia model and strongly supports the etiological role of a genetic impairment of GSH synthesis in schizophrenia.

3.2.2 Cognitive Functions

The effect of a transient GSH deficit during development (with or without induced hyperdopaminergy) on cognitive functions was evaluated in the adolescence and adulthood using various behavioral tests, including Morris water maze (Morris, 1984; Whishaw and Pasztor, 2000), Homing board task (Schenk, 1989; Rossier and Schenk, 2003), radial maze (Lavenex and Schenk, 1996) and a novel object recognition test (Ennaceur and Delacour, 1988). Rats with GSH deficit (with or without induced hyperdopaminergy) during the development displayed normal motor development and adult-like walking, suggesting that the treatment was not associated with unspecific toxic consequences affecting the motor system. By contrast,

several aspects of cognitive functions were affected in the adolescence and, especially, adulthood despite the fact that GSH levels were back to normal.

3.2.2.1 Sensory Integration Deficit Transitory GSH deficit alone during brain development impaired spatial memory performance of the young rats in the water maze (Cabungcal et al., 2007). Moreover, a more severe impairment became evident in adult rats that were trained to acquire diffuse visual and olfactory cues in a Homing board test. Indeed, place learning was impaired when only distant visual cues were available. While place learning was not affected when only one olfactory cue was present, such ability was impaired when five different olfactory cues were present at the same time on the Homing board (Cabungcal et al., 2004a,b). Furthermore, rats that had a transient GSH deficit during development also demonstrated working memory errors and deteriorated performance in a radial maze test when each arm of the maze was marked by a specific olfactory cue. These results support the hypothesis that a GSH deficit during a critical developmental phase can lead later to a dysfunction in integrating many pieces of sensory information important for spatial representation. These studies suggest that the observed impairments are not attributable to sensory defects but rather to problems at the level of information integration. The olfactory deficit observed in the animal model is consistent with the reported olfactory recognition impairment in schizophrenia (Moberg et al., 1999; Coleman et al., 2002; Malaspina et al., 2002; Corcoran et al., 2005).

3.2.2.2 Memory Deficit In an object recognition memory test, adult ODS rats, mutants that cannot synthesize ascorbic acid, failed to accurately discriminate familiar from novel objects when a transient GSH deficit together with an excess of extracellular DA had been induced during development. Such impairment in object recognition was not observed in nonmutant rats, which have the ability to synthesize ascorbic acid and partly compensate for the diminished antioxidant capacity of the GSH system. These results indicate that a strong disturbance of the antioxidant systems during development can cause a deficit in episodic memory. This effect appeared earlier and was more important in male (day 65) than in female (day 94) rats (Castagne et al., 2004a,b). Such an observation is of interest in view of the fact that the first psychotic episode appears about 5 years later in woman than in man. The gender difference could be related to the known antioxidant effect of estrogens (Kume-Kick et al., 1996; Kume-Kick and Rice, 1998; Behl and Manthey, 2000).

Thus, a GSH deficit has consequences consistent with the concept of functional disconnectivity, as hypofunction of NMDA-R and alteration of dopamine signaling have been observed. When imposed on animals during development, a GSH deficit induces also a structural disconnectivity, as revealed by the decrease in dendritic spines and parvalbumin-immunoreactivity of inhibitory interneurons in the prefrontal cortex. Finally, a transient GSH deficit during brain development causes deficits in visual recognition and olfactory integration.

In conclusion, GSH-deficient models reveal some morphological, physiological and behavioral anomalies analogous to those observed in patients. Results at the genetic and functional levels provide also evidences for a link between schizophrenia and GCL genetic variations, affecting the function of the encoded proteins in their ability to promote GSH synthesis when challenged by an oxidative stress. They support the new concept that a dysregulation of GSH metabolism is one of the vulnerability factors contributing to the development of the disease, at least in a subgroup of patients.

4 Therapeutic Perspectives

4.1 Clinical Trial with *N*-Acetyl Cysteine (NAC)

Overall, the results of both clinical and experimental research have strengthened the validity of the GSH hypothesis of schizophrenia and justified a clinical trial with NAC. NAC is a safe, orally bioavailable donor of cysteine, the rate-limiting substrate of GSH. Given orally, NAC is quickly absorbed, and plasma peak concentration of cysteine is reached within 120 min (Borgstrom et al., 1986; Olsson et al., 1988; Borgstrom

and Kagedal, 1990). NAC crosses the blood–brain barrier (Farr et al., 2003), and cysteine can be used in the brain as GSH precursor. Indeed, animal studies have shown that systemic administration of NAC protects brain against GSH depletion (Ercal et al., 1996; Aydin et al., 2002; Fu et al., 2006; Kamboj et al., 2006). NAC has been used in clinic as an antioxidant since the 1950s, first indicated for chronic respiratory diseases and later approved as antidote for paracetamol intoxication. Various mechanisms may underlie NAC's redox and antioxidant actions: (a) promotion of GSH biosynthesis in vivo; (b) direct inactivation of reactive oxygen substances through its thiol group, producing NAC disulfides; and (c) induction of oxidative stress-related genes expression via stimulation of transcription factors such as NF-κB and AP1 (Cotgreave, 1997).

In a double-blind, multicenter, randomized, placebo-controlled study, 140 patients with treatment-refractory schizophrenia were treated with NAC (2 g/day) as an add-on to their antipsychotic maintenance medication over a 24-week interval followed by a 4-week washout. NAC administration moderately improved the clinical outcomes on the basis of the Clinical Global Impression (CGI) Severity and Improvement scales, and reduced positive and negative symptoms scores (based on the Positive and Negative Syndrome Scale (PANSS)). In addition, the severity of abnormal movements measured by the Barnes Akathisia Scale was diminished. The moderate effects of NAC treatment on a refractory cohort of patients indicate that NAC is an effective add-on strategy for chronic schizophrenia, (Berk et al., 2008).

4.2 NAC Improves Mismatch Negativity in Schizophrenia Patients

In the context of the multicenter clinical trial with NAC (Berk et al., 2008), we studied the auditory-related brain potentials (auditory-evoked potentials, AEPs) with EEG measurements in the Lausanne subgroup of patients to assess the effect of NAC on MMN which is impaired in schizophrenia (Shelley et al., 1991; Javitt et al., 1993; Catts et al., 1995; Shutara et al., 1996; Javitt et al., 1998). MMN is an electrophysiological response to infrequent target stimuli interspersed among frequent standard stimuli that typically peaks ~100 ms post stimulus onset. Patients exhibit deficits in low-level processing, including pitch discrimination, which is manifested in AEPs as an impaired MMN. NAC (2 g/day for 60 days) was administered to schizophrenia patients using a double-blind cross-over protocol. Analyses of AEPs recorded at protocol onset indicated that patients ($n = 11$) were significantly impaired in generating the MMN relative to age-matched controls ($n = 11$). Administration of NAC ($n = 7$) versus placebo ($n = 7$) revealed the efficacy of this GSH precursor in improving MMN generation mechanisms (Lavoie et al., 2007).

NMDA-R antagonists have been shown to block MMN generation (Umbricht et al., 2000), and NMDA-R dysfunction has been linked to the underlying pathophysiology of schizophrenia. Combined with the observation that GSH deficit leads to NMDA-R hypofunction (Steullet et al., 2006), the improvement of MMN by NAC is likely to reflect an amelioration of NMDA-R function.

These results provide the first evidence in a clinical setting that a GSH precursor can improve MMN in schizophrenia, likely via re-establishment of NMDA-R function. However, the mechanisms underlying this improvement are not yet fully understood. Moreover, as we presently know that the key synthesizing enzyme GCL is dysregulated, the use of a direct precursor such as NAC might not be the most efficient treatment.

5 GSH Deficit in the Broader Context of Schizophrenia Research

We propose that a redox/antioxidant dysregulation due to GSH deficit could represent a vulnerability factor at the early phase of brain development in at least a subgroup of schizophrenia patients. Combined with other genetic factors and environmental factors, such as stress, obstetrical complications or viral infections, it could favor the development of the disease. The role of the GSH deficit proposed allows integration in a causal way many phenomenological aspects of schizophrenia. It is compatible with both the DA and the glutamate/NMDA hypotheses and with the neuropathological observations. In contrast to

neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, in which GSH deficit appears to be secondary to an excess of ROS, the GSH deficit observed in schizophrenia is of primary origin, due to a genetic impairment of GSH synthesis.

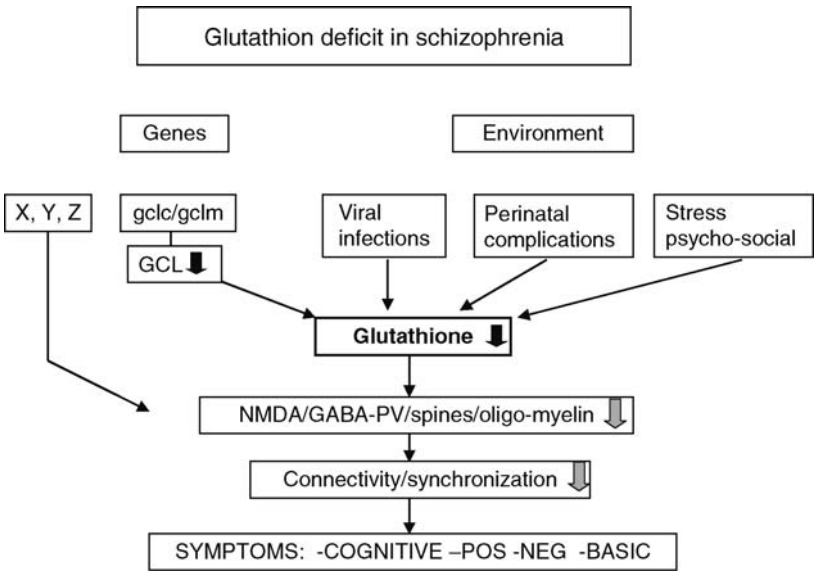
Life event stresses, through the hypothalamic–pituitary–adrenal (HPA) axis stimulation, induce important DA release (Piazza and Le Moal, 1996; Barrot et al., 2001; Ganguli et al., 2002). This could result, when combined with a GSH deficit, in an increase in ROS and thus in oxidative damage to lipid, protein and DNA (Liu et al., 1996), leading in turn during brain development and maturation to progressive structural and functional disconnectivity. This hypothesis is strongly supported by the decrease in dendritic spines on pyramidal cells and the reduction of parvalbumin immunoreactive profiles in GABAergic interneurons of the anterior cingulate cortex that were observed in rats with a transient GSH deficit combined with an excess of extracellular DA during their early postnatal development. Moreover, the effect of an acute GSH deficit on the function of NMDA-R and on DA signaling indicates that a GSH deficit during adulthood could also contribute to the symptomatology of the illness. Our studies suggest that the GSH deficit observed in schizophrenia patients is due to a genetic anomaly affecting at least one GSH-related enzyme, GCL, the rate-limiting enzyme of GSH synthesis. This genetic susceptibility, in convergence with environmental stressors during a particular period of ontogeny, may lead to additional disturbances within subsequent developmental processes. This is compatible with the neurodevelopmental theory of schizophrenia (Lewis and Levitt, 2002). Indeed, the potential genetic origin of the GSH deficit is consistent with the idea that the structural disconnectivity takes place during brain maturation. The premorbid troubles exacerbate at late adolescence when stress enhances DA release. It is indeed established that the brain DA innervation increases towards the end of puberty and that sociopsychological stresses induce a DA discharge. As the onset of the first disease outbreak is frequently triggered by a psychosocial stress situation, it is tempting to speculate that a crisis situation might cause a massive DA discharge which outruns an already impaired GSH antioxidant/redox system and precipitates the psychotic symptoms. Most interestingly, in male rat, 1 h restrain stress induces a deficit in elevated plus maze performance and a 50% brain GSH decrease, an effect which is reversed by pretreatment with NAC (Chakraborti et al., 2007). Moreover, there might exist "critical periods" essential for the normal development of anterior cortical areas (prefrontal, anterior cingulate, anterior temporal) as has been shown in the visual system (for review see Hensch (2005). When such critical periods are perturbed by some key factors, it could lead to detrimental consequences for the functions, which depend on appropriate anterior cortical wiring. In other words, the convergence between genetic susceptibly and environmental stressors taking place at specific periods during brain development could induce the alterations responsible for the disease.

Environmental risk factors for schizophrenia, such as viral infections as well as obstetrical and perinatal complications, can themselves impact the GSH metabolism. Thus, various viral infections affect the GSH system (Buhl et al., 1989; Eck et al., 1989; Nucci et al., 2000). Very interestingly, the injection of the bacterial endotoxin lipopolysaccharide (LPS) during pregnancy in rats triggered an oxidative stress in the fetuses' hippocampus, as evidenced by a rapid rise in protein carbonylation as well as by decreases in α-tocopherol levels and the ratio of reduced/oxidized forms of glutathione (GSH/GSSG). This also led to the impairment of NMDA synaptic currents and long-term potentiation in CA1, as well as spatial recognition in 28-day-old offsprings. These oxidative stress biochemical markers and the delayed detrimental effects were restored by pretreatment with NAC, the GSH precursor. Oxidative stress may thus participate in the neurodevelopmental damage induced by a prenatal LPS challenge (Lante et al., 2007). Furthermore, a deficit of GSH biosynthesis as well as of GCLM mRNA and protein was observed in HIV-1 tat transgenic mice model, resulting in an increased sensitivity of GCL to feedback inhibition by GSH (Choi et al., 2000). This is precisely the enzymatic subunit for which the gene has been shown to be associated with the disease. During preeclampsia, one of the perinatal complications that show increasing risk for schizophrenia, GSH levels are lower than in normal pregnancy (Chen et al., 1994; Kharb, 2000). These above risk factors by their direct interference on the GSH metabolism could therefore be highly detrimental for subjects with a genetic susceptibility linked to the synthesis of GSH (*Figure 2.5-3*).

In addition, GSH dysregulation might play a role in the framework of the "Single-Carbon Hypothesis" of schizophrenia originally proposed by Smythies et al. (Smythies et al., 1997). In the transmethylation pathway, methionine is converted to homocysteine providing methyl groups to DNA, lipids and proteins.

Figure 2.5-3

Proposed model of genetic and environmental factors contributing to GSH deficit and neurobiological anomalies leading to schizophrenia phenotypes. Evidences are given in the text (*Section 2.5.5*)



Homocysteine can be either re-methylated to methionine through activation of methionine synthase, which depends on folate and vitamin B12, or metabolized to cystathionine and cysteine through the transsulfuration pathway. Cysteine can then be used as a precursor of GSH (Vitvitsky et al., 2006). Thus, homocysteine is in a central position, going either to transmethylation or to transsulfuration and GSH synthesis. Deth and collaborators proposed that methionine synthase could act as a "redox sensor." Under oxidative stress conditions, methionine synthase is inactivated, allowing homocysteine to be shunted into the transsulfuration pathway to increase GSH synthesis and thus neutralize oxidative stress (Muratore et al., 2006; Powercharnitsky et al., 2006; Deth et al., 2007). This mechanism is of particular interest in the perspective of schizophrenia, as hyperhomocysteinemia has been reported in subgroups of patients (Regland et al., 1995; Levine et al., 2002; Muntjewerff et al., 2006). Such a hyperhomocysteinemia could be related to a partial block of the transmethylation and/or the transsulfuration pathway. In this context, a GSH deficit due to the impairment of GCL could very well interfere with the transsulfuration pathway and inhibit the methionine synthase affecting the transmethylation pathway. This mechanism is likely to be exacerbated by an enhancement of oxidative stress during the acute phases of psychosis. The neural toxicity of homocysteine, mediated by its product homocysteic acid, could then be responsible for some of the failures of brain functions. Furthermore, Kety and collaborators showed that a diet enriched in methionine exacerbates schizophrenia symptoms (Park et al., 1965). This exogenous methionine overloads the methionine cycle, thereby contributing to homocysteine excess. It should also be noted that anomalies of the folate system (Carney, 1967; Young and Ghadirian, 1989) and of the DNA methylation have been reported in schizophrenia patients (Abdolmaleky et al., 2004, 2005; Petronis, 2004). Summing up, a deficit in GSH could strongly contribute to the dysfunction in regulation of key methylation processes.

Furthermore, some comorbidities seem to share potential genetic vulnerability factors with schizophrenia. An increased risk of myocardial infarction has been described in schizophrenia patients (Enger et al., 2004). Both GCLC and GCLM genes contain DNA polymorphisms which increase the susceptibility to coronary endothelial vasomotor dysfunction and myocardial infarction (Nakamura et al., 2002; Koide et al., 2003). The mutations associated with the myocardial infarction were described in promoter sequences of both GCL genes. It was shown that these alterations decreased the responsiveness to oxidative

stress. Indeed, schizophrenia may inherently share predisposing factors with cardiovascular illnesses, diabetes and metabolic syndrome: Insulin resistance, impaired fasting glucose, impaired glucose tolerance and abnormal body fat distribution are all more prevalent in drug-naïve and first-episode schizophrenia patients (i.e., prior to antipsychotics drug treatment) compared to age-, sex- and lifestyle-matched healthy controls (Ryan et al., 2003; Thakore, 2005). Bahn et al. also reported an increase of glucose levels in the CSF of drug-naïve patients (Holmes et al., 2006). Interestingly, increased production of ROS and oxidative stress has been implicated as one essential mechanism for induction of both micro- and macrovascular complications (Haidara et al., 2006). In particular, abnormal intracellular GSH redox status plays an important role in reducing insulin sensitivity in type 2 diabetes mellitus. GSH infusion increased both intraerythrocytic GSH/GSSG ratio and insulin-mediated glucose uptake (Paolisso et al., 1992; De et al., 1998).

Thus, a redox/antioxidant dysregulation could be a "hub" of vulnerability (Mirnics et al., 2006) that contributes to the development of various diseases, including cardiovascular diseases, diabetes and major psychiatric disorders. These considerations also emphasize the role of a general metabolic component in the pathophysiology of schizophrenia, which most likely requires brain-specific risk factors to evolve. Moreover, the convergence of such redox/ antioxidant dysregulation "hub" with other tissue-specific risk factors would eventually determine the specificity of the effects. The relative weight of this vulnerability factor among many others remains to be assessed.

Acknowledgments

We would like to thank all the patients and controls subjects who participated in the studies. Our thanks also go to the following collaborators who were involved in the project: O. Boulat, V. Castagne, P. Deppen, F. Gheorghita, G. Grima, J. P. Hornung, M. Knyazeva, M. L. Matthey, R. Meuli, M. Murray, M. Preisig, V. Ruiz, M. Saraga, F. Schenk, A. Solida, J. Sandell and M. Tosic. We are grateful to Prof P. Magistretti for his constant support. We also thank the technical collaborators N. Ballanfat, B. Benz, M. Blanc, V. Perroud and S. Reymond. This work has been supported by the Swiss National Foundation, Novartis Research Foundation, the "Loterie Romande," NARSAD, Foundation Pro Scientia et Arte and the Foundation Alamaya for research on schizophrenia.

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Section 3

Anatomical Systems

3.1 Hippocampus

S. Heckers

D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_3.1,

Abstract: Schizophrenia is associated with structural and functional abnormalities in a variety of neural circuits. This chapter reviews studies of the hippocampus in schizophrenia.

The original finding was significantly smaller overall hippocampal volume in schizophrenia. Many studies have confirmed this original finding and have demonstrated it at the initial stage of the illness. While less prominent when compared to neurodegenerative illnesses, the volume difference is comparable to major depression and posttraumatic stress disorder.

More recent studies have explored region- and cell-specific alterations of hippocampal anatomy as well as functionally selective impairments of hippocampal function. Several studies have identified a decreased expression of the genes and proteins that characterize hippocampal interneurons. In addition, studies of regional cerebral blood flow have identified overall greater hippocampal activity and specific abnormalities of hippocampal recruitment during the performance of memory tasks.

Taken together, the evidence for a specific deficit of hippocampal interneurons is accumulating. Together with the neuroimaging findings, this pattern points to a disinhibition of hippocampal pyramidal cells in schizophrenia. Future studies will explore the molecular mechanisms of hippocampal dysfunction in schizophrenia and it's impact on the cognitive and social function in schizophrenia.

List of Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; ERC, entorhinal cortex; FEP, first-episode psychosis; fMRI, functional magnetic resonance imaging; GABA, gamma-aminobutyric acid; GAD, glutamic acid decarboxylase; PET, positron emission tomography; PHC, parahippocampal cortex; PRC, perirhinal cortex; rCMRglc, regional cerebral glucose metabolic rates; vGAT, vesicular gamma-aminobutyric acid transporter; vGT, vesicular glutamate transporter

1 Introduction

Two engines propel schizophrenia research: the study of the human brain and the exploration of abnormal mental states. Emil Kraepelin pioneered the former approach, showcasing the finding of cortical neuron loss in dementia praecox with photomicrographs in the later editions of his psychiatry textbook (Kraepelin, 1913). Eugen Bleuler was the champion of the latter approach, emphasizing cognitive and affective changes in schizophrenia (the most memorable being his four A's: autism, association, affectivity, and ambivalence) (Bleuler, 1911).

Studies of the hippocampus in schizophrenia recapitulate this pattern: structural changes of the hippocampus are now one of the most consistent findings in the entire schizophrenia research literature, while a separate line of investigation (and speculation) has provided evidence for a role of the hippocampus in the generation of psychotic symptoms and cognitive deficits (Heckers, 2001; Heckers and Konradi, 2002).

The hippocampus is a fitting place to look for a neural substrate of schizophrenia. The human hippocampus receives highly processed, multimodal sensory information via two inputs from the entorhinal cortex (ERC), compares the two inputs, and returns information to several cortical areas. This connectivity pattern is crucial for at least two brain functions that are considered to be abnormal in schizophrenia, i.e., memory and affect regulation.

Here, we review the abnormalities of hippocampal structure and function in schizophrenia and consider how they can give rise to memory deficits and psychotic symptoms. While it is unlikely that the hippocampus is the sole locus of pathology in schizophrenia, there is now compelling evidence that (1) hippocampal volume and shape are abnormal early on in the disease process, (2) specific regions of the hippocampus show alterations of neuronal gene expression, protein expression, and cell number, and (3) cognitive deficits and the experience of psychotic symptoms are linked to abnormalities of hippocampal function.

2 Hippocampal Volume and Shape

The initial evidence for abnormalities of the hippocampus in schizophrenia came from postmortem and structural imaging studies. A study exploring volume changes in limbic brain regions reported

that the hippocampus is 40% smaller in patients with schizophrenia compared with control subjects (Bogerts et al., 1985). Recent meta-analyses (Nelson et al., 1998; Wright et al., 2000) and reviews (Dwork, 1997; McCarley et al., 1999; Heckers, 2001) have now concluded that smaller hippocampal volume, while less dramatic than originally reported (on an average around 5%), is a robust finding in schizophrenia.

A considerable strength of structural neuroimaging studies is the ability to study hippocampal volume at the onset and throughout the course of the illness. Such studies have provided clear evidence that the hippocampus is smaller in patients with schizophrenia early in the disease process (first-break psychosis) (Honea et al., 2005; Steen et al., 2006; Velakoulis et al., 2006; Vita et al., 2006). This is a reassuring finding, since it makes it unlikely that hippocampal volume decrease is merely an effect of treatment or long-term illness (Scherk and Falkai, 2004, 2006).

However, it is not clear at which point, during the transitions from asymptomatic predisposition through the prodromal stage to apparent psychosis, the hippocampal volume change in schizophrenia occurs. Some studies have reported that smaller hippocampal volume predicts whether at-risk offspring of affected parents will develop schizophrenia (Lawrie et al., 1999; Pantelis et al., 2000). This has not been confirmed by a recent, large study (Velakoulis et al., 2006) and remains a matter of significant debate (Lawrie, 2007; Tebartz van Elst et al., 2007). At this point, it is reasonable to assume a progression of hippocampal volume reduction throughout the disease process. This may occur before, during, or after the presentation of psychotic features (Giedd et al., 1999; Velakoulis et al., 1999, 2006; Wood et al., 2000) and may be the consequence of the experience of stress as result of the illness (Arango et al., 2001; Phillips et al., 2006).

Several investigators have used structural MRI to study regionally selective changes of hippocampal volume and shape (Suddath et al., 1990; Rossi et al., 1994; Csernansky et al., 1998; Velakoulis et al., 2001; Wang et al., 2001; Weiss et al., 2005; Goldman et al., 2007). In the context of the distinct afferent and efferent connections (Goldman-Rakic et al., 1984; Barbas and Blatt, 1995) and the evidence for a functional segregation of the anterior and posterior hippocampal formation (Lepage et al., 1998; Strange et al., 1999), this finding could indicate that some but not all hippocampal functions are impaired in schizophrenia.

The hippocampus is not smaller in all patients with schizophrenia, i.e., hippocampal volume alone is not a biological marker and cannot be used to make the diagnosis of schizophrenia. Furthermore, several other diseases, most notably unipolar depression (Campbell and Macqueen, 2004), PTSD (Smith, 2005), and alcoholism (Geuze et al., 2005) are also associated with reduced hippocampal volume. Does smaller hippocampal volume tell us something about a unique pathology in schizophrenia or is hippocampal volume decrease the final common pathway of several pathological conditions?

Finally, the hippocampus is not the only brain region that is smaller in schizophrenia (Honea et al., 2005; Steen et al., 2006; Velakoulis et al., 2006; Vita et al., 2006). Especially those brain regions that are closely connected with the hippocampus (i.e., amygdala, ERC, perirhinal cortex (PRC), and parahippocampal cortex (PHC)) appear to be affected similarly in schizophrenia (Wright et al., 2000; Sim et al., 2006) (*Figure 3.1-1*).

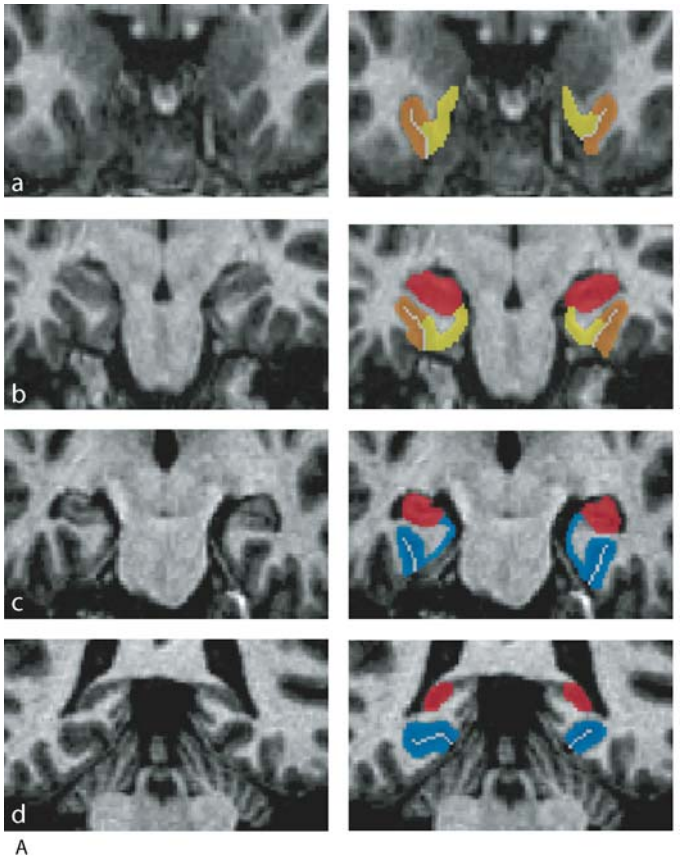
Despite the considerable evidence reviewed above, the significance of smaller hippocampal volume in schizophrenia remains unclear. Under the best of circumstances, hippocampal volume studies will help to diagnose schizophrenia (Davatzikos et al., 2005) and will have predictive power for illness progression and outcome. Additional information about the chemical constituents of the hippocampus, e.g., those measured by proton spectroscopy (Steen et al., 2005), could help to clarify the underlying pathological process. However, it is clear already now that a more detailed analysis of the hippocampus at the level of cellular organization and molecular mechanisms is required to appreciate fully a potential role of the hippocampus in schizophrenia.

3 Cellular Mechanisms of Hippocampal Dysfunction in Schizophrenia

Each human hippocampus contains ~10 million neurons (West and Gundersen, 1990). The majority of hippocampal neurons (about 90%) are large, pyramidal-shaped neurons (principal cells) that use

Figure 3.1-1

Medial temporal lobe volumes in schizophrenia. (A) Four coronal slices through regions of the medial temporal lobe at the level of the (a) amygdala, (b) uncus, (c) body, and (d) tail of the hippocampus (Entorhinal cortex (ERC) in yellow, perirhinal cortex (PRC) in orange, hippocampus in red and parahippocampal cortex (PHC) in blue), (B) Volume of the hippocampus and three regions of the parahippocampal gyrus: ERC, PRC, and PHC in control and schizophrenia subjects. Each circle indicates one subject. The individual volumes are centered around each of the four average regional volumes of the control group



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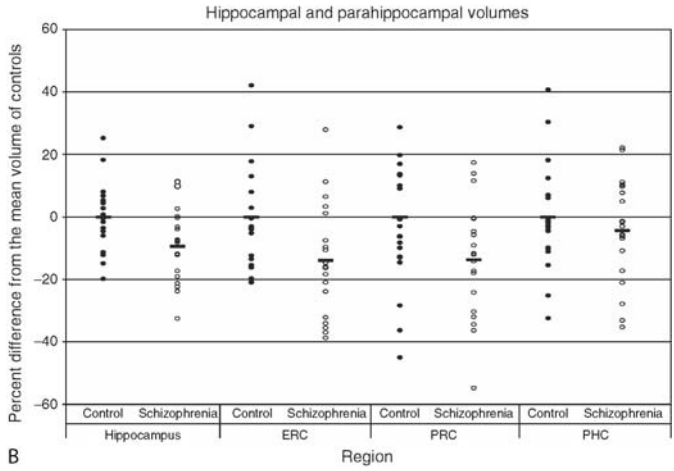


B

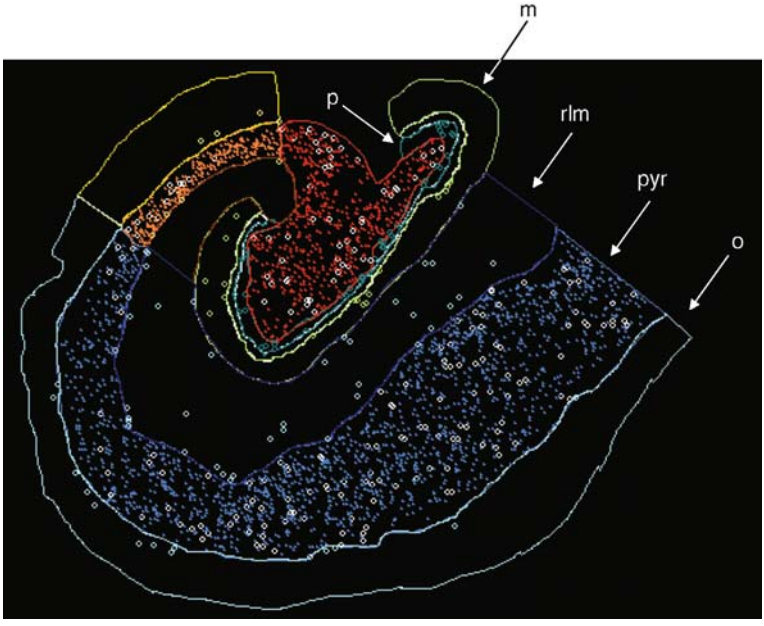
glutamate as their neurotransmitter and provide the excitatory drive in the hippocampus. The remaining 10% of hippocampal neurons are smaller, nonpyramidal neurons (nonprincipal cells) that use gamma-aminobutyric acid (GABA) as their neurotransmitter and provide the inhibitory tone in the hippocampus (Olbrich and Braak, 1985; Freund and Buzsaki, 1996). The pyramidal cell layer is the primary location of hippocampal neurons, whereas the two other layers of the hippocampus (i.e., the stratum oriens and the stratum radiatum/lacunosum/moleculare) contain few neurons. Subtle differences in the cellular architecture of the three-layered hippocampus give rise to the four sectors of the cornu ammonis and the dentate gyrus (*Figure 3.1-2*).

Most studies of overall cell density have reported no significant change in schizophrenia (Dwork, 1997; Harrison, 1999; Arnold, 2000). Two studies provided estimates of total hippocampal cell number and found no changes in any of the four sectors of the cornu ammonis in schizophrenia (Heckers et al., 1991; Walker et al., 2002). The finding of normal total cell number is important for the interpretation of decreased hippocampal volume reported by neuroimaging studies of patients with schizophrenia. In contrast to neuropsychiatric disorder such as temporal lobe epilepsy and dementia, smaller hippocampal volume is not the macroscopic correlate of pyramidal cell loss in schizophrenia.

Is there any evidence for deficits in subsets of hippocampal neurons? Here, we will briefly review the evidence for an abnormality of the GABAergic and glutamatergic neurons in the hippocampus in schizophrenia (reviewed by others (Benes, 1999; Arnold, 2000; Benes and Berretta, 2001; Harrison and Eastwood, 2001; Harrison, 2004) and in this volume (Kristiansen et al.)).

Figure 3.1-2

Pyramidal and nonpyramidal cells in the human hippocampus. The graph displays the exhaustive count of all pyramidal neurons (dots) and nonpyramidal neurons (open circles) in a 10-μ-thick, Nissl-stained section of the CA1 (blue), CA2/3 (yellow), and CA4 (red) regions of the cornu ammonis and the polymorph (p) and molecular (m) layers of the dentate gyrus (green). The pyramidal neurons are limited to the pyramidal cell layer (pyr), whereas nonpyramidal neurons are also located in the neighboring stratum oriens (o) and stratum radiatum/lacunosum/moleculare (rlm). The nonpyramidal neurons in the pyramidal cell layer are shown in white open circles to distinguish them better from the surrounding pyramidal neurons



3.1 GABAergic Hippocampal Neurons

Initial studies of GABAergic neurotransmission in schizophrenia focused on postsynaptic receptors, located on pyramidal and nonpyramidal cells, and revealed a regionally specific upregulation of GABA(A) receptor binding in sectors CA2–4, but not CA1 (Benes et al., 1996, 1997). The marked increase of the GABA(A) receptor in CA2/3 was found primarily on interneurons, indicating a decreased GABAergic regulation by other interneurons (Benes, 1999).

Recent studies have focused on the hallmark feature of GABAergic neurons, i.e., glutamic acid decarboxylase (GAD), the enzyme that converts glutamate to GABA. Two isoforms of *GAD* are known: the gene *GAD1* codes for GAD67 and the gene *GAD2* codes for GAD65. An initial in-situ hybridization study of GAD mRNA expression in the hippocampus in normal controls, patients with schizophrenia, and patients with bipolar disorder revealed significant decreases of *GAD2* (and to a lesser degree *GAD1*) mRNA expression in bipolar disorder and less significant changes in schizophrenia (Heckers et al., 2002). A subsequent gene-expression microarray study confirmed the decreased expression of *GAD1* and *GAD2* in bipolar disorder and did not find any changes in schizophrenia (Konradi et al., 2004). Finally, a large-scale postmortem study of *GAD1* mRNA expression in 32 patients with schizophrenia and 76 normal control subjects revealed decreased expression in schizophrenia in the dorsolateral prefrontal cortex, but no changes in the hippocampus (Straub et al., 2007).

These studies of hippocampal *GAD* mRNA expression in schizophrenia have to be reevaluated in light of a recent study using laser-capture microdissection and microarray profiling (Benes et al., 2007). Benes et al. revealed that changes of hippocampal GAD67 expression in schizophrenia are regionally specific; while the expression is normal in the large sector CA1, it is significantly decreased in the small, but functionally crucial, sector CA2/3.

Additional evidence for selective changes in hippocampal interneurons in schizophrenia comes from the study of calcium-binding proteins that are differentially expressed in essentially nonoverlapping subpopulations of interneurons (Seress et al., 1993; Freund and Buzsaki, 1996). These subpopulations of neurons create a dynamic, spatiotemporal control of hippocampal cell firing, which gives rise to several brain states crucial for normal cognition (Somogyi and Klausberger, 2005). An initial study of neuronal density revealed a significantly decreased density of parvalbumin-positive neurons in all hippocampal regions, while the density of calretinin-positive cells was normal (Zhang and Reynolds, 2000). The finding of decreased parvalbumin expression has now been corroborated by further studies and provides evidence for a subtype-specific abnormality of interneurons in schizophrenia (Eyles et al., 2002; Torrey et al., 2005).

3.2 Glutamatergic Hippocampal Neurons

Glutamate controls the excitation of neurons and glia through the activation of various glutamate receptors. Most studies of glutamatergic neurons in schizophrenia have focused on the expression of these receptor complexes. Glutamate receptors can be ion channels (three ionotropic glutamate receptor types assembled from various subunits: *N*-methyl-D-aspartate, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate) or G-protein coupled receptors (eight metabotropic glutamate receptors) (Ozawa et al., 1998).

The expression of various glutamate receptor subtypes is altered in the hippocampus in schizophrenia (Meador-Woodruff and Healy, 2000; Kristiansen et al., this volume). In a number of studies, the AMPA subunits GluR1 and GluR2 were decreased in the hippocampus and the parahippocampal gyrus (Harrison et al., 1991; Eastwod et al., 1995; Eastwood et al., 1997). In concordance, ligand binding to AMPA receptors was decreased (Kerwin et al., 1990). The kainate receptor subtypes GluR6 and KA2 were also significantly reduced in the schizophrenic hippocampus (Porter et al., 1997). Studies on kainate receptor density, conducted with radiolabeled kainate, demonstrated a decrease in the hippocampus (Deakin et al., 1989; Kerwin et al., 1990).

Initial studies of the NMDA receptor, which focused on the PCP binding site located inside the ion channel, found no marked changes in the hippocampus in schizophrenia (Kornhuber et al., 1989;

Meador-Woodruff and Healy, 2000). A study of the NMDA receptor subunits NR1, NR2A, and NR2B found an increase of NR2B mRNA and a decrease of NR1 mRNA in the hippocampus in schizophrenia.(Gao et al., 2000), but this has not been replicated in subsequent studies (see Kristiansen et al., this volume).

The balance of GABAergic and glutamatergic neurotransmission in the hippocampus in schizophrenia was explored in studies of the expression of the two modulatory proteins – complexin I (presumably reflecting on the integrity of GABAergic neurons) and complexin II (presumably reflecting on the integrity of glutamatergic neurons). While complexin II expression was found to be more reduced than complexin I expression in schizophrenia (Harrison and Eastwood, 1998; Eastwood and Harrison, 2000), this did not correlate with similar changes of the vesicular gamma-aminobutyric acid transporter (vGAT) and vesicular glutamate transporter (vGT) (Sawada et al., 2005). However, the complexin II/I ratio correlated inversely with the degree of cognitive impairment antemortem (Sawada et al., 2005), providing intriguing evidence that glutamatergic dysfunction in the hippocampus will lead to cognitive deficits in schizophrenia.

3.3 Additional Evidence

While most studies have explored abnormalities of GABAergic and glutamatergic neurotransmission, additional evidence suggests further molecular changes of the hippocampus in schizophrenia. First, there are abnormalities of serotonergic, cholinergic, and dopaminergic neurotransmission in the hippocampus in schizophrenia (see Kristiansen et al., this volume). The most compelling evidence exists, arguably, for changes in cholinergic neurotransmission, including the decreased expression and binding to the alpha-7 nicotinic receptor and the M1/M4 muscarinic receptors (see Kristiansen et al., this volume).

A recent line of evidence has implicated abnormalities of mitochondria in the mechanisms of both schizophrenia and bipolar disorder. While some have reported abnormal expression of nuclear genes coding for proteins involved in mitochondrial energy metabolism in bipolar disorder but not schizophrenia (Konradi et al., 2004), others have provided evidence for mitochondrial pathology in both disorders (Altar et al., 2005).

Taken together, there is evidence for cellular and molecular abnormalities of the hippocampus in schizophrenia. These changes lead neither to an overall decrease in the number of neurons nor to an overall decrease of either GABAergic or glutamatergic neurotransmission. Rather, hippocampal pathology in schizophrenia seems to be selective for subtypes of neurons and for regions within the cornu ammonis. Such a pattern of cell- and region-specific pathology could be related to some of the recently identified genetic mechanisms of schizophrenia and could give rise to selective deficits of hippocampal function in patients with schizophrenia.

4 The Genetic Basis of Hippocampal Abnormalities in Schizophrenia

While a genetic basis of schizophrenia is now firmly established, it is less clear which regions of the brain are affected by potential changes in protein expression and function (Harrison and Weinberger, 2005; Owen et al., 2005). Several potential risk genes for schizophrenia are expressed in the hippocampus and it is, therefore, appropriate to speculate about their role in the mechanisms of abnormal hippocampal structure and function in schizophrenia. Here, we will briefly review the evidence for effects of three schizophrenia risk genes, i.e., neuregulin-1 (*NRG-1*), disrupted in schizophrenia 1 (*DISC-1*), and dystrobrevin-binding protein 1 (*DTNBP-1*), also known as dysbindin-1, in the hippocampus.

The *NRG-1* gene (located on chromosome 8p22) and the gene for one of its receptors, *ErbB4* (located on chromosome 2q34), have both been associated with schizophrenia (Owen et al., 2005). *NRG1* and *ErbB4* are expressed in the hippocampus (Law et al., 2004; Mechawar et al., 2007) and are of particular interest since they regulate GABAergic neurotransmission (Woo et al., 2007) and affect the function of the alpha-7 nicotinergic receptor (Chang and Fischbach, 2006) located on hippocampal interneurons. *NRG-1* is known

to affect long-term potentiation of hippocampal synapses and has effect on dendritic growth and plasticity (Li et al., 2007).

The disrupted in schizophrenia 1 (*DISC-1*) gene was originally identified in a single pedigree with prominent psychiatric history and has been subsequently associated with several aspects of the schizophrenia phenotype (Roberts, 2007). Although the expression of DISC-1 mRNA was not found to be abnormal in schizophrenia, the expression of several molecules in the *DISC-1* pathway was decreased in schizophrenia (Lipska et al., 2006). While the exact mechanisms of DISC-1 in schizophrenia remain unclear, hippocampal volume and function are under considerable control by the *DISC-1* gene (Callicott et al., 2005).

The dystrobrevin-binding protein 1 (DTNBP-1), also known as Dysbindin-1, has been associated with schizophrenia in several studies (Harrison and Weinberger, 2005). The presynaptic dysbindin-1 expression was found to be reduced in intrinsic, glutamatergic terminals of the hippocampus in schizophrenia (Talbot et al., 2004). This has been interpreted as contributing to glutamatergic dysfunction in the polysynaptic pathway in the hippocampus. Taken together, it is likely that some of the genetic effects of NRG-1, DISC-1, and DTNBP1 in schizophrenia are conveyed through abnormalities of the hippocampus.

5 Hippocampal Function and Schizophrenia

Adult relatives of patients with schizophrenia who do not develop the full-fledged picture of schizophrenia (but might show more subtle signs of psychopathology) have smaller hippocampal volumes (Seidman et al., 1999, 2002; Honea et al., 2005; Boos et al., 2007). These studies provide evidence for the hypothesis that a smaller hippocampus is an endophenotype (i.e., a brain abnormality linked to a genetic risk factor for schizophrenia) (Tsuang, 2000). While these more recent studies are in contrast to an earlier study of monozygotic twins discordant for schizophrenia (Suddath et al., 1990), they challenge the notion that hippocampal pathology may explain the core clinical features of the illness. This is intriguing, since a growing volume of literature has linked hippocampal dysfunction to the cognitive deficits and psychotic symptoms seen in schizophrenia. We will review this evidence next.

The advent of functional neuroimaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) has provided researchers with the tools to study hippocampal function in patients with schizophrenia. Most investigators have explored a role of the hippocampus in the encoding and retrieval of memory (Achim and Lepage, 2005; Boyer et al., 2007). The unique role of the hippocampus within the memory system is the ability to disambiguate relationships between items and sequences of events, necessary for relational, episodic, and autobiographical memory (Eichenbaum, 2004). However, the hippocampus is also crucial for other cognitive and motor functions (Bast and Feldon, 2003). This has led to the study of hippocampal mechanisms with regard to various brain functions in schizophrenia, ranging from sensory gating (Tregellas et al., 2007) to decisional capacity (Eyler et al., 2007).

Functional imaging studies, linking schizophrenia to a functional abnormality of the hippocampus, have pursued three different kinds of questions:

1. Is resting hippocampal activity abnormal in schizophrenia?
2. Is the experience of psychotic symptoms such as delusions and hallucinations associated with abnormalities of hippocampal function?
3. Is the activity of the hippocampus abnormal during the performance of cognitive tasks, especially in the domain of memory?

5.1 Hippocampal Activity at Rest in Schizophrenia

Two different patterns of abnormal hippocampal activity at rest have been described in schizophrenia: lower regional cerebral glucose metabolic rates (rCMRglc) in the hippocampus (Buchsbaum et al., 1992; Tamminga et al., 1992; Nordahl et al., 1996) and increased medial temporal lobe regional cerebral blood

flow (rCBF) (Friston et al., 1992; Kawasaki et al., 1992, 1996; Liddle et al., 1992; Medoff et al., 2001; Lahti et al., 2003; Malaspina et al., 2004). While it is not easy to reconcile these findings, more recent studies have demonstrated that increased hippocampal rCBF in schizophrenia is normalized in patients treated with dopamine D2 antagonists (Medoff et al., 2001; Lahti et al., 2003; Malaspina et al., 2004), potentially obscuring abnormal patterns of resting activity. Furthermore, it is likely that some of the abnormalities of hippocampal activity at rest are related to the unique composition of the various patient samples. For example, Gur et al found increased left temporal metabolism in patients with negative symptoms as well as those with severe delusions and hallucinations (Gur et al., 1995). Similarly, when resting rCBF values were correlated with clinical symptoms, increased left medial temporal lobe blood flow in schizophrenia was associated with more severe psychopathology in general (Friston et al., 1992) or with more prominent positive symptoms (delusions and hallucinations) (Liddle et al., 1992). These studies have been interpreted as evidence that hippocampal hyperactivity (or the lack of inhibition) is involved in the pathogenesis of delusions and hallucinations.

5.2 Hippocampal Activity During the Experience of Psychosis

Several neuroimaging studies have demonstrated the activation of a network of brain regions during the experience of auditory hallucinations in patients with schizophrenia (Weiss and Heckers, 1999). These regions include those involved in the processing of auditory information and language as well as the hippocampal formation. Notably the studies by Silbersweig et al. (1995) and Dierks et al. (1999) have documented activation of the hippocampal formation during the experience of auditory hallucinations. It is unclear whether the hippocampal activation seen in these studies occurs early on in the generation of the hallucinatory experience or whether it is involved in the top-down processing of representations generated in the primary and secondary auditory cortices, e.g., to monitor the source of an auditory representation.

5.3 Hippocampal Activity and Cognitive Function in Schizophrenia

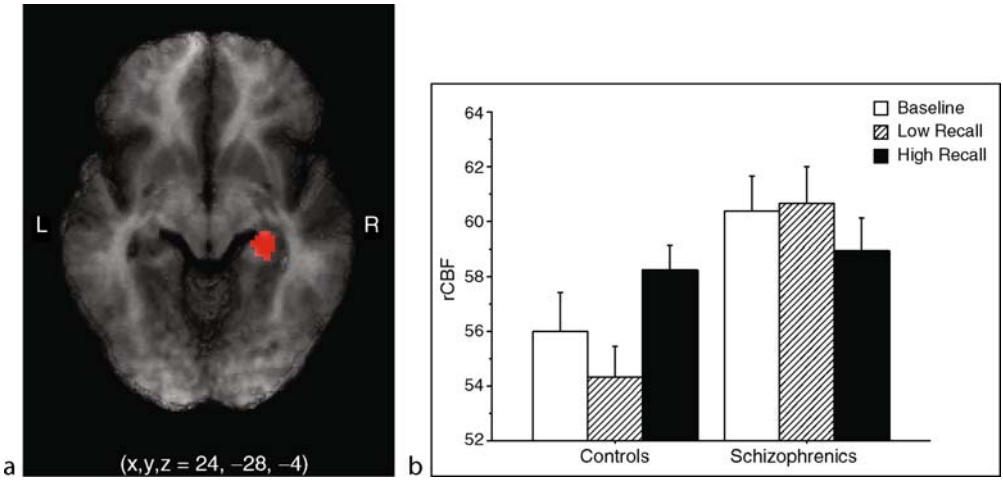
The ability to encode new information into memory and to retrieve previously stored information from memory is impaired in schizophrenia. Several models have proposed abnormalities of cortical–hippocampal circuitry as the mechanism for the abnormal mental representations seen in schizophrenia (Roberts, 1963; Venables, 1992; Hemsley, 1993; Talamini et al., 2005; Siekmeier et al., 2007).

Such models can now be tested using functional neuroimaging experiments. We provided the first evidence for abnormal hippocampal recruitment during memory retrieval in schizophrenia (Heckers et al., 1998, 1999) (*Figure 3.1-3*). While normal subjects activated a right frontal-temporal network to retrieve previously studied words, patients with schizophrenia failed to recruit the hippocampus but showed robust and even increased activation of prefrontal regions. Compared to the control group, hippocampal activity was continuously increased in schizophrenia and was not modulated by environmental contingencies. Increased hippocampal activity at baseline and impaired recruitment during episodic memory retrieval might represent the functional correlate of an abnormal cortico–hippocampal interaction in schizophrenia (Fletcher, 1998).

This initial finding of abnormal hippocampal function during memory retrieval in schizophrenia has been clarified by several subsequent studies. First, patients with schizophrenia rely less on the recruitment of the hippocampus and show more widespread activation of the prefrontal cortex during the retrieval of previously learned information (Weiss et al., 2003). Second, the ability to classify new items as previously not experienced is impaired in schizophrenia and is associated with decreased activation and smaller volume of the hippocampus (Weiss et al., 2004). Third, hippocampal recruitment in schizophrenia is impaired specifically during a relational, but not during a nonrelational memory task (Ongur et al., 2006).

Figure 3.1-3

Abnormal recruitment of the hippocampus during memory retrieval in schizophrenia. The right hippocampal region indicated in red in (a) showed increased relative cerebral blood flow (rCBF) during high accuracy recall when compared with both lexical retrieval at baseline and low accuracy recall in healthy control subjects ("controls" in (b)). This normal pattern was absent in the schizophrenia group and all three recall conditions were associated with higher rCBF in the hippocampus ("schizophrenics" in (b))



These findings of impaired hippocampal activation during memory retrieval in chronic patients with schizophrenia have now been complemented by a study of hippocampal-based encoding of memory in first-episode psychosis (FEP) patients (Achim et al., 2007). While some hippocampal functions remained intact, the FEP patients demonstrated a selective deficit to engage hippocampal-dependent relational binding when presented with arbitrary stimulus pairs, resulting in poorer subsequent recognition performance.

In addition to the abnormalities of hippocampal function during the performance of memory tasks as summarized above and reported by others (Ragland et al., 2001; Jessen et al., 2003), studies have revealed abnormal patterns of hippocampal activity in schizophrenia. For example, patients with schizophrenia demonstrate significantly greater activation of the hippocampus while passively viewing facial expressions (Holt et al., 2006). Furthermore, healthy subjects demonstrate significant habituation of hippocampal activity to the repeated presentation of fearful faces, whereas patients with schizophrenia do not demonstrate such habituation (Holt et al., 2005) (*Figure 3.1-4*).

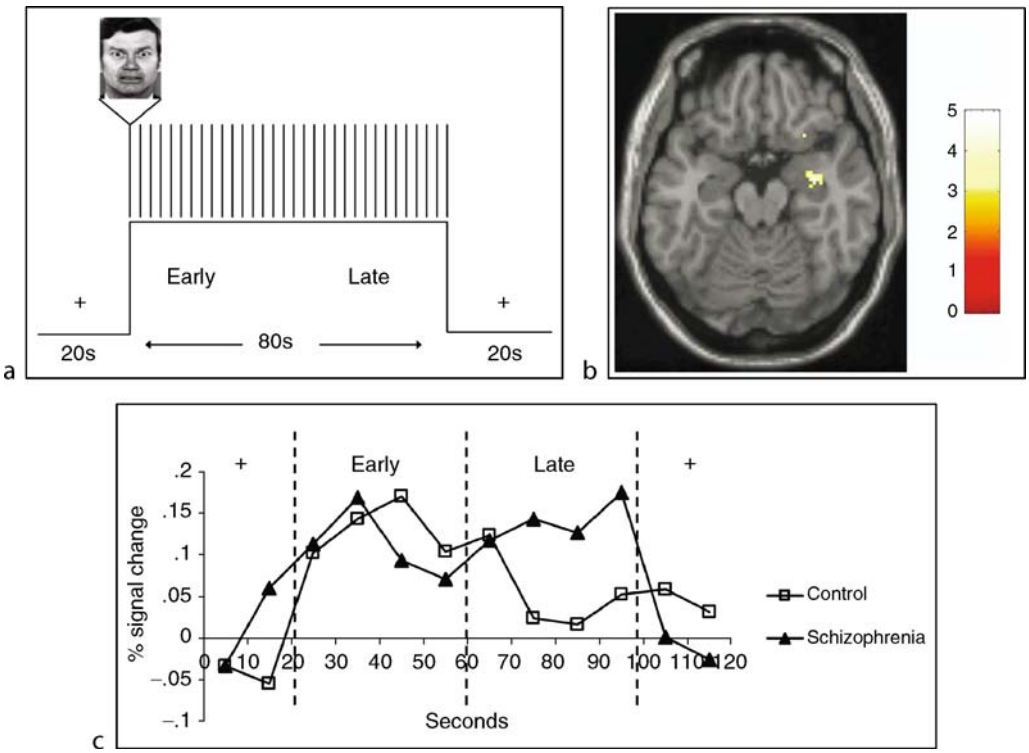
In summary, functional neuroimaging studies have reported increased blood flow in the hippocampus in schizophrenia, which is associated with higher levels of psychopathology and the experience of psychosis (i.e., delusions and hallucinations). The evidence of abnormal hippocampal activity is particularly strong for the domain of memory, with several studies revealing specific abnormalities of hippocampal recruitment during the performance of memory tasks. Future studies need to explore whether such abnormalities of hippocampal function, demonstrated so far in small samples of subjects, can explain the memory deficits in most patients with schizophrenia and, most importantly, whether they can explain the social dysfunction resulting from memory deficits in schizophrenia (Green, 1996; Eyler et al., 2007).

6 Conclusion: The Role of the Hippocampus in Schizophrenia

Over the last 20 years, the hippocampus has been studied extensively in patients with schizophrenia. This is a remarkable change from the preceding 80 years of schizophrenia research, which had focused primarily on

Figure 3.1-4

Decreased habituation of hippocampal activity during passive viewing of faces in schizophrenia. During fMRI scanning, fearful and happy faces were presented repeatedly to healthy control and schizophrenia subjects. One vertical line in the top part of (a) indicates one scan with five presentations of a face. Habituation of the hippocampus was measured by comparing the hemodynamic response occurring during the early and late portions of an 80-s block. Right hippocampal habituation to fearful faces was significantly greater in control subjects than in the schizophrenia patients (b). There were no significant differences between the control and schizophrenia subjects in the first half of the block, suggesting a sustained activation in response to fearful faces in schizophrenia (c)



the cerebral cortex and the thalamus (Heckers, 1997). In addition, the growing awareness of significant memory deficits in schizophrenia has focused attention on potential hippocampal mechanisms (Weiss and Heckers, 2001).

While the macroscopic changes of hippocampal volume and shape and the abnormal patterns of hippocampal activity and task-related recruitment are now firmly established, a comprehensive hippocampal model of schizophrenia is still lacking (Heckers, 2001). The most compelling models have proposed that impaired information processing (e.g., the inability to ignore irrelevant stimuli), memory deficits, and psychotic symptoms in schizophrenia are caused by hippocampal dysfunction or hyperactivity (Bickford-Wimer et al., 1990; Adler and Waldo, 1991; Krieckhaus et al., 1992; Venables, 1992; Hemsley, 1993; Port and Seybold, 1995). These models have recently been supported by neural network models of hippocampal dysfunction in schizophrenia (Talamini et al., 2005; Siekmeier et al., 2007). Some have also proposed a hippocampal mechanism for hyponatremia/polydipsia in patients with schizophrenia (Goldman and Mitchell, 2004; Goldman et al., 2007).

What is required now is the clear demonstration that these findings are relevant for the diagnostic classification of schizophrenia. If psychiatric disorders other than schizophrenia are also associated with

abnormalities of the hippocampus (Geuze et al., 2005), then we need to explore possible overlap and/or differences between them at the level of the hippocampus. Finally, a more detailed analysis of the cellular and molecular mechanisms is required to identify targets for drug development (Dhikav and Anand, 2007; Newton and Duman, 2007) and illness prevention.

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3.2 Early-Stage Visual Processing Deficits in Schizophrenia

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_3.2,

Abstract: *Purpose of review*: While cognitive deficits, including memory, executive functioning, and attentional deficits, are a core feature of schizophrenia, recent work has also shown basic sensory processing dysfunction as well. Deficits are particularly prominent in the visual system and may be related to cognitive deficits and outcome. This chapter reviews studies of early-stage visual processing in schizophrenia. These studies reflect the growing interest and importance of sensory processing deficits in schizophrenia.

Recent Findings: The visual system is divided into magnocellular and parvocellular pathways, which project to dorsal and ventral visual areas. Recent electrophysiological and behavioral investigations have found preferential magnocellular/dorsal stream dysfunction, with some deficits in parvocellular function as well. These early-stage deficits appear to be related to higher level cognitive, social, and community function. Postmortem and MRI studies provide anatomical support for early visual processing dysfunction.

Summary: These findings highlight the importance of sensory processing deficits, in addition to higher cognitive dysfunction, for understanding the pathophysiology of schizophrenia. Understanding the nature of sensory processing deficits may provide insight into mechanisms of pathology in schizophrenia, such as *N*-methyl-D-aspartate (NMDA) dysfunction or impaired signal amplification, and could lead to treatment strategies, including sensory processing rehabilitation, that may improve outcome.

List of Abbreviations: ISI, interstimulus interval; LGN, lateral geniculate nucleus; M, magnocellular; MST, medial superior temporal area; MT, medial temporal area; NMDA, N-methyl-D-aspartate; P, parvocellular; ssVEP, steady-state visual evoked potential; TE, temporal lobe; TEO, temporal occipital area; tVEP, transient visual evoked potential; VEP, visual evoked potential; V1, primary visual cortex

1 Introduction

When Bleuler first described schizophrenia, among the issues he noted was that sufferers were "flooded with an undifferentiated mass of incoming sensory data" (Bleuler, 1950). While a great deal of focus in schizophrenia has been on higher cognitive deficits (Goldman-Rakic, 1994; Goldberg and Gold, 1995; Weinberger and Gallhofer, 1997; Green, 1998), there is a rich but underappreciated clinical literature on perceptual deficits (Arieti, 1955, 1962; Conrad, 1958; McGhie and Chapman, 1961; Chapman, 1966; Matussek, 1987). In first person accounts, dating back decades (McGhie and Chapman, 1961), patients often complained of visual distortions as among the earliest symptoms of schizophrenia. Similarly, in symptom inventories, subjective complaints regarding visual disturbances were as common as complaints regarding higher-order processes such as attention or memory (McGhie and Chapman, 1961; Cutting and Dunne, 1989; Klosterkotter et al., 2001). Complaints included changes in visual perception such that vision is less clear, muted or blurry (Freedman and Chapman, 1973), or more acute (Freedman, 1974), that "everything is in bits" (McGhie and Chapman, 1961), and that "the organization of things was different" (Cutting and Dunne, 1989). Indeed, in a study of people with prodromal symptoms of schizophrenia, visual perceptual problems were actually more sensitive than other symptoms such as problems of thought and language, and ideas of reference in terms of predicting who will develop schizophrenia (Klosterkotter et al., 2001).

This chapter focuses on the growing evidence for perceptual deficits in schizophrenia, which have become increasingly well-documented in the visual system (Green et al., 1994b; Cadenhead et al., 1998; Chen et al., 1999c, 2008; Schwartz et al., 1999; Braus et al., 2002; Doniger et al., 2002; Li, 2002; Schechter et al., 2003; Keri et al., 2004; Butler et al., 2005, 2007; Dakin et al., 2005; Kim et al., 2005; Slaghuis et al., 2007; Lalor et al., 2008; Martinez et al., 2008; Vohs et al., 2008; Yeap et al., 2008a) and may contribute to higher level cognitive impairments and community outcome (Kee et al., 1998; Brenner et al., 2002; Gold et al., 2002; Sergi and Green, 2003; Butler et al., 2005; Keri et al., 2005b; Revheim et al., 2006; Sergi et al., 2006). An early report by (Saccuzzo et al., 1974) of visual backward masking dysfunction in schizophrenia was particularly important, not only because it indicated deficits in the earliest components of visual information processing (Schuck and Lee, 1989; Green et al., 1994a, b) but also because it

suggested dysfunction of a particular visual pathway – the psychophysically defined transient visual pathway (Breitmeyer and Ganz, 1976; Merritt and Balogh, 1989; Schuck and Lee, 1989; Slaghuis and Curran, 1999).

The classic transient/sustained psychophysical dichotomy has been supplanted over recent years by anatomically and physiologically based distinctions between magnocellular and parvocellular pathways, which roughly correspond to the properties of transient and sustained pathways respectively. The magnocellular system, in general, conducts low-resolution visual information rapidly to cortex and is involved in attentional capture and the processing of overall stimulus organization (Merigan and Maunsell, 1993; Steinman et al., 1997; Vidyasagar, 1999; Fenske et al., 2006). The parvocellular system, in contrast, conducts high-resolution visual information to cortex and is involved in the processing of fine-grained stimulus configurations and object identification (Merigan and Maunsell, 1993; Norman, 2002) (*Figure 3.2-1*). A focus of this review is to examine the differential functional roles of magnocellular and parvocellular systems in visual pathway dysfunction, including their contribution to "upstream" cognitive impairments and outcome.

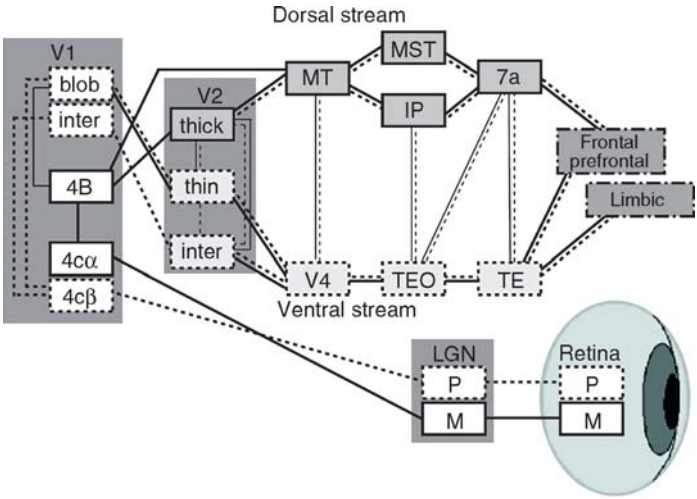
Figure 3.2-1

***Left* panel shows an image with high spatial frequencies filtered out so that the image only contains low spatial frequencies. This biases processing towards the magnocellular pathway and results in a low-resolution image; middle panel shows the same image but with low spatial frequencies filtered out so that it only contains high spatial frequencies. This biases processing towards the parvocellular pathway and results in only fine detail being present; *right* panel shows the intact picture with all spatial frequencies present. Reprinted with permission from Bar (2004)**

Both the magnocellular and parvocellular pathways begin in the retina, and project by means of the lateral geniculate nucleus (LGN) to primary visual cortex (striate cortex, V1) (*Figure 3.2-2*). These pathways have very different properties that can be manipulated to preferentially activate the magnocellular or parvocellular components of the system. For instance, magnocellular neurons are more sensitive than parvocellular neurons to low luminance contrast (Tootell et al., 1988; Kaplan, 1991). In addition, magnocellular cells are activated vigorously by stimulus elements that are relatively large, whereas parvocellular cells are activated more strongly by stimulus elements that are relatively small (Jindra and Zemon, 1989; Dacey and Petersen, 1992). Magnocellular cells are also relatively unresponsive to chromatic (color)

Figure 3.2-2

A schematic of the visual system. M, magnocellular; P, parvocellular; LGN, lateral geniculate nucleus; V1, primary visual cortex; MT, medial temporal area; MST, medial superior temporal area; TEO, temporal occipital area; TE, temporal lobe. This figure was made by Dr. Charles Schroeder



contrast, while parvocellular cells are not (Kaplan, 1991). It should be noted that there is also a third system of "koniocellular" neurons in the LGN with very small cell bodies (Hendry and Reid, 2000). These cells are thought to drive a third visual pathway whose function is not well understood but may be involved in the integration of somatosensory-proprioceptive information.

Magnocellular information is conveyed preferentially to parieto-occipital cortex and other dorsal stream visual areas that are involved in motion and space perception (the "where" pathway) (Merigan and Maunsell, 1993; Tootell et al., 1998b) (*Figure 3.2-2*). Parvocellular information is conveyed preferentially to temporo-occipital cortex and other ventral-stream areas that are involved in color and form perception (the "what" pathway) (Merigan and Maunsell, 1993; Tootell et al., 1998b). Crossover occurs at multiple levels, including from higher-order dorsal stream to higher-order ventral stream areas (Schroeder et al., 1998; Lamme and Roelfsema, 2000; Chen et al., 2007). Transmission is faster through the dorsal stream, perhaps permitting it to prime ventral stream areas. A fundamental role of the magnocellular system/dorsal stream may be to "spotlight" relevant information for transmission to ventral stream areas so that crossover inputs from dorsal stream may modulate activity within ventral stream structures (Vidyasagar, 1999; Doniger et al., 2002; Fenske et al., 2006; Laycock et al., 2007).

In accord with the original theory of "transient channel" dysfunction in schizophrenia, deficits are particularly prominent in processes, such as motion detection, velocity discrimination, spatial localization, trajectory, and eye-tracking tasks that depend mainly upon magnocellular input to the dorsal visual stream, and in the detection of low contrast and large stimuli (Green et al., 1994b; Stuve et al., 1997; Cadenhead et al., 1998; Chen et al., 1999a, 2004; Li, 2002; Brenner et al., 2003; Kim et al., 2006; Butler et al., 2007; Slaghuis et al., 2007). However, deficits have been observed as well even in the processing of parvocellular-biased stimuli (Slaghuis, 1998; Butler et al., 2005; Delord et al., 2006). In addition, while some ventral stream object identification deficits have also been found, several studies provide evidence that the latter are due to aberrant crossover input from dorsal to ventral stream areas (Schwartz et al., 1999; Doniger et al., 2002) and from aberrant feed-forward magnocellular input to frontal areas that subsequently impairs frontal feedback to ventral areas (Fenske et al., 2006; Laycock et al., 2007). Advances in understanding visual processing dysfunction in schizophrenia have occurred in part due to the use of basic neurophysiological properties of magnocellular and parvocellular neurons to

selectively activate these pathways. Recent papers examining early visual processing dysfunction, with an emphasis on those related to magnocellular and parvocellular as well as dorsal and ventral stream processing, are reviewed.

2 Electrophysiological Studies

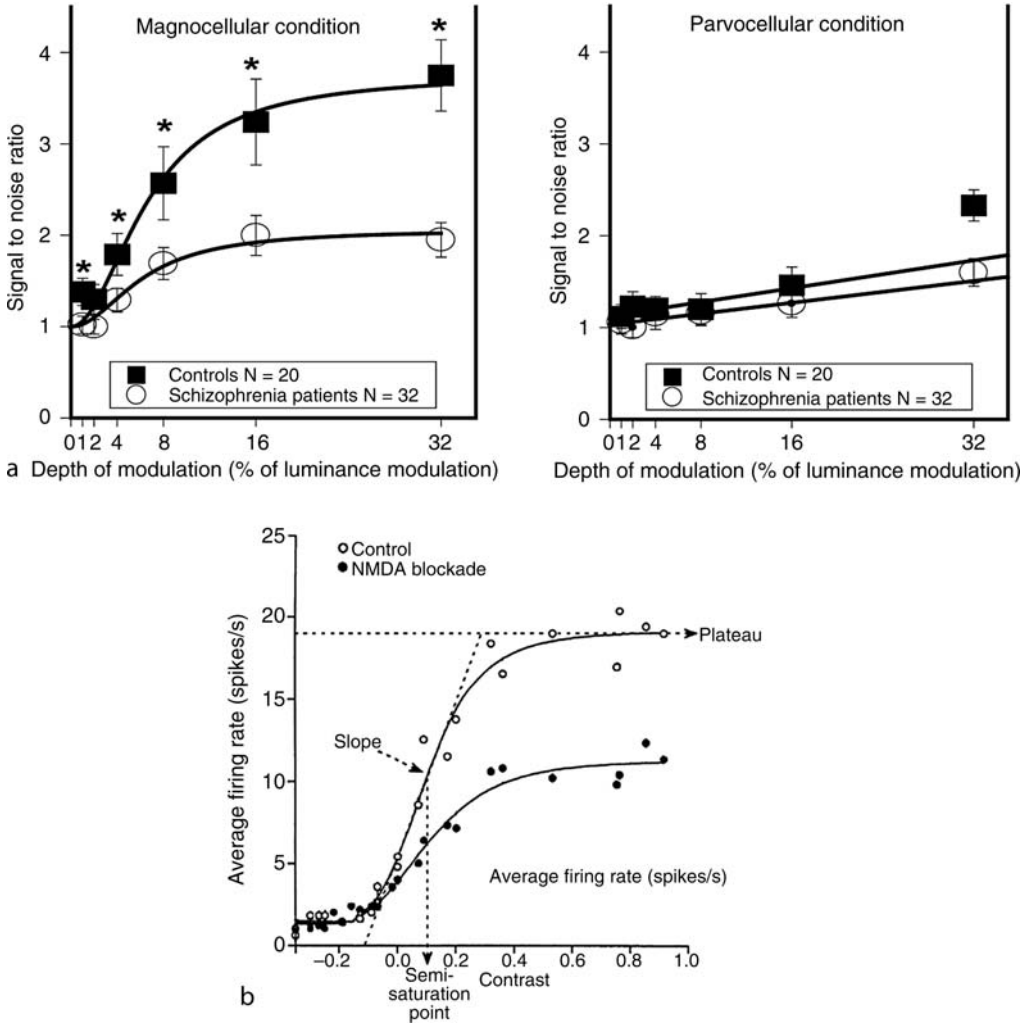
A primary approach to analyzing the integrity of visual processing is the use of visual-evoked potentials (VEPs). An advantage of VEPs is that they are nonbehavioral, and hence provide an objective measure of brain function. In the steady-state VEP (ssVEP) approach, stimuli are presented rapidly and may produce habituation of the higher-level cortical response, and thus increase sensitivity for evaluating lower level responses. In this approach, physical stimulus features such as luminance contrast can be manipulated to preferentially activate the magnocellular and parvocellular components of the system. ssVEP studies provide a definitive demonstration of magnocellular dysfunction in schizophrenia.

In one ssVEP study, Butler et al. (2005) used luminance contrast to bias processing toward the magnocellular versus parvocellular pathway. In the magnocellular-selective condition, stimuli appeared and disappeared: a manipulation that preferentially engages the magnocellular system. Conversely, in the parvocellular-selective condition, stimuli were modulated around a high 48% level of luminance contrast ("pedestal") that saturates the magnocellular response, and hence isolates the additional parvocellular activation (Greenstein et al., 1998; Butler et al., 2001). Patients generated evoked potentials that analysis of variance showed were significantly reduced in response to magnocellular, but not parvocellular, biased stimuli (*Figure 3.2-3a*). In terms of the pattern of responses in the magnocellular condition, controls showed a steeply rising increase in response to low luminance contrast stimuli (~1–10% contrast), which reached a saturation-level once luminance contrast reached ~16–32%, similar to what is seen in single-cell recordings from magnocellular neurons in monkey LGN (Kaplan, 1991). However, responses of patients showed much shallower gain at low luminance contrast and a much lower plateau, indicating a decreased signal amplification. Visual pathways within the brain use glutamate as their primary neurotransmitter. The decreased contrast gain and plateau in the magnocellular-biased ssVEP contrast response curve for schizophrenia patients in this study closely resembles results of the microinfusion of an *N*-methyl-D-aspartate (NDMA) antagonist into cat LGN and visual cortex (*Figure 3.2-3b*) (Fox et al., 1990; Kwon et al., 1992), consistent with glutamatergic theories of schizophrenia (Javitt and Zukin, 1991; Olney and Farber, 1995; Tsai and Coyle, 2002; Stone et al., 2007) (Also see Javitt, this volume). As seen in *Figure 3.2-3a*, in controls, but not patients, a deviation from linear gain was found in the parvocellular response at 32% depth of modulation. This suggests that the critical issue underlying early-stage visual dysfunction in schizophrenia may be the process involved rather than the specific pathway. Thus, the capacity of neuronal systems for nonlinear gain (i.e., amplification) may be reduced in schizophrenia. In the visual system, nonlinear gain is more characteristic of the magnocellular, than parvocellular, system. However, deficits would also be seen in the parvocellular pathway under conditions that drive this system into a nonlinear gain process.

In a second ssVEP study, Kim et al. (2005) utilized windmill-dartboard and partial-windmill stimuli to investigate responses across harmonic levels (*Figure 3.2-4*). In most studies utilizing ssVEPs, the first harmonic response, extracted by Fourier transform, is used to analyze data. The first harmonic refers to responses at the stimulus input frequency. However, second harmonic responses can also be extracted. The second harmonic refers to responses at twice the stimulus input frequency. The windmill-dartboard condition used in this study elicits apparent changes in form, but not motion, and produces ssVEP responses with a dominant first harmonic and an attenuated second harmonic, whereas the partial-windmill condition elicits apparent motion and produces an ssVEP that contains a dominant second harmonic response. Second harmonic responses are preferentially elicited by achromatic (McKeefry et al., 1996) and low spatial frequency (Murray et al., 1983; Grose-Fifer et al., 1994) stimuli, and are thus thought to be mediated primarily by magnocellular system activity. In contrast, first harmonic responses are elicited by contrast above the magnocellular-specific range of function (Zemon and Ratliff, 1982), which indicates a dominant role of the parvocellular pathway in the generation of the first harmonic (Tootell et al., 1998a).

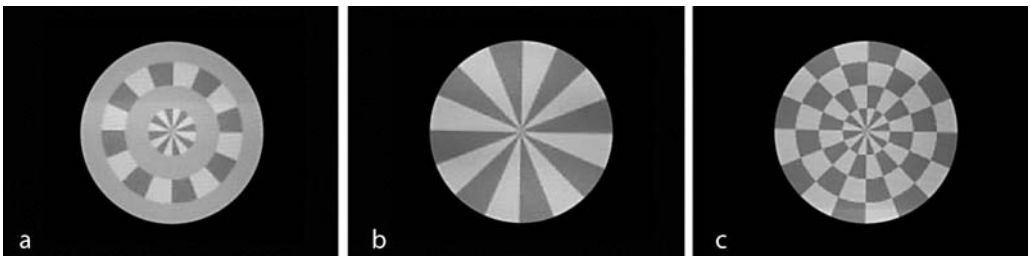
Figure 3.2-3

(a) Steady-state visual evoked potential (ssVEP) signal-to-noise ratios for patients with schizophrenia and healthy controls in test conditions using luminance contrast to emphasize magnocellular or parvocellular visual pathways. For the magnocellular condition, analysis of variance showed a significant between-group effect. For the parvocellular condition, while analysis of variance did not show a significant between-group effect, a significant between-group difference was observed at 32% depth of modulation. *$p < 0.05$; (b) Characteristic nonlinear contrast response functions of cat LGN (open circles) showing the effect of microinfusion of an N-methyl-D-aspartate (NMDA) antagonist (closed circles). The NMDA antagonist produced decreased contrast gain (initial slope) and decreased plateau indicating decreased signal amplification. The decreased contrast gain and plateau in the magnocellular-biased ssVEP contrast response curve for schizophrenia patients closely resembles the results seen following NMDA antagonist administration. Panel A reprinted with permission from Butler et al. (2005). Panel B reprinted with permission from Kwon et al. (1992)



Schizophrenia patients showed reduced amplitude and coherence of second harmonic responses in both conditions, but intact first harmonic responses in the windmill-dartboard condition (*Figure 3.2-5*). This indicates a significant loss in the magnocellular pathway, which contributes to the generation of the second harmonic component under these conditions.

Figure 3.2-4
(a) The partial-windmill condition. The pattern elements in the central disk and second annulus contrast reverse to produce a partial windmill. (b, c) Windmill-dartboard condition. The windmill-dartboard stimulus has two distinct phases: windmill (shown in (b), and dartboard, shown in (c). The contrast of the first and third annuli are held constant. Contrast reversal of the pattern elements in the central disk and second annulus result in the change of appearance from a windmill to a dartboard. Reprinted with permission from Kim et al. (2005)



Transient VEP (tVEP) studies have also addressed early visual cortical responses in schizophrenia. In this approach, stimuli are presented more slowly and responses are analyzed in the time domain. Several early components have been assessed. P1 amplitude, which occurs at a latency of about ~100 ms, has a dorsal stream as well as a ventral stream generator (Martinez et al., 1999; Di Russo et al., 2002). The dorsal generator is driven primarily by magnocellular input and the ventral generator by parvocellular input (Martinez et al., 2001). The N1, which peaks ~150–200 ms, appears to reflect primarily ventral stream sources (Allison et al., 1999; Bentin et al., 1999; Doniger et al., 2002). Most studies have found P1 deficits in schizophrenia (Matsuoka et al., 1996; Basinska, 1998; Foxe et al., 2001, 2005; Doniger et al., 2002; Spencer et al., 2003; Schechter et al., 2005; Yeap et al., 2006, 2008a, b; Butler et al., 2007; Haenschel et al., 2007; Lalor et al., 2008; Vohs et al., 2008), although some have not (Strandburg et al., 1994; Alain et al., 1998; Bruder et al., 1998; van der Stelt et al., 2004; Johnson et al., 2005). In contrast, the N1 is intact in many (Foxe et al., 2001, 2005; Doniger et al., 2002; van der Stelt et al., 2004), though not all (Alain et al., 1998; Bruder et al., 1998), studies.

While the impaired P1 is of particular interest because it has a dorsal stream generator that is driven predominantly by magnocellular input, considerable controversy surrounds whether dorsal stream dysfunction is due to intrinsic cortical dysfunction or to impaired magnocellular input to cortex. Many studies to date have used stimuli chosen for cognitive, rather than psychophysical, content. Butler et al. (2007) recently examined this question, using magnocellular-biased low spatial frequency (LSF) and parvocellular-biased high spatial frequency (HSF) gratings. P1 and N1 were both reduced in amplitude to magnocellular-biased LSF stimuli but were intact to parvocellular-biased HSF stimuli (*Figure 3.2-6*). This study demonstrates that patients with schizophrenia show significant and substantial deficits in early visual processing affecting the subcortical magnocellular pathway that lead to secondary impairment in the activation of cortical visual structures within both the dorsal and ventral stream pathways. Although deficits in visual processing have frequently been construed as resulting from failures of top-down processing (Cohen and Servan-Schreiber, 1992; Weinberger and Gallhofer, 1997; van der Stelt et al., 2004), the present findings argue strongly for bottom-up rather than top-down dysfunction at least within the early visual pathway. These findings also support ssVEP studies showing that patients have severe deficits even in response to very primitive visual stimuli. Moreover, this study (Butler et al., 2007) using low and high spatial frequency gratings, suggests that the choice of stimulus may be critical in demonstrating P1 deficits.

Neural oscillations have also been examined in EEG studies in schizophrenia (for reviews see Javitt et al., 2008; Uhlhaas et al., 2008). Gamma band (>30 Hz) measures have been of particular interest in schizophrenia, because they have been proposed to mediate perceptual feature binding. Several recent studies have reported decreased early visual-evoked gamma oscillations in schizophrenia (Spencer et al., 2003; Spencer et al., 2004; Wynn et al., 2005; Spencer, 2008). However, deficits have also been found at lower

Figure 3.2-5

Patients with schizophrenia showed significantly reduced second harmonic responses but intact first harmonic responses. This finding of a differential deficit may indicate a significant loss in the magnocellular pathway. Bi, binocular eye condition; Dom, dominant eye condition; ND, nondominant eye condition. a, $P < 0.01$; b, $P < 0.05$. Reprinted with permission from Kim et al. (2005)

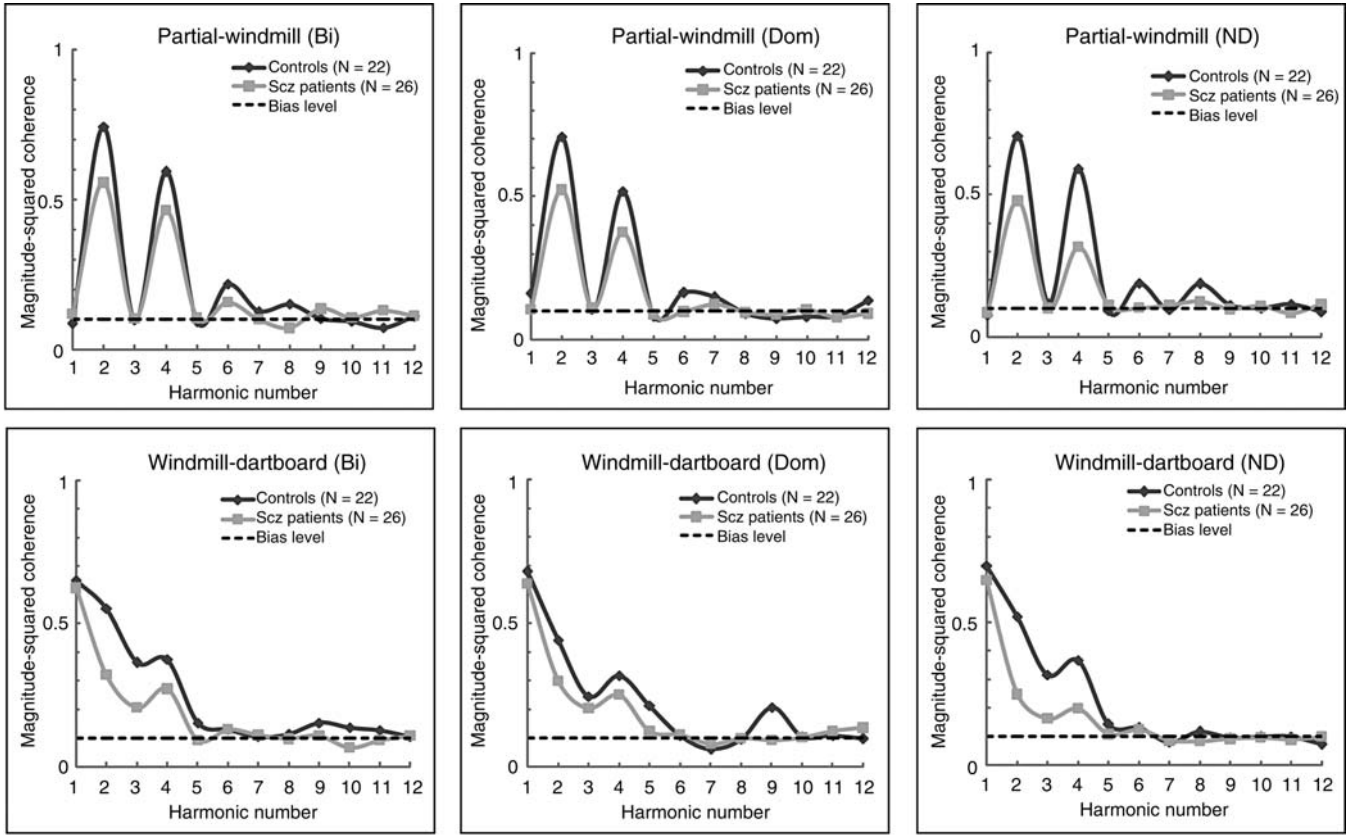
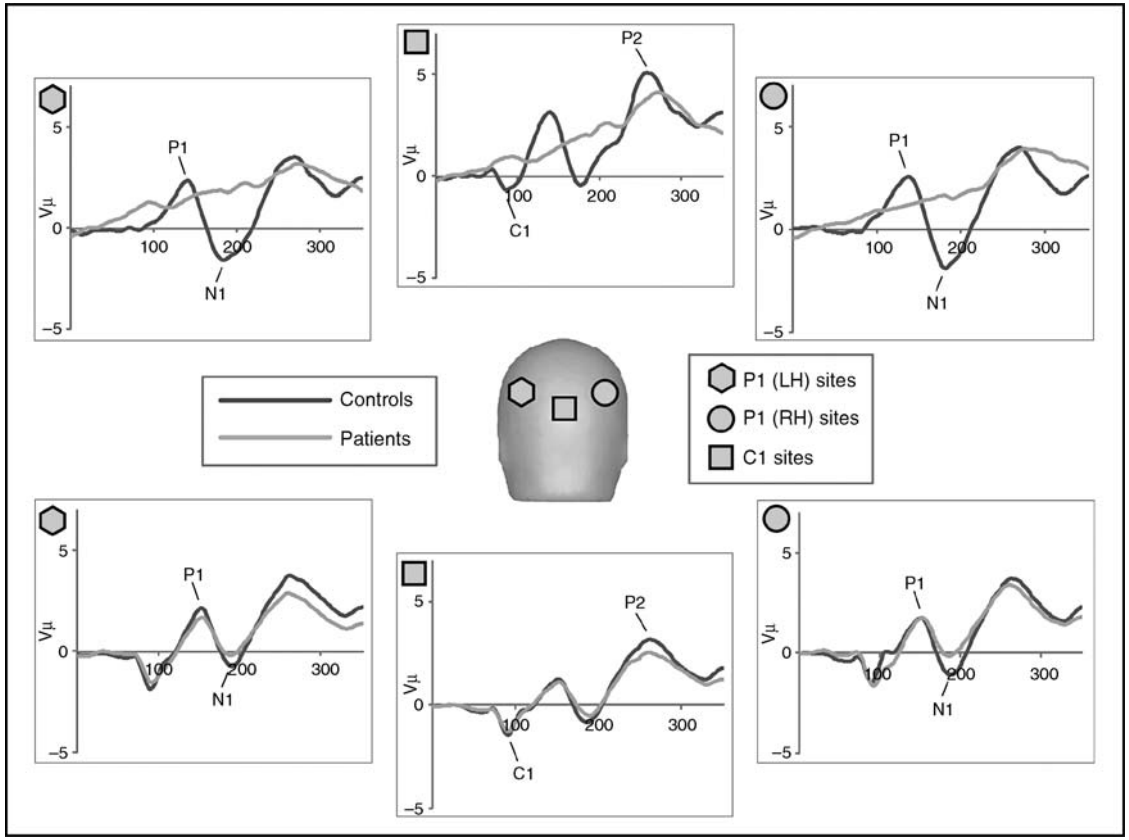


Figure 3.2-6

Group averaged voltage waveforms for controls (n = 16) and patients (n = 18) in response to low (1 cycle/degree) spatial frequency (*top*) and high (5 cycles/degree) spatial frequency (*bottom*) gratings. Waveforms are the mean of the response at six electrode sites used for P1 and N1 in each hemisphere and C1 centrally. A hexagon indicates the location of the six electrode sites in the *left* hemisphere used for P1. A *square* indicates the location of the six electrodes in the *right* hemisphere used for P1 and a circle indicates the location of the six electrodes used for C1. Reprinted with permission from Butler et al. (2007)



beta range frequencies (Uhlhaas et al., 2006a). In addition, studies using ssVEPs, in which brain activity is driven by the frequency of the presenting stimuli, show reduced amplitudes at high (beta/gamma-range) frequencies (Krishnan et al., 2005) as well as at lower (theta/alpha) driving frequencies (Jin et al., 2000; Butler et al., 2001; but see Krishnan et al., 2005). Thus, deficits in neural synchrony have been found in schizophrenia at gamma as well as lower frequencies. In ssVEP studies, the deficits may be dependent on the stimulus used, with greater deficits seen to magnocellular- versus parvocellular-biased stimuli (Butler et al., 2005) and greater deficits in even versus odd harmonics in the windmill-dartboard paradigm (Kim et al., 2005).

In conclusion, ssVEP and tVEP studies provide a strong support of early visual cortical dysfunction, and in particular, provide evidence for preferential magnocellular dysfunction in schizophrenia. These deficits impact on perceptual organization (see section on "Effects on Higher Level Function").

3 Behavioral Studies

Visual pathway function has also been studied using behavioral methods. While studies using luminance and chromatic contrast to bias processing toward magnocellular and parvocellular pathways have generally shown preferential magnocellular dysfunction, studies utilizing stimulus size to bias processing have been more equivocal. One method of studying stimulus size assesses the amount of contrast (i.e., contrast threshold) needed to detect stimuli of varying sizes with large (i.e., low spatial frequency) stimuli biasing processing toward the magnocellular pathway and small (i.e., high spatial frequency) stimuli biasing processing toward the parvocellular pathway. Lower thresholds indicate better performance. One issue is that high spatial frequency stimuli can produce low luminance contrast thresholds. Since the magnocellular system responds well to low luminance contrast (~1–10% contrast), parvocellular neurons do not start responding until stimuli reach higher contrast (~10%), whereas contrast threshold levels below 10% will be biased toward the magnocellular system regardless of spatial frequency. In addition, responses to spatial frequency are not specific to striate versus extrastriate areas (Tootell et al., 1998b).

Several recent studies have used contrast to examine magnocellular and parvocellular function. One study used backward masking (Schechter et al., 2003). Backward masking refers to a paradigm in which a target (e.g., a letter) is presented first and then a mask (e.g., a cross of Xs that spatially overlaps the target) follows. The mask interferes with processing of the target. In one version of the paradigm, the duration of the interstimulus interval (ISI) is increased to determine how much time participants needed between the target and mask in order to successfully identify the target. Schechter et al. (2003) used low luminance contrast to bias processing toward the magnocellular system and chromatic contrast at isoluminance to bias processing toward the parvocellular system. For schizophrenia patients, backward masking dysfunction (i.e., increased ISIs between target and mask needed for target identification) was found when low luminance, but not chromatic, contrast stimuli were used as masks supporting a role of magnocellular-system dysfunction in backward masking deficits in schizophrenia. In a vernier acuity task, Keri and colleagues (Keri et al., 2004; Keri et al., 2005a, b) used low contrast and low spatial frequency to bias processing toward the magnocellular system and high contrast and chromatic contrast at isoluminance to bias processing toward the parvocellular system. Patients showed deficits in vernier acuity in the low spatial frequency and low contrast conditions, but not in the high contrast and isoluminant color contrast conditions, consistent with magnocellular dysfunction. In a far-out jerk paradigm, Slaghuis and Thompson (2003) examined the ability of moving objects in the periphery to decrease detection of a central object. This far-out jerk response is likely mediated by long-range transient, or magnocellular, function. Patients with predominantly negative, rather than positive, symptoms of schizophrenia showed a decreased far-out jerk response, indicating magnocellular dysfunction.

Most backward masking studies in schizophrenia have been done using masks that spatially overlap the targets (Saccuzzo and Braff, 1986; Green et al., 1994a; Butler et al., 1996; Cadenhead et al., 1998; Rund et al., 2004). However, Rassovsky et al. (2004) examined masking in schizophrenia using masks that surround, rather than overlap, the target. This technique limits the mechanism of masking to interruption, in which

the fast transient (e.g., magnocellular) response to the mask interrupts the slower sustained (e.g., parvocellular) response to the target and interferes with target processing. In contrast, when the mask spatially overlaps the target, masking occurs both by integration (e.g., responses to target and mask fuse) and by interruption. Rassovsky et al. (2004) found that patients with schizophrenia showed deficits in masking by interruption, consistent with magnocellular dysfunction (Breitmeyer and Ganz, 1976; Green et al., 1994b).

A recent backward masking study (Luber et al., 2007) examined the performance of individual patients. While the patient group as a whole showed backward masking deficits compared with controls, this deficit was primarily due to the approximately 25% of patients who continued to have difficulty identifying the target letter even at very long ISIs. These patients may have long-duration early perceptual deficits. If these patients were removed from the analysis, patients did not differ from controls. This highlights an important consideration in schizophrenia research, which is the variability of performance within patients on most tasks and the need to understand differences in subpopulations of patients. However, as the authors themselves point out, this does not negate the numerous studies showing backward masking deficits. As discussed later (see Patient Characteristics), Slaghuis (2004) only found backward masking deficits in patients with predominantly negative, not positive, symptoms. Again, stimulus properties and task demands are also important in understanding the results of studies. Others (e.g., Cadenhead et al., 1998) found backward masking deficits in a target location (magnocellular/dorsal stream-biased) task but not a target identification (parvocellular/ventral stream-biased) task. The conditions used by Luber et al. (2007) were not designed to target magnocellular/dorsal stream versus parvocellular/ventral stream function.

While studies using stimulus size to bias processing have consistently found contrast threshold deficits to low spatial frequency stimuli, reflecting magnocellular dysfunction (Slaghuis and Thompson, 2003; Chen et al., 2004; Slaghuis, 2004; Butler et al., 2005), results are less clear at medium to higher spatial frequencies. Slaghuis and colleagues (Slaghuis and Thompson, 2003; Slaghuis, 2004) reported a threshold detection deficit at medium and higher spatial frequencies of 4 and 8 c/deg, indicative of parvocellular dysfunction. However, Butler et al. (2005) reported a deficit at spatial frequencies up to 7 cycles/degree but not at higher spatial frequencies of 10 and 21 cycles/degree, suggesting intact parvocellular function. Differences in absolute contrast levels at medium and high spatial frequencies may underlie variant findings.

Spatial frequency studies looking at discrimination, rather than contrast thresholds, have also been carried out and consistently found impaired spatial frequency discrimination to low spatial frequency gratings (O'Donnell et al., 2002; Kiss et al., 2006). Deficits were restricted to low spatial frequencies in one of these studies (Kiss et al., 2006) and were also seen at medium but not high spatial frequencies in the other (O'Donnell et al., 2002).

Contrast detection has also been utilized to examine whether visual processing deficits are related to early-stage visual dysfunction. Slaghuis (2004) found that backward masking dysfunction was related to deficits in contrast threshold, which supports a role of early-stage visual processing deficits in backward masking dysfunction in schizophrenia. They comment that an earlier study by Keri et al. (2000) did not find such a relationship, which may have been due to measurement of contrast thresholds in central vision and backward masking in four separate parafoveal locations in that study, whereas Slaghuis (2004) measured both in the same central retinal location.

Chen and colleagues have found motion processing deficits in schizophrenia for velocity discrimination (Chen et al., 1999a, c, 2004, 2006b) and have also used contrast to examine whether this deficit is intrinsic to dorsal stream motion areas or due to impaired early-stage input (Chen et al., 2004). They pointed out that neurophysiological and lesion studies show that a velocity discrimination deficit may be related to early-stage motion processing (i.e., LGN, striate cortex) if it is contrast-dependent and later stage processing (i.e., extra-striate) if it is contrast-independent. Thus, they assessed velocity discrimination at high and low contrast, using each participant's contrast detection threshold to equate contrast levels. Patients showed an impaired velocity discrimination that did not improve with high contrast, whereas the performance of controls did improve with increased contrast. The contrast-independence of the deficit in schizophrenia indicates that it is mediated by later-stage extra-striate areas.

Kim et al. (2006) also examined low-level contributions to motion perception deficits. They found that schizophrenia patients showed deficits in velocity discrimination for both incoherent and coherent motion. However, when coherent motion performance was measured at individually determined incoherent motion thresholds, accuracy levels for patients were similar to those of controls suggesting that the decoding of coherent from incoherent motion, a process that takes place within dorsal stream regions such as MT (medial temporal area), is relatively intact. In addition, velocity discrimination correlated significantly with magnocellular-biased ssVEP responses. This study suggests that motion processing deficits in schizophrenia may be largely attributable to impaired bottom-up input to motion processing areas such as MT, rather than to local, intrinsic dysfunction within these regions, although this is still unclear (Chen et al., 2004).

There are also deficits in gain control and integration of stimuli in schizophrenia that may involve aberrant lateral interactions and/or impaired visual pathway input (Silverstein et al., 2000; Doniger et al., 2002; Must et al., 2004; Dakin et al., 2005; Johnson et al., 2005; Uhlhaas et al., 2006b; Kurylo et al., 2007; Butler et al., 2008), although more work is needed to understand the substrates of gain control and integration.

4 Medication Effects

An unresolved issue is the degree to which medication may affect visual processing. Chen et al. (2003a) have suggested that decreased contrast detection thresholds are related to medication such that they are found only in patients taking typical, rather than atypical, antipsychotics. However, in other studies, ssVEP and contrast detection deficits, as well as visual masking dysfunction, were observed even in patients receiving atypical antipsychotics alone (Rassovsky et al., 2004; Butler et al., 2005), suggesting that patient characteristics rather than medication type might be the primary predictor of visual dysfunction. In addition, decreased magnocellular-biased vernier acuity, as well as aberrant lateral interactions and low spatial frequency discrimination deficits, was found in patients who had been off medications for several weeks (Keri et al., 2004; Must et al., 2004; Kiss et al., 2006). Recently, performance on contrast sensitivity, form discrimination in noise, and dot motion discrimination were found not to differ between medicated patients with schizophrenia and those withdrawn from medication, supporting visual deficits regardless of medication status (O'Donnell et al., 2002).

5 Patient Characteristics

Several recent studies have examined early visual processing in various groups of participants. Within schizophrenia groups, Slaghuis and colleagues (Slaghuis and Thompson, 2003; Slaghuis, 2004) found that deficits in contrast detection, backward masking, and far-out jerk tasks are more prominent in patients with predominantly negative, rather than positive, symptoms of schizophrenia. A number of other studies have reported that backward masking deficits are more pronounced in patients with persistent negative symptoms and poor functional outcome than in higher functioning patients (Saccuzzo and Braff, 1981; Green and Walker, 1984; Braff, 1989; Weiner et al., 1990; Slaghuis and Bakker, 1995; Cadenhead et al., 1997).

6 Endophenotypes

A growing number of studies have begun to examine whether early visual processing deficits are endophenotypes of schizophrenia (See DeLisi this volume). Endophenotypes are biological markers of the disease; are familial (e.g., are seen in first degree relatives of patients with schizophrenia); and can be used as phenotypes in genetic analyses.

Most of the emphasis to date on studies of first-degree relatives has been behavioral. For instance, psychophysical studies show decreased magnocellular-mediated vernier acuity in both patients and first-degree relatives (Keri et al., 2004, 2005a). Backward masking deficits, particularly those involving "transient visual channels," were seen in siblings of patients with schizophrenia (Green et al., 2006). In addition, velocity discrimination deficits were found in both patients and first-degree relatives (Chen et al., 1999b). While coherent motion thresholds were not impaired in first-degree relatives (Chen et al., 2005), although they are impaired in patients with schizophrenia (Chen et al., 2003b, 2005; Kim et al., 2006), Keri et al. (2006) found that children of mothers with schizophrenia showed developmental abnormalities in coherent motion detection and concluded that this may be a characteristic feature of schizophrenia vulnerability.

Less work has been done using electrophysiology. A recent study showed that, like patients with schizophrenia, first degree relatives had decreased P1 amplitude to isolated check stimuli (Yeap et al., 2006). The deficit was localized to midline regions in early visual sensory cortices and regions of the dorsal visual stream. The effect size was quite large ($d = 0.9$) providing further support for magnocellular/dorsal stream dysfunction as a possible endophenotype. Importantly, P1 deficits in schizophrenia have been associated with a dysbindin risk haplotype (Donohoe et al., 2008). However, two studies of first-degree relatives using different paradigms did not find P1 deficits (Katsanis et al., 1996; Sponheim et al., 2006).

Bedwell and colleagues have used fMRI to look at early visual processing in first-degree relatives of patients with schizophrenia. They reported decreased magnocellular/dorsal stream function under neutral (nonred) light conditions using fMRI (Bedwell et al., 2004), further supporting decreased magnocellular pathway function as a possible endophenotype. They also examined the effect of diffuse red light, which is thought to suppress magnocellular activity, and found that a subset of nonpsychotic first-degree relatives had an effect opposite to that of controls in two studies – an increased fMRI response to red light (Bedwell et al., 2006) and increased accuracy of target location in a backward masking task on a red background (Bedwell et al., 2003), whereas controls had decreased responses in both studies. These studies did not include patients with schizophrenia, and hence, it is unclear if relatives perform similarly to patients. However, it is tempting to speculate that there may be a magnocellular deficit in first-degree relatives and that they are using a different process to compensate for the deficit.

Early-stage visual processing dysfunction also appears to be a trait rather than a state marker, because for instance, P1 deficits are found in early-onset schizophrenia (Haenschel et al., 2007) as well as in first-episode patients (Yeap et al., 2008b) and are unrelated to chronicity of illness (Yeap et al., 2008a).

Several studies have looked at schizophrenia spectrum disorders and results have been less clear than for first-degree relatives. For instance, coherent motion thresholds, P1 amplitude, contrast sensitivity, and form and trajectory discrimination were not impaired (Farmer et al., 2000; O'Donnell et al., 2006; Vohs et al., 2008) in schizotypal personality disorder. However, a backward masking deficit was found in schizotypal personality disorder (Cadenhead et al., 1999).

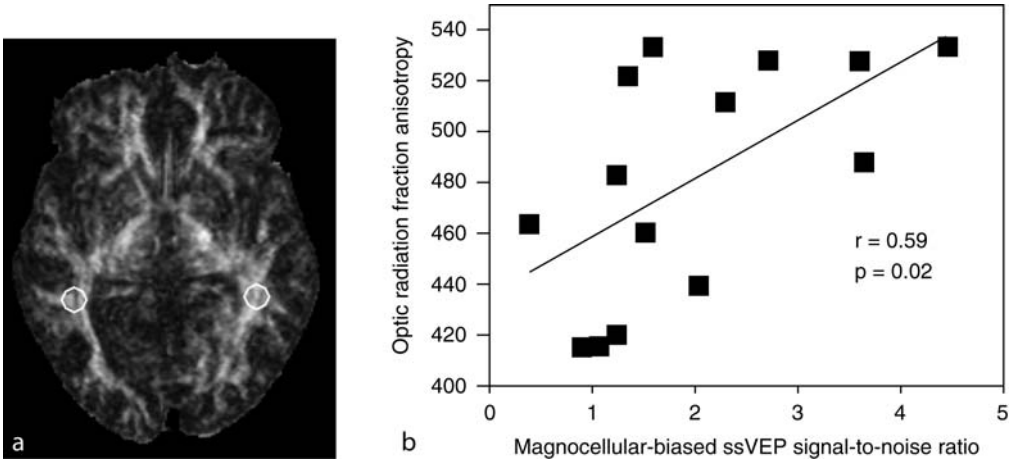
Thus, first-degree family studies suggest that early-stage visual processing deficits are promising endophenotypes associated with schizophrenia liability, but results are less clear with schizotypal personality disorder. As suggested by Vohs et al. (2008), some cases of schizotypal personality disorder may represent a phenocopy that is not related to the genetic liability for schizophrenia.

7 Anatomical Studies

Inputs to primary visual cortex project from LGN to V1 via optic radiations. These white matter tracts can be examined by using the technique of diffusion tensor imaging. Recently, decreased integrity of occipital white matter adjacent to the corpus callosum, in the region of the optic radiations, has been found in adults with schizophrenia (Ardekani et al., 2003; Butler et al., 2006), in agreement with an earlier study (Agartz et al., 2001). Decreased occipital white matter integrity has also been found in adolescents with early onset schizophrenia (Kumra et al., 2004). In addition, a significant relationship has been found between decreased magnocellular-biased ssVEP responses and decreased white matter integrity in the optic

Figure 3.2-7

(a) Fractional anisotropy image with circles representing regions of interest based on their placement in the optic radiations on the *b* = 0 image (not shown). (b) Scatter plot showing the relationship between magnocellular-biased steady-state visual evoked potential responses and fractional anisotropy of optic radiation white matter tracts for patients with schizophrenia. Fractional anisotropy measures range from 0 to 1000, with 0 representing complete isotropic diffusion (no directional selectivity) and 1000 representing complete anisotropy. Reprinted with permission from Butler et al. (2005)



radiations, but not in higher level visual areas (Butler et al., 2005) (*Figure 3.2-7*). The latter finding provides direct support for the hypothesis that magnocellular dysfunction occurs at the earliest stages of visual responsivity.

Postmortem work is also supportive of deficits in visual cortex. Selemon et al. (1995) found a 10% increase in neuronal density in area 17 (i.e., V1), similar to the 17% increase found in prefrontal cortex in the same brains from people with schizophrenia. This was interpreted as reflecting a decrease in dendritic and axonal arborization resulting in a decrease in neurophil. More recently, Dorph-Petersen et al. (2007) found a significant reduction in total neuron number (25%) and volume (22%) in area 17, although no changes in neuronal density or cortical thickness were found, and concluded that patients with schizophrenia have a smaller cortical area allocated to primary visual perception. Cell number and volume of the magnocellular and parvocellular layers in LGN, however, were not abnormal in schizophrenia, suggesting that visual processing deficits in schizophrenia are not due to a decreased neuronal number in LGN (Selemon and Begovic, 2007).

In addition, some (Renshaw et al., 1994; Martinez et al., 2008), but not other (Braus et al., 2002; Barch et al., 2003) functional imaging studies provide evidence for altered activation of occipital cortex in schizophrenia. However, with the exception of the study by Martinez et al. (2008), stimuli were not designed to bias processing toward the magnocellular versus parvocellular pathway. Martinez et al. (2008) found a decreased activation of V1, V2, as well as multiple regions of parietal and temporal lobes in response to magnocellular-biased low spatial frequency (i.e., larger size) gratings but not in response to parvocellular-biased high spatial frequency (i.e., smaller size) gratings in patients with schizophrenia versus controls. The decreased activation in patients in response to low spatial frequency stimuli was particularly prominent in the low luminance contrast condition that further biased processing toward the magnocellular pathway.

Thus, there appear to be alterations in both gray and white matter in low-level visual areas in schizophrenia and in two studies that examined the relationships with visual pathway function, anatomical deficits were related to magnocellular dysfunction (Butler et al., 2005; Martinez et al., 2008).

8 Effects on Higher Level Function

A growing number of studies have focused on the impact of low-level visual deficits on higher-level cognitive dysfunction and outcome in schizophrenia (Brenner et al., 2002; Sergi and Green 2003; Butler et al., 2005; Keri et al., 2005b; Revheim et al., 2006; Sergi et al., 2006; Laycock et al., 2007). A major focus of interest in cognitive neuroscience is the ability of magnocellular projections to dorsal stream and frontal cortical areas to influence later parvocellular input to ventral stream object processing areas (Givre et al., 1994; Schroeder et al., 1998; Vidyasagar, 1999; Lamme and Roelfsema, 2000; Bar, 2003; Chen et al., 2007; Fenske et al., 2006; Sehatpour et al., 2006). Transmission is faster through the dorsal stream, which would permit it to prime ventral stream areas (Schmolesky et al., 1998; Schroeder et al., 1998; Foxe and Simpson, 2002). Recent physiological evidence for priming was found by Schroeder and colleagues (Chen et al., 2006a). V1, V2, and dorsal stream areas showed a feed-forward profile of cortical laminar activation in which initial excitation was produced in layer 4 (granular lamina). However, in ventral stream regions V4 and IT, initial excitation was produced outside layer 4 in the supra- and infra-granular layers, which excludes the possibility of initial feed-forward activation. Thus, lateral connections from dorsal areas such as V5 may be responsible for the initial input to ventral regions. Later activation of layer 4 in the ventral stream is provided by slower parvocellular projections.

A fundamental role of the magnocellular system/dorsal stream may be to produce a low resolution template of the visual scene that influences perceptual processes such as categorization of natural images, object recognition, and perceptual grouping in the ventral occipito-temporal cortex by allowing parvocellular fine-detailed input to be utilized more effectively (Givre et al., 1994; Nowak and Bullier, 1997; Schmolesky et al., 1998; Schroeder et al., 1998; Vidyasagar, 1999; Schwartz et al., 2001; Chen et al., 2006a; Laycock et al., 2007). This has been referred to as the "frame and fill" model of visual processing (Schroeder et al., 1998). Magnocellular deficits would result in an impairment not only of dorsal stream functions such as motion perception and object location but also of later ventral stream processing in LOC. As reviewed by Laycock et al. (2007), the "magnocellular disadvantage" would be expected to have an impact on higher cognitive processes and even anomalous experiences. Keri et al. (2005b) have suggested that in addition to purely visual deficits, impaired magnocellular function and attendant lack of attentional feedback to other brain areas would play a role in symptomatology.

Javitt and colleagues examined the relationships between magnocellular/dorsal stream dysfunction and perceptual organization in schizophrenia. Using a perceptual closure paradigm, fragmented line drawings of objects were shown (Doniger et al., 2002). High density ERPs were recorded, including P1, which has a dorsal occipito-parietal generator, N1, which has a ventral occipito-temporal generator, and Ncl (cl = closure negativity), which has a later ventral generator and is related to the perception of fragmented images. Patients with schizophrenia had an impaired ability to recognize fragmented objects as well as a decreased Ncl. The amplitude of the dorsal stream P1 component was significantly reduced in patients, whereas the ventral N1 was intact. Doniger et al. (2002) concluded that the decreased P1 is reflective of impaired magnocellular input, whereas the intact N1 suggests that the initial stages of ventral stream processing are relatively preserved. The impaired magnocellular dorsal stream function in schizophrenia may lead to secondary dysregulation of ventral stream object recognition areas and hence the Ncl and perceptual closure deficit.

Several behavioral studies have also found evidence for contributions of magnocellular impairment to later-stage perceptual deficits. Keri et al. (2005b) found that magnocellular pathway dysfunction (impaired vernier performance) was related to deficits in perceptual organization and natural scene categorization. They also assessed anomalous visual experiences and found that the best predictor of these experiences was the perceptual organization deficit. Schwartz and colleagues (Schwartz et al., 1999) found that patients with schizophrenia perform more poorly than controls on a motion defined letter recognition task. These results lend further support for "frame and fill" deficits in schizophrenia, with impaired magnocellular pathway/dorsal stream functioning resulting in abnormal recurrent processing within dorsal stream and secondary dysregulation of the ventral stream object recognition areas.

Revheim et al. (2006) showed that a subgroup of patients with dyslexia also had decreased contrast sensitivity in response to magnocellular-biased stimuli, further supporting the idea that impairments in early-stage visual processing may have profound effects on higher-level perceptual processes. Relationships have also been found between early visual processing and abnormal neurological signs, particularly sensory integration deficits (Cimmer et al., 2006) as well as neuropsychological tests of complex visual processing and working memory (Brenner et al., 2002; Butler et al., 2005; Kelemen et al., 2007). Indeed, a recent study found a P1 amplitude reduction in patients during encoding and retrieval of a working memory task and concluded that early-stage visual processing deficits contribute to working memory dysfunction in schizophrenia (Haenschel et al., 2007). A relationship between early visual processing and a measure related to outcome (the Problem Solving Factor of the Independent Living Scale; Loeb, 1996) has been reported (Butler et al., 2005).

Effects of early visual processing deficits on social cognition have been another focus of efforts. Social cognition studies in schizophrenia have examined social perception, emotion perception, and theory of mind (Kerr and Neale, 1993; Penn et al., 1997; Kohler et al., 2003; Sergi et al., 2006). Correlations have been found between performance on backward masking and other early visual processing tasks and emotion and social cue perception in schizophrenia (Corrigan et al., 1994; Addington and Addington, 1998; Kee et al., 1998; Sergi and Green, 2003). Magnocellular dysfunction may produce emotion perception deficits because of difficulty in detecting contrast in certain parts of the face and/or difficulty in forming a low-resolution template of a face.

Green and colleagues have suggested that social perception mediates the influence of neurocognition on functional status (Green et al., 2000). Using structural equation modeling in a large group of patients, they recently reported that social perception mediates the influence of early visual processing on functional status in schizophrenia (Sergi et al., 2006).

Relationships between early visual processing, particularly involving the magnocellular system, and higher-level cognitive function and outcome are thus promising areas of research and may help in understanding the pathophysiology of schizophrenia.

9 Conclusions

Results from behavioral and electrophysiological studies support early visual processing dysfunction in schizophrenia, preferential deficits being found in the magnocellular pathway, although parvocellular deficits have been found as well. Preferential magnocellular dysfunction may provide a substrate for dorsal stream dysfunction as well as higher level cognition deficits and outcome. Structural deficits in occipital cortex and optic radiations, and their relationship to early visual processing deficits document the importance of subcortical as well as cortical dysfunction in schizophrenia. Deficits in magnocellular processing may reflect more general impairments in neuronal systems functioning, such as deficits in nonlinear amplification, and may thus represent an organizing principle for predicting neurocognitive dysfunction in schizophrenia. These low-level visual processing deficits also may have profound consequences for cognitive function, including social perception, and outcome.

Acknowledgments

This review was supported in part by USPHS grant RO1 MH66374 (PDB).

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3.3 Auditory Cortex Anatomy and Asymmetry in Schizophrenia

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Abstract: The features and progression of the neuropathology of schizophrenia are still poorly defined. Structural and electrophysiological finds indicate that auditory areas of the superior temporal gyrus (STG) may be especially affected. In vivo structural imaging has found a decreased gray matter volume in the STG that is more consistent in the caudal STG, and may be more pronounced in the left hemisphere. Located on the caudal STG are Heschl's gyrus (HG) and the adjacent planum temporale (PT). These regions also have a reduced volume in schizophrenia, suggesting that early stages of auditory sensory processing, possibly including the primary auditory cortex, are involved in schizophrenia pathology. Relatively few postmortem studies in auditory cortex are available, but the initial findings suggest that these areas have anatomical changes comparable to those found in frontal and other regions of the cerebral cortex.

List of Abbreviations: A1, primary auditory cortex; HG, Heschl's gyrus; paAe, external parakoniocortex; paAi, internal parakoniocortex; paAr, rostral parakoniocortex; PT, planum temporale; R, rostral auditory area of monkey; RT, rostrotemporal auditory area of monkey; SI, sulcus intermedius; STG, superior temporal gyrus; TA, auditory area TA of von Economo; TB, auditory area TB of von Economo; TC, auditory area TC of von Economo; Tpt, temporo-parietal area; vMGB, ventral subdivision of the medial geniculate body

1 Introduction

There are compelling reasons to look into auditory cortex for neuropathology in schizophrenia. Historically, investigators have focused on the frontal and limbic areas of cortex. However, accumulating evidence has demonstrated that anatomical and functional changes may be present across the entire cerebral cortex. Irregularities seen with evoked potential studies have been demonstrated even in early stages of sensory information processing. In visual modalities, schizophrenia patients have a reduced P1 amplitude (Butler et al., 2007). In the auditory cortex, short latency N1 and mismatch negativity responses are altered, and source localization has placed the origins of these responses in either the primary or the nearby secondary auditory cortex (Javitt, 2000). These findings in the auditory cortex may correspond to the structural MRI findings of reduced cortical volume in the auditory regions of the superior temporal gyrus (STG) that are as pronounced as any other area of the cerebral cortex (Shenton et al., 2001).

Practical considerations also point to the auditory cortex as an appealing place to investigate the neuropathology of schizophrenia. Comparisons with nonhuman primates suggest that anatomical criteria can be used to identify and map auditory areas in humans with reasonable precision. Because the superior temporal plane contains both the primary cortex and successive levels of the auditory association cortex, these stages of cortical information processing, with their distinctive thalamic and cortical connections, can be separately evaluated for pathological changes. Additionally, the auditory cortex and the surrounding language areas are known to have pronounced hemispheric asymmetries. In schizophrenia, both functional and anatomical asymmetries appear to be altered. For example, in vivo imaging methods have repeatedly shown altered asymmetry of the middle and superior temporal gyri with respect to resting glucose metabolism (Gur and Chin, 1999) and with respect to cortical activation seen with fMRI during semantic and prosodic language discrimination tasks (Dollfus et al., 2005; Sommer et al., 2001, 2007; Weiss et al., 2006; Woodruff et al., 1997). The cause of altered language asymmetry in schizophrenia is unknown. Crow has argued that failure of language lateralization may be a key causative factor in the pathology of schizophrenia (Crow, 1990, 1997).

To date, most anatomical studies of the auditory cortex in schizophrenia have evaluated the gross structural features of the superior temporal plane, including Heschl's gyrus (HG) and the planum temporale (PT). This approach has provided striking evidence for altered cortical volumes and some evidence for altered hemispheric asymmetry. More recently, several groups have begun to apply postmortem cytoarchitectonic methods, with the expectation that this approach will more precisely identify the neural circuitries that are affected. The goal of this review is to provide a brief overview of our current understanding of the anatomical correlates of schizophrenia pathology in the auditory areas of the superior temporal region.

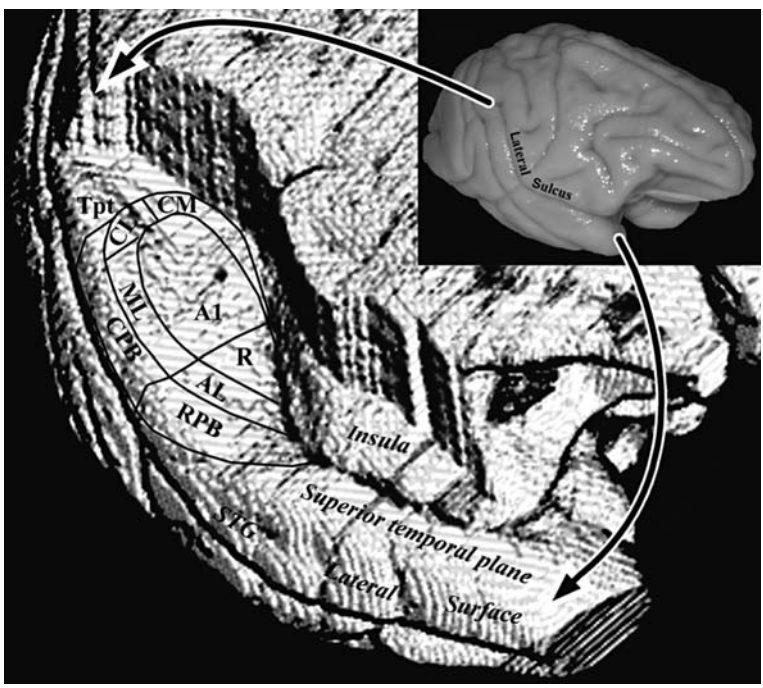
2 Cytoarchitectonic Organization of Auditory Areas

2.1 Monkey Auditory Areas

Our current understanding of the anatomical components of the human auditory cerebral cortex relies heavily on comparisons with animals, and especially monkeys. In macaques, there are at least two areas of primary auditory cortex (A1 and R, see *Figure 3.3-1*), as defined by organized cochleotopic maps and sharply tuned frequency responses (Fu et al., 2003; Kosaki et al., 1997; Merzenich and Brugge, 1973). Studies in new world monkeys have supported the existence of a third primary-like area (RT) located rostral to R (Bendor and Wang, 2006; Hackett and Kaas, 2004; Morel and Kaas, 1992). However, the rostral areas R and RT are relatively inaccessible by invasive recording methods, and their precise borders and function are not well characterized. Tract tracing studies have demonstrated differential connectivity of rostral and

Figure 3.3-1

Monkey auditory areas, parcellated by the nomenclature of Hackett and Kaas (2004), are shown schematically on a 3-dimensional reconstruction of a monkey brain made from block-face video images taken during histological sectioning. The parietal and frontal opercula were cut away to reveal the surface of the lateral sulcus. Monkeys have 2 clearly identified primary auditory areas. Area A1 is located on the superior temporal plane just caudal to the insula, and area R abuts its anterior border and extends rostrally into the ventral circular sulcus, which forms the ventral border of the insula. Surrounding the primary areas are "belt" areas that are first order auditory association area. The belt has been subdivided into several distinct areas on the basis of electrophysiology and anatomical connections. Belt areas include the caudal medial area (CM), the caudal lateral area (CL) and the medial lateral area (ML). There is also evidence for another belt area (AL, not shown) medial to area R. Lateral to the belt areas on the superior temporal gyrus are parabelt areas, that are considered higher order association areas largely devoted to auditory processing. Parabelt has been divided into caudal parabelt (CPB) and the rostral parabelt (RPB). Area Tpt is an area with multisensory responses at the caudal end of the lateral sulcus



caudal auditory areas (de la Mothe et al., 2006a; Romanski et al., 1999), and it has been suggested that the different areas may be functionally specialized, analogous to the "what" and "where" pathways of visual processing (Rauschecker, 1998). In macaque monkeys, the largest primary auditory area, A1, is located along the relatively flat superior temporal plane, with lower frequencies represented rostro-laterally and higher frequencies represented caudo-medially. Area R abuts the rostral border of A1 and has reversed tonotopic order, with higher frequencies found more rostrally. The precise border of the two areas is not readily distinguished by anatomical methods. The cochleotopically organized auditory input to these primary areas is mainly from the ventral division of the medial geniculate nucleus (vMGB).

In macaques, the primary areas of cortex can be readily distinguished from adjacent areas by several histochemical methods. In particular, parvalbumin immunoreactivity is enriched in the axons of thalamic relay cells of the vMGB, resulting in a region of intense labeling that corresponds quite precisely with physiologically defined primary auditory cortex (Fu et al., 2003; Hackett et al., 1998; Jones, 1998; Jones et al., 1995; Kosaki et al., 1997). The dense parvalbumin label colocalizes neatly with the high density of cells in primary areas seen with Nissl stains, and also with the dense acetylcholinesterase and cytochrome oxidase labeling in these areas (Hackett et al., 2001; Morel et al., 1993; Smiley et al., 2007b). In macaques, these labels are dense throughout A1 and R, and extend rostrally along the lateral bank of the ventral circular sulcus.

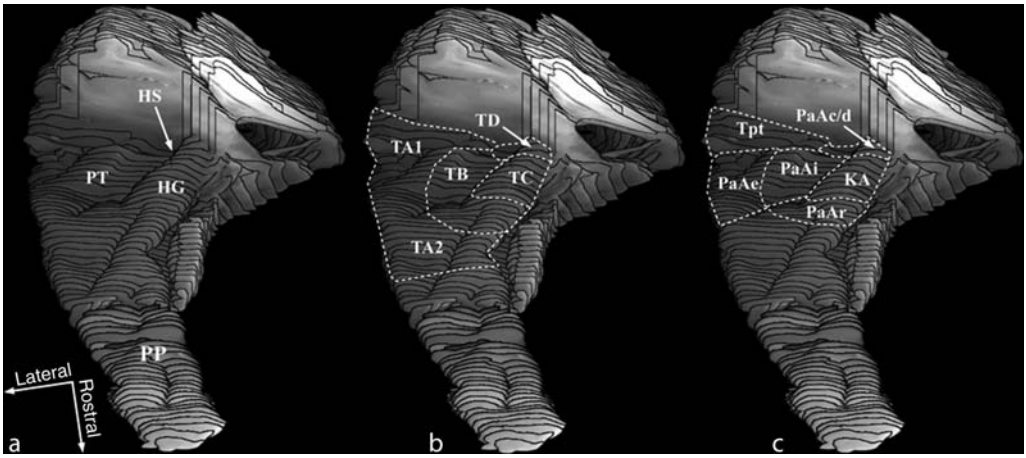
Hackett and Kaas (2004) have used the term "core" for the primary auditory areas, and have divided the adjacent areas of auditory cortex into a narrow "belt" or first level auditory association cortex and then a "parabelt" or second level auditory association cortex. These are bordered in turn by higher order multisensory association areas, including the insular and retroinsular cortex medially, the temporoparietal temporal area (Tpt) caudally, and the superior temporal sulcus laterally, that vary in the extent of auditory and other sensory modalities that are processed. An identifying feature of the auditory belt and parabelt areas is that they receive dense thalamocortical projections from the dorsal and medial divisions of the medial geniculate nucleus, whereas the surrounding areas do not (de la Mothe et al., 2006b; Hackett, 2007). While belt and parabelt areas have been traditionally considered unimodal association cortex, it is now clear that they have some multisesensory responses (reviewed in (Ghazanfar et al., 2005)). The belt is the nearly exclusive source of cortical association connections of the primary auditory cortex, and as such appears to be an obligatory synaptic relay for most information transfer between primary auditory cortex and downstream association areas (Hackett et al., 1998). In monkeys, the belt has been subdivided into five or more distinct areas on the basis of physiology and connections (Hackett and Kaas, 2004; Rauschecker and Tian, 2004). In contrast to the core regions, these lack sharply tuned frequency responses and a highly organized cochleotopic organization. They can be distinguished from the core, and from parabelt, in Nissl sections, and typically have a density of parvalbumin labeling that is intermediate between that of the core and the parabelt (Smiley et al., 2007b). The parabelt in macaque monkeys lies on the lateral surface of the STG lateral to the belt. It has been divided into rostral and caudal subdivisions, but their border is not readily distinguishable by anatomy. The Parabelt can be distinguished from more lateral temporal areas in Nissl stains and by slightly greater parvalbumin staining (e.g., (Smiley et al., 2007b)).

2.2 Human Auditory Areas

In humans, cytoarchitectonic studies have identified the primary auditory cortex on HG (*Figure 3.3-2*), that is, the most anterior transverse gyrus of the superior temporal plane (Brodmann, 1909; Campbell, 1905; Galaburda and Sanides, 1980; Hackett, 2007; Morosan et al., 2001; Rivier and Clarke, 1997; von Economo, 1929). In general, anatomical methods located the primary cortex on the caudal-medial half of HG, and this interpretation is confirmed by depth electrode recordings in surgical patients that demonstrated short-latency auditory-evoked responses at this location (Howard et al., 2000; Liegeois-Chauvel et al., 1991, 2001, 2004). Similar to monkeys, the primary auditory cortex in human is characterized by a high density of cells especially in the upper layers, by a high density of parvalbumin immunolabeling in the middle layers, and by comparatively dense cholinesterase, myelin, and cytochrome oxidase staining (e.g., Hackett et al., 2001; Rivier and Clarke, 1997). Although cortex with this appearance occupies much of

Figure 3.3-2

The approximate location of the human auditory areas described von Economo (1929) and by Galaburda and Sanides (1980). (a) A 3 dimensional reconstruction of the superior temporal gyrus shows the location of Heschl's gyrus (HG) and the planum temporale (PT), separated by Heschl's sulcus (HS). The view in this image is from a dorsal and rostral perspective of the superior temporal plane. The reconstruction was made from 1 mm spaced histological sections that were imaged with a video camera while being sectioned with a microtome. (b) The approximate location of auditory areas described by von Economo (1929) are overlayed on the reconstruction. The primary auditory cortex (TC) is located caudally on Heschl's gyrus, and is surrounded laterally and rostrally by first order association cortex area TB. Lateral to TB is areas TA that is thought to correspond to higher order auditory association cortex, similar to the parabelt of monkeys. Area TA also extends onto the lateral surface of the superior temporal gyrus (not shown). Area TD is a distinct area at the caudal edge of TC. (c) A somewhat more detailed schematic was proposed by Galaburda and Sanides (1980). The primary kortex (KA) is surrounded by beltlike areas, including a rostral parakonicortex (paAr), and laterally an internal parakoniocortex (paAi). A second level of auditory association cortex located lateral to paAi was the external parakoniocortex (paAe). Caudal to KA is area paAc/d. Area Tpt is located on the caudal portion of the planum temporale, and also extends onto the lateral surface of the superior temporal gyrus (not shown)



the caudal-medial half of Heschl's gyrus, it is not completely homogeneous. There are patches with especially high neuron density, and there are regularly spaced bands seen with cholinesterase and cytochrome oxidase staining (Clarke and Rivier, 1998; von Economo, 1929). Galaburda (Galaburda and Sanides, 1980) distinguished medial and lateral parts of the primary auditory cortex, but did not emphasize this distinction in later studies (Cipollini and Pandya, 1989; Galaburda and Pandya, 1983). In monkeys there is some support for a medial-lateral division of primary auditory cortex, based on myelin staining and connections seen with tract-tracing (de la Mothe et al., 2006a; Pandya and Sanides, 1973; Smiley et al., 2007b).

At the caudal-medial end of HG, the primary auditory cortex borders on a cortex that has comparatively larger cells in layer III but still has some features of primary cortex, including relatively dense parvalbumin staining and a high density of supragranular cells. von Economo (1929) named this area "TD," distinct form the primary cortex (his TC), and stated that its appearance was consistent with either the primary auditory cortex or an auditory association area. Later authors reached similar conclusions about this small region (Galaburda and Sanides, 1980; Hackett et al., 2001; Morosan et al., 2001). Depth recordings have provided tentative evidence that this area may be a separate primary cortex on the basis of its short latency auditory-evoked responses (Liegeois-Chauvel et al., 2001; Pantev et al., 1995). Functional imaging studies have also revealed some evidence for multiple areas of primary cortex, similar to monkeys (e.g., Formisano et al., 2003).

Surrounding the primary auditory cortex in humans are areas that are thought to correspond to the belt and parabelt auditory association areas found in monkeys. On its lateral border, A1 is typically bordered by Heschl's sulcus, and different authors have varied with respect to the identity of the cortex at and near the fundus of the sulcus. This cortex retains the high density of parvablumin and acetylcholinesterase staining seen in the primary cortex, but these are restricted to a narrower layer III–IV band in the middle of the cortex. It lacks the striking high density of small neurons seen in the primary cortex, but is clearly distinguishable from the neatly laminated and highly columnar cortex just lateral to the sulcus. Thus, this cortex has features consistent with primary cortex and with belt cortex, and its cytoarchitectonic identification is complicated by the anatomical distortion typically seen in the fundus of sulci. While some studies have included this cortex in delineations of primary cortex (Brodmann, 1909; Galaburda and Sanides, 1980; Morosan et al., 2001; Rademacher et al., 1993), others separated it as a distinct area possibly corresponding to the auditory belt of monkeys (von Economo and Koskinas, 1925).

Lateral to Heschl's sulcus, depth recordings have identified areas along the length of the Heschl's sulcus and extending onto the anterior portion of HG that have longer latency responses with less distinct tonotopic organization than found in A1 e.g., (Binder et al., 2000; Howard et al., 2000; Liegeois-Chauvel et al., 2004). Cytoarchitectonic studies have provided varying degrees of detail in parcellation of the areas around HG. Among the most detailed and commonly used nomenclature for these areas are those of von Economo (1925, 1929) and Galaburda and Sanides (1980). (For an overview of additional nomenclatures see Hackett, 2007). von Economo, using Nissl stained tissue, described area TB, which surrounds HG laterally and extends onto the anterior part of HG. In particular, TB has a characteristic striking regularity of minicolumns that are especially obvious in layers III–V, with large pyramidal cells in lower layer III and regular laminar spacing. Von Economo referred to the striking appearance of minicolumns as an "organ-pipe formation." Lateral to area TB is area TA, which has less homogeneous minicolumns and less neatly organized cortical lamination. Von Economo placed area TA mainly on the lateral surface of the STG, except rostrally (area TA2) where it wrapped onto anterior HG, and caudally (area TA1) where it extended up onto the surface of the superior temporal plane caudal to area TB.

A slightly different parcellation was provided by Galaburda and Sanides (1980), who separated the cortex caudal-lateral to HG into three subdivisions. The area nearest to HG, their internal parakoniocortex (paAi), appears to be anatomically similar to TB of von Economo (mentioned earlier), but does not extend so far laterally, covering only the medial part of the PT. They state that paAi is similar in appearance to area paAlt of monkeys (Pandya and Sanides, 1973) or roughly the lateral belt of Hackett et al. (2001). Medially, they show paAi abutting the primary auditory cortex at the fundus of the Heschl's sulcus. Rostrally, like area TB of von Economo, another belt-like area (paAr) extends onto the rostral part of HG. Lateral to paAi, Galaburda and Sanides describe external parakoniocortex (area paAe) that is similar to paAi, but with a thinner layer IV and larger cells in layer V, and they suggest that this corresponds approximately to the auditory parabelt of monkeys (TS_3 of Pandya and Sanides, 1973). Caudal and lateral to paAe, they describe area Tpt that has an even thinner layer IV and less distinct lamination patterns at the III–IV and IV–V borders. They considered Tpt to be a "transitional type of cortex" between the specialized auditory areas and the association cortex of the inferior parietal lobule.

More recent studies have employed additional histochemical labels to parcellate auditory areas (Clarke and Rivier, 1998; Hutsler and Gazzaniga, 1996; Morosan et al., 2005; Nakahara et al., 2000; Rivier and Clarke, 1997; Sweet et al., 2005). The application of parvablumin and acetylcholinesterase in humans shows distinct areas in the PT with features similar to the monkey belt and parabelt association areas. However, this region is considerably larger and more complex in humans, and detailed parcellations are somewhat hindered by the complex and variable sulcal and gyral patterns found in some brains.

3 Cytoarchitectonic Asymmetries of Human Auditory Areas

While numerous studies have documented gross structural differences between the left and right lateral sulcus (see below), comparatively few studies have measured the asymmetry of cytoarchitectonically defined areas in the superior temporal plane. Galaburda et al. exhaustively analyzed 3 brains (only Tpt

was analyzed in a fourth brain) in Nissl stained histological sections (Galaburda and Sanides, 1980; Galaburda et al., 1978). They found consistent right > left volume asymmetries of areas PaAi and PaAe, and consistent left > right asymmetry in area Tpt. The much larger left Tpt prompted the authors to suggest that this area may be an anatomical correlate of Wernicke's speech area. Earlier hemispheric comparisions by von Economo and von Horn (1930) stated that area TB (similar to PaAi and PaAe of Galaburda) appeared to be larger on the left. Both von Economo et al. and Galaburda et al. concluded that the primary auditory cortex was approximately symmetrical, although von Economo et al. noted that the left side had a larger central area of complete granulation (i.e., having a high density of small neurons). Replication of these studies is difficult, given that identification of area borders was based on qualitative criteria in Nissl-stained material.

More recently, Rademacher et al. applied quantitative criteria in Nissl stained sections to identify the borders of primary auditory cortex in 27 postmortem brains (14 females), and also concluded that there is not a consistent hemispheric asymmetry (Rademacher et al., 2001a, b). Their delineation of primary cortex included an expanded area that extended across Heschl's sulcus. They did not measure the volume of the smaller area that corresponds to the classical primary auditory cortex restricted to HG (their Te1.0) (Morosan et al., 2001).

There is evidence that the auditory areas of the left and right hemispheres have some differences in cytoarchitectonic organization. Hutsler et al. reported left > right size of large pyramidal cells in primary auditory regions and in lateral regions of the STG (auditory association cortex) in 7 brains (5 females) (Hutsler, 2003; Hutsler and Gazzaniga, 1996). Anderson et al. (1999) measured the volume of both gray and white matter of STG at the level of the PT, in 16 brains (all males). They replicated the commonly found left > right asymmetry of gray matter, but also showed that the white matter asymmetry is disproportionately greater than that of gray matter. They additionally used electron microscopy in 8 of these brains, and found approximately 20% left > right myelin sheath thickness. The authors suggest that white matter differences, including thicker myelin and possibly greater axon number in the left hemisphere could provide a mechanism of language specialization by allowing greater processing speed that has been suggested to be a fundamental left hemisphere specialization for language (Tallal et al., 1993). A similar left > right volume asymmetry of white matter, disproportionate to the gray matter asymmetry, has also been demonstrated for HG (Dorsaint-Pierre et al., 2006; Golestani et al., 2007; Penhune et al., 1996).

There are reports of left > right spacing of "minicolumns." Minicolumns are perpindicular stacks of neurons arranged between myelin bundles, and are especially obvious in layers III/IV in temporal areas. Although anatomically defined, they are presumed to represent functional domains, possibly being subcomponents of larger, functionally defined, columns (or "macrocolumns") that are thought to be fundamental functional units of the cortex (Jones, 2000). Seldon (1981) reported that the distance between minicolumns was 18% greater in the left auditory areas than in the right, in primary and secondary auditory areas (areas TC, TB and TA of von Economo). He interpreted these studies in light of a subsequent Golgi study of 3 brains that showed only slight hemispheric differences in the tangential spread of pyramidal cell dendrites (Seldon, 1982). From these comparisons, he argued that pyramidal cell dendrites in left auditory areas contact fewer neighboring minicolumns, possibly resulting in more segregated responses in the left hemisphere. Interestingly, a similar conclusion was derived from lipophilic dye injections in human postmortem STG (Galuske et al., 2000). Small dye injections revealed connections with surrounding cell clusters, and fewer clusters were contacted in the left hemishere than in the right. A similar asymmetry was not seen in HG. Again, the authors suggested that this difference might facilitate finer-grained feature analysis in the left hemisphere. Subsequent measurements of minicolumn spacing also reported left > right asymmetry, by as much as 15%, in 14 brains (9 males), although the precise source of sampled tissue on the PT was unclear (Buxhoeveden and Casanova, 2000; Buxhoeveden et al., 2001). When Seldon revisited this issue and sampled tissue on the PT adjacent to HG in 10 brains (5 males), the asymmetry was only 5%, but statistically significant and similar in males and females (Seldon, 2006). More recently, Chance et al. (Chance et al., 2006a) measured minicolumn spacing on Heschls' gryus and the adjacent PT in 17 brains (7 males). Although the hemispheric differences did not reach significance, they report left > right asymmetries in the PT of about 5% in males and 2% in females, and left > right asymmetries on the order of 2% were found in HG. Taken together, these studies seem to provide roughly

consistent evidence for hemispheric asymmetry of spacing in the PT, although more recent studies suggest the hemispheric difference may be quite subtle.

The spacing of minicolumn could be affected by various mechanisms. Von Economo (1929) suggested that the perpendicular bundles of myelinated fibers might contribute to their arrangement. Seldon has proposed a "balloon model" which states that because the left hemisphere is more myelinated, the increased white matter volume causes an outward and tangential expansion of cortex (Seldon, 2005). An interesting feature of his model is the suggestion that minicolumns spacing may reflect myelination processes that continue into adulthood. Other authors have speculated that minicolumns may reflect early arrangement of neurons around radial glial that occurs early in corticogenesis (Buxhoeveden and Casanova, 2002). It has also been pointed out that minicolumn spacing is greater in gyral crests, and would therefore be altered by processes that cause increase gyrification (Welker, 1984). It is additionally possible that minicolumn width is affected by other dynamic processes that change the volume of the cortical neuropil, including dendritic and axonal proliferation and glia size and number.

4 Altered Cellular Anatomy of Auditory Areas in Schizophrenia

Cytoarchitectonic studies of schizophrenia auditory areas are still comparatively few in number, in contrast to the more intensively studied frontal, cingulate, and medial temporal areas. In some cases, the auditory cortex has been compared with other cortical regions to determine whether changes are regionally specific. For example, Konapaske et al. measured the density of chandalier cell synaptic cartridges immunoreactive for the GABA transporter, and found evidence that these were modestly reduced in auditory association cortex, similar to their reduction in prefrontal area 46 (Konopaske et al., 2006). Sweet et al. reported a 10–13% decrease in neuron volume, in pyramidal neurons that were selected by their characteristic pyramidal shape from layers III of both primary auditory and first order ("belt") auditory association cortex, evaluating the left hemisphere of 18 schizophrenia patients and 18 matched controls (Sweet et al., 2004). This change in auditory cortex was comparable to reduced pyramidal cell size in prefrontal area 9 in the same sample of brains (Pierri et al., 2001), suggesting that the pathological processes that effect pyramidal cell size are similar across areas of cortex, including the primary sensory cortex. Other studies of primary and secondary auditory areas in schizophrenia did not find reduced size, but all neurons were included without selecting by shape (Beasley et al., 2005; Smiley et al., 2002). In other cortical areas, findings of reduced neuron volume have been inconsistent. Measurements that included all Nissl-stained neurons found a decreased soma size especially in the large pyramidal neurons in layers III and V, in area 9 of the prefrontal cortex (Rajkowska et al., 1998) and in the anterior cingulate cortex (Chana et al., 2003). Other studies that measured all neurons in the prefrontal cortex did not find significant changes (Benes et al., 1986; Cotter et al., 2002; Cullen et al., 2006), but Cullen et al. found a reduced left > right asymmetry when selecting pyramidal shaped cells. The less-than-perfect agreement between studies is perhaps to be expected considering the rather subtle (usually 5 to 10%) decrease in cell size seen even when sampling just large pyramidal cells, and the small sample size of most studies (usually 10–20 subjects per group). Studies that used immunostained sections to measure the size of pyramdal cells generally have not shown significant reductions (Law and Harrison, 2003; Miguel-Hidalgo et al., 2005), but this might be explained by the sampling bias of the labeling method (Pierri et al., 2003), or by the distortion of cell size caused by immunohistochemical staining (Maldonado-Aviles et al., 2006).

Neuron density measured by Cotter et al. in HG and in the adjacent PT (Beasley et al., 2005; Cotter et al., 2004) was unchanged compared with controls, as it was in their studies of prefrontal area 9 and cingulate area 24 in the same brains (Cotter et al., 2001a, 2002). Smiley et al. (2002) also found neuron density in the primary auditory cortex and in the PT to be unchanged from control levels, using stereological methods. An increased neuron density has been reported in areas 9 and 46 of the prefrontal cortex, using unbiased stereological methods (Selemon et al., 1998, 2003), and in the cingulate cortex, using 2-dimensional counting methods (Chana et al., 2003). However, other studies in the prefrontal and cingulate cortex found neuron density to be lower or unchanged, using either stereological methods (Cullen et al., 2006; Thune et al., 2001) or nonstereological methods (Akbarian et al., 1996; Benes et al., 1986; Cotter et al., 2002; Radewicz et al., 2000).

Cotter et al. also measured the density of glial cells in Nissl-stained sections in layers III and V of HG in schizophrenia and control brains, and found evidence for a decrease of about 8% in schizophrenia (Cotter et al., 2004). Previously in the same brains, they had found a significant decrease of up to 34% of glia density in layers V and VI in area 9, and a slight decrease in these layers in the cingulate cortex (Cotter et al., 2001b, 2002). Other studies have found some evidence for disrupted glia in the prefrontal cortex (e.g., Benes et al., 1986; Rajkowska et al., 2002), but there are also negative studies, and the literature is complicated by the difficulty of confidently identifying subtypes of glial cells (e.g., Webster et al., 2001).

Cortical thickness has also been evaluated in the auditory cortex in postmortem schizophrenia brains. Smiley et al. (2007a) found evidence for reduced thickness specific to the supragranular layers of HG and the PT of the left and right hemispheres. The size of the reduction was approximately 3–7% of the upper layers, and reached statistical significance only in the left hemisphere PT. Similar width measurements by Cotter and collaborators (Beasley et al., 2005) did not report significant changes, but it is interesting that their data from a sample of mixed left and right hemispheres show a nonsignificant 8% decrease in the supragranular layers. They stated that they found no significant changes in HG (Cotter et al., 2004). Chance et al. (2004) measured cortical width in the walls and depths of Heschl's sulcus and did not find a significant difference in schizophrenia. Postmortem studies in other cortical areas have also sometimes found a reduced thickness. For example, significant decreases of about10% were found in the anterior cingulate cortex, but not in prefrontal area 9 (Kreczmanski et al., 2005). Selemon et al. (1995, 1998) found a nonsignificant decrease of about 8% in prefrontal areas 9 and 46. However, Radewicz (Radewicz et al., 2000) reported no change in area 9, the anterior cingulate, or in the STG. Postmortem findings of reduced cortical thickness are consistent with MRI findings of thinner cortex in several cortical areas including the superior temporal plane (Cannon et al., 2002; Kuperberg et al., 2003; Narr et al., 2005; Thoma et al., 2004; Thompson et al., 2004).

Conclusions: At present, the precise cellular/cytoarchitectonic changes that occur in schizophrenia are not clearly established in any part of the brain. Relatively initial investigations of the primary and secondary auditory cortex have provided evidence that some changes found in other areas, such as decreased size of large neurons, cortical thinning, and changes in GABAergic synapses, are also likely to be present even in the early stages of cortical processing of auditory information. At present, most findings need to be considered with caution until they are further replicated.

5 Structural Asymmetry of Auditory Regions in Normal and Schizophrenia Brains

Measurements of the size of HG, the PT, and the whole STG do not correspond precisely to functional areas. However, they do provide an index of the hemispheric asymmetry of this region, and have been used to investigate language asymmetry and the presence of pathology. The asymmetric shape of the posterior Sylvian fissure has been known at least since the studies of Eberstaller in 1884, who noted that it more often turns up dorsally on the right side, whereas it more often extends more caudally on the left (see Harrington, 1998). In 1936, Pfeiffer reported that the surface area of the PT was larger on the left side. Geschwind and Levitsky replicated this surface area asymmetry in a sample of 100 cadaver brains, finding that the PT was larger on the left in 65% of the brains, about equal in 24%, and larger on the right in 11% (Geschwind and Levitsky, 1968). They emphasized that this anatomical asymmetry could be the basis for language asymmetry. Support for this idea came from the observation of similar asymmetries in this region in great apes but not in monkeys (LeMay and Geschwind, 1975). Additionally, human fetal brains were found to have HG asymmetries at least as early as the third trimester of pregnancy, suggesting that these hemispheric differences may be genetically programmed (Chi et al., 1977). These observations have greatly influenced the thinking about the anatomical origins of human language. However, recent findings have suggested that these ideas may need to be modified. While some early studies found correlations between language dominance and PT asymmetry (Foundas et al., 1994; Ratcliff et al., 1980), recent functional imaging studies have not supported this finding (Dorsaint-Pierre et al., 2006; Eckert et al., 2006; Josse et al., 2003). Additionally, while anatomical asymmetry of this region may be present very early in development, there

are recent findings that the shape and asymmetry of the lateral sulcus changes during adolescent development, and that the asymmetry of the lateral sulcus might at least partially reflect the growth of parietal and temporal areas surrounding the lateral sulcus (Sowell et al., 2002, 2003).

5.1 Structural Asymmetry of Heschls' Gyrus

Heschl's gyrus contains the primary auditory cortex on its caudal-medial portion. The gyrus originates medially at the caudal end of the insula, and typically courses across the superior temporal plane at about a 45° angle from the rostral-caudal axis, but the angle can vary from nearly rostral-caudal to nearly medial-lateral. It is bordered caudal-laterally by Heschl's sulcus, which is often the deepest sulcus on the superior temporal plane. It is bordered rostral-medially by the anterior limiting sulcus. In many cases HG is easy to identify, but in some cases its precise borders are ambiguous, giving rise to discrepancies between different studies. There are 3 obvious sources of ambiguity. The first is deciding which gyrus is HG when multiple gyri are present. In some cases, there is a gyrus rostral to HG, running along the base of the insula. Usually, this gyrus is thinner and shorter and has a more rostral-caudal course than HG, but sometimes the distinction is less obvious. In addition, it is common to see one or more "transverse" gyri located caudal to HG. In histological preparations, HG is easily identified by the presence of the primary auditory cortex. In other preparations, the widely used convention is to select the gyrus whose medial termination is at, or just caudal to, the caudal end of the insula (Barta et al., 1995; Frangou et al., 1997; Steinmetz et al., 1989).

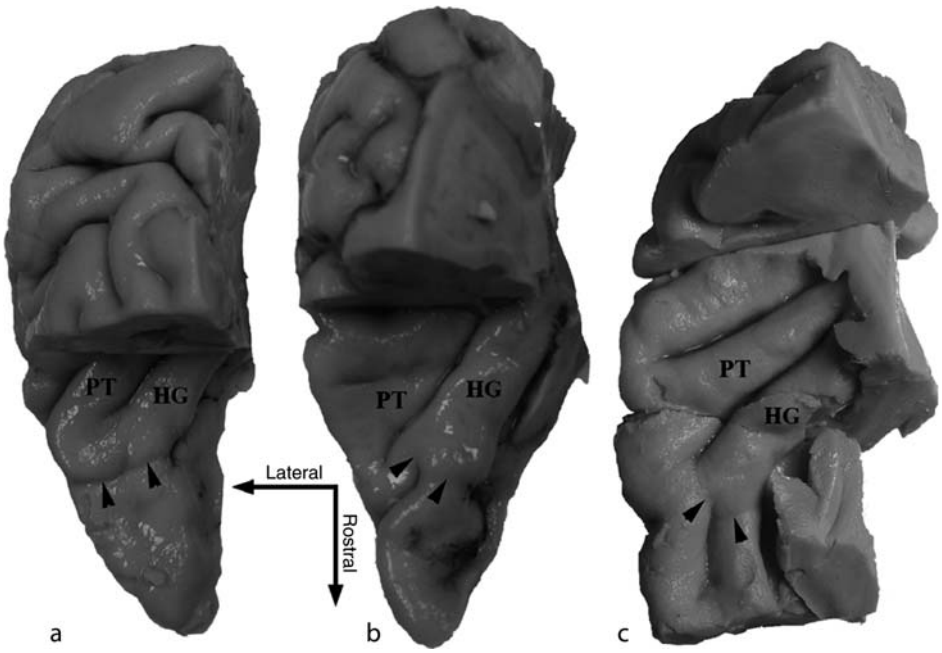
A second ambiguity is that HG often has a "sulcus intermedius" (SI) coursing along the length of its gyral surface. The SI can vary from a shallow groove to a full sulcus, and investigators need to establish criteria to distinguish a SI from a sulcus that separates HG from a second transverse gyrus (i.e., Heschl's sulcus). A commonly used rule is that of Steinmetz et al. (adapted from Pfeifer, 1920) (1989, 1990), which states that any sulcus that extends medially to the "immediate retroinsular region" at the depth of the lateral sulcus should be considered the lateral border of HG (i.e., Heschl's sulcus). Nearly all studies have applied this rule of retroinsular origin, but there are various methods on how to handle unusual formations of Heschl's sulcus, and whether to include the cortex on both sides of the SI. By the method of Steinmetz, the cortex on both sides of a SI (that does not reach the retroinsular region) is included in HG. Barta et al. (1995) largely adapted the method of Steinmetz, but added the additional rule that if the medially defined stem of HG fused laterally with a second transverse gyrus, then the lateral border of HG should be switched over to the sulcus lateral to that gyrus. Another commonly used variant adapts the criteria of Steinmetz, but excludes from HG any cortex on the lateral side of the SI (Kim et al., 2000b; Rojas et al., 1997). An alternative method was applied by Penhune et al. (1996), who considered both sides of the SI to be part of HG as long as the SI did not extend more than 50% of the length of HG. If greater than 50%, then the SI was re-categorized as Heschl's sulcus.

A third source of ambiguity is in the definition of the anterior-lateral end of HG (*Figure 3.3-3*). In cases where the gyrus extends clearly to the crest of the lateral sulcus, this border is simply at the crest of the lateral sulcus. However, it is not uncommon for the gyrus to extend rostrally and terminate without reaching the lateral edge of the sulcus. One solution is to extrapolate the course of Heschl's sulcus and the anterior limiting sulcus to the gyral surface, but this will clearly exaggerate the volume of the sulcus in cases where HG is directed most rostrally (Steinmetz et al., 1990). A solution proposed by Barta et al. was to terminate the gyrus at the most rostral extent of clearly identified Heschls' sulcus (Barta et al., 1995; Kim et al., 2000b). An alternative method is to terminate the gyrus when it is no longer distinguishable from the rostral temporal plane (Penhune et al., 1996; Rojas et al., 1997). In my experience, using postmortem samples, there is usually a reasonably clear rostral end of the gyrus where its dorsal surface steps down to join the rostral temporal plane.

Clearly the different methods described above may give rise to inconsistent measurements by different researchers. Besides the divergent definitions, there is the additional consideration that the criteria are somewhat subjective, are likely to be interpreted differently by different researchers, and their application will vary depending upon the resolution of the anatomical preparation.

Figure 3.3-3

Examples of the rostral anatomy of Heschl's gyrus are shown in three examples of dissected tissue from the superior temporal plane. In these photographs, the viewer's perspective is approximately perpendicular to the superior temporal plane. In most cases, the rostral end of Hesch's gyrus can be visualized as a distinct step down or ridge, where HG meets the cortex of the rostral superior temporal plane. Heschl's gyrus (HG) is separated from the planum temporale (PT) by Heshcls' sulcus (HS). In these examples, Heschl's sulcus does not extend to the lateral crest of the lateral sulcus, and the border between HG and PT must be determined by extending a line from the front end of HG to the crest of the lateral sulcus. Figures (a) and (c) show examples of multiple transverse gyri crossing the superior temporal plane



Left > right asymmetry in the size of HG has been reported in several studies. Penhune et al. (Penhune et al., 1996) used structural MRI and found a clear left > right asymmetry in 31 of 40 normals, in contrast to a clear right > left in only 3 subjects. Separating the cortex from white matter, they found that the asymmetry was mainly due to white matter volume, although there was a parallel but nonsignificant asymmetry in gray matter volume. A similar asymmetry, but statistically significant for both gray and white matter, was found in MRI studies of epileptic patients (n = 44; Dorsaint-Pierre et al., 2006) or subjects selected for high or low performance on a verbal recognition task (Golestani et al., 2007). Additionally, there have been several recent reports of left > right gray matter volumes using postmortem tissue (Chance et al., 2006b) and in vivo imaging (Hirayasu et al., 2000; Salisbury et al., 2007; Takahashi et al., 2006). Other MRI studies that measured gray matter volume did not find consistent asymmtry (e.g., (Frangou et al., 1997; Knaus et al., 2006; Kulynych et al., 1995; Rademacher et al., 2001a; Schneider et al., 2002)). In part, this inconsistency might be due to the relatively small effect size of the gray matter asymmetry, but it can also be attributed to the variety of methods used. For example, it is notable that several studies that reported asymmetry used the method of Penhune et al. to delineate HG (Dorsaint-Pierre et al., 2006; Golestani et al., 2007; Penhune et al., 1996).

There is no consistent evidence for the hemispheric asymmetry of the number of transverse gyri crossing the PT. Typically, there are one or two clearly defined gyri per hemisphere, but there may be as many as five, and it is not uncommon to see complex folding that are not easily defined as tranverse gyri.

Recent studies have not found left–right differences in the number of transverse gyri (Leonard et al., 1998; Musiek and Reeves, 1990; Penhune et al., 1996; Rademacher et al., 2001b), but earlier studies counted more gyri on the right (Campain and Minckler, 1976; von Economo and von Horn, 1930). Leonard et al. (1998) has suggested that this discrepancy may be due in part to the different criteria used to count gyri that are partially separated by the sulcus intermedius.

5.2 Heschl's Gyrus Structural Changes in Schizophrenia

Heschl's gyrus traditionally received less attention than the PT in schizophrenia research. However, a number of recent in vivo MRI studies have reported reductions of gray matter volume in schizophrenia, although some earlier studies did not find this change (see *Table 3.3-1* for references). Among the studies that reported significant decreases, the change was usually bilateral although slightly greater on the left (about 16% on average, *Table 3.3-1*) compared with the right (about 11%). It is striking that in most of these studies with positive findings, the methods used to define HG showed a sizeable left > right volume of HG, usually about 20% greater on the left. In most cases, the hemispheric asymmetry was slightly and nonsignificantly reduced in schizophrenia, with the exception of one study of schizotypal patients that found a reduced volume only in the left-hemisphere (Dickey et al., 2002). In contrast to these positive

Table 3.3-1
Volume measurments of Heschl's gyrus in schizophrenia

	N sz	N cnt	Def.[a]	% L	% R	Comments
Positive results						
Salisbury et al. (2007)	20	32	HG1	−20	−15	Decrease correlates with mismatch negativity
Takahashi et al. (2006)	65	72	HG1	−12	−10	
Takahashi et al. (2006)[c]	*39*		*" "*	*−9*	*−4*	*schizotypals*
Sumich et al. (2005)	25	0		na	na	Lft. correlates with pos. symptoms First episode.
Crespo-Facorro et al. (2004)	30	30	HG3	−6	−13	
Kasai et al. (2003b)	13	22	HG1	−23	−18	First episode
Sumich et al. (2002)	21	20	HG2	−16	−17	
McCarley et al. (2002)	15	18	HG1	−18	−11	First episode
Hirayasu et al. (2000)	20	20	HG1	−18	−11	Bilateral reduction in HG compared to controls, and compared to bipolars
Rojas et al. (1997)	19	21	HG5	na[b] na	na na	Paranoid schizorphenia, ~20% decrease in males but not females
Dickey et al. (2002)	*21*	*22*	*HG1*	*−21*	*1*	*schizotypals*
Negative results						
Yamasue et al. (2004)	13	19	HG1	15	0	Chronic illness
Kwon et al. (1999)	16	16	HG1	5	8	Chronic illness
Frangou et al. (1997)	32	39	HG2	12	4	
Barta et al. (1997)	*28*	*32*	HG1	−4	−11	

[a]Definition of Heschl's gyrus: HG1: Barta et al. (1995); HG2: Steinmetz et al. (1989); HG3: Kim et al. (2000b); HG4: Larsen et al. (1989); HG5: Rojas et al. (1997)

[b]na: volumes not available

[c]Entries in italics are studies of schizotypal patients

findings, the studies that did not find volume reductions in schizophrenia also did not report hemispheric asymmetry (*Table 3.3-1*). Thus, it is possible that differences in anatomical criteria are a source of the inconsistency among findings in schizophrenia.

Most studies of HG volume in schizophrenia used mainly or exclusively male subjects. One study, at least, found similar volume reductions in both male and female subjects with schizophrenia (Takahashi et al., 2006). In contrast, a study of paranoid schizophrenia patients by Rojas et al. (1997) found reduced HG in males but not in females.

Correlations with behavioral changes and symptoms provide insights into the significance of HG volume changes. Sumich et al. (2005) reported a correlation of smaller volume with hallucinations and delusions in first episode patients, and Kasai et al. (2003) found a correlation with suspicious thinking, but other studies have not found similar correlations (Crespo-Facorro et al., 2004; Hirayasu et al., 2000; Kwon et al., 1999). A link between HG and positive symptoms is also suggested by fMRI studies that found an increased activation in the region of HG, along with several other brain areas, during auditory hallucinations (Bentaleb et al., 2002; Dierks et al., 1999; McGuire et al., 1993). A decreased HG volume and cortical width has also been found to correlate with electrophysiological deficits (McCarley et al., 2002; Thoma et al., 2004). In particular, a decreased mismatch negativity response to repeated tone stimuli was correlated with decreased left HG volumes and cortical width (Salisbury et al., 2007). Mismatch negativity to tones occurs early in cortical sensory processing, and has been localized at or near HG, suggesting that HG abnormalities may be related to low-level sensory gating deficits (Javitt et al., 2000).

The specificity of HG volume changes to schizophrenia is supported by parallel measurements in patients with affective psychosis or mania, where decreased HG volume was not found (Hirayasu et al., 2000; Kasai et al., 2003b; McCarley et al., 2002; Salisbury et al., 2007). It is undetermined whether the volume change in schizophrenia precedes the onset of psychosis. Although some studies found a decreased volume at early onset of schizophrenia (Hirayasu et al., 2000; McCarley et al., 2002; Salisbury et al., 2007; Sumich et al., 2005), there is evidence that the volume deficit worsens with disease progression and drug treatment (Crespo-Facorro et al., 2004; Kasai et al., 2003b; Salisbury et al., 2007).

5.3 Structural Asymmetry of the Planum Temporale

The PT is defined as the cortex on the lower bank of the lateral sulcus (i.e., the superior temporal plane) caudal and lateral to HG. The shape of this region of the cortex is quite variable. In some cases, it is a relatively flat planar surface extending to the caudal terminus of the lateral sulcus. In other cases, the surface may contain one or more transverse gyri caudal to Heschl's gyri, or other complex folding patterns, and it is sometimes invaginated by small sulci extending up along the lateral surface of the STG. Extending caudally, the lateral sulcus sometimes branches into two clearly defined sulci: one coursing dorsally (the "posterior ascending" branch) and one extending approximately caudally (the "posterior descending" branch). It has been estimated that as many as 80% of hemispheres have a branched sulcus (Witelson and Kigar, 1992). However, this estimate depends on the qualitative determination of a clearly defined sulcus, and in some cases the descending branch is poorly defined. Independent of this branching pattern, it is common in both the hemispheres to see the caudal part of the sulcus angle upward toward the parietal cortex, and this upward angulation is more common and more pronounced in the right hemisphere in comparison with the left (Eberstaller, 1884; Sowell et al., 2002).

Various methods have been used to define the borders of the PT for quantification. Its anterior-medial border is the caudal-lateral border of HG, as discussed earlier. Behind Heschl's sulcus, its medial border is the fundus of the lateral sulcus. Its lateral border is the crest of the lateral sulcus where it converges with the lateral surface of the STG. The greatest variability in definition has been at the caudal border. In many studies, only the rostral portion of the lateral sulcus (the "horizontal limb") was included, whereas the caudal cortex, beyond the upward angulation, was excluded. In cases where the PT did not angle upward, the entire ventral surface to the caudal end of the sulcus is included. This is commonly referred to as the "knife cut method" referring to a planar cut along the horizontal surface of the lateral sulcus that excludes the caudal ascending branch (or "vertical limb"), and this was the method used in the pioneering study of

Geschwind and Levitsky (1968). In practice, of course, the upward angulation of the cortex is often irregular and gradual, and placement of this border will vary according to laboratories and preparations. This ambiguity was partially overcome by the refinement of Steinmetz et al., in which the caudal border was defined by a line drawn from the caudal-medial termination of Hechl's sulcus to the point of upward angulation of the lateral sulcus seen on the lateral brain surface (Steinmetz et al., 1989, 1990). This method probably has been the most commonly used, but alternative methods have also been applied. For example, in cases where the PT branches caudally, some early studies measured the cortex in the descending branch (Steinmetz et al., 1991; Witelson and Pallie, 1973). However, nearly all subsequent studies of the caudal PT included the ascending branch and excluded the descending branch. Another suggestion has been to terminate the PT at the coronal section at the end of Heschl's sulcus (Zetzsche et al., 2001). These various methods have been reviewed previously (Barta et al., 1995; Beaton, 1997; DeLisi et al., 1997; Honeycutt et al., 2000; Shapleske et al., 1999; Zetzsche et al., 2001).

It needs to be emphasized that these different definitions of the caudal border of the PT do not reflect functional or cytoarchitectonic boundaries (Witelson et al., 1995). For example, in my experience, the cytoarchitectonically defined auditory association areas may extend into either the ascending branch or the descending branch of the caudal sulcus, or may even be split between them. Additionally, the use of different definitions of the PT can give rise to dramatically different results. For example, methods that include only the PT rostral to its upward angulation (e.g., the knife-cut method) consistently reveal a left > right asymmetry (Eckert et al., 2006; Honeycutt et al., 2000; Shapleske et al., 1999). However, if the PT is terminated at a coronal plane at the caudal end of Heschl's sulcus, thus including all cortex lateral to HG, then a right > left asymmetry is revealed (Harasty et al., 2003; Zetzsche et al., 2001). Alternatively, most studies that include all cortex to the terminal end of the lateral sulcus (i.e., the horizontal limb plus the ascending limb) have usually found little or no asymmetry in the PT (Loftus et al., 1993; Steinmetz et al., 1990; Witelson and Kigar, 1992; Zetzsche et al., 2001).

5.4 Planum Temporale Structural Changes in Schizophrenia

Studies of altered PT structure in schizophrenia have reported a reduced or reversed hemispheric asymmetry, or bilateral reductions in volume. Not all laboratories replicate these findings, and the literature is difficult to decipher because of the various methods used by different laboratories. The following discussion reviews these findings in the context of the different methods used. *Table 3.3-2* provides an overview of studies that used volume measurements. A more complete review of earlier studies that used length and surface area measurements is available elsewhere (Shapleske et al., 1999, 2001).

A few studies measured the length of the lateral sulcus at the lateral surface of the brain. While earlier studies found altered hemisheric asymmetry (Falkai et al., 1992; Hoff et al., 1992), this was not replicated in later studies (Bartley et al., 1993; Shapleske et al., 2001).

Many of the earlier structural MRI studies measured the surface area of the PT, typically measuring only the horizontal limb. Two groups reported a striking surface area reduction of left PT. Rossi et al. measured the surface area of the horizontal limb of the PT, and reported a reversal of the normal left > right asymmetry, due to a 12% reduction on the left and an 8% increase on the right (Rossi et al., 1992). In a follow up study using a large samples, the same group did not replicate this reversal of asymmetry, but they did find a correlation between reduced asymmetry and thought disorder (Rossi et al., 1994). About the same time, another group also found schizophrenia brains to have a striking reversal of the normal surface area asymmetry, again measuring only the horizontal limb (Barta et al., 1997; Petty et al., 1995). Curiously, there was not a corresponding change in cortical volumes, and the authors suggested that these changes might be caused by large changes in cortical thickness. In contrast to these two positive findings, several other studies that measured the surface area of the PT horizontal limb in schizophrenia did not find significant changes (DeLisi et al., 1994, 1997; Jacobsen et al., 1997; Kleinschmidt et al., 1994; Kulynych et al., 1995).

More recent studies have tended to measure the cortical volume of the PT, and these can be divided into those that measured only the horizontal limb and those that measured the whole PT (horizontal plus

Table 3.3-2
Volume measurments of the planum temporale in schizophrenia

	N sz	N cnt	Def.[a]	% L	% R	Comments
Whole planum studies - positive results						
Takahashi et al. (2006)	65	72	HG1,PT3	−20	−13	Bilateral reduction, greater on Lft.
Takahashi et al. (2006)[c]	*39*		*" "*	*−21*	*−13*	*schizotypals*
Yamasue et al., 2004	13	19	HG1,PT3	−31	0	Lft. correlates with mismatch negativity
Kasai et al. (2003b)	13	22	HG1,PT3	−27	3	First episode
McCarley et al. (2002)	15	18	HG1	−21	10	First episode
Hirayasu et al. (2000)	20	20	HG1,PT3	−22	7	First episode
Kwon et al. (1999)	16	16	HG1,PT3	−28	4	Chronic illness
Whole planum studies – negative results						
Meisenzahl et al. (2002)	30	30	HG2,PT3	3	−2	
Horizontal limb studies – positive results						
Sumich et al. (2002)	25	16	HG2,PT1	−22	−17	First episode
Falkai et al. (1995)						
Males	12	10	HG4,PT2	−19	21	Postmortem brains
Females	12	14	" "	−23	2	
Horizontal limb studies – negative results						
Crespo-Facorro et al. (2004)	30	30	HG3,PT2	−3	−5	
Sallet et al. (2003)	40	20	HG?,PT2	−3	5	
Goldstein et al. (2002)	40	48	HG1,PT1	na[b]	na	
Meisenzahl et al. (2002)	30	30	HG2,PT2	7	−1	Study also listed above
Shapleske et al. (2001)	74	32	HG1,PT1	−5	−3	
Frangou et al. (1997)	32	39	HG2,PT1	−3	−10	
Barta et al. (1997)	28	32	HG1,PT1	−5	−17	

[a]Definition of planum temporale (for HG see Table 1)
PT1: Barta et al. (1995); Steinmetz et al. (1989)
PT2: knife cut method Geschwind and Levitsky, (1968); Kim et al. (2000b); Larsen et al. (1989)
PT3: whole planum (ascending plus descending limbs)
[b]na: volumes not available
[c]Entries in italics are studies of schizotypal patients

vertical limbs, see *Table 3.3-2*). Falkai et al. (1995) measured the horizontal limb in postmortem brains and found a loss of asymmetry due to a 20% reduction in volume in the left hemisphere (Falkai et al., 1995). In males, but not females, there was a 22% increase in the right PT compared with controls. More recently, a structural MRI study of first episode patients found a bilateral reduction of the PT that reached significance only in the left hemisphere (Sumich et al., 2002). It is not stated why this study did not see the expected asymmetry in their control group. Several other studies that measured the cortical volume in the PT horizontal limb did not find significant differences (Barta et al., 1997; Crespo-Facorro et al., 2004; Frangou et al., 1997; Goldstein et al., 2002; Meisenzahl et al., 2002; Sallet et al., 2003; Shapleske et al., 2001).

Fewer studies have measured the volume of the combined horizontal and vertical limbs of the PT. A series of studies from McCarley's group have consistently found a significant loss of PT asymmetry due to decreased left PT volumes (Hirayasu et al., 2000; Kasai et al., 2003b; Kwon et al., 1999; McCarley et al., 2002). In each of these studies, the schizophrenia subjects had a 20–30% reduction in left PT volume, but slightly larger right PT volumes compared with control subjects. Similar findings were obtained in both

chronic and first episode patients, and interestingly, a follow up study on the same subjects showed evidence that this volume reduction is progressive over the first 1.5 years after the disease onset (Kasai et al., 2003b). Recently, another research group measured the whole PT and also found about a 20% reduction in the left hemisphere, but the right hemisphere reduction was nearly as that of the left (Takahashi et al., 2006). In contrast to these positive findings, another study did not find altered volumes or an asymmetry in the whole PT (Meisenzahl et al., 2002). The reason for the discrepant findings is not obvious, but it is notable that in the studies with positive findings the control groups have a striking hemispheric asymmetry that was not found by Meisenzahl et al.

5.5 Superior Temporal Gyrus Structural Changes and Schizophrenia

In monkeys, the gyral surface of the caudal part of the STG contains higher order auditory association areas and these are bordered caudally and laterally by multisensory areas with widespread cortical connections. The rostral part of the STG is less directly connected with early auditory areas, and unlike the caudal STG, has intense connections with medial temporal and ventral frontal regions (Pandya, 1995). In humans, like monkeys, the caudal STG is preferentially activated by complex auditory stimuli. The caudal STG is activated by word and language comprehension tasks, and this activation is usually greater in the left hemisphere (Binder et al., 2000; Vigneau et al., 2006). Similar to monkeys, the cortex around the caudal STG in the dorsal bank of the superior temporal sulcus and in the most caudal parts of STG has complex multisensory responses (Beauchamp et al., 2004; Foxe et al., 2002).

The anatomical definition of STG used for volumetric studies in schizophrenia varies between studies. Nearly all authors include all cortex from the fundus of the lateral sulcus to the fundus of the superior temporal sulcus, thus including HG and the PT, although a few authors used an alternative method and subdivided this region in order to separately evaluate the cortex outside of the lateral sulcus (Takahashi et al., 2006; Kim et al., 2000, 2003). Rostrally, it is common to extend the STG to the temporal pole or alternatively to the temporal "stem" (i.e., rostral extent of the white matter connection between the temporal and frontal lobes). Caudally, the border of STG has been defined by the termination of the lateral sulcus, or by some other external landmarks such as the fornix, the trigone of the lateral sulcus, or the caudal end of the corpus callosum. Separation of the caudal from rostral STG has been done by using landmarks such as the mammilary bodies or the rostral end of Heschl's sulcus, or by simply using the geometric middle. *Table 3.3-3*, which summarizes STG volume studies in schizophrenia, does not tabulate methods because nearly all laboratories have developed slightly different boundaries for the STG. A few laboratories used the method of Shenton et al. (1992), which includes all cortex from the temporal stem rostrally to most caudal coronal section that also includes the fornix.

In a comprehensive review of structural MRI studies of schizophrenia between 1988 and 2000, Shenton et al. found that 23 of 27 studies reported a significant decrease of the volume of the STG, and it was concluded that this was one of the most consistent structural changes of any brain region (Shenton et al., 2001). Subsequent to that review, at least 6 MRI studies also reported volume decrease (*Table 3.3-3*) and one did not (Buchanan et al., 2004). Three studies that used voxel-based morphometry found significant decreases of gray matter density in this region (Borgwardt et al., 2007; Pantelis et al., 2003; van Haren et al., 2007). One postmortem volumetric study also reported a decreased volume (Highley et al., 1999). Nearly all volumetric studies have found reductions in the range of 5–15%. The findings are similar across genders (Flaum et al., 1995; Gur et al., 2000a; Highley et al., 1999; Pearlson, 1997). The specificity of this finding to schizophrenia is supported by negative findings in patients with affective psychosis (Hirayasu et al., 1998; Kasai et al., 2003b; Keshavan et al., 1998; Pearlson, 1997; Schlaepfer et al., 1994) and by similar reductions in unmedicated schizotypal patients (Dickey et al., 1999; Takahashi et al., 2006). Additionally, studies of at-risk populations and of nonpsychotic relatives also have found similar reductions in the STG (Borgwardt et al., 2007; Pantelis et al., 2003).

A somewhat less consistent observation is that the STG volume loss is significantly greater in the left hemisphere compared with the right. This inconsistency might be partially attributed to the part of the STG that was evaluated. Measurements restricted to the posterior STG seem to more commonly find a left > right

Table 3.3-3
Volume measurements of the superior temporal gyrus in schizophrenia

			Whole STG		Posterior STG		Anterior STG		
	N sz	N cnt	% L	% R	% L	% R	% L	% R	Comments
Left > Right volume decrease									
Takahashi et al. (2006)	65	72	–		−25*	−16	1	−14	Left ant. correlates with pos. symptoms.
""*Schizotypals*[b]	*39*	*" "*	–	–	*−34**	*−20*	*−5*	*−13*	*Schizotypals, unmedicated.*
Onitsuka et al. (2004)	23	28	−15*	−3	–	–	–	–	Left correlates with positive symptoms.
Kasai et al. (2003a)	13	14	−13*	−7	−14*	−7	−11	−6	First episode.
McCarley et al. (2002)	15	18	–	–	−15*	−5	−4	−1	First episode.
Rajarethinam et al. (2000)	20	20	−9	−4	−6	−5	−10*	−4	Lft. ant. correlates with hallucinations. Lft. post. correlates with thought disorder.
Levitan et al. (1999)	30	0	na[a]	na	na	na	na	na	Lft. ant. correlates with aud. hallucinations.
Dickey et al. (1999)	*16*	*14*	*−9**	*−4*	–	–	–	–	*Schizotypal, all male.*
Highley et al. (1999)									
Men	14	16	−12*	−1	–	–	–	–	Postmortem brains.
Women	13	13	−14*	−4	–	–	–	–	
Hajek et al. (1997)	10	10	na	na	na	na	na	na	Lft. Correlates with aud. evoked potentials.
Hirayasu et al. (1998)	17	18	−11	−16	−14*	−4	–	–	First episode or within 8 months of first episode
Pearlson et al. (1997)									
Men	32	43	–	–	−13*	4	−11*	−7*	
Women	14	17	–	–	−9*	16	−8*	−9*	
Tune et al. (1996)	14	15	−23*	−14	–	–	–	–	Trend level decrease on Rt.
Menon et al. (1995)	20	20	–	–	−24*	−11*	–	–	Reduction significantly greater on Lft. Lft. post. correlates with delusions.

continued

Table 3.3-3 (continued)

			Whole STG		Posterior STG		Anterior STG		
	N sz	N cnt	% L	% R	% L	% R	% L	% R	Comments
Shenton et al. (1992); McCarley et al. (1993)	15	15	−15*	–	−15*	–	−15*	–	Lft. correlates with thought disorder and P300 deficit (McCarley et al., 1993).
Barta et al. (1990)	15	15	–	–	–	–	−10*	−12	Lft. ant. correlates with aud. hallucinations
Left = or < Right volume decrease									
Kim et al. (2003)	25	25	–	–	−9	−13*	–	–	First episode, neuroleptic naive. Rt. post. correlates positively with neg. symptoms. Lft.ant.correlates negatively with neg. symptoms
Matsumoto et al. (2001)	40	40	−6	−12*	–	–	–	–	Early onset schizophrenia (∼16 y.o.). Rt. correlates with thought disorder and hallucinations.
Sanfilipo et al. (2000b)	53	20	−5*	−7*	–	–	–	–	
Gur et al. (2000)	100	110	−7*	−9*	–	–	–	–	Young adults with mild to moderate symptoms
Niemann et al. (2000)	20	20	–	–	–	–	–	–	First episode.
Bryant et al. (1999)	59	37	−7	−6*	–	–	–	–	
Holinger et al. (1999)	8	10	−9	−15*	−15*	−16*	13	−11	Lft. handed males. Rt. correlates negatively with thought disorder.
Marsh et al. (1997)	52	44	–	–	−5*(L + R)		−4*(L + R)		Control brains from Sullivan et al. (1998)
Sullivan et al. (1998)	71	73	–	–	−9*(L + R)		−11*(L + R)		
Keshavan et al. (1998)	17	17	−9*	−9*	–	–	–	–	First episode, neuroleptic naive.
Marsh et al. (1997)	56	52	−9*	−9*	–	–	–	–	Severe chronic illness with early onset

Table 3.3-3 (continued)

			Whole STG		Posterior STG		Anterior STG		
	N sz	N cnt	% L	% R	% L	% R	% L	% R	Comments
									Lft. and Rt. correlate with pos. symptoms
Flaum et al. (1995)									
Men	70	45	−3*	−5*	–	–	–	–	
Women	32	42	−9*	−6*	–	–	–	–	
Zipursky et al. (1994)	22	20	−5	−1	–	–	–	–	Similar decrease in frontal and parietal cortex
No significant change									
Buchanan et al. (2004)	44	34	−1(L + R)		–	–	–	–	
Haversman et al. (1999)	30	17	−3	−5	–	–	–	–	No correlations with aud. hallucinations.
Roy et al. (1998)	22	15	6(L + R)		–	–	–	–	
Woodruff et al. (1997)	42	43	na	na	na	na	na	na	Decreased inter-reginal correlations in patients.
Reite et al. (1997)	20	20	na	na	na	na	na	na	Trend level reductions in males but not females.
Kulynych et al. (1996)	12	12	−12	0	–	–	–	–	All males with prominent positive symptoms
Vita et al. (1995)	19	15	–	–	5	−2	–	–	Measured 3 sections at caudal STG.
DeLisi et al. (1994)									
Men	50	24	1	−3	–	–	–	–	First episode.
Women	35	16	−3	−5	–	–	–	–	

*Statistically significant difference between groups

[a]na: volumes not available

[b]Entries in italics are studies of schizotypal patients

asymmetry, compared with measurements of the whole STG or anterior STG (*Table 3.3-3*). There is some evidence that the altered asymmetry in this region may be gender-dependent (Bryant et al., 1999), but most studies found similar reductions in both men and women (Flaum et al., 1995; Gur et al., 2000b; Highley et al., 1999; Pearlson, 1997). Taken together, literature shows a trend for greater volume reductions in the left posterior STG, but there are also findings of reduced volume in the anterior STG and in the right hemisphere.

Early MRI studies showed a correlation of left hemisphere STG volumes with positive symptoms (Barta et al., 1990; Shenton et al., 1992) and several subsequent studies have replicated this finding (Kim et al., 2000b, 2003; Nestor et al., 2007; Onitsuka et al., 2004; Rajarethinam et al., 2000), although some studies did not find correlations with symptoms (Gur et al., 2000b; Havermans et al., 1999; Matsumoto et al., 2001; Sanfilipo et al., 2000). A study of only left handed males found a correlation between thought disorder and the right STG volume, suggesting that the hemispheric specificity of volume changes may be related to handedness (Holinger et al., 1999).

6 Conclusions

Both functional imaging and anatomical findings have shown changes in regions of the auditory cerebral cortex, and evidence for altered hemispheric asymmetry. At present, the cause and cellular basis of these changes are poorly understood. Only a limited number of postmortem cytoarachitectonic studies in the superior temporal cortex of schizophrenia brains are available, and even fewer have made hemispheric comparisons. These few studies have provided some evidence for cellular and neurochemical changes similar to those found in prefrontal and other areas of the cortex. However, the most extensive anatomical evidence for change in this region comes from volumetric studies in nearly all cases from in vivo imaging. There are now a reasonably large number of studies that have evaluated volumes of HG, the PT, and the STG. Although there are some contradictory findings, most studies have found decreased volumes in these structures, usually in the range of about 5 to 15%. Decreased volumes of HG are generally as large or even larger than in more lateral auditory regions, suggesting that primary auditory areas may be equally involved in these changes. Not all studies have found left > right decreases in volume, but this is a common finding. In HG, most studies reported bilateral volume decreases, but it is notable that the decreased volume is often modestly more pronounced in the left hemisphere. In the PT, there are also reports of either left only or bilateral volume deficits. In the whole STG, the left hemisphere selectivity of volume deficits is more consistent in those studies that separately focused on the caudal portion of the STG, which is anatomically apposed to the PT and is responsive to complex auditory stimuli.

Taken together, these findings suggest that schizophrenia pathology is commonly expressed in the auditory cortex and it is likely expressed more in the left hemispheres. In this context, there is clearly an opportunity for postmortem studies to look into this region to more clearly identify the features of this pathology. Hemispheric comparisons are rarely made in postmortem schizophrenia brains, because most brain collection centers process the two hemispheres differently. However, hemispheric comparisons have the advantage that confounding histological variables such as postmortem degradation and fixation artifacts are largely obviated.

Currently, there is a commonly expressed view that decreased cortical volume in schizophrenia is the result of a reduction of cortical neuropil, presumably due to an atrophy of neural dendrites and axons (e.g., (Selemon and Goldman-Rakic, 1999)). A direct prediction of this model is that neuron density should be increased in proportion to the decrease in cortical volume. While there is some evidence for increased density in the prefrontal cortex (Selemon et al., 1995, 2003), there are also a number of negative findings (Akbarian et al., 1995; Cotter et al., 2002; Cullen et al., 2006; Thune et al., 2001). In auditory areas, the available studies have not shown an altered neuron density, even with hemispheric comparisons (Beasley et al., 2005; Cotter et al., 2004; Smiley et al., 2002). An alternative hypothesis is that reduced cortical volume is accompanied by a loss of neuron number, but this issue remains to be addressed in the auditory cortex. In other brain areas, there is some evidence for decreased neuron number, although the differences are often subtle and statistically nonsignificant (Benes et al., 1986; Dorph-Petersen et al., 2007; Stark et al., 2004; Thune et al., 2001). Changes in total cell number in the cerebral cortex have been difficult to determine, because these measurements require a clearly identified reference volume.

Evidence from in vivo studies suggests that gray matter volume changes in schizophrenia might be caused by heterogeneous and possibly dynamic processes. Volume decreases appear to be present before the onset of psychosis, in as much as they are present in populations at risk for schizophrenia (Borgwardt et al., 2007; Pantelis et al., 2003) in nonpsychotic relatives of patients with schizophrenia (e.g., (Cannon et al., 2002; Job et al., 2003)) and in schizotypal patients (Dickey et al., 1999; Takahashi et al., 2006). However, there is also evidence for progressive decrease of gray matter that occurs after the onset

of psychosis (Crespo-Facorro et al., 2004; Kasai et al., 2003b; Salisbury et al., 2007). Furthermore, gray matter volume may be prone to dynamic process that occur as a result of changes in the psychotic state and as a result of antipsychotic medications (Garver et al., 2000; Lieberman et al., 2005; McClure et al., 2006). Thus, it is possible that the in vivo volume changes in the auditory cortex may be partially due to transient changes that might not persist after death, and for this reason, may not be detected in postmortem studies.

Acknowledgements

This study was supported by grains to the author from NIH (MH 067138) and from the Stanley Medical Research Institute.

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3.4 The Role of Prefrontal Abnormalities in Schizophrenia

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_3.4,

Abstract: Cognitive deficits are a key and persistent feature of schizophrenia. A large neurophysiological and neuropsychological literature has highlighted the role of the dorsolateral prefrontal and dorsal anterior cingulate cortices in mediating a number of these cognitive deficits, which include the executive functioning domains of working memory, error monitoring, and response inhibition. Many of these deficits appear to arise from abnormal GABAergic cells and their interaction with glutamatergic systems. Patients with schizophrenia also have profound deficits in social aspects of cognition, mainly subserved by orbitofrontal brain regions. However, these deficits are only poorly understood, and their neural basis remains elusive. In this chapter, we review behavioral, neuroimaging, and pharmacogenetic evidence for the role of dorsofrontal brain regions in executive dysfunction in schizophrenia. We then turn to a discussion of the role of inferior frontal cortex abnormalities and their relevance to social cognition including facial emotional processing, decision-making, theory of mind deficits, and impulsivity and aggression.

List of Abbreviations: ACC, Anterior cingulated cortex; AX-CPT, AX-Continuous Performance Test; BOLD, Blood-oxygen-level dependent; COMT, Catechol – O-methyltransferase; CPT, Continuous Performance Test; CSF, Cerebrospinal Fluid; dACC, Dorsal Anterior Cingulate; DLPFC, Dorsolateral Prefrontal Cortex; DMN, Default Mode Network; dMPFC, Dorsomedial Prefrontal Cortex; DTI, Diffusion Tensor Imaging; ERP, Event-related potential; FA, Fractional Anisotropy; fMRI, Functional Magnetic Resonance Imaging; GABA, γ-Aminobutyric acid; GAD, Glutamic Acid Decarboxylase; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT1A, Serotonin receptor 1A; 5-HT, 5-hydroxytryptamine (serotonin); 5-HTTPLR, Serotonin transporter polymorphism; MAOA, Monoamine Oxidase Inhibitor A; mRNA, Messenger ribonucleic acid; NMDA, N-methyl-D-aspartic acid; OFC, Orbitofrontal Cortex; PCP, Phencyclidine; PET, Positron emission tomography; PFC, Prefrontal Cortex; SPECT, Single photon emission computed tomography; TOM, Theory of Mind; TPH, Tryptophan hydroxylase; vlPFC, Ventrolateral Prefrontal Cortex; WCST, Wisconsin Card Sorting Test; WM, White Matter

1 Introduction

Schizophrenia is a devastating mental illness that affects approximately 1% of the population across cultures. It is characterized by positive symptoms, such as delusions and hallucinations, and negative symptoms, such as social withdrawal and apathy, as well as pervasive cognitive deficits. In general, positive symptoms have been found to respond well to first generation antipsychotic medications. Negative symptoms and cognitive deficits have proven much more difficult to treat. For this and other reasons, a considerable amount of effort has gone toward understanding the nature of cognitive deficits in schizophrenia.

Although the neurobiology of schizophrenia is complex, there is widespread acceptance of the idea that functions mediated by prefrontal regions are abnormal in this disorder, and that these abnormalities are related to the cognitive deficits seen in patients. More than 20 years ago, Weinberger et al. (1986) found that, unlike healthy controls, medication-free patients with schizophrenia failed to increase regional cerebral blood flow selectively in dorsolateral prefrontal cortex (DLPFC) while they performed the Wisconsin Card Sorting Test. In a companion study, Berman et al. (1986) found, additionally, that there were no differences between patients and controls in DLPFC regional cerebral blood flow when subjects were scanned while performing a visual continuous performance test that was thought to less clearly involve function in that region. A similar pattern of findings was obtained by Berman et al. (1988). These seminal findings helped to set the stage for an explosion in research devoted to understanding the role of prefrontal cortical abnormalities in schizophrenia. Other studies, described in the following section, have provided evidence that dysfunction in the dorsal anterior cingulate (dACC) is associated with schizophrenia. Relatively little attention has focused on the role of ventral prefrontal regions, including orbitofrontal cortex (OFC) (Waltz and Gold, 2007), however, despite their role in social function (Penn et al., 1997), object recognition (Bar et al., 2006), and decision making strategies (Bechara et al., 1999, 2000a) and in impulsive/aggressive behaviors, domains that are all abnormal in schizophrenia (Steadman et al., 1998). In this chapter, we review the roles of DLPFC and dACC in cognitive deficits in schizophrenia and then move on to a

consideration of data supporting the role of the OFC in schizophrenia, particularly in the development of aggression.

The argument being made here is not that frontal regions are the only ones in which abnormalities are observed in schizophrenia, rather that frontal abnormalities are important and have played an important role in the neurobiological theories of the syndrome. Medial temporal lobe abnormalities, including reduced hippocampal volumes (Bogerts et al., 1990, 1993), are well established. These deficits appear to be present in unmedicated first-episode patients (Szeszko et al., 2003) and to primarily affect the anterior portion of the hippocampus (Bilder et al., 1995; Szeszko et al., 2003). In addition, there is mounting evidence (see Javitt, Chapter 1.3 and Butler, Chapter 3.2, this Volume), that patients with schizophrenia have sensory processing deficits involving dysfunctions of occipital and auditory cortices. Although nonfrontal deficits are not the focus of this chapter, they are important for two reasons: (1) they provide support for the NMDA receptor hypothesis of schizophrenia (Javitt and Zukin, 1991) and (2) they may upwardly generalize to higher levels of cognition, potentially serving as the basis for those deficits. For example, Leitman et al. (2005) showed that poor tone matching performance predicted poor tune processing and voice discrimination. One mechanism by which these deficits may upwardly generalize involves disconnection between brain regions, as suggested by Leitman et al.'s. finding that performance on prosody tasks correlated with fractional anisotropy (FA) in areas near the acoustic radiations, as well as in the dorsal auditory pathways. However, it remains the case that a very large literature has evolved examining the role of prefrontal deficits in schizophrenia, and it is to this that we turn.

2 Dorsolateral Prefrontal Cortex (DLPFC)

2.1 Prefrontally-mediated Cognitive Abnormalities

The prefrontal cortices subserve a variety of cognitive functions, including planning, abstract reasoning, selective attention, decision-making, cognitive flexibility and inhibitory control, traditionally considered "executive" functions, or in current parlance, "cognitive control" functions. The Wisconsin Card Sorting Test (WCST), AX-Continuous Performance Task (AX-CPT), and Stroop task are three of the most widely used measures of executive function. Relative to healthy controls, patients with schizophrenia typically show impaired performance on these tests (e.g., fewer categories achieved on the WCST, poorer response inhibition on the AX-CPT, and greater interference on standard Stroop color-naming tasks).

By their nature, executive functions are quite complex. For this reason, computational (Cohen et al., 1996)/cognitive neuroscience (Carter et al., 2000; MacDonald and Carter, 2002; Carter and Barch, 2007) approaches have gained prominence in research focused on elucidating the role of abnormalities in this domain in schizophrenia.

Deficits in contextual processing (Cohen and Servan-Schreiber, 1992; Servan-Schreiber et al., 1996; Cohen et al., 1999a) may serve as an underlying explanation for performance deficits in multiple cognitive domains. Cohen and colleagues have focused on the role of abnormalities in dopamine neurotransmission in contextual processing (Cohen and Servan-Schreiber, 1992, 1993), which they argue are responsible for the deficits seen in working memory, attention, and language processing in patients with schizophrenia (Cohen et al., 1996). Dopaminergic tone serves, they propose, to increase the signal-to-noise ratio of neural processing in the frontal lobe (Cohen and Servan-Schreiber, 1993; Braver et al., 1999). Consistent with this notion, the dopamine agonist d-amphetamine enhances performance on spatial working memory and Stroop tasks as well as language production in patients (and controls) (Barch and Carter, 2005). This thread has been picked up as well by Winterer et al. (Winterer and Weinberger, 2004; Winterer et al., 2006), who have examined the genetics of dopamine-related cognition (see section on COMT).

Patients with schizophrenia perform poorly on tasks requiring cognitive control and contextual information processing (Stroop, AX-CPT, go/no-go). In the typical AX-CPT task (Rosvold et al., 1956), participants are shown a series of letters, presented one at a time. The letter "X" is designated as a target, but only when it is immediately preceded by a valid cue (letter "A"); "X" preceded by an invalid cue (a letter other than "A"; BX trials), and other letters preceded by "A" (AY trials) or any other letter (BY trials) are

designated nontargets. When AX trials occur much more frequently than AY trials (as in the AX-70 variant (Cohen et al., 1999b; Javitt et al., 2000), the letter "A" becomes a potent prime for the target response. Patients with schizophrenia show deficits on this task (Javitt et al., 2000; Barch et al., 2001). In particular, they make commission errors, responding incorrectly to uncued, nontarget "X"s on BX trials.

In a forced choice (target/non-target) AX-CPT task, medication-naive patients with schizophrenia show decreased DLPFC activation task conditions requiring context processing (AX, BX) but intact activation in posterior and inferior PFC (Barch et al., 2001). Using the same paradigm in medicated, chronic schizophrenics, MacDonald and Carter (2003) found a decreased DLPFC activation in patients, but noted that controls showed an increased DLPFC activation only when the task requires maintaining context representations to overcome an automatic response (e.g., BX trials). On a go/no-go variant of the AX-CPT in which subjects are to respond only on target trials and withhold responding on nontarget trials, schizophrenics show increased error rates (both false alarms and misses) as well as a decreased frontocentral ERP activity (Javitt et al., 2000). Dias et al. (2003) showed that dACC and DLPFC activity (ERP) is greater in association with withholding a response compared to initiating a response (in controls).

Several studies of cognitive control have implicated both anterior cingulate and DLPFC. Using fMRI, Kiehl et al. (2000) observed an increased activation in rostral cingulate and left lateral frontal cortex on no-go errors. Liddle et al. (2001) reported ACC activation during both go and no-go trials and DLPFC and ventrolateral PFC (vlPFC) activation during no-go trials, suggesting that cingulate was important for monitoring, while DLPFC was important for inhibition. Fellows and Farah concluded that dACC is not necessary for cognitive control, as patients with dACC lesions perform normally on both Stroop and go/no-go tasks (Fellows and Farah, 2005).

Kerns used a Stroop task in conjunction with fMRI. In healthy controls, conflict-related ACC activity predicted both greater prefrontal activity and behavioral adjustments (Kerns et al., 2004). Schizophrenic subjects showed a decreased dACC activation associated with both conflict and error-related processing (Kerns et al., 2005). Data obtained from healthy controls do not support a primary role for dACC in error detection. During a novel task that allows subjects the explicit option of error avoidance by rejecting trials they cannot complete within the available time, ACC activation was greatest for rejected trials (Magno et al., 2006), as was DLPFC activation. These authors concluded that their data are consistent with ACC having a role in performance monitoring and DLPFC in implementing behavioral changes to avoid errors.

2.2 Working Memory

Working memory refers to the ability to maintain information over a brief time interval so that it can be used in subsequent cognition operations (Baddeley, 1981). In the 1980s, Goldman-Rakic (1987) discovered that neurons in monkey DLPFC fire during the delay period of a spatial working memory antisaccade task. This firing rate is decreased when errors are made, and the decrease is correlated with the magnitude of the error. Studies such as these highlighted the critical role of the DLPFC in working memory (Smith and Jonides, 1997; Miller, 2001). Patients with schizophrenia show marked working memory deficits (Park and Holzman, 1992; Keefe et al., 1995; Rushe et al., 1999), and many have proposed that impairments in working memory are central to the cognitive deficits seen in schizophrenia (Goldman-Rakic, 1994; Silver et al., 2003). Poor-performing patients with schizophrenia show a reduced frontal activation during working memory tasks (Perlstein et al., 2001, 2003). Moreover, patients whose performance was more similar to controls show a heightened DLPFC activation (Manoach et al., 2000; Callicott et al., 2003; Manoach, 2003), perhaps suggesting inefficient processing in that region.

2.3 DLPFC Neuroanatomical Findings

In an elegant series of studies, Lewis et al. ((2005) for a review) have found lamina-specific abnormalities in parvalbumin-containing (Woo et al., 1997; Lewis et al., 2001) GABA-ergic chandelier cells (Lewis, 1998; Pierri et al., 1999; Volk et al., 2002) in the DLPFC. These inhibitory chandelier cells receive input from dopaminergic neurons and have unique axonal cartridges that powerfully inhibit the axon initial segment of

excitatory pyramidal output neurons in layer III of the cortex (Somogyi, 1977). Although chandelier cells are not reduced in number in schizophrenia, the expression of parvalbumin (Hashimoto et al., 2003) and GAD67 (an enzyme involved in GABA synthesis; Akbarian et al., 1995) mRNA is downregulated. Additional abnormalities in this region include reduced dendritic spine density (Glantz and Lewis, 2000, 2001; Kolluri et al., 2005) and somal size (Pierri et al., 2001, 2003) in layer III pyramidal cells in prefrontal cortex.

These abnormalities have been hypothesized to underlie disruptions in synchronous neural firing in the 50 Hz (gamma) range. Gamma oscillations are induced and maintained during working memory delay periods (Tallon-Baudry et al., 1998), and their amplitude increases with working memory load (Howard et al., 2003). Moreover, in schizophrenia, the amplitude of gamma oscillations is reduced during the delay period (Cho et al., 2006).

Importantly for the foregoing, firing in at least certain populations of GABA neurons induces gamma activity (Cobb et al., 1995). One of the affected populations of neurons might be those that contain parvalbumin – i.e., the chandelier cells mentioned earlier. Thus, the disruption of this population that is seen in schizophrenia may be causally related to reduced gamma activity, and thereby, to working memory deficits. These deficits may also be related to abnormalities in glutamatergic function and dopaminergic function (Laruelle et al., 2003; Lewis et al., 2003; Moghaddam and Jackson, 2003), both of which are also well known to be abnormal in schizophrenia. In sum, then, Lewis et al.'s work provides a reasonably worked-out mechanism for the neural basis of working memory deficits in schizophrenia.

3 Dorsal Anterior Cingulate (DACC)

3.1 DACC Neuroanatomical Findings

Even in first episode patients, anterior cingulate volumes are reduced in schizophrenia, possibly more apparent in men (Szeszko et al., 1999, 2000; Yamasue et al., 2002), (see Dwork et al., 2008 for a review). Reduced gray matter deficits have been found in anterior cingulate in populations at high risk for the disorder (Job et al., 2003), as well as in those with child onset schizophrenia (Vidal et al., 2006). These reductions, as well as those in other regions, appear to be associated with poorer performance on emotion attribution tasks (Fujiwara et al., 2007; Yamada et al., 2007). Cortical thickness also appears to be reduced in patients compared with healthy controls (Wang et al., 2007). Moreover, there appear to be reductions in cortical thickness in the dACC in relatives of patients with schizophrenia, as well as a decreased right cingulate volume (Goghari et al., 2007), suggesting decreased neuropil in this region both in patients and in those at risk for the disorder.

4 Genetic Variants and DLPFC

4.1 Catechol-O-methyltransferase (COMT) and Schizophrenia

Catechol-O-methyltransferase (COMT) is an enzyme that breaks down dopamine. A $Val^{158}Met$ substitution on chromosome 22q11 codes for a polymorphism that has been associated with 3–4 fold variation in COMT activity (Lachman et al., 1996). COMT appears to be particularly important in regulating dopamine activity in PFC. The $Val^{158}Met$ polymorphism has a more limited effect on dopamine in the striatum and its relevance in other regions has not been extensively studied (see Tunbridge et al., 2006). The *met* allele is associated with less COMT activity in the blood, less rapid breakdown of dopamine, and hence with higher dopaminergic tone in PFC. Associations have been demonstrated between COMT genotype and performance on PFC-mediated tasks such as the WCST (Egan et al., 2001; Malhotra et al., 2002) and working memory tasks (Goldberg et al., 2003; Ho et al., 2005). In addition, COMT genotype has been related to DLPFC activation during working memory tasks. In general, the low activity *val* allele has

been associated with poorer performance and greater activation and thus appears to confer inefficient PFC processing.

However, for some task domains, performance is superior in patients with the *val* allele. In a computerized Competing Programs task that required alternation between two rules of responding - imitation and reversal - *met* homozygotes were better at acquiring the imitation rule than *val* homozygotes, but were worse at switching from imitation to reversal (Nolan et al., 2004). Val homozygotes had poorer imitation performance and slower reaction times. Nolan et al. suggested that the *met* allele is associated with better cognitive performance on tasks requiring cognitive stability as opposed to cognitive flexibility. In addition, the *met* allele has been associated with aggressive behavior. The low activity *met* allele was associated with history of violence in patients with schizophrenia (Strous et al., 1997, 2003; Lachman et al., 1998; Nolan et al., 2001; Han et al., 2004; Volavka et al., 2004), as well as in nonpsychiatric criminal populations (Kotler et al., 1999), and in animal studies (Gogos et al., 1998). Thus, the cognitive and behavioral effects of COMT genotype cannot be summed up simply as *met*/good and *val*/bad.

4.2 COMT and Electrophysiology

One of the hypothesized actions of dopamine is the modulation of signal-to-noise ratio in the brain. Using an electrophysiological measure of neuronal response variability, Winterer et al. (2004) showed that prefrontal noise predicts poor performance on measures of prefrontal cognition (*n*-back working memory, WCST, CPT) and genetic risk for schizophrenia. They hypothesized that prefrontal noise is partially dependent on cortical dopamine signaling and subsequently showed that it is related to COMT genotype. *Val* homozygosity was associated with the greatest amount of prefrontal noise in patients with schizophrenia, healthy relatives, and unrelated controls (Winterer et al., 2006). This modulation of the signal-to-noise ratio may mediate the observed effects of COMT genotype on cognitive stability.

4.3 COMT and Structure

Relatively few studies have examined the structural brain differences among COMT genotype groups in schizophrenia (Van Haren et al., 2008). Ohnishi et al. (2006) found that across patients and controls, *val/val* individuals had reduced volumes of the left anterior cingulate and right middle temporal gyrus compared with individuals with a *met* allele. Patients with the *val/val* genotype had reduced volumes in the dACC bilaterally, as well as in the left amygdala-uncus, right middle temporal gyrus, and left thalamus compared with patients with a *met* allele. These results suggest that the $val^{158}met$ COMT polymorphism may be associated with morphological abnormalities in schizophrenia. In a sample of patients with schizophrenia and healthy controls, Ho et al. (2005) found differences between COMT genotype groups in frontal gray matter, white matter, and CSF volumes. However, in a sample of subjects at high risk for schizophrenia, *val* homozygotes had reduced dACC volumes compared with subjects with at least one *met* allele (McIntosh et al., 2007). Taylor et al. (2007) found that in healthy controls, *val/val* homozygotes had significantly smaller hippocampal and temporal lobe volumes than *met* carriers.

Although the COMT val158met polymorphism has received considerable attention as a candidate gene for schizophrenia, its role remains controversial. Both positive and negative findings have been reported in case-control studies, but meta-analyses do not support a single-locus association with schizophrenia risk (see Craddock et al., 2006; Williams et al., 2007), despite robust evidence for its functional influences on PFC.

5 Prefrontal White Matter Abnormalities

Diffusion tensor imaging (DTI) is a MRI method that allows examination of white matter integrity by measuring the directional diffusion of water. When there are no structural boundaries, water diffuses in all directions equally. When there are structural barriers, including myelin and cell membranes, diffusion tends

to follow the direction of those boundaries (anisotropic diffusion). Fractional anisotropy (FA) is a measure derived from DTI that quantifies the degree of anisotropic diffusion, with higher values reflecting more anisotropic diffusion, and has been taken as a measure of white matter integrity. FA has been found to be reduced in prefrontal white matter (WM) in schizophrenia among other regions (Buchsbaum et al., 1998; Lim et al., 1999; Agartz et al., 2001), with no concomitant reduction of white matter (WM) volume (Lim et al., 1999). We have found reduced FA in pericingulate WM among other regions (Ardekani et al., 2003), as has Buchsbaum et al. (2006). We have also found that neuropsychological performance is correlated with FA in WM in task-relevant regions in patients with schizophrenia (Lim et al., 2006). Attentional and executive functions, as measured by the digit symbol and digits backward scores from the Wechsler Adult Intelligence Scale-III (Wechsler, 1997), were associated with prefrontal WM FA.

6 Inferior Prefrontal Cortex

6.1 Introduction

Although a great deal of attention has been focused on the role of the DLPFC and the dACC in schizophrenia, abnormalities have also been observed in dorsomedial prefrontal (dMPFC) and OFC. The OFC is involved in multisensory integration of qualities related to taste, smell, and flavor (de Araujo et al., 2003; Rolls, 2006). It also appears to be important for the integration of multisensory information to evaluate competing reward outcomes (Wallis, 2007). Additionally, and possibly relatedly, it plays a role in higher level social functioning including moral reasoning (Anderson et al., 1999; Greene et al., 2001) and risky decision making (Tranel, 1994; Bechara et al., 2000b). Clearly dMPFC and OFC support diverse, and in many cases, complex functions. For this reason, and also because it is a particularly difficult region to evaluate using psychophysiological and neuroimaging methods, its role in schizophrenia remains poorly understood.

6.2 Inferior Frontal Cortex and Social Cognition

Patients with schizophrenia have significant deficits in social cognition (Penn et al., 1997) that predict poor clinical outcome (Vauth et al., 2004; Green, 2006). Deficits in social cognition may also lead to misinterpretations of the intention of others, which could cause conflicts, particularly in patients who have delusions and hallucinations or impulse control problems.

For example, patients with schizophrenia have difficulty processing facial expressions (Hall et al., 2004). Recent evidence suggests that the OFC receives information from the magnocellular visual pathway and that this information modulates processing taking place in the fusiform face area (Bar et al., 2006; Kveraga et al., 2007). In this way, OFC appears to exert a top-down influence on visual recognition processes, possibly extending to facial recognition. Patients also appear to have problems with interpreting emotional prosodic utterances (Leitman et al., 2005, 2007; Hoekert et al., 2007), suggesting that their difficulties in perceiving emotion extends to the auditory domain. Leitman et al's. (2007) finding that FA in OFC is correlated with better prosodic performance is consistent with the idea that this region influences emotional processing.

Nonemotional aspects of social cognition are also disrupted in schizophrenia. Patients also have problems with understanding the implications being made in conversations (Tenyi et al., 2002) and in theory of mind (TOM) processes that are related to inferring the thought processes of others (Brune, 2005; Leitman et al., 2006). A meta-analysis of neuroimaging studies suggests that abnormalities in ventromedial prefrontal cortex, as well as in dMPFC, are associated with TOM deficits both in patients and those at high risk for the illness (Brunet-Gouet and Decety, 2006). Thus, patients with schizophrenia show patterns of behavior similar to those of patients with ventromedial prefrontal, but not DLPFC, lesions on an affectively laden TOM task (Shamay-Tsoory et al., 2007). In addition, healthy relatives of patients with schizophrenia show a reduced activation in PFC in response to visually presented jokes compared with individuals without ill relatives (Marjoram et al., 2006).

It is perhaps relevant that the so-called default mode network (DMN) (Raichle et al., 2001), which reflects activity in the brain at rest, includes medial prefrontal regions, especially because this network is putatively involved in self-referential cognitive processes (Gusnard et al., 2001) that would be necessary to impute the intentions of others. There may be a split in localization between affective and nonaffective social cognition tasks that might map onto a distinction from the developmental literature between hot vs. cold executive processes (Zelazo and Muller, 2002; Castellanos et al., 2006). Hot executive functions are those that have an affective component, whereas cold executive functions lack this aspect. Unlike relatively abstract tasks commonly used to measure executive function, such as the WCST or Stroop tasks, measures of hot executive function involve decisions about emotional or social events or have meaningful consequences for the subject. A meta-analysis of such processes suggested that ventromedial regions, especially, are involved in processing of hot executive functions (Krain et al., 2006).

6.3 Inferior Frontal Cortex and Reversal Learning

Still another cognitive domain in which the OFC plays a critical role is reversal learning (Rolls et al., 1994; O'Doherty et al., 2001). In reversal learning, the subject is initially rewarded for choosing one stimulus over another. However, the reward contingencies are later reversed. OFC lesions impair reversal learning (Fellows and Farah, 2003), and in fMRI studies, better performance on the reversal aspect of this task has been associated with activation in vlPFC and medial prefrontal areas (Cools et al., 2001, 2002, 2007). Patients with schizophrenia show deficits in reversal learning (Elliott et al., 1995; Pantelis et al., 1999; Lee et al., 2007; Waltz and Gold, 2007). These deficits are not attributable to deficient acquisition of the initial reward contingencies, for which patients and controls did not differ (Waltz and Gold, 2007). To highlight the relevance of OFC deficits in schizophrenia, it is perhaps significant that reversal learning is impaired in rats treated with phencyclidine (PCP), an NMDA antagonist, and this performance deficit is alleviated by atypical, but not typical, antipsychotic medications (Idris et al., 2005; Abdul-Monim et al., 2006). As alluded to earlier, NMDA receptor abnormalities are the basis for one of the most compelling models of the neurophysiological basis of schizophrenia (Javitt and Zukin, 1991).

7 Aggression and Violence and the Prefrontal Lobe

7.1 Introduction

Compared with persons without a psychiatric diagnosis, the one year prevalence of violence is five to six times higher in patients with serious mental illness in the community (Swanson et al., 1990). The risk for aggression is increased further in patients with severe mental illness who are hospitalized (Tardiff et al., 1997; Owen et al., 1999; Barlow et al., 2000) and in patients with comorbid schizophrenia and substance abuse (Steadman et al., 1998; Elbogen & Johnson, 2009). Thus, patients with schizophrenia represent a group at elevated risk for violence.

Some of the posited causes of violence, such as poor social cognition, impulsivity, and psychopathy, involve brain regions such as the OFC that function poorly in schizophrenia. Since the time of Phineas Gage (Harlow, 1868; Damasio et al., 1994), dysfunction in the frontal and temporal lobes has been associated with violent behavior (Virkkunen and Linnoila, 1992; Convit et al., 1996; Krakowski, 1997; Scarpa and Raine, 1997) (see Volavka, 2002 for a review). Neuroimaging studies of violent behavior are limited in scope and number, but they similarly implicate prefrontal and subcortical circuitry (Bassarath, 2001; Dolan, 2002). It is distinctly possible that the OFC's role in aggression is a consequence of its roles in other cognitive functions. Indeed, Blair et al. have suggested that psychopathy, an important risk factor for aggression (Hare, 1981, 1999; Hart, 1998; Hemphill et al., 1998), is associated with failures in reversal learning (Blair and Cipolotti, 2000; Mitchell et al., 2003), deficits in TOM processes (Richell et al., 2003), and impaired social cognition (Blair et al., 1997; Stevens et al., 2001).

7.2 COMT and Aggression

As mentioned earlier, COMT genotype has been related to aggressive behavior in patients with schizophrenia. The *met* allele has been associated with history of aggressive and dangerous behavior (Strous et al., 1997; Lachman et al., 1998) and with violent suicide attempts (Nolan et al., 2000). COMT genotype has also been related to variations in limbic and prefrontal reactivity to emotional stimuli in two recent fMRI studies. Smolka et al. (2005) reported an increased *met* allele dose-dependent activation in ventrolateral PFC and the hippocampus in response to unpleasant visual stimuli. Using fMRI in conjunction with an emotional face processing task, Drabant et al. (2006) observed *met* allele dose-related effects on BOLD response in the hippocampus and ventrolateral PFC. Of particular interest, they also reported that *met* homozygotes showed an increased functional connectivity between the amygdala, OFC/vlPFC, and hippocampal formation that was significantly negatively correlated with scores for Novelty Seeking. Although it is attractive to speculate that the COMT *met* allele may confer a predisposition toward inflexible processing of affective information that can, in some contexts, increase the risk for aggression, these two studies examined healthy controls; it remains to be seen how this polymorphism may relate to brain differences in violent patients with schizophrenia.

Additionally, there may be important differences between lateral and medial orbitofrontal function, lateral regions being more involved in inhibiting previously rewarded options, and medial regions being more involved in selecting and updating stimulus–response associations on the basis of their reward value, as well as to visceral aspects of cognition (i.e., gut feelings) (Elliott et al., 2000). It seems likely that studies of OFC function in schizophrenia would benefit from a consideration of this differentiation.

7.3 Serotonin and Aggression

One of the most consistent findings in the impulsive aggression literature is the association between low levels of brain serotonin (5HT) and aggression, dating back to the reports of Asberg et al. (1976) (Asberg, 1976) that reduced 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) correlated with the presence and lethality of suicide attempts in patients with major depression. This finding has been highly replicable (e.g., Brown et al., 1982). Low CSF 5-HIAA also is associated with aggression (Brown et al., 1979, 1982; Linnoila et al., 1983, 1989; Virkkunen et al., 1987a, b; Coccaro et al., 1989; Virkkunen and Linnoila, 1990).

There is evidence from PET studies of reduced $5HT_{1A}$ receptors in aggressive individuals as measured using pharmacological challenges (Coccaro et al., 1990; Cleare and Bond, 2000). Moreover, serotonin transporter availability is reduced in the anterior cingulate in patients with impulsive aggression relative to age- and sex-matched controls (Frankle et al., 2005).

Genetic studies also implicate the serotonin system in aggression. The so-called "L" allele of the tryptophan hydroxylase (TPH) gene has been associated with suicide in offenders (Nielsen et al., 1994, 1998) and with violence in schizophrenia (Nolan et al., 2000). We are aware of no studies examining the relationships between TPH genotypes and brain structural or functional measures. However, the short allele of the serotonin transporter polymorphism genotype has been associated with heightened amygdala responsivity to fearful and angry facial expressions in healthy controls (Hariri et al., 2005).

7.4 MAO-A and Aggression

Monoamine oxidase inhibitor A (MAOA) is an enzyme that breaks down monoamines – dopamine and norepinephrine – in the synaptic cleft. The low activity variant of a polymorphism in the gene for MAOA has been associated with aggression, especially in men. In a recent study, Meyer-Lindenberg et al. (2006) found that the low activity genotype was associated with reduced gray matter density in the anterior cingulate and lateral OFC (this latter finding in men only). Men with this genotype also showed an

increased activation in both the amygdala and the hippocampus, as measured using functional MRI, relative to men with the high activity genotype during an emotional memory test, and showed a reduced activation in anterior cingulate during a response inhibition task. The authors related their findings to the role of MAOA in aggression and impulsivity, although they did not actually examine these behaviors. Thus, a critical study in this area will be to study brain activation and structure in aggressive individuals with the high-versus-low activity genotype.

7.5 Summary of Pharmacogenetics and Aggression

Thus far, the pharamacological and pharmacogenetic studies point to brain chemical systems that may be important in the neural basis of impulsive aggression. However, because the field of pharmacogenetics is new, many gaps exist in the literature. Thus, few studies have directly examined the relationship between genes and their role in brain prefrontal structure and activity in impulsive aggression. This remains an important area for research.

7.6 Imaging and Aggression

7.6.1 PET

Raine et al. (1994, 1997) examined regional cerebral glucose metabolism in 41 accused murderers pleading not guilty by reason of insanity and 41 age- and sex-matched controls using performance on a continuous performance task (CPT). They found that the murderers showed a reduced glucose metabolism in prefrontal and superior parietal cortices, angular gyrus, and corpus callosum, with abnormal metabolic asymmetries (right > left) in the amygdala, thalamus, and medial temporal lobe during the CPT.

In a reanalysis of these data, Raine et al. found that the reduction in frontal activation was much more pronounced in murderers whose crimes had an affective rather than predatory basis (Raine et al., 1998). Affectively motivated crimes are generally considered to have a more impulsive quality than predatory murders that are, by definition, planned. Davidson et al. (2000) have postulated that impulsive violence results from a breakdown in the brain's ability to regulate negative affect. Thus, affective crimes may provide a window into the brain mechanisms underlying impulsive aggression.

The PET and SPECT data are generally consistent with the rest of the literature in showing that frontal and temporal dysfunction are related to violent behavior and psychopathy.

7.6.2 MRI

7.6.2.1 Structural Studies Patients with antisocial personality disorder have been shown to have reduced prefrontal gray matter volumes (Raine et al., 2000). In addition, these individuals exhibit abnormalities in the corpus callosum (Raine et al., 2003), showing an increased callosal length and white matter volume, but a decreased thickness. They also showed evidence of increased functional efficiency of interhemispheric interaction, which was not, however, associated with their anatomical measures. Raine et al. interpreted their results as indicative of either abnormal white matter pruning or increased myelination in the callosum.

In studies of patients with schizophrenia, the results are rather different. Hoptman et al. (2005) found that among chronic treatment-resistant patients, larger left gray and bilateral white matter OFC volumes were associated with higher levels of aggression. They suggested that this relationship, and similar ones with cognitive and psychiatric symptoms, may be related to iatrogenic effects of long term exposure to typical antipsychotics, similar to those seen in caudate (Chakos et al., 1994, 1998; Keshavan et al., 1994). Analogous results, albeit more closely related to motor impulsivity, were obtained by Antonucci et al. (2006) in a nonselected group of psychiatric patients. Recently, Rusch et al. (2008) found that inferior frontal white

matter volumes were higher in patients with schizophrenia who had a suicide attempt than in those who did not, and that current self-aggression in patients was positively correlated with white matter volumes in the same region. It should be noted that aggression in schizophrenia has also been associated with increased putamen and decreased hippocampal volumes compared with healthy controls (Barkataki et al., 2006), with higher putamen volumes in violent than nonviolent patients. Hoptman et al. (2006) found that within patients, larger caudate volumes were associated with increased levels of violence.

7.6.2.2 White Matter Integrity The findings from a variety of neuroimaging modalities suggesting that frontal and temporal lobe dysfunction is associated with impulsive and violent behavior leaves the possibility open that these behaviors may result from an impaired connectivity between these regions. In their literature review, Davidson et al. proposed that violent behavior was related to dysfunction in a circuit involving orbitofrontal cortex, the anterior cingulate, and the amygdala (Davidson et al., 2000). Impaired connectivity among these regions, resulting in poorer communication among them, might be observed as an alteration in white matter integrity.

Consistent with this hypothesis, in a study of 14 men with schizophrenia, our group found significant negative correlations between the Motor Subscale of the Barratt Impulsiveness Scale (Barratt and Stanford, 1995) and right inferior frontal white matter FA, and significant positive correlations between aggressive attitudes and mean diffusivity, perhaps suggestive of reduced barriers to diffusion, in right inferior frontal white matter regions (Hoptman et al., 2002). To our knowledge, these are the first data that show a relationship between integrity of inferior frontal white matter and impulsivity or aggressive attitudes. However, we have also published work showing that increased orbitofrontal volumes are associated with higher levels of aggression (as well as poorer cognitive function) in the same population of treatment-resistant patients. This finding may be attributable to ineffective long-term treatment with typical antipsychotic medication, because these medications can be associated with regional volumetric enlargements at least in the caudate nucleus (Heckers et al., 1991; Keshavan et al., 1994; Chakos et al., 1994, 1995).

8 Conclusions

It is clear that schizophrenia involves dysfunction in a number of cognitive domains and that many of these are associated with abnormalities in DLPFC and dACC. Such deficits include contextual processing, working memory, and certain kinds of response inhibition. These deficits may be related, especially in the case of the DLPFC, to GABA-ergic/glutamatergic dysfunction and possibly to genetic factors. More recently, abnormalities also have been noted in social cognition, TOM, and reversal learning processes that are subserved by OFC and medial PFC. Additionally, recent explorations have begun to uncover the connections between the structure and function of the prefrontal cortex and aggression and violence in schizophrenia. It is clear that a deeper understanding of the specific nature of these deficits is needed, but they nevertheless point to the importance of considering inferior frontal regions in cognitive/behavioral deficits in schizophrenia.

Acknowledgment

MJH gratefully acknowledges the support provided by NIMH grant RO1 MH64783.

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Section 4

Research Methodology

4.1 Magnetic Resonance Spectroscopy

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_4.1,

List of Abbreviations: ACC, anterior cingulate cortex; Amyg, amygdala; ATP, adenosine triphosphate; BG, basal ganglia; Cho, choline-containing compounds; Cr, creatine and phosphocreatine; Ctrl, control; DI, duration of illness; DLPFC, dorsolateral prefrontal cortex; FE, first episode; FL, frontal lobe; fMRI, functional magnetic resonance imaging; GABA, γ-aminobutyric acid; Glu, glutamate; Gln, glutamine; Glx, glutamate, glutamine, and GABA; GM, gray matter; GPCh, glycerophosphatidylcholine; GPEth, glycerophosphatidylethanolamine; ^{1}H-MRS, proton magnetic resonance spectroscopy; Hippo, hippocampus; HR, high-risk; LMTL, left medial temporal lobe; mI, *myo*-inositol; MRI, magnetic resonance imaging; NAA, N-acetylaspartate; NMDA, N-methyl-D-aspartate; NTP, α, β and γ nucleoside triphosphates; PCP, phencyclidine; PCr, phosphocreatine; PDE, phosphodiesters; PFC, prefrontal cortex; Pi, inorganic phosphorus; PME, phosphomonesters; ^{31}P MRS, ^{31}phosphorus magnetic resonance spectroscopy; Pt, patient; PtdCh, phosphatidylcholine; PtdEth, phosphatidylethanolamine; RF, radiofrequency; SNR, signal-to-noise ratio; STG, superior temporal gyrus; SZ, schizophrenia; TE, spin-Echo time; TL, temporal lobe; VOI, volume (voxel) of interest; WM, white matter

1 Principles of Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy is the study of the magnetic properties and energies of atomic nuclei. The NMR phenomenon of absorption and emission by nuclei of electromagnetic energy when placed in an external magnetic field was discovered independently in 1946 by Bloch and Purcell (Bloch et al., 1946; Purcell et al., 1946). The application of NMR as a spectroscopy tool was made possible by the discovery of chemical shift effects where nuclei absorb and emit energy at different resonance frequencies according to their chemical environment (Proctor and Yu, 1950), (Dickinson, 1950). The introduction of pulsed NMR and Fourier transform in 1966 (Ernst and Anderson, 1966) enabled the modern NMR experiments. In vivo NMR (MRS) and MRI became possible after the introduction in 1973 of magnetic field gradients in addition to the static magnetic field (Lauterbur, 1973: Mansfield and Grannell, 1973). The magnetic field gradients create position-dependent magnetic fields that allow mapping the resonances from nuclei in molecules of interest to a specific anatomical location and creating an image. For a detailed and readable introduction to the field of in vivo NMR/MRS, the reader is referred to the excellent textbook by Robin de Graaf (de Graaf, 2008); the texts by Slichter (Slichter, 1996) and Ernst (Ernst et al., 1990) provide descriptions of the NMR phenomena in more rigorous quantum mechanics terms. NMR can only be applied to nuclei that have a net spin, such as ^{1}H, ^{13}C, ^{31}P, ^{19}F, and ^{7}Li. Each of these nuclei is characterized by an intrinsic gyromagnetic ratio (γ), which combined with the natural abundance of the nucleus gives the relative NMR sensitivity (set to one for the proton). Each nucleus with a spin resonates at a specific NMR frequency (Larmor frequency) that is proportional to its gyromagnetic ratio and the strength of the external magnetic field (B_0). For the proton at 1.5 T, the NMR resonance frequency is 63.9 MHz, and 127.7 MHz at 3T. A radiofrequency (RF) excitation pulse induces in a nucleus an electromotive force (signal) that is detected by the receiving coil; the change of the electromotive force over time is called free induction decay (FID). This FID time-domain data are converted to frequency data (a spectrum) by a Fourier transformation (Bracewell, 1965). Nuclei in nature exist in certain chemical environments: they are surrounded by electrons and form molecules; electrons thus reduce (shield) the magnetic field that is sensed by a nucleus and this causes the chemical shift phenomenon. The Larmor frequency (ν) is related to the gyromagnetic ratio (γ), the external magnetic field (B_0), and a shielding or screening constant (σ):

$$\nu[\mathrm{Hz}] = (\gamma/2\pi)B_0(1-\sigma)$$

Chemical shifts are typically not expressed in Hz, but as "ppm" (parts per million) in comparison with a reference compound. Tetramethylsilane is used as a reference compound in ^{1}H MRS and its chemical shift is set to zero.

$$\delta[\text{chemical shift}] = (\nu - \nu_{\mathrm{ref}})/\nu_{\mathrm{ref}} \times 10^{6}$$

The signal from nuclear magnetic resonance in metabolites of interest is also affected by alterations in the spin–lattice (T_1) longitudinal relaxation time constant of the metabolite. Typically, T_1 relaxation times of the ^{1}H metabolites NAA, Cr, and TMAs at 1.5 T are approximately between 1.0 and 1.5 s which differ depending on brain region, tissue type, and field strength. To fully recover the MR signal, the pulse repetition time (TR) must be at least five times T_1. If this condition is not met, then the observed MR signal will have a signal amplitude less than that of the fully relaxed MR signal; this is referred to as partial saturation. The rotational mobility of the observed metabolites is characterized by a correlation time, and is inversely proportional to the spin–spin transverse relaxation time constant (T_2). The T_2 is an exponential time constant that characterizes the decay of the MR signal or free-induction decay (FID), including the decay of a spin– echo signal. This implies that freely mobile molecules with a short correlation time will have a longer observed FID signal (longer T_2), whereas less mobile and/or larger molecules will have a much shorter observed FID signal (shorter T_2). If the echo time (TE) is short, as in ^{1}H MRS of glutamate and GABA, the resulting spectrum will not only include narrow line shapes, but also relatively broader line shapes underlying the narrower peaks due to macromolecules (Behar et al., 1994), and these should be taken into account in the analysis of short TE ^{1}H MRS metabolites (Bartha et al., 1999). Both the T_1 and the T_2 relaxation times can differ between study groups and they have been found to be prolonged in schizophrenia (Andreasen et al., 1991; Williamson et al., 1992; Pfefferbaum et al., 1999b) in both gray and white matter, and this would affect signal amplitudes. Signal-to-noise (SNR; sensitivity) and metabolite peak separation (spectral resolution) in MRS increase linearly with increased B_0 and can be improved by an increase of the external magnetic field, but an equally important SNR and separation factor is the ability to achieve maximal magnetic field homogeneity by "shimming," since local magnetic field inhomogeneities widen and distort the spectral lines from their ideal forms (Drost et al., 2002).

The postprocessing and quantification steps of MRS include the removal of unwanted water and/or lipid signal, baseline correction (particularly for short TE spectra), fitting the spectrum with the appropriate modeling functions to deconvolve overlapping peaks, correcting for relaxation and saturation, curve fitting, and calculation of concentrations. Discrepancies in reported metabolite values between investigators are often due to differences in postprocessing (Drost et al., 2002) and an emphasis has been placed on the automation and improvement of these steps to achieve consistency between studies (Stanley et al., 2000). Recommendations are to use processing methods that incorporate a priori knowledge (Provencher, 1993; Bartha et al., 1999) of the resonances of metabolites, particularly at short echo ^{1}H MRS and to combine MRS with tissue segmentation in the VOI and to correct for the voxel gray matter/white matter/CSF composition. Additionally, it is recommended to obtain and report absolute metabolite concentration levels instead of expressing results as metabolite ratios, although it is not clear which of the proposed methods (unsuppressed water signal as an internal standard, external reference standard, phantom replacement method) provides the most accuracy and reliability (Stanley et al., 2000; Jansen et al., 2006).

MR spectra are of value if they can be spatially localized to anatomical locations in the brain. The volume of interest (VOI) localization techniques are based on the combination of frequency-selective RF pulses and static field gradients, and selection is achieved by applying slice selective pulse clusters to prepare spins along each of the three orthogonal axes in turn. The VOI is formed at the intersection of the prepared slices; this results in VOIs that are cuboidal in shape and it is not possible for the VOI to follow the complex geometry of brain regions of interest, which indicates that even in the minimal achievable voxel volumes (1.5 cm^3 for ^{1}H MRS at 4 T (Theberge et al., 2004)), there will be partial volume effects. The advantage of the VOI approach is that it enables a greater homogeneity in the magnetic field. This is important at short TEs, and in turn, leads to an improved water suppression and spectral resolution. MR spectroscopic imaging (MRSI), or chemical shift imaging (CSI), uses slice selection and spatial encoding techniques drawn from MRI to collect spectra simultaneously from a multidimensional array of voxels. Simultaneous spatial and spectral resolution allows multivoxel MRS and creation of metabolite maps, which is important in the study of schizophrenia where localized hypotheses based on just several VOIs may not represent the complexity of brain changes, and a whole-brain approach may be more informative. MRSI creates challenges such as considerable magnetic field inhomogeneity, spectral degradation due to intervoxel contamination, long data acquisition time periods, processing and analysis of the large multidimensional

datasets (de Graaf, 2008). MRSI requires long TE to simplify the spectra and reduce the lipid and macromolecule signal, but this requirement makes it impossible to use MRSI for important short-TE metabolites, such as Glu and Gln (Drost et al., 2002).

2 Proton (^{1}H) Magnetic Resonance Spectroscopy

The proton nucleus is the most sensitive nucleus for MRS with the highest intrinsic MRS sensitivity (gyromagnetic ratio) and a natural abundance of >99.9%, and it is present in nearly all metabolites of biological interest. *Figure 4.1-1a* illustrates a typical ^{1}H MRS at a field strength of 1.5 T acquired at long spin– echo time (TE = 135 ms at 1.5 T). The most prominent resonance in brain tissue is from the methyl group of N-acetylaspartate (NAA) at 2.01 ppm. The other singlet resonance is from phosphocreatine and creatine (PCr + Cr) at 3.02 PPM; PCr + Cr is also called "total creatine" or just Cr. Trimethylamines or "choline-containing compounds" (TMA or tCho) produce another single resonance that consists of free choline, glycerophosphorylcholine (GPC), and phosphorylcholine (PC). Metabolites that have protons interacting with other protons through the chemical bonds in a molecule (J-coupling) form multiple peaks that can only be resolved with short echo times (*Figure 4.1-1b*, TE = 20 ms at 1.5 T). These metabolites include glutamate, glutamine, GABA, *myo*-inositol, *scyllo*-inositol, aspartate, NAAG, taurine, and glucose (de Graaf, 2008). MRS at ultra-high magnetic fields improves the signal-to-noise ratio (SNR) and spectral (peak) separation (Ugurbil et al., 2003), as shown in *Figure 4.1-1c* (7 T, TE = 6 ms).

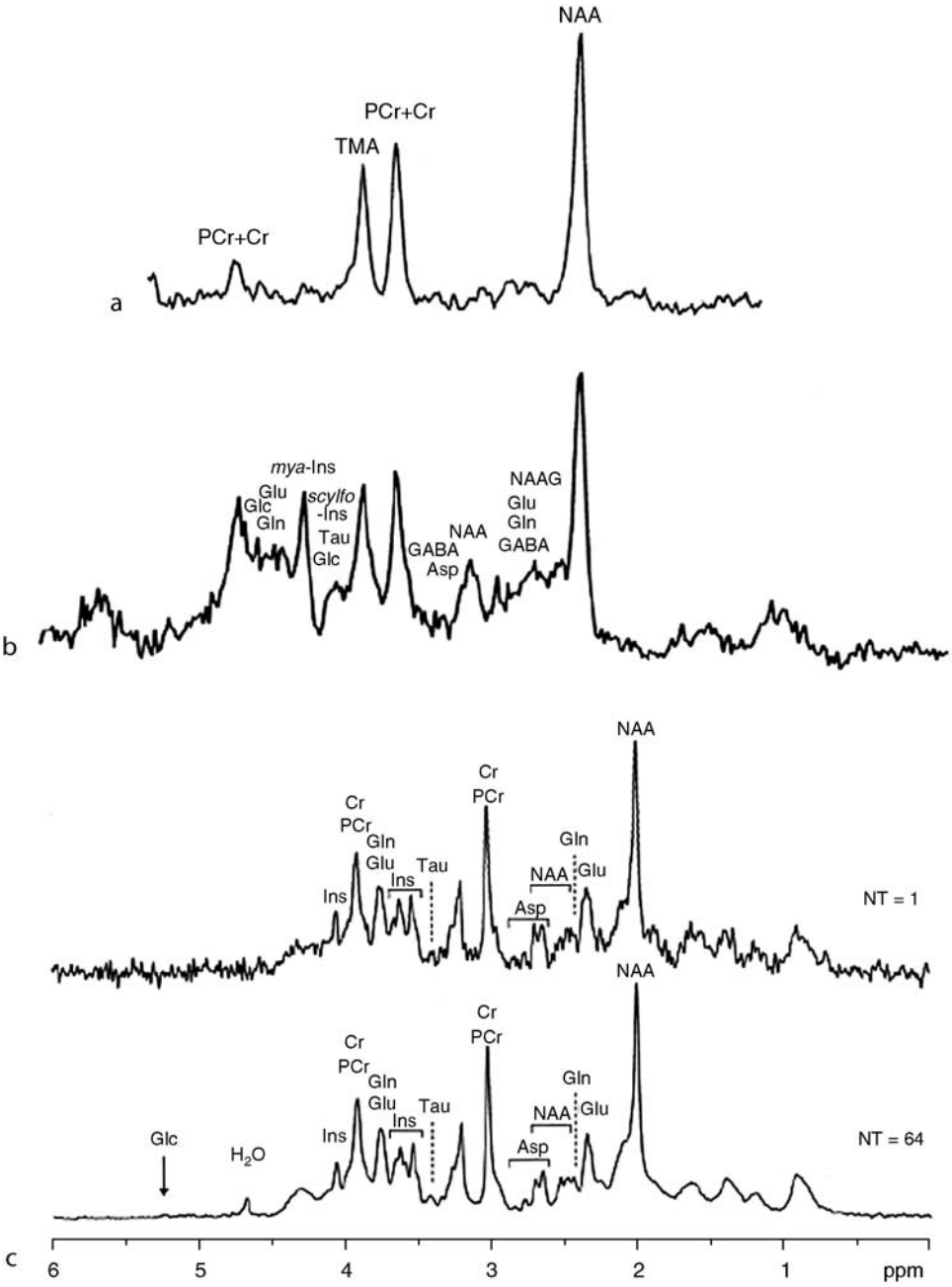
The focus of this chapter is on NAA, glutamate, glutamine, and GABA, but other ^{1}H MRS metabolites have also been shown to be involved in SZ. The Cho peak is an indicator of cell density and may reflect alterations in phospholipid membrane formation (Bustillo et al., 2002). Several studies have shown increased Cho signals in the basal ganglia of medicated (Fujimoto et al., 1996; Shioiri et al., 1996) and antipsychotic-naïve (Bustillo et al., 2002) SZ patients, suggesting changes in phospholipid membrane metabolism. Additionally, increased Cho and Cho/Cr have been found in the anterior cingulate (Yamasue et al., 2002; O'Neill et al., 2004), and frontal lobes (Block et al., 2000; O'Neill et al., 2004) of patients with SZ. However, choline compounds have been found to be decreased in the thalamus of chronically medicated patients (Ende et al., 2001, 2003) and the authors interpreted this as decreased gliosis and synthetic activity in this region of the brain. Creatine and phosphocreatine play a role in maintaining energy stores in the brain (Malhi et al., 2002) and the Cr peak, which consists of signals from both Cr and PCr, can be used as a marker for changes in neuronal energy metabolism. O'Neill and colleagues reported an increase of the Cr signal in the anterior cingulate of patients, suggesting abnormalities in neuronal energy demand in this brain region (O'Neill et al., 2004). In contrast, reductions in Cr have been found in the DLPFC of first-episode and chronic patients, as well as ultrahigh risk subjects (Wood et al., 2003), suggesting a hypometabolic state both before the onset of psychosis and during illness progression (Ohrmann et al., 2007). Myo-Inositol (mI) is thought to maintain cell osmolality and is considered to be a glial cell marker (Malhi et al., 2002). Szulc and coworkers found an increase in mI and NAA in the thalamus of schizophrenic patients after treatment with the second-generation antipsychotic risperidone (Szulc et al., 2005), although there was no comparison with placebo or a first generation antipsychotic. Myo-Inositol and Cho were elevated and NAA was decreased in the left thalamus of SZ patients and this was interpreted to agree with histologic and imaging findings of reduced neuronal density and volume in this region (Auer et al., 2001).

2.1 Biology of N-acetylaspartate (NAA) and Magnetic Resonance Spectroscopy of NAA

N-acetylaspartate (NAA) is an "enigmatic molecule" (Moffett et al., 2007) present at exceptionally high (>10 mM) concentrations in the brain, with controversial biological roles that also produces a strong signal in ^{1}H MRS. Please refer to a recent comprehensive review by Moffett, Ross, and colleagues regarding the neurobiology and MRS literature on NAA (Moffett et al., 2007). One important comment by these experts is that "unfortunately, spectroscopic studies have dramatically outnumbered studies into the basic

Figure 4.1-1

Single-voxel in vivo ^{1}H spectra of human brain tissue acquired with the STEAM sequence with different echo times and different field strength. (a) and (b) field strength 1.5 T, VOI in the left prefrontal region (8 cm^3). (a) TE = 135 ms, and (b) TE = 20 ms. TMA, trimethylamines, same as Cho, choline-containing compounds (From Stanley et al., 2000, with permission). (c) field strength 7 T, VOI over the occipital lobe, largely over gray matter (8 cm^3), TE = 6 ms, for single shot (NT = 1) and for 64 averages (NT = 64) (from Ugurbil et al., 2003 with permission)



biochemistry of NAA in the brain, and this disparity has complicated the interpretation of MRS results in various disease states due to the lack of basic knowledge on NAA function and metabolism" (Moffett et al., 2007). NAA is synthesized in neurons and its immunoreactivity is present predominantly in neurons and their processes, with higher concentrations in gray matter than in white matter; oligodendrocytes have low, but demonstrable, NAA levels (Moffett et al., 1995, 2006). The major hypotheses for the function of NAA in the brain have relevance to models of schizophrenia. NAA is an immediate precursor for the enzyme-mediated biosynthesis of the important neuronal dipeptide N-acetylaspartylglutamate (NAAG) and NAA availability may limit the rate of NAAG synthesis. NAAG is liberated by neurons after membrane depolarization and acts as a highly selective agonist of the presynaptic autoreceptor type 3 metabotropic glutamate receptor (mGluR3); at low levels, NAAG is an NMDA receptor antagonist. Taken together, low central NAAG levels may antagonize the effect of glutamate at NMDA receptors and may decrease its agonistic effect on presynaptic mGluR3; both activities could increase glutamate release, similar to the increase demonstrated in the PCP model of schizophrenia (Tsai, 2005). It has also been proposed that NAA and NAAG, and their intermediates, including acetate, aspartate, and glutamate, are cycled between neurons and glia as a mechanism of intercellular signaling (Baslow and Guilfoyle, 2006) and that the NAA-NAAG system is involved in the bioenergetics of neuronal activity and cognition, and in neuronal – astrocyte – vascular interactions that match blood flow to neuronal activity (Baslow and Guilfoyle, 2007). NAA provides a critical source of acetate for myelin lipid synthesis in oligodendrocytes. NAA is catabolized in oligodendrocytes to acetate and aspartate by the enzyme aspartoacylase (ASPA) and ASPA expression is increased in early development (Kirmani et al., 2003) in parallel with myelination; it is thought that this increase becomes critical during times of peak postnatal myelin synthesis in order to liberate the acetate from NAA for the synthesis of acetyl CoA, which is necessary for the synthesis of certain myelin-associated lipids. Deficiency in NAA-derived acetate for myelin lipid synthesis could thus explain the findings in the rare but fatal genetic disorder known as Canavan disease where a mutation in the gene for ASPA results in an inability to catabolize NAA, leading to an increased level of NAA in the brain (Matalon and Michals-Matalon, 1998) and may explain the progressive, fatal leukodystrophy in affected infants. There is now an increased research interest in white matter and myelin pathology of schizophrenia (Lim, 2007) and a recent study (Tang et al., 2007) that simultaneously employed ^{1}H MRS and diffusion tensor imaging (DTI) found that NAA was significantly reduced in the SZ patient population in the medial temporal regions and DTI anisotropy indices were also reduced in the same regions. These decreases were correlated, suggesting an in vivo relationship between NAA decrease and decreased coherence and myelination of axonal bundles in schizophrenia.

In the early years of MRS, reduced levels of NAA were interpreted to represent irreversible loss of neurons, but later studies showed that decreases in regional NAA levels can also represent reversible neuronal or mitochondrial dysfunction (Clark, 1998, Demougeot et al., 2004). In humans, NAA is decreased in brain ischemia, brain injury, brain cancer, multiple sclerosis, epilepsy, alcohol dependence, and Alzheimer disease (reviewed by (Moffett et al., 2007)). People with multiple sclerosis recovered their NAA levels during remission or in response to interferon beta treatment (Narayanan et al., 2001); patients with ALS recovered their NAA levels in corticomotor neurons after riluzole therapy (Kalra et al., 1998); NAA is also reduced in alcohol dependence and may recover after prolonged abstinence (Parks et al., 2002). The decrease of NAA in the brain appears to be a dynamic reversible process that depends on neuronal "health and well-being." In this sense, NAA is currently thought to be a marker of neuronal "viability and integrity," and it is potentially misleading to describe NAA as a structural marker. Regional decreases in NAA may be due to many factors that have been proposed in schizophrenia pathogenesis, including neuronal death, decreased neuronal volume, decreased neuropil, and myelin abnormality; a chronic hypoxic state (Prabakaran et al., 2004); and a downstream response to neurotransmitters, for example, the finding that in people with SZ lower NAA in the DLPFC was related to greater amphetamine-induced release of dopamine (Bertolino et al., 2000a).

The NAA peak at 2.02 ppm (measured at long TE) contains contributions from other compounds, most notably NAAG, that constitute 10–15% of the signal ascribed to NAA, and hence, the reductions in NAA reported in studies may be due to a parallel decrease of NAA and NAAG, or of either compound alone (Moffett et al., 2007). NAAG may be the biologically more important metabolite to measure with ^{1}H MRS,

but its determination is more difficult (short TE, higher field strength); in a postmortem HPLC study, both NAAG and NAA were found to be decreased in the temporal lobe in SZ (Nudmamud et al., 2003). No in vivo MRS studies on both NAAG and NAA in SZ have been published.

2.2 *N*-acetylaspartate (NAA) in Schizophrenia

NAA is abundant in the brain and its biology is relevant to schizophrenia research. Consequently, there is a considerable body of research on NAA in SZ, with a number of reviews on the topic (Kegeles et al., 1998; Bertolino et al., 1999; Stanley et al., 2000; Keshavan et al., 2000; Lyoo and Renshaw, 2002; Abbott and Bustillo, 2006). In spite of the extensive work, there are still controversies in the literature. Steen and colleagues conducted the first meta-analysis of the SZ NAA literature, which included 64 studies that met their criteria (1,256 patients and 1,209 healthy controls) (Steen et al., 2005). The authors concluded that there is consistent evidence that NAA is reduced in a broad range of tissues in the SZ brain, including reductions of greater than $\geq$5% in the hippocampus and in both the cortical gray matter (GM) and the white matter (WM) of the frontal lobe. There is no evidence to support a hypothesis that NAA levels are reduced to a different degree in frontal lobe GM and WM nor is there robust evidence of a difference in NAA levels between patients with first-episode and chronic SZ. Of note, most studies were significantly underpowered (only 19 subjects per group), and Steen and colleagues recommended sample sizes in the order of 39 participants per cell, at least until spectral and spatial sensitivities improved. Only three of the 64 studies included enough subjects to have 80% power to detect a 10% NAA reduction in patients, and no studies were adequately powered to detect a 5% NAA reduction with 80% power. Importantly, most studies (77%) focused on patients with chronic SZ. There were only 14 studies with first-episode or childhood-onset subjects (13% of all SZ subjects), and the authors conclude that (by June 2004) "relatively little is known about whether the brain of a typical newly diagnosed patient is different from normal" with regard to NAA level.

We examine studies of NAA in SZ published since June 2004 in the context of the findings and recommendations of the meta-analysis (See *Table 4.1-1* for a list of NAA studies). The uncertainty about the decrease of NAA in the course of illness was addressed in several cross-sectional studies. There was no difference in NAA level in the DLPFC between 21 healthy controls and 18 first-episode patients, but there was a significant reduction of NAA in the 21 chronic (Duration of Illness (DI) = 6.9 years) patients compared with healthy controls and with first-episode patients without correlation with DI (Ohrmann et al., 2005). Molina and colleagues found the same differences in the NAA/Cr ratios when they compared 16 recent-onset patients (DI = 1.8 $\pm$ 0.6 years), 19 chronic patients (DI = 9.7 $\pm$ 6.1 years), and 20 healthy controls, but the longer DI was related to lower NAA/Cr (Molina et al., 2005). The advantages of a FE longitudinal design are clear, but only two studies of this type have been reported. Bustillo and coworkers (Bustillo et al., 2007) studied 32 minimally treated schizophrenia patients (mean age 24.7 years) and 21 matched healthy subjects with single-voxel proton magnetic resonance spectroscopy (^{1}H-MRS) of the frontal and occipital lobes, the caudate nucleus, and the cerebellum. The patients were blindly randomized 1:1 to haloperidol or quetiapine and the average duration of follow-up was 9 months; field strength = 1.5 T. At baseline, prior to treatment, NAA was lower in schizophrenia subjects than that in controls, but only when the four regions were combined in the analysis. The longitudinal analyses found no effects of time of follow-up and no group effects on NAA concentrations; the NAA levels in the 4 VOI were not significantly different, and the "global" NAA level in the patient group continued to be slightly lower. The other longitudinal study by Theberge and colleagues (also discussed in section 3.2 on Glu) examined 2 VOI in the left anterior cingulate and the left thalamus in 16 FE patients (mean age = 25 years) 16 healthy participants (mean age 29 years); field strength = 4 T (Theberge et al., 2007). At baseline the healthy participants (slightly older) actually had a slightly lower level of NAA than the patients in the cingulate VOI; at 30 months of follow-up there were no between-group NAA differences, and no significant changes in the cingulate or the thalamic NAA level between baseline at follow-up scans. At baseline patients and controls did not differ in gray matter volume determined by voxel-based morphometry, but, by the 30-month follow-up MRI, the patients had a significant gray matter loss over controls. The two longitudinal studies

Table 4.1-1
^{1}H-MRS studies of N-acetyl aspartate in schizophrenia

Author, Year	Sample size	Methods	Summary of findings
Auer et al. (2001)	Pt = 20M,12F Ctrl = 9M,8F	1.5 T, VOI	↓ NAA in thalamus and ↑ Cho and mI in parietal WM in patients; ↑ Cr in WM of patients positively correlated with Brief Psychiatric Rating Scale
Aydin et al. (2007)	FE = 8M,4F Pt = 11M,5F Ctrl = 18M,10F	1.5 T, VOI	↓ NAA in corpus callosum of chronic and FE patients; patient NAA concentrations negatively correlated with Brief Psychiatric Rating Scale, SANS and SAPS
Bartha et al. (1999)	FE = 9M,2F Ctrl = 9M,2F Antipsychotic-naïve	1.5 T, VOI	Found no difference between first-episode patients and controls in NAA, GM or WM in left medial TL
Bertolino et al. (1998)	Pt = 9M,3F Ctrl = 9M,3F	1.5 T, MRSI	↓ NAA/Cr and NAA/Cho in hippocampus and DLPFC of patients; no significant differences in the thalamus, putamen, occipital cortex, ACC, posterior cingulate, frontal WM or STG
Bertolino et al. (2001)	Pt = 18M,3F	1.5 T, MRSI	↑ NAA levels in DLPFC of patients while on antipsychotics; no treatment effects in thalamus, putamen, occipital cortex, ACC, posterior cingulate, frontal WM or STG
Bertolino et al. (2000)	Pt = 11M,2F Ctrl = 8M,5F	1.5 T, MRSI	NAA levels in DLPFC of patients correlated with activation of DLPFC, TL and inferior parietal cortex during Wisconsin Card Sort Test; no correlation in the ACC and STG
Bertolino et al. (1998)	Pt = 11M,3F Ctrl = 11M,3F Childhood-onset	1.5 T, MRSI	↓ NAA/Cre in Hippo region and DLPFC of patients; no differences in the thalamus, putamen, occipital cortex, ACC, posterior cingulate, prefrontal WM or STG
Bertolino et al. (1996)	Pt = 8M,2F Ctrl = 8M,2F	1.5 T, MRSI	↓ NAA/Cre and NAA/CHO in Hippo region and DLPFC of patients, no differences found in the thalamus, putamen, occipital cortex, ACC, posterior cingulate, prefrontal WM or STG
Block et al. (2000)	Pt = 18M,7F Mixed Diag = 6M,7F Fam = 19M,16F Ctrl = 7M, 12F	1.5T, VOI	↓ NAA/CHO ratio in left FL of patients as compared with unaffected family members and controls; no metabolite differences found in BG
Braus et al. (2001)	Pt = 11M,12F	1.5 T, MRSI	Significant correlation between duration of atypical meds and NAA signal in ACC in patients on atypical antipsychotics; typical neuroleptic users showed progressive decrease in NAA levels in ACC
Braus et al. (2002)	Pt = 9M,12F	1.5 T, MRSI	↑ NAA in ACC and fewer errors on Wisconsin Card Sort Task in patients treated with atypical meds compared with patients treated with typical antipsychotics
Brooks et al. (1998)	Pt = 9M,7F Ctrl = 6M,6F Children	1.5 T, VOI	↓ NAA/Cr in FL of children with schizophrenia spectrum disorders; no medication effects or other metabolite differences found
Bustillo et al. (2001)	Pt = 25M,13F Ctrl = 11M,10F	1.5 T, VOI	↓ NAA in left FL of haloperidol-treated patients, clozapine-treated patients showed intermediate levels compared with controls and haloperidol group

Table 4.1-1 (continued)

Author, Year	Sample size	Methods	Summary of findings
Bustillo et al. (2007)	Pt = 26M,6F Ctrl = 18M,3F Minimally treated	1.5 T, VOI	Global ↓ NAA levels in patients prior to beginning antipsychotic medication; no changes in NAA in the FL, occipital lobe, caudate nucleus or cerebellum after beginning treatment
Bustillo et al. (2002)	Pt = 8M,3F Ctrl = 10M,1F Antipsychotic-naive	1.5 T, VOI	↑ Cho in caudate nucleus of patients, no differences in other NAA or Cr in caudate nucleus
Callicott et al. (2000)	Pt = 30M,6F Ctrl = 45M,28F	1.5 T, MRSI	Patients showed a negative correlation between NAA/Cr ratio in FL and negative symptom ratings
Callicott et al. (1998)	Pt = 43M,47F Family = 25M,60F Ctrl = 42M,66F	1.5 T, MRSI	↓ NAA/Cr in Hippo area of both patients and their unaffected siblings as compared with healthy controls suggesting heritability of trait
Cecil et al. (1999)	Pt = 12M,6F Ctrl = 15M,9F	1.5 T, VOI	↓ NAA/Cr in TL and FL of patients; Cho/Cr ↑ in FL, ↓ in TL of patients
Chang et al. (2007)	Pt = 23 Ctrl = 22	4.0 T, VOI	↓ NAA, mI, ↑ Glu,Gln in FL, TL and occipital lobe of elderly patients with schizophrenia
Deicken et al. (2001)	Pt = 20M Ctrl = 15M	1.5 T, MRSI	↓ NAA and Cr in anterior cerebellar vermis of schizophrenic patients; no relation between NAA levels and DI or medication dose in patients
Deicken et al. (2000)	Pt = 17M Ctrl = 10M	1.5 T, MRSI	↓ NAA in left and right thalami of patients; no differences in Cho and Cr concentrations
Deicken et al. (1999)	Pt = 24M,6F Ctrl = 12M,6F	1.5 T, MRSI	↓ NAA in Hippo of patients; no correlation between Hippo volume and NAA levels in patients or controls
Deicken et al. (1997)	Pt = 21M,3F Ctrl = 11M,4F	1.5 T, MRSI	↓ NAA in left FL of patients; no association between NAA and medication dose or DI; no differences in Cho and Cr levels
Deicken et al. (1998)	Pt = 24M,6F Ctrl = 12M,6F	1.5T, MRSI	↓ NAA in left and right Hippo of patients; trend toward ↑ Cr in left Hippo as compared with the right in patients
Deicken et al. (1997)	Pt = 22M,4F Ctrl = 12M,4F	1.5 T, MRSI	↓ NAA in left and right ACC of schizophrenic patients; no correlation between NAA and medication dose or DI
Delamillieure et al. (2002)	Pt = 14M,3F Ctrl = 11M,3F	1.5T, VOI	No difference in the metabolites (NAA, mI, Cho) in thalamus, PFC or Hippo between groups
Eluri et al. (1998)	Pt = 5M,7F Ctrl = 5M,3F	1.5 T, VOI	↓ NAA/Cr ratio in the pons of patients; found no difference in cerebellum
Ende et al. (2001)	Pt = 10M,5F Ctrl = 8M,7F	1.5 T, MRSI	↓ NAA and Cho in mediodorsal region of thalamus of patients; NAA correlated in left and right thalamus
Ende et al. (2003)	Pt = 8M,5F Ctrl = 8M,5F	1.5 T, MRSI	↓ NAA in Hippo and thalamus of patients; ↓ Cho in thalamus of patients; no differences between groups in putamen
Ende et al. (2000)	Pt = 9M,10F Ctrl = 11M,5F	1.5 T, MRSI	↓ NAA in ACC of patients; patients on typical psychotics show lower NAA signal than patients on atypical medications; Age-corrected NAA correlates significantly with duration of illness
Ende et al. (2005)	Pt = 12M,2F Ctrl = 8M,6F	1.5 T, MRSI	↓ NAA in cerebellar cortex and vermis of patients
Fannon et al. (2003)	Pt = 26M,11F Ctrl = 17M,8F	1.5 T, VOI	↓ NAA/Cr in Hippo of antipsychotic naïve patients at baseline; no between group differences in Hippo, PFC or BG at follow-up

continued

Table 4.1-1 (continued)

Author, Year	Sample size	Methods	Summary of findings
Fujimoto et al. (1996)	Pt = 14M Ctrl = 10M,2F	2.0 T, VOI	↓ NAA/Cho ratio bilaterally in the BG of patients; ↑ Cho in left BG of patients
Fukuzako (2000)	Pt = 34M,30F Ctrl = 25M,26F	2.0 T, VOI	↓ NAA/Cr ratio in left medial TL of patients; subjects with family history of schizophrenia showed greater deficit than subjects without history
Fukuzako et al. (1999)	Pt = 20M,20F Ctrl = 20M,20F	2.0 T, VOI	↓ NAA/Cr ratio in left medial TL of patients; ↓ NAA/Cr and Cho/Cr ratios in patients with disorganized and undifferentiated types of schizophrenia than with paranoid schizophrenia
Fukuzako et al. (1995)	Pt = 8M,22F Ctrl = 8M,22F	2.0 T, VOI	↓ NAA/Cr and NAA/Cho in FL and TL of patients; ↑ Cho/Cr in left TL of patients
Gimenez et al. (2003)	FE = 11M Ctrl = 11M Antipsychotic-naïve	1.5 T, VOI	Negative correlation between NAA/Cho ratio in BG and procedural learning in antipsychotic-naïve patients
Hagino et al. (2002)	Pt = 11M,2F Ctrl = 11M,2F	1.5 T, VOI	↓ NAA/Cho in left inferior FL of patients; verbal memory deficits correlated with NAA/PCr ratio
Heimberg et al (1998)	Pt = 24M Ctrl = 39M	1.5 T, VOI	↓ Inositol/Cr in left TL of patients; ↓ NAA/Cr in left thalamus of patients; ↑ NAA in left FL and ↓ Cho in left BG of patients on atypical medications
Jakary et al. (2005)	Pt = 22M Ctrl = 22M	1.5 T, MRSI	↓ NAA in thalamus of patients; left thalamic NAA level negatively correlated with illness duration; no other metabolite differences found
Jessen et al. (2006)	Pt = 19M,2F Ctrl = 17M,14F HR = 9M,10F	1.5 T, VOI	↓ NAA/Cr and NAA/Cho in left FL and NAA/CR in ACC in patients and at-risk subjects; ↓ NAA/Cho, ↑Cho/Cr in ACC of converters to schizophrenia then in non-converters; no differences in superior TL
Keshavan et al. (1997)	HR = 6M,5F Ctrl = 7M,5F	1.5 T, VOI	Trend toward ↓ NAA/Cho in ACC of offspring of persons with schizophrenia
Lim et al. (1998)	Pt = 10M Ctrl = 9M	1.5 T, MRSI	↓ GM volume but not NAA in patients in PFC, TL, parietal or occipital lobes; ↓ WM NAA but not volume in same regions of patients
Maier et al. (1996)	Pt = 20M,6F Ctrl = 22M,16F	1.5 T, VOI	Found age related Cho changes in Hippo of patients but not controls; no difference in NAA concentrations in Hippo
Miyaoka et al. (2005)	Pt-GS = 7M,8F Pt = 8M,7F Ctrl = 8M,7F Gilbert's syndrome	1.5 T, VOI	↓ mI/Cr in Hippo, cerebellar vermis and BG of patients with Gilbert's syndrome (GS); ↓ NAA/Cr in BG and Hippo of patients with and without GS compared with controls
Molina et al. (2005)	FE = 10M,6F Pt = 13M,6F Ctrl = 11M,9F	1.5 T, VOI	↓ NAA/Cr ratio in left DLPFC of chronic patients; inverse relation between left side NAA/Cr and disease duration; no difference found in right DLPFC
Nasrallah et al. (1994)	Pt = 7M,4F Ctrl = 7M,4F	1.5 T, VOI	↓ NAA in right Hippo/Amyg region of patients; differences in left Hippo/Amyg not significant
Ohara et al. (2000)	Pt = 9M,1F Ctrl = 9M,1F	1.5 T, VOI	No difference in NAA/Cr, NAA/Cho, Cho/Cr ratios between groups in the lenticular nuclei
Ohrmann et al. (2007)	FE = 10M,5F Pt = 14M,6F Ctrl = 13M,7F	1.5 T, VOI	↓ NAA, Glu, Gln, Cho in DLPFC of chronic patients; ↓ Cr in DLPFC of chronic and first-episode patients

Table 4.1-1 (continued)

Author, Year	Sample size	Methods	Summary of findings
Ohrmann et al. (2005)	FE = 13M,6F Pt = 15M,6F Ctrl = 13M,8F	1.5 T, VOI	↓ NAA, Glu, Gln in DLPFC of patients with chronic schizophrenia as compared with controls and first-episode patients; metabolite deficits not correlated with medication or DI
Omori et al. (2000)	Pt = 12M,8F Ctrl = 10M,6F	1.5 T, VOI	↓ NAA/Cr, Cho/Cr ratios in thalamus of patients; no differences observed in FL
O'Neill et al. (2004)	Pt = 7M,4F Ctrl = 10M,10F Childhood-onset	1.5 T, MRSI	↑ Cr in superior ACC of patients; ↑ Cho in ACC, FL and caudate head of patients; ↓ NAA in thalamus of male patients but not female; no differences in striatum, parietal cortex or parietal or frontal WM
Pae et al. (2004)	Pt = 11M,13F Ctrl = 10M,10F	1.5 T, VOI	↓ NAA/Cr ratio in FL of patients; no laterality; no change in metabolic ratios with antipsychotic treatment
Shimizu et al. (2007)	Pt = 11M,8F Ctrl = 12M,6F	1.5 T, VOI	↓ NAA/Cr in posterior cingulate gyrus of patients; Controls show age-related decline in NAA/Cr, ratio low in patients at all ages
Shioiri et al. (1996)	Pt = 12M,9F Ctrl = 12M,9F	1.5 T, VOI	↑ Cho and Cho/NAA in left BG of patients; neuroleptic dosage correlated positively with NAA and negatively with Cho/NAA
Sigmundsson et al. (2003)	Pt = 24M,1F Ctrl = 22M,4F	1.5 T, VOI	Significant negative correlation between NAA concentration and severity of symptoms; correlation between NAA concentration and social functioning; no differences in metabolites found in DLPFC
Stanley et al. (2007)	FE = 13M,5F Ctrl = 39M,22F Antipsychotic-naive	1.5 T, VOI	↓ NAA in left DLPFC of patients; younger patients showed deficit as compared with young controls while older patients and controls did not differ in NAA in DLPFC
Szulc et al. (2005)	Pt = 10M,4F Not medicated	1.5T, VOI	↑ NAA, mI in thalamus of patients after treatment with Risperidone; pre-treatment positive symptoms correlated with NAA in FL; negative symptoms correlated with Glx in TL
Szulc et al. (2007)	Pt = 40M,18F Ctrl = 13M,8F	1.5 T, VOI	No difference in NAA in FL, TL or thalamus in patients on typical or atypical antipsychotics; ↓ NAA in thalamus of patients on typical antipsychotics compared with controls
Tanaka et al. (2006)	Pt = 10M,4F Ctrl = 10M,3F	1.5 T, VOI	↓ NAA in left FL of patients; NAA reduction correlated with severity of negative symptoms and poorer Wisconsin Card Sort performance
Tang et al. (2007)	Pt = 29M,13F Ctrl = 23M,17F	3.0 T, MRSI	↓ NAA and diffusion tensor imaging (DTI) anisotropy in medial temporal WM in patients; no differences in DLPFC or occipital WM
Theberge et al. (2004)	FE = 14M,5F Antipsychotic-naïve	4.0 T, VOI	Negative correlation between thalamic NAA and duration of prodromal symptoms; correlation between Cho and duration of psychosis in thalamus and ACC
Thomas et al. (1998)	Pt = 7M,6F Ctrl = 6M,6F Adolescents	1.5 T, VOI	↓ NAA/Cr in FL gray matter of patients; No differences between groups in occipital GM
Tibbo et al. (2000)	Pt = 12M Ctrl = 12M	3.0 T, VOI	No differences in NAA/Cr or Cho/Cr in cerebellar vermis between patients and controls

continued

Table 4.1-1 (continued)

Author, Year	Sample size	Methods	Summary of findings
Wood et al. (2003)	FE = 36M,20F HR = 17M,13F Ctrl = 13M,8F	1.5 T, VOI	No differences between first-episode patients and controls in left medial temporal region or DLPFC; ↑ NAA/Cr, Cho/Cr ratios in left DLPFC of high risk subjects as compared with controls
Wood et al. (2006)	FE = 29M,17F	1.5 T, VOI	↓ NAA/Cr ratio in left prefrontal cortex related to poorer outcome measures in first-episode patients
Wood et al. (2007)	Pt = 15M Ctrl = 14M	3.0 T, VOI	↓ NAA concentrations bilaterally in ACC of patients; no difference in Glu, Gln
Yamasue et al. (2002)	Pt = 10M,5F Ctrl = 9M,4F	1.5 T, VOI	↓ NAA/Cho, ↑ Cho/Cr in ACC of patients; GM correlated positively with NAA/Cho in ACC of patients; negative association between NAA/Cho and severity of blunted affect symptom in schizophrenics
Yasukawa et al. (2005)	Pt-GS = 8M,7F Pt = 8M,7F Ctrl = 10M,10F Gilbert Syndrome	1.5 T, VOI	↓ NAA/Cr, Cho/Cr, mI/Cr in ACC, thalamus and insular cortex of GS patients compared with controls and non-GS patients; ↓ NAA/Cr, Cho/Cr, mI/Cr in ACC and insular cortex of non-GS patients compared with controls
Yurgelun-Todd et al. (1996)	Pt = 15M,1F Ctrl = 12M,2F	1.5 T, VOI	↓ NAA/Cr in left and right TL of schizophrenic patients
Zabala et al. (2007)	Pt = 7M,1F Psychosis = 10M,5F Ctrl = 23M,10F Adolescents	1.5 T, VOI	↓ NAA/water in left DLPFC of patients with schizophrenia as compared with non-schizophrenia psychosis patients and healthy controls; No differences in other metabolites or in right DLPFC

strengthen the view that there are no changes in NAA during the progression of illness, at least in the first 2 1/2 years. Both studies may be underpowered, but the SZ group in the Bustillo study has a total of 32 subjects; although it was underpowered to detect the difference between the haloperidol and quetiapine groups. Both of these studies have methodological strengths: they reported metabolite concentrations, which is advantageous to NAA/Cr ratios, since metabolites can vary independently and Cr levels exhibited an age effect (Pfefferbaum et al., 1999a); they corrected the reported NAA values for the tissue composition of the VOI after segmentation to quantify gray matter (81% water content); white matter (71%) and CSF (100%) and the Theberge report examined longitudinal changes in metabolites in conjunction with gray matter volume changes. The question about changes in NAA with SZ progression may be answered by longitudinal study designs with longer follow-up time and larger sample size; if one assumes a parallel in NAA decease and brain tissue decrease, future studies should be sufficiently powered to detect NAA decrease proportional to the progressive brain tissue loss estimated from several SZ longitudinal anatomical MRI studies, which is ~0.5% per year compared with ~0.2% per year for healthy individuals (Hilleke et al., 2008). Additionally, it may be advantageous to incorporate an MRSI (in conjunction with anatomical MRI) rather than a VOI approach into longitudinal studies, since our current hypotheses about specific regional changes in SZ are not well defined, and since the sensitivity of ^{1}H MRSI has increased in recent years. The assumption of a parallel decrease in NAA and brain volume was challenged by a cross-sectional study (Molina et al., 2006) that showed no association between DLPFC gray matter volumes and NAA ratios in 17 first-episode, 17 chronic SZ and 50 control subjects, although the chronic SZ patients had decreased NAA, gray matter and increased CSF in the chosen VOI in the DLPFC, compared with the FE and the control subjects; the authors comment that their finding may be limited to this brain area (*Table 4.1-1*).

The conversion from "ultra high risk" (UHR) of SZ to overt illness is of considerable interest and ^{1}H MRS can play a role in the understanding of this process, and hopefully, finding interventions to prevent the development of SZ or to attenuate the symptoms before they have led to full-blown illness and impaired psychosocial function. One study (Wood et al., 2003) assessed the left medial temporal and left DLPFC

regions of 56 patients in their first episode of a psychotic disorder, 30 young people at UHR of developing psychosis, and 21 healthy controls, using proton MRS. No differences were detected between the first episode and control groups for NAA/Cr in either region of interest. There was a significant elevation of NAA/Cr in the DLPFC region of the UHR group, but it did not discriminate between those UHR individuals and who later became psychotic ($N = 6$) and those who did not. Another group that studies the conversion from the high-risk prodromal state to full SZ (Jessen et al., 2006) reported ^{1}H MRS findings with VOI's in the left frontal lobe, the anterior cingulate gyrus, and the left superior temporal lobe, with 21 patients with full disease, 10 subjects with "early at risk syndrome," 9 subjects with "late at risk syndrome," and 31 healthy control subjects. Subjects were followed longitudinally for a mean duration of 11.6 months to detect conversion to SZ. There was a significant reduction of the metabolic ratios NAA/Cr and NAA/Cho in the left frontal lobe and of NAA/Cr in the anterior cingulate gyrus in both at-risk groups and in the schizophrenic patients, as compared with healthy controls. Based on this study, low NAA was interpreted as a vulnerability indicator. The at-risk subjects who converted to schizophrenia within the observation period had a higher Cho/Cr and a lower NAA/Cho ratio in the anterior cingulate gyrus compared with non-converters; the elevated Cho in the anterior cingulate was interpreted as a predictor for conversion to the full disease. NAA/Cr did not differ between converters and nonconverters and there were no medication effects. This study is in contrast with the findings by Wood and coworkers of increased NAA/Cr in the UHR group; it is notable that the healthy group in Wood's study was significantly older than the FE and the UHR groups (mean ages 34.1, 21.7, and 19.5 years respectively), whereas in Jessen's study, the ages were more comparable, being 34.8 years for the healthy group, 33.4 for the SZ group, 27.0 for the "early at risk" and 28.7 years for the "late at risk." While it is difficult to closely match the subject ages in a prodrome study, large age differences may affect comparisons, as there is fair agreement in the literature that NAA levels decrease with age (Minati et al., 2007) and Angelie and colleagues (Angelie et al., 2001) have estimated that in healthy individuals, in one decade, the cortical NAA concentration decreases by 2.15%, creatine increases by 3.86%, and the NAA/Cr ratio decreases by 4.6%. The age difference between the healthy group and the UHR group in Wood's report is 14.6 years, and although they entered age as a covariate in their analysis, it remains a potential confounder in their analysis.

The reduction of NAA level in the prefrontal cortex in SZ has been associated with more severe negative symptoms (Callicott et al., 2000), poor working memory (Bertolino et al., 2000b) and longer duration of illness (Stanley et al., 1995), which suggest that low NAA may be related to worse concomitant psychosocial outcome, but until recently there was no information on the important question whether decrease in NAA may be predictive of poor outcome in the future. Wood and colleagues addressed this question by measuring NAA, Cho and Cr in the left prefrontal cortex and the left mediotemporal lobe in 46 subjects with FE psychosis, and assessing psychosocial outcome measures after 18 months of follow-up (Wood et al., 2006). The finding was that a lower NAA/Cr ratio in the prefrontal cortex was strongly related to poorer future outcome, and that the NAA/Cr ratio explained 17–30% of the variance in outcome. This interesting work indicates that NAA decrease may be an early marker of poor prognosis across the first years of illness, and this may have practical significance in the planning of services and treatments.

3 ^{1}H MRS, Focus on Glutamate, Glutamine and GABA

3.1 Biological Role of Glutamate and the NMDA Receptor Hypofunction Hypothesis of Schizophrenia

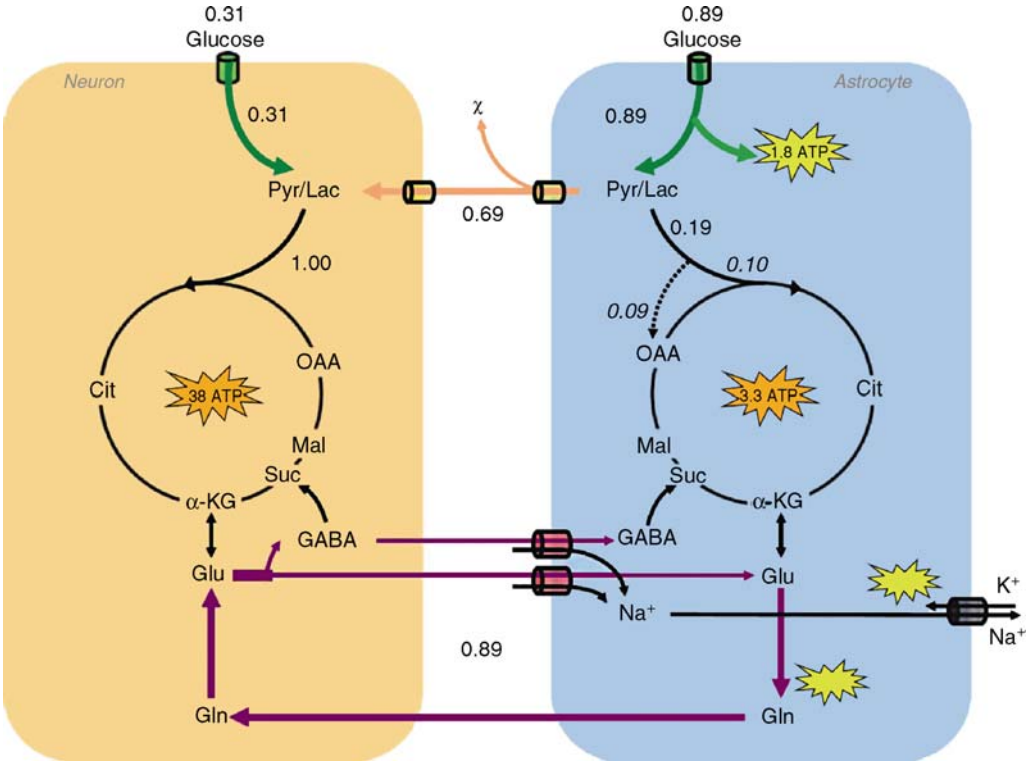
Glutamate (Glu) is the most abundant amino acid in the brain and plays a major role as an excitatory neurotransmitter in the cerebral cortex (Erecinska and Silver, 1990), which is rich in glutamatergic neurons. Following the release of glutamate from the presynaptic terminal to the synaptic cleft, excess glutamate is taken up by surrounding astrocytes and is converted to glutamine (Gln). Gln is then released, taken up by the presynaptic neuron, and is reconverted to glutamate. This Glu–Gln cycle is coupled to the synthesis of GABA from Glu, and to glucose utilization in the brain; the set of concentrations of Glu, Gln, and GABA,

and the kinetics of the enzymes involved, are consistent with a model in which alterations in glial-neuronal interactions and brain activity could change the concentrations of glutamate, glutamine, and GABA, and vice versa (Hyder et al., 2006) (see *Figure 4.1-2* and Section 4.1.5.1).

The glutamate NMDA receptor hypofunction hypothesis of schizophrenia was proposed by Olney (Olney and Farber, 1995; Olney et al., 1999; see also Javitt in this Volume) on the basis of his experimental work on the pathomorphological neurotoxic syndrome induced in the adult rat brain by phencyclidine (PCP) and other NMDA antagonists, and as a synthesis of earlier findings that the dissociative anesthetics PCP and ketamine induce a schizophrenia-like psychotic state in human subjects through blockade of NMDA glutamate receptors. The dopamine hypothesis continues to be the most influential, as well as the longest enduring, model of schizophrenia (Kapur and Mamo, 2003) and it explains the psychotic symptoms and their response to treatment by (mostly) D_2-receptor blocking agents, but it has difficulty explaining the negative and cognitive symptoms that are resistant to treatment with D_2-receptor blocking antipsychotics, as well as the structural brain abnormalities in schizophrenia. The NMDA glutamate receptor dysfunction model better accounts for these facts. It states that NMDA receptor hypofunction, the condition induced in

Figure 4.1-2

The revised model for coupling of brain glucose metabolism and neuronal activity. Glial uptake of ~0.89 glucose equivalents (green) produces ~1.8 glycolytic ATP (yellow) to fuel ion pumping and glutamine synthesis. The glial tricarboxylic acid cycle consumes ~0.19 glucose equivalents in total, where pyruvate carboxylase flux itself provides ~0.09 glucose equivalents, yielding ~3.3 oxidative ATP (orange) in astrocytes. The remaining glial lactate (~0.70) is transported to neurons (~0.69) and blood ($x \approx 0.01$). For ~1.00 glucose equivalents to be oxidized in neurons, an additional ~0.31 glucose uptake is needed in neurons, yielding ~38 oxidative ATP (orange) in neurons. This process in neurons and glia results in the cycling of ~0.89 glucose equivalents of neurotransmitter glutamate (~82%) and GABA (~18%) (from Hyder et al., 2006, with permission)



the human or animal brain by an NMDA antagonist drug, might also be viewed as a model for a disease mechanism that explains the symptoms and natural course of schizophrenia and that the disease mechanism itself might involve dysfunction of the NMDA receptor or downstream effects that can be modeled by blocking NMDA receptors. In Olney's model, NMDA receptor hypofunction of receptors on GABAergic inhibitory interneurons results in a downstream hyperglutamatergic state. Over time, the unrestrained release of glutamate would lead to postsynaptic excitotoxic changes, which could account for the poor outcome, volumetric tissue reductions and perhaps lower NAA and Glx in chronic patients. There are several lines of evidence in support of this model:

1. Ketamine and PCP are use-dependent noncompetitive antagonists of the NMDA receptor that bind to the intrachannel site of the receptor and prevent calcium ion flux (Javitt and Zukin, 1991); in humans they cause a syndrome that is clinically indistinguishable from schizophrenia and that involves not only positive (psychotic) symptoms, but also, negative symptoms (blunted affect, avolition), thought disorder and cognitive impairments.
2. NMDA receptor antagonists induce schizophrenia-like deficits in early sensory information processing, such as abnormal mismatch negativity, abnormal visual P1-evoked potentials and deficits in continuous performance tasks (Javitt et al., 2008).
3. Repeated subcutaneous injections of NMDA channel blockers cause neurodegenerative changes in rat cortex (posterior cingulate/ retrosplenial cortex, anterior cingulate, hippocampus and amygdala), which roughly coincide with the structural changes seen in schizophrenia (Olney and Farber, 1995).
4. Ketamine causes increase of cortical glutamate in animals and humans. Repeated administration of ketamine or PCP causes an increase in cortical glutamate, detected using microdialysis studies in rodents. It appears that the site of NMDA blockade is regionally distinct from the site of the resultant glutamate release since injection of the selective and specific NMDA receptor antagonist MK-801 into the anterior nucleus of the thalamus induced cortical degeneration in a pattern indistinguishable from systemic administration, while injection directly into cortical regions did not lead to any neurodegenerative changes (Sharp et al., 2001). In an exciting confirmation of animal studies, Rowland and colleagues were able to demonstrate with ^{1}H-MRS at 4T a significant increase of glutamine in the anterior cingulate cortex following ketamine administration to healthy human volunteers (Rowland et al., 2005).
5. Genetic evidence that supports the glutamate model has gegun to accumulate. For example, GRM3 is a heteroceptor that modulates NMDA-receptor transmission and a polymorphism in this gene was significantly associated with schizophrenia (Egan et al., 2004). In addition, healthy subjects that carried the risk allele of this schizophrenia susceptibility gene exhibited inefficient prefrontal cortical fMRI activation and reduced working memory performance (Egan et al., 2004). Furthermore, a ^{1}H MRSI study showed a significant reduction of N-acetylaspartate levels in the right DLPFC in this group (Marenco et al., 2006), although no Glu/Gln MRS findings have been reported. The glutamate NMDA receptor hypofunction hypothesis is not mutually exclusive with the dopamine model of schizophrenia, and a recent study (Tan et al., 2007) demonstrated the possibility for a system-level interaction between the dopaminergic and glutamatergic systems in human cortical circuits implicated in working memory dysfunction; the finding was that the COMT-Val allele (putatively associated with reduced prefrontal dopamine) and the GRM3-A allele (putatively related to suboptimal glutamatergic function) interacted to give disproportionately inefficient DLPFC fMRI activation in a working memory task, compared with the effect of each allele separately (epistasis). This finding is consistent with results from animal experiments and current models of working memory, reviewed by Seamans and colleagues (Seamans and Yang., 2004).

3.2 ^{1}H MRS Studies of Glutamate and Glutamine in Schizophrenia

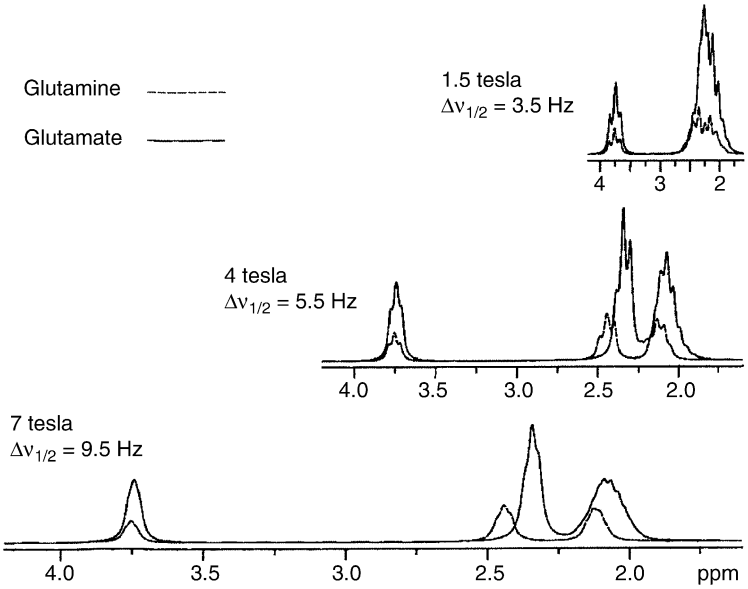
Among the metabolites that can be measured with MRS, glutamate, glutamine and GABA provide the most direct link to the study of neuronal and glial function, and they are central to current models of

schizophrenia. Unfortunately, they are also among the most difficult metabolites to measure: J-coupling complicates their resonances so that instead of well-resolved single peaks the signals are distributed among triplet resonances or in some cases even more complex patterns; many of the resonances of glutamate, glutamine, and GABA in vivo overlap with one another or with other peaks. For that reason, most studies report peak areas for a single, irregular combined resonance in the vicinity of 2.4 ppm called "Glx." At the short echo times normally used to detect Glx in psychiatric research, macromolecules make a major contribution to the peak intensity at glutamate, glutamine, and GABA locations (Hwang et al., 1996; Hofmann et al., 2001; Kassem and Bartha, 2003). Separation of Glu and Gln is important and Gln concentration may be more indicative of neurotransmission status than the concentration of Glu, since it has been estimated that 80% of stimulus-released neurotransmitter glutamate is derived from glutamine and this smaller "neurotransmitter" pool of glutamate does not equilibrate quickly with the larger "metabolic" pool of cellular glutamate. The ratio of the intracellular to extracellular concentration of glutamate is 2000–5000:1, whereas that for glutamine is 30–70:1 (Erecinska and Silver, 1990) and the in vivo concentration of glutamate is greater than glutamine ($\sim$ 3:1). Thus, in the context of in vivo ^{1}H MRS, glutamine may prove to be more sensitive than glutamate, or the combined Glx peak, as an indicator of glutamatergic neurotransmission (Bartha et al., 1997). Most human ^{1}H MRS studies are currently conducted at field strengths of 1.5–4 T. As illustrated in *Figure 4.1-3*, the separation of Glu and Glx is suboptimal even at 4 T. Postprocessing of raw data can ameliorate, but cannot resolve this problem, since the Glu-H4 and Gln-H4 protons become separate resonances only at magnetic field strengths of 7 T or higher (Tkac et al., 2001). Because of the issues of resolution, particularly at 1.5 T, signal-to-noise ratios, and macromolecular contamination, many of the glutamate and glutamine measurements made in psychiatric research should be viewed carefully.Δ

In spite of the difficulties and caveats in the spectroscopy of Glu and Gln, the MRS study of these metabolites in schizophrenia has both furthered our knowledge and added some controversies. The earliest

Figure 4.1-3

Simulated ^{1}H NMR spectra of glutamine and glutamate at different magnetic field strengths. Linewidths ($\Delta\nu_{1/2}$) corresponded to values typical for very well shimmed volumes of the human brain. The concentration ratio [Glu]/[Gln] was set to 3:1. Frequency scale (Hz) is identical in all three spectra (From Tkac et al., 2001, with permission)



study (Bartha et al., 1997) to focus on Glu and Gln measurement in a single VOI at 1.5 T reported a significant increase in glutamine level in the medial prefrontal cortex of first-episode never treated schizophrenic patients compared with controls (7.2 ± 3.7 vs 3.7 ± 2 units, $N = 10$; df = 15; $p = 0.03$) without significant difference in NAA, Glu, Cho, and PCr + Cr. This was interpreted to suggest a hyperglutamatergic state early in the illness, consistent with the NMDA hypothesis. To address questions of disease progression, 18 first-episode patients, 21 chronic patients, and 21 age-matched controls were studied on a 1.5 T magnet with a volume of interest in the DLPFC (Ohrmann et al., 2005); chronic patients, but not first-episode patients had significant decreases in both Glx and NAA, consistent with a progressive course of illness that may involve the glutamate system. In a later report, the same group (Ohrmann et al., 2007) examined an overlapping sample of subjects and found that in both patient groups, lower NAA, but not Glx levels in the left DLPFC significantly correlated with poorer performance in verbal learning and memory; this does not appear to be consistent with the a continuous hyperglutamatergic state.

Theberge and colleagues conducted several seminal studies at 4 T field strength, voxel size of 1.5 cm^3 with more advanced postprocessing methods. In their first study (Theberge et al., 2002) of 21 young never-treated patients with schizophrenia (duration of illness = 1.7 years) and 21 healthy volunteers, the patients had elevated Gln with normal Glu and NAA in both the left anterior cingulate and left anterior thalamus (the only VOI studied). This study, similar to the very first Glu study (Bartha et al., 1997) suggests an increased glutamatergic neurotransmission early in the illness, which is not attributable to medication or chronicity. This group then studied (Theberge et al., 2003) 21 patients with chronic schizophrenia (duration of illness = 15.6 years) and 21 controls. Glu and Gln were reduced in the anterior cingulate, but Gln was increased in the left thalamus. They concluded that decreased levels of these metabolites in the anterior cingulate of chronic patients could be related to neurodegeneration or the effects of chronic medication, whereas increased glutamatergic turnover persisted in the thalamus while the glutamatergic system "burned out" earlier in the cingulate, suggesting disease progression or antipsychotic medication effects. This work was continued with one of the few longitudinal studies in the MRS field in which Theberge and colleagues examined Glu, Gln, and gray matter volume in the left anterior cingulate and thalamus in 16 patients with first-episode schizophrenia at baseline, and then after 10 and 30 months of antipsychotic treatment and in 16 healthy participants on two occasions 30 months apart (Theberge et al., 2007). They reported higher Gln levels in the anterior cingulate and thalamus of never-treated patients, consistent with their first study (12 out 16 subjects overlapped with the cohort of their 2002 study). At month 30 of follow-up, the anterior cingulate Gln level continued to be increased, which is in contrast with their 2003 study of patients with longer duration of illness. The level of glutamine in the thalamus was significantly reduced after 30 months, but did not decrease below the healthy control level; parietal and temporal lobe gray-matter loss was correlated with thalamic glutamine loss. There were no changes in Glu and Gln over 30 months in the healthy group. They conclude that elevated glutamine in never-treated patients followed by decreased thalamic glutamine and gray-matter loss in connected regions could indicate either neurodegeneration or a plastic response to reduced subcortical activity. These three higher field studies are limited in the number of voxels that were examined, and by the lack of a "reference" VOI where there would be no expected changes in Glu/Gln on theoretical grounds (e.g., occipital cortex).

Recent studies have begun to address the possibility of using Glu/Gln MRS measures as potential endophenotypic markers or as predictors of conversion to overt disease: a comparison between 20 adolescents with high genetic risk for schizophrenia (had a parent with schizophrenia) and a group of 22 adolescents at low genetic risk (no parent with a history of schizophrenia) (Tibbo et al., 2004) found significantly higher glutamate/glutamine (Glx) in the right medial frontal lobe (the only VOI measured) of the high-risk group and this was interpreted to support a neurodevelopmental hypotheses for schizophrenia. People with schizophrenia perform poorly on the continuous performance test (CPT) that measures sustained attention and involves the rapid presentation of visual stimuli where the subject needs to identify target items among foils. CPT has been studied extensively as a cognitive endophenotype, and the NMDA antagonist ketamine worsens performance on CPT in healthy individuals (Umbricht et al., 2000). A study of Glu and Glx at 3 T of 15 adult siblings of individuals with schizophrenia (high genetic risk group) and 14 healthy volunteers (Purdon et al., 2008) found only a trend correlation ($r = 0.32$; $p = 0.096$) between

worse performance on CPT and higher glutamate level in the medial frontal lobes, and no between-group difference in the correlation coefficients. After median stratification, the high-glutamate group contained a larger proportion of high-risk subjects (67% vs. 29%), and also who scored lower on the CPT. Endophenotypic differences are often quite subtle and larger sample sizes may be needed to detect them, but this study suggests that regional glutamate changes, in conjunction with a cognitive measure, may have a role as an endophenotypic marker for schizophrenia (*Table 4.1-2*).

Recent years have seen an increasing interest and research in white matter abnormalities in schizophrenia that was made possible by: (1) the development of magnetic resonance methods to study white matter in vivo, such as diffusion tensor imaging (DTI) and tractography, magnetization transfer imaging (MTI), T2 relaxography, and MRS (Wozniak and Lim, 2006; Lim, 2007) (2) an orientation toward circuit-based hypotheses of schizophrenia that emphasize structural and functional disconnectivity (Andreasen, 1999; Davis et al., 2003); (3) implication of oligodendrocyte-related genes in schizophrenia (Karoutzou et al., 2008) and the convergence of data that may link schizophrenia susceptibility genes involved in glutamate transmission, the control of synaptic plasticity, dopaminergic transmission, oxidative stress, and oligodendrocyte viability (Carter, 2006). MRS has made considerable contributions to the study of neurological demyelinating disorders, such as multiple sclerosis, but there have been few MRS studies of white matter in schizophrenia. A recent study by Chang and colleagues (Chang et al., 2007) makes an important contribution and supports a connection between abnormal glutamate–glutamine homeostasis and white matter neurochemical abnormalities. Compared with the 22 healthy subjects, the 23 elderly schizophrenia participants had higher Glx (+28.7%, $p = 0.0016$), lower NAA (−12.6%, $p = 0.0008$) and lower myoinositol (−16.4%, $p = 0.026$) in all of the three white matter VOI: frontal, temporal, and occipital. This was interpreted as a white matter abnormality distinct from the findings in demyelinating or inflammatory disorders that is related to either excess neuronal glutamate release or glial dysfunction in glutamate reuptake. This study also illustrates the technical issues and compromises involved in MRS experiments. Even on a 4 T magnet, with the echo time chosen, they were able to resolve only a combined Glx peak, but were able to determine reliably NAA, mI, and Cho. The value of this study is in the combined measurement of Glu/Gln and markers indicative of neuronal viability (NAA), decreased glial content or function (mI) and cell membrane constituents, and phospholipid metabolites (Cho).

3.3 Biological Role of γ-aminobutyric Acid (GABA) and Involvement in Schizophrenia

GABA is the major inhibitory neurotransmitter in the central nervous system. It is synthesized predominantly by inhibitory interneurons, such as the cortical basket cells which form axo-somatic synapses with pyramidal cells, and the chandelier cells which form axo-axonal synapses with pyramidal cells (Benes and Berretta, 2001). Chandelier and basket cells are electrophysiologically characterized as "fast-spiking" neurons that exhibit repetitive firing by synaptic activation of depolarized potentials, short duration action potentials, and relatively negative resting potentials. They are connected to each other by both chemical synapses and electrical synapses, and it has been proposed that they form networks that might contribute to the synchronization of electrical activity in cortical neurons (Gibson et al., 1999). Synchronous oscillations in a wide range of frequencies are thought to link spatially distributed neuronal assemblies into functionally integrated and specialized networks. Neural synchrony has been shown to play a role in perceptual integration, working memory, and selective attention (Tallon-Baudry and Bertrand, 1999; Fries et al., 2001; Palva et al., 2005), and there is considerable evidence of deficits in perceptual integration, working memory, and attention in schizophrenia (Perlstein et al., 2001; Uhlhaas and Silverstein, 2005). This is consistent with findings of abnormal neural gamma band (30–100 Hz) synchronization (Spencer et al., 2003) and lower frequency than healthy controls of synchronous oscillations (Spencer et al., 2004) during tasks of perceptual integration, and reduced EEG power at 40 Hz with delayed synchronization in schizophrenia (Kwon et al., 1999). Neural synchrony between inhibitory GABA interneurons is established through glutamatergic excitation from pyramidal neurons in the hippocampus (Jensen and Lisman, 1996; Lisman et al., 2005). Neurophysiological studies with electrodes in the DLPFC of behaving monkeys have shown that working memory depends on the coordinated and sustained firing of groups of

Table 4.1-2
^{1}H-MRS studies of glutamate and glutamine in schizophrenia

Author, Year	Sample size	Methods	Summary of findings
Bartha et al. (1997)	Pt = 8M,2F Ctrl = 8M,2F Antipsychotic-naïve	1.5 T, VOI	↑ Gln in left medial PFC of patients who had not been previously treated; No other metabolite differences found
Berger et al. (2008)	E-EPA = 8M, 4F Placebo = 12 M First Episode	3.0 T, VOI	↑ glutathione in left and right TL; ↑ Gln,Glu in left TL of patients treated with ethyl-eicosapentaenoic acid; brain metabolite changes correlated with improvement of negative symptoms
Chang et al. (2007)	Pt = 23 Ctrl = 22	4.0 T, VOI	↑ Glu,Gln ↓ NAA, mI in FL, TL and occipital lobe of elderly patients
Choe et al. (1996)	Pt = 17M,17F Ctrl = 10M,10F	1.5 T, VOI	↓ Glx/Cr in PFC in patients after treatment with neuroleptics; No significant lateral metabolic effect in either group
Goff et al. (2002)	Pt = 10M,4F	1.5 T, VOI	↑ Glu/Cr in ACC of patients showing improvement in negative symptoms with olanzapine; ↑ serum glutamate concentrations after switch from conventional agents to olanzapine
Kegeles et al. (2000)	Pt = 10M Ctrl = 10M	1.5 T, VOI	↑ Glx/Cho in right Hippo of patients as compared with controls
Ohrmann et al. (2007)	FE = 10M,5F Pt = 14M,6F Ctrl = 13M,7F	1.5 T, VOI	↓ Gln,Glu, NAA, Cho, in DLPFC of chronic patients as compared with first-episode patients and healthy controls; ↓ Cr in first-episode and chronic patients compared with controls
Ohrmann et al. (2005)	FE = 13M,6F Pt = 15M,6F Ctrl = 13M,8F	1.5 T, VOI	↓ Glx, NAA in DLPFC of patients with chronic schizophrenia as compared with controls and first-episode patients not correlated with duration of illness or medication
Purdon et al. (2008)	HR = 2M,13F Ctrl = 3M,11F	3.0 T, VOI	More HR subjects showed high glutamate levels in medial FL than controls; high glutamate subjects scored lower of continuous performance test
Rowland et al. (2005)	Ctrl = 10M Crossover design Ketamine/placebo	4.0, VOI	↑ Gln in ACC after ketamine infusion; no change in Glu, NAA, Cho, Cr, after ketamine infusion
Theberge et al. (2003)	Pt = 20M,1F Ctrl = 20M,1F	4.0 T, VOI	↓ Gln, Glu in left ACC of patients; ↑ Gln in left thalamus of patients
Theberge et al. (2007)	FE = 14M,2F Ctrl = 14M,2F	4.0 T, VOI	↑ Glu in ACC and thalamus of antipsychotic-naïve patients; glutamine levels decreased in patients after 30 months treatment; GM loss in patients at 30 months treatment
Theberge et al. (2002)	FE = 14M,7F Ctrl = 14M,7F Antipsychotic-naïve	4.0 T, VOI	↑ Gln in left ACC and left thalamus of patients
Tibbo et al. (2004)	HR = 7M,13F Ctrl = 9M,13F	3.0 T, VOI	↑ Gln, Glu in right medial FL of adolescents at high-risk for schizophrenia
Van Elst et al. (2005)	Pt = 13M,8F Ctrl = 23M,10F	2.0 T, VOI	↑ Glu in DLPFC and Hippo of patients; ↑ prefrontal Glu associated with poor global functioning

pyramidal neurons that have excitatory glutamatergic outputs on GABA interneurons, and that GABA-mediated inhibitory effects on pyramidal neurons are essential for the synchronization of the pyramidal neuron activity that maintains working memory. It has been proposed that GABA interneuron inhibition may control both which DLPFC pyramidal neurons are activated during spatial working memory (spatial tuning) and when they are active during the different phases of working memory (temporal tuning) (Constantinidis and Wang., 2004). Direct application of the GABA antagonist bicuculline to the DLPFC of monkeys induced deficits in the performance of a delayed response task (Sawaguchi et al., 1989), and when applied iontophoretically to functionally characterized neurons of the DLPFC, in combination with microelectrode recordings, resulted in the loss of established spatial tuning during a working memory task and created significant spatial tuning in units that were untuned prior to bicuculline application (Rao et al., 2000). Thus, impairments in GABAergic inhibition in the DLPFC could explain the disturbances in working memory in people with schizophrenia. This is supported by the widely replicated finding of decreased mRNA for the 67 kDa form of glutamic acid decarboxylase (GAD67, the key enzyme in GABA synthesis) in the DLPFC in postmortem tissue detected by in situ hybridization-histochemistry (Akbarian et al., 1995) or by microarray expression profiling (Mirnics et al., 2000). Further studies have found decreased mRNA and/or protein in temporal cortex, cerebellum, and anterior cingulate cortex; these findings are not specific to schizophrenia: a decreased GAD 67 expression was also reported in bipolar disorder and autism (Akbarian and Huang, 2006). It was also established that in schizophrenia, 25–30% of the GABA inhibitory interneurons (the chandelier cells) in the DLPFC do not express a GAD 67 transcript. Moreover, such neurons have undetectable levels of the GABA membrane reuptake transporter GAT1 mRNA; this is accompanied by $GABA_A$ receptor upregulation, and a decrease in neuropil (Lewis et al., 2005). In a combined biochemical and in situ hybridization analysis of DLPFC postmortem tissue, the cases with schizophrenia had a significant decrease in tissue content of GABA, accompanied by increased mRNA for $GABA_A$ receptor and decreased mRNA for GAT1 (Ohnuma et al., 1999). This is consistent with the early biochemical findings of decreased GABA concentration in postmortem brains of schizophrenics (Spokes et al., 1980; Korpi et al., 1987) that gave rise to the current hypothesis of the role of GABAergic inhibitory interneurons in schizophrenia. The most likely pathogenetic mechanism that leads to a decreased GABA signaling in a subgroup of inhibitory interneurons in DLPFC is a reduced glutamatergic excitatory drive from projections to the DLPFC from the mediodorsal nucleus of the thalamus and the hippocampus (Lewis et al., 2005). These findings of molecular and cell biology studies underscore the potential value of ^{1}H MRS studies to determine both Glu/Gln and GABA in regions of interest.

3.4 ^{1}H MRS of GABA in Schizophrenia

The in vivo measurement of GABA presents considerable challenges. In terms of MRS sensitivity, it has a low concentration in the brain of less than 1 mM, and has a complicated, multiresonance spectrum that is overlapped by the dominant creatine (Cr), *N*-acetylaspartate (NAA), and glutamine/glutamate (Glx) resonances; even at 9.4 T, there is a prominent overlap of the C4 3.01 GABA triplet with creatine. Various techniques for spectral editing have been developed that utilize the phenomenon of spin–spin (scalar) coupling (interaction between nuclei with magnetic moments through electrons in chemical bonds) (de Graaf, 2001). GABA can be detected at 7 T at 2.28 ppm in human brain without spectral editing, thanks to the increased peak separation at higher field strength, particularly for J-coupled metabolites (Tkac et al., 2001).

To date, there are no published in vivo ^{1}H MRS studies of GABA in schizophrenia. There are, however, reports of a highly significant (52%) reduction in occipital cortex GABA levels in people with depression (Sanacora et al., 1999), and from the same group, a 22% reduction in occipital cortex GABA concentration in people with panic disorder, compared with controls (Goddard et al., 2001). These studies were done with a 2.1T magnet in a 13.5 cm^3 volume of interest with a homonuclear J-editing procedure to separate the GABA C4 triplet resonance at 3.01 ppm from overlapping resonances. The authors (Goddard et al., 2001) note that, under the same conditions, their group did not observe low occipital cortex GABA levels in schizophrenia subjects (unpublished results). The occipital lobe was chosen as a region of interest because of technical considerations (it is easier to conduct shimming there); current hypotheses of schizophrenia do not consider the occipital lobe, and there are no data to date to implicate this region in schizophrenia.

Alterations of GABA in the occipital lobe are also not consistent with the histopathological and functional brain abnormalities in major depression. Hasler and colleagues were able to obtain GABA measurements from two frontal regions of interest that have been shown to be structurally or functionally abnormal on depression, and found that unmedicated symptomatic patients with major depression had low GABA and Glx levels the dorsomedial/dorsal anterolateral prefrontal region (Hasler et al., 2007), whereas subjects with remitted major depression had normal prefrontal GABA levels (Hasler et al., 2005). The study of MRS of GABA in schizophrenia thus remains an area of great promise, considering the solid data of abnormal GABA signaling in schizophrenia.

Given the difficulties with in vivo GABA MRS, and the lack of published studies in schizophrenia, it may be informative to consider the ^{1}H MRS (NMR) GABA findings in postmortem brain tissue, since high-resolution MRS of brain extracts can completely resolve complex peaks into their individual components and can provide absolute quantification of metabolites at far lower concentrations than the detection limit of clinical MRS systems. This method allowed the reliable determination of sixteen metabolite concentrations, including GABA, glutamate, and glutamine in samples from frontal cortex, thalamus, and cerebellar vermis from 8 schizophrenics and 10 controls with a 300.5 MHz (7 T) NMR spectrometer. There were no significant between-group differences in GABA and glutamine concentrations in any of these areas and only a trend increase of glutamate in the thalamus in the schizophrenia group (Omori et al., 1997). Another study examined GABA in postmortem tissue as part of a broader metabolomics approach with high-resolution ^{1}H NMR on a 700 MHz (16.4 T) with a method that allows analysis of intact brain tissue (high-resolution magic angle spinning (HRMAS)) (Prabakaran et al., 2004). They studied prefrontal cortex tissue (Brodmann area 9) from white and gray matter of 10 schizophrenia and 10 control individuals and found a decrease of the GABA level (0.6-fold or 40%) and an increase of the glutamate level (1.35 fold or 35%) in the schizophrenia frontal lobe samples. Interestingly, these changes were more pronounced in white matter; the changes in gray matter were in the same direction, but did not reach statistical significance. This is consistent with the findings of Chang and colleagues (Chang et al., 2007), discussed in section 3.2, and points to the importance of white matter pathology in schizophrenia.

4 ^{31}Phosphorus Magnetic Resonance Spectroscopy (^{31}P MRS)

4.1 ^{31}P Metabolites and their Biological Significance

^{31}Phosphorus has a natural abundance of 100%, but it has a lower gyromagnetic ratio than ^{1}H and its NMR sensitivity is only 7% of ^{1}H. The chemical shift dispersion of a ^{31}P NMR spectrum is fairly large (30 ppm) and this allows good spectral resolution. Phosphorus-containing compounds are involved in the structure of membranes and in high-energy phosphate metabolism; ^{31}P MRS can thus provide in vivo information about the integrity of neuronal cell membranes and brain bioenergetics. The following metabolites are detectable by ^{31}P MRS: phosphomonoesters (PME), phosphodiesters (PDE), phosphocreatine (PCr), α, β and γ nucleoside triphosphates (NTP), and inorganic phosphate (Pi). Intracellular ADP, pH, and Mg^{2+} levels can be deduced indirectly (Gupta et al., 1984), (Pettegrew et al., 1988). The PME peak includes the phospholipids phosphatidylethanolamine (PtdEth) and phosphatidylcholine (PtdCh), which are associated with cell membrane precursors. The phospholipids in the PDE peak, glycerophosphatidylcholine (GPCh) and glycerophosphatidylethanolamine (GPEth), are associated with membrane breakdown.

4.2 Early Studies of Phospholipid Changes in Schizophrenia and the Membrane Phospholipid Hypothesis for the Pathogenesis of Schizophrenia

An influential early study (Pettegrew et al., 1991) found that the PMEs were decreased and the PDEs were increased in the prefrontal cortex of drug-naïve first-episode patients with schizophrenia. This was interpreted as a higher membrane turnover at the onset of schizophrenia, or as a decreased synthesis and an

increased breakdown of membrane lipids in schizophrenia. Similar results were reported for the left temporal lobes of first-episode patients (Fukuzako et al., 1999a); the same group also reported that treatment with haloperidol reduced the excess of PDE in the left temporal lobe (Fukuzako et al., 1999b). These findings were confirmed in a group of patients with chronic schizophrenia who had higher PDE and lower PME in their frontal lobes, but normal levels in their parietal lobes (Deicken et al., 1994). Low in vivo PMEs in the prefrontal cortex of first-episode patients were strongly correlated with low red cell membrane phospholipid polyunsaturated fatty acids (Yao et al., 2002). These in vivo abnormalities and the close relationship between changes in membrane phospholipids in brain and in peripheral tissue provide support for the membrane phospholipid hypothesis (Horrobin, 1998) as a biochemical basis for the neurodevelopmental concept of schizophrenia. This concept (Weinberger, 1996) postulates a neurodevelopmental, possibly genetic, abnormality that occurs in utero or during childhood and results in abnormal neural networks through disturbances in neuronal migration, synapse formation, and pruning. In late adolescence and early adulthood, often after some precipitating factors, this mostly latent neurodevelopmental abnormality may cause the behavioral, thought, cognitive, and motivational signs described as schizophrenia. Horrobin proposes that phospholipid metabolism is (genetically) altered in schizophrenia with an increased rate of loss of arachidonic acid, doxohexanoic acid and dihomgamma-linolenic acid from membrane glycerophospholipids. This, in turn, changes functions of bioactive lipids as second messengers and affects receptor activity, neurotransmitter release, neuronal migration, pruning, and synaptic plasticity. The ^{31}P MRS findings from the 1990s are one of the foundations of membrane phospholipid hypothesis, which is also supported by lipid abnormalities in peripheral tissues, phospholipids analysis of postmortem brain tissue, epidemiological and nutritional data regarding deficits of essential fatty acids and incidence of schizophrenia, increased activity of the lipid-metabolizing phospholipases A2, and (modest) symptom improvement after supplementation of the diet with essential fatty acids (Berger et al., 2006). This hypothesis suggests a generalized lipid metabolism abnormality. However, two ^{31}P MRS studies of the temporal lobe found no changes in PME and PDE, but found changes in high-energy phosphates (Calabrese et al., 1992), (Deicken et al., 1995a). It also appears that the pattern of changes in phospholipids may differ with the progression of illness or with exposure to antipsychotics: in a cross-sectional study of 11 drug-naive first-episode, 8 newly diagnosed medicated, and 10 chronic medicated patients with schizophrenia, PMEs were decreased in the DLPFC of all the three groups, but PDEs were increased only in the drug-naive first-episode patients (Stanley et al., 1995). So far, there have been no longitudinal ^{31}P MRS studies that could provide a direct way to address changes in PDE and PME over time. The low PME and high PDE pattern does not appear to be specific to schizophrenia, since it was found in the frontal lobes in patients with bipolar disorder (Deicken et al., 1995b).

4.3 Recent ^{31}P MRS Studies of Phospholipids in Schizophrenia

The MRS methodology in the early and mid-nineties did not have sufficient spectral resolution to separate in vivo the components of the PME peak (PtdEth and PtdCh) and of the PDE peak (GPCh and GPEth). The introduction of the method of ^{1}H-decoupled two-dimensional ^{31}P chemical shift imaging made it possible to separate these peaks at 1.5 T (Potwarka et al., 1999a), and it was applied to study chronic medicated schizophrenic patients and healthy controls (Potwarka et al., 1999b). In patients, the authors found an increase of the mobile phospholipid (MP) peak area (an additional peak in the PDE part of the spectrum) in the prefrontal area, but not in the motor area or parietooccipital cortex. The MP accounted for the overall increase of PDE, but GPCh and GPEth were not increased. This may explain some of the controversial findings of earlier studies and is an argument against a generalized lipid metabolism abnormality in schizophrenia. The MP signal is associated with phospholipid-containing vesicles that carry neurotransmitters, and an increased signal may reflect changes in the relative concentrations or size of the phospholipid vesicles and changes in the regulation of neurotransmitter activity.

The typical field strength (1.5 T) used in the 1990s did not allow for good spatial resolution (^{31}P has low NMR sensitivity), and voxel volume was typically about 50 cm^3. Jensen and colleagues improved on previous studies, and reported on in vivo ^{31}P MRS on a 4 Tesla MR scanner with people with schizophrenia

(Jensen et al., 2006); at the higher field strength the effective voxel volume was 15 cm^3 (nominal volume 5.4 cm^3) and the PtdEth, PtdCh, and GPCh and GPEth peaks were resolved; this study is discussed in section 4.4 on high-energy phosphates. One study by this group (Jensen et al., 2002) examined 11 people with chronic schizophrenia and 11 matched controls, and based the voxels of interest on a group of hypotheses that postulate that schizophrenia may be caused by abnormal functioning of the DLPFC, anterior cingulate, thalamus, cerebellum, and hippocampus and of the neuronal circuitry that connects these brain regions (Lipska and Weinberger., 1993; Jones, 1997; Benes, 1998; Andreasen 1999; Selemon and Goldman-Rakic, 1999). They found that differences in membrane phospholipids were present in all brain areas of interest. GPEth was decreased in the anterior cingulate, right prefrontal cortex, and left thalamus but was increased in the left hippocampus and left cerebellum compared with controls. PtdEth and GPCh were decreased in the right prefrontal region and PCh was decreased in the anterior cingulate cortex. No significant differences in membrane phospholipid levels were seen between groups in the parieto-occipital and posterior cingulate regions, which are not thought to be involved in the pathogenesis of schizophrenia. The differences in PDEs and PMEs appear to be regionally diverse and do not appear to be consistent with a simple model of generalized decrease in PME and increase in PDE. Another study by the same group (Jensen et al., 2004), focused on patients with first-episode schizophrenia and the choice of voxels, was driven by the hypothesis that early in the illness an increased membrane breakdown may occur in the anterior cingulate gyrus. Similar to their previous study, their hypothesis also included decreased membrane metabolite levels in hippocampal, thalamic, and cerebellar regions, consistent with neurodevelopmental abnormalities. The finding in first-episode schizophrenia was an increased level of PDE (GPCh) in the anterior cingulate, suggesting an increased membrane breakdown. No significant PDE or PME differences were found in the other regions of interest. The authors commented that the membrane breakdown in the anterior cingulate suggested a neurodegenerative process that may be caused by increased glutamate activity in this region early in the illness. Their findings are consistent with the results of a ^1H MRS study that found increased glutamate metabolites in the left anterior cingulate (Theberge et al., 2002) and with the anatomical MRI findings of reduced tissue volume in this region. This is an interesting convergence of findings and ideas, since increased glutamate-mediated activity in the anterior cingulate (glutamate hypothesis of schizophrenia) may cause an increased neuronal membrane breakdown (membrane phospholipid hypothesis).

This research group (Theberge et al., 2004) then proceeded to the next step in the analysis of the relationship between glutamate status and phospholipid changes in the anterior cingulate gyrus by conducting a study of both ^1H (voxel = 1.5 cm^3) and ^{31}P MRS voxel = 15 cm^3) at 4 T in 9 patients with chronic schizophrenia with left cingulate and left thalamus as regions of interest. They found no difference in glutamine and PtdEth concentrations between the groups. There was a highly positive correlation ($r = 0.81$) in the left anterior cingulate of patients between levels of glutamine and PtdEth, but was not significantly different from the same correlation in the healthy controls. This correlation may reflect a physiological link between the glutamate neurotransmission system and the regulation of neuronal cell membrane synthesis and breakdown. This could occur through the excitotoxic effects on membranes caused by abnormal glutamatergic signaling. Conversely, a dysfunction in neuronal cell membranes and changes in ion fluxes may alter glutamate neurotransmitter levels (Magistretti et al., 1999). The parallel study of two biologically important types of MR spectra is an approach with great promise, but involves the difficulties in interpretation and compromises caused by the considerable difference in the minimal achievable voxel volumes (at 4 T) of 1.5 cm^3 for ^1H and 15 cm^3 for ^{31}P. *Table 4.1-3*

4.4 High-energy Phosphates in Schizophrenia

^{31}P MRS levels of phosphocreatine (PCr), inorganic phosphate (Pi), and α, β and γ nucleoside triphosphates (NTP), predominantly α, β and γ adenosine triphosphate (ATP), reflect the energy metabolism in the brain. Research of high-energy compounds in SZ was stimulated by early findings of frontal lobe hypometabolism ("hypofrontality") at rest in schizophrenia (Brodie et al., 1984; Alavi et al., 1986) measured with (18)F-fluorodeoxyglucose positron emission tomography (FDG-PET). The MRS findings

Table 4.1-3
^{31}P-MRS studies of phosphorus metabolites in schizophrenia

Author, Year	Sample size	Methods	Summary of findings
Calabrese et al. (1992)	Pt = 10M,1F Ctrl = 9M	2.0 T, VOI	Patients showed ↑ PCr/β-ATP and PCr/Pi in right TL as compared with left and ↑ β-ATP in left TL as compared with right; No asymmetries found in TL of controls
Deicken et al. (1994)	Pt = 20M Ctrl = 16M	2.0 T, MRSI	↓ PCr and ↑ PDE in left and right FL in patients; ↓ Pi in left FL in patients; No differences in left or right parietal regions
Fukuzako et al. (1999a)	FE = 10M,7F Ctrl = 10M,7F Antipsychotic-naïve	2.0 T, MRSI	↑ PDE, ↓ PME in TL of drug-naïve patients; ↑ PCr in left TL of patients
Fukuzako et al. (1999b)	Pt = 7M,6F Ctrl = 7M,6F Not medicated	2.0 T, MRSI	↑ PDE in TL of patients prior to medication; haloperidol reduced excess PDE in the left TL of patients
Fukuzako et al. (2002)	Pt = 6M,5F Ctrl = 11M,9F	2.0 T, MRSI	↓ PME in left TL of patients with schizotypal personality disorder
Gangadhar et al. (2004)	Pt = 15M,4F Ctrl = 21M,10F	1.5 T, VOI	↓ PCr/P and PCr/ATP ratios in BG in patients
Hinsberger et al. (1997)	Pt = 8M,2F Ctrl = 9M,1F	2.0 T, VOI	↓ PMEs and ↑ intracellular magnesium in left PFC of patients; No correlation between metabolites and GM volumes
Jayakumar et al. (2003)	Pt = 15M,5F Ctrl = 21M,9F	1.5 T, VOI	↑ PME/PDE ratios in BG of patients; lower ratios associated with lower positive and negative symptom scores
Jayakumar et al. (2006)	Pt = 10M,2F Ctrl = 6M,7F Antipsychotic-naive	1.5 T, VOI	↓ PCr/P, PCr/ATP in caudate nucleus of patients; smaller caudate volume in patients; Age at onset of psychosis positively correlated with PCr/P ratio in left caudate nucleus
Jensen et al. (2002)	Pt = 10M Ctrl = 10M	4.0 T, MRSI	↑GPEth in left Hippo and cerebellum, ↓GPEth in ACC, right PFC and left thalamus, ↓ PtdEth and GPCh in right PFC, ↓ PCh in ACC of patients; No difference between groups in posterior cingulate and parieto-occipital regions
Jensen et al. (2004)	FE = 13M,2F Ctrl = 13M,2F	4.0 T, MRSI	↑ GPCh, ATP, Pi and PCr in anterior cingulate of subjects with first-episode schizophrenia; No difference P-metabolites in thalamus, Hippo, cerebellum, parieto-occipital cortex or PFC
Jensen et al. (2006)	Pt = 11M,1F Ctrl = 9M,2F	4.0 T, MRSI	↓ ATP levels in GM, ↑ ATP levels in white matter in fronto-temporal-striatal region of patients with first-episode schizophrenia; No metabolite differences found between groups in FL
Keshavan et al. (1993)	FE = 7M,2F Antipsychotic-naïve	1.5 T, VOI	PDE concentration significantly correlated with volume of rostral quartile of corpus callosum
Keshavan et al. (2003)	HR = 8M,8F Ctrl = 29M,8F	1.5 T, MRSI	High risk subjects with psychopathology (HR-P) had ↓ freely mobile PME in the PFC; ↑ broad signal under PDE and PME peaks in HR-P subjects
Klemm et al. (2001)	HR = 4M,10F Ctrl = 4M,10F	1.5 T, VOI	↓ PME/PDE ratio, ↑ PDE in FL of subjects with at least one parent or sibling with schizophrenia
Pettegrew et al. (1991)	FE = 7M,4F Ctrl = 6M,4F Antipsychotic-naïve	1.5 T, VOI	↓ PME, Pi in DLPFC of patients; ↑ PDE, ATP in DLPFC of patients
Potwarka et al. (1999)	Pt = 10M,1F Ctrl = 10M,1F	1.5 T, VOI	↓ Pi, PCh in PFC in patients; ↑ mobile phospholipid peak area in PFC of patients; No differences found in parieto-occipital region

Table 4.1-3 (continued)

Author, Year	Sample size	Methods	Summary of findings
Riehemann et al. (2002)	Pt = 42M,30F Ctrl = 19M,13F	1.5 T, VOI	↓ pH in right PFC of patients taking clozapine
Riehemann et al. (1999)	Pt = 31M,20F Ctrl = 19M,13F	1.5 T, VOI	↑ Pi, ↓ PCr in FL of female controls as compared with male controls; no gender difference in patients
Rzanny et al. (2003)	HR = 8M,10F Ctrl = 8M,10F	1.5 T, VOI	↑ PDE and linewidth of broad component in DLPFC of group at high-risk of schizophrenia because of affected family members
Shioiri et al. (1994)	Pt = 16M,10F Ctrl = 16M,10F	1.5 T, VOI	↓ PME in FL of patients with high negative symptom scores on the BPRS compared with low scoring patients and controls
Shioiri et al. (1997)	Pt = 22M,14F Different subtypes	1.5 T, VOI	↓ PME in FL of patients with disorganized schizophrenia; ↓ PME level associated with ↑ motor retardation score; ↑ PDE level associated with ↑ emotional withdrawal and blunted affect
Smesny et al. (2007)	FE = 7M,5F Pt = 8M,11F Ctrl = 15M,16F Not medicated	1.5 T, MRSI	↓ PME, PDE, PCr, Pi in PFC and medial TL, caudate nucleus, thalamus and anterior cerebellum of first-episode and chronic patients; changes associated with BPRS scores
Stanley et al. (1995)	FE = 8M,3F Pt = 17M,1F Ctrl = 17M,4F Antipsychotic-naïve	2.0 T, VOI	↓ PME in left DLPFC of first-episode drug naïve and chronic medicated patients; ↑ PDE in DLPFC of drug-naïve patients compared with controls
Theberge et al. (2004)	Pt = 9M Ctrl = 8M	4.0 T, VOI	Positive correlation between Gln and PtdEth in left ACC of patients; negative correlation between NAA and GPCh in left thalamus of patients; no significant correlations in these regions in controls
Volz et al. (1998)	Pt = 18M,8F Ctrl = 11M,12F	1.5 T, VOI	PCr concentration, PCr/ATP ratio in FL negatively correlated with Wisconsin Card Sort Task score in controls
Volz et al. (1999)	Pt = 5M,3F	1.5 T, VOI	↑ PDE in left FL of patients after initiation of neuroleptic treatment; No changes in other metabolites in left or right FL
Volz et al. (1997)	Pt = 10M,4F Ctrl = 7M,6F	1.5 T, VOI	↓ PDE in DLPFC of patients; ↑ PME/PDE in DLPFC of patients
Volz et al. (1998)	Pt = 31M,19F Ctrl = 20M,16F	1.5 T, VOI	↓ PDE, ↑ PCr and PCr/ATP ratio in DLPFC of patients; ATP positively correlated with chlorpromazine-equivalent dose in patients
Yacubian et al. (2002)	Pt-drug naïve = 9M,9F Pt = 21M,14F Ctrl = 15M,20F	1.5 T, VOI	↓ PDE in left FL of drug-naïve patients; ATP correlated with negative symptoms and neuropsychological impairment in both patient groups
Yao et al. (2002)	FE = 6M,5F Ctrl = 6M,5F Antipsychotic-naïve	1.5 T, MRSI	↓ PDE, PME in PFC of patients; Changes associated with ↓ red blood cell membrane fatty acid content; No correlations found in occipital, inferior parietal, superior temporal regions or BG

have been inconsistent, perhaps due to differences in MRS methodology and patient groups studied (Keshavan et al., 2000); Pi was found to be decreased and β-ATP to be increased in the frontal lobe, which was interpreted as a decreased ATP utilization with a decreased production of Pi (Pettegrew et al., 1991). Another study found decreased frontal Pi and PCr, and no SZ difference in β-ATP (Deicken et al., 1994). Kato and colleagues found an increased PCr in the left frontal cortex and interpreted this as an indicator of a decreased energy demand in this cortical region, since PCr serves as a buffer of ATP and when ATP is not consumed by neuronal activity, PCr is increased (Kato et al., 1995). This is in agreement with a later study of the DLPFC with 50 patients and 36 controls (Volz et al., 1998) that found higher PCr and PCr/ATP in the SZ group with no difference in Pi or α, β or γ – ATP. Yacubian and colleagues studied 53 patients, of whom 18 were drug naïve and 35 healthy controls with a VOI in the left frontal lobe, and found no between-group differences in high-energy phosphates, but found that γ-ATP correlated positively with negative symptoms scores (both BPRS and NSRS) in drug-naïve patients ($r = 0.64$, $p < 0.01$) and that β-ATP correlated negatively with performance in the WCST in previously medicated patients ($r = -0.46$, $p < 0.01$) (Yacubian et al., 2002).

Jensen and coworkers conducted a methodologically sound study at 4 Tesla where they used high-resolution ^{31}P MRSI [chemical shift imaging (CSI)] to obtain spectra from multiple voxels with effective volumes of 15 cm^3 each from 12 FE SZ patients and 11 matched controls; this was combined with anatomical MRI, tissue segmentation, and tissue regression analysis to examine regional between-group differences in gray and white matter of PDEs, PMEs, and high-energy phosphates (Jensen et al., 2006). In this study no group differences were found in PDE or PME, or in PCr, but β-ATP in the fronto-temporal-striatal region was significantly higher in white matter and lower in gray matter in patients compared with controls. According to the authors, the results of their study support a possible widespread tissue-specific pathology compared with matched healthy controls, possibly indicating a global shift in brain energetic functioning upon onset of symptoms. They comment that inconsistencies between studies may be explained by use of mixed tissue voxels, as opposed to tissue regression analysis, in addition to differences in spectral resolution, patient populations, and postprocessing.

5 Future Directions

5.1 Research Directions

The natural abundance of the nonradioactive stable carbon-13 (^{13}C) isotope is 1.108% and this isotope has a low relative gyromagnetic ratio ($\gamma^{13}C/\gamma^{1}H = 0.251$), both of which contribute to low sensitivity; advantageous properties are the broad spectrum range of 200 ppm and narrow line widths (de Graaf, 2008). The low MRS sensitivity of ^{13}C can be overcome by higher field strength, specialized sequences, and foremost, by enrichment of the metabolic substrates with ^{13}C, e.g., [1-^{13}C]glucose and [1-^{13}C]acetate. The intravenous infusion of ^{13}C-enriched substrates results in the time-dependent appearance of the ^{13}C-label in metabolite pools, thereby allowing flux quantification under steady state metabolic conditions (Rothman et al., 2003). ^{13}C MRS studies that combine infusion of both [1-13C]glucose and [2-13C] acetate have allowed the simultaneous study of metabolic fluxes through the Glu–Gln, GABA–Gln, and glucose oxidation cycles (Hyder et al., 2006) (*Figure 4.1-2*). The findings from a number of ^{13}C MRS studies on rats and humans are that the neurotransmitter cycles are tightly coupled to each other and to glucose oxidation, and that fluxes through glutamatergic and GABAergic systems (and neurotransmitter concentrations) change commensurately with neuronal activity (Lebon et al., 2002; Rothman et al., 2003; Patel et al., 2005; Hyder et al., 2006). Current models of schizophrenia emphasize abnormalities in glutamatergic and GABAergic neurotransmission and disturbances in brain energy metabolism (Prabakaran et al., 2004), but ^{1}H MRS can only provide static measures of Glu, Gln, and GABA levels that cannot address potential dynamic disturbances in the interrelated glutamate–glutamine cycle, the GABA–glutamine cycle, and in the coupling of neurotransmitter cycling to neuroenergetics. ^{13}C MRS has the potential to study these dynamic processes directly, but no studies of people with schizophrenia have been published to date. Several practical issues that have slowed progress in this area are the long scanning time required to collect data, need for two

IV lines and a two-hour infusion, high cost of ^{13}C-enriched substrates, and sample size considerations: studies with healthy volunteers had $N < 10$, but this may not be enough to detect differences in the metabolic models between groups of patients and healthy volunteers.

Signal-to-noise ratio and metabolite peak separation in ^{1}H MRS increase linearly with the increase of B_0 and they can be improved by conducting studies in humans at higher magnetic fields; for ^{31}P these gains may be better than linear (Tkac et al., 2001; Ugurbil et al., 2003). The higher SNR and spectral separation are accompanied by a number of engineering and technical challenges related to obtaining magnetic field homogeneity and postprocessing, but higher fields, such as 7T or 9.4 T will allow experiments to be conducted faster and on smaller VOI's. Regardless of that, MRS will remain a rather crude tool: its sensitivity for ^{1}H metabolites will still be in the millimolar, to, at best, high micromolar range, and even the smallest VOI cannot follow the spatial complexity of brain structures. The upper limit of the magnetic field in human imaging depends on concerns about patient safety such as RF tissue heating; magnets at 3 T are FDA approved, and at the time of writing, the FDA categorized clinical MR systems with a static magnetic field of less than or equal to 8 T as posing "nonsignificant risk" for patients over 1 month old (www.fda.gov). Initial in vivo MRI studies at 9.4 T of 44 healthy volunteers indicate that safe and successful human head imaging is feasible at this field strength (Vaughan et al., 2006). A major advantage of ultrahigh magnetic field imaging is that it makes possible novel multimodal MRS approaches or considerably expands the scope of such approaches to human imaging. Prior to the introduction of ^{1}H MRS at 7 T, functional MRS in humans was limited to the study of lactate increase during visual stimulation; the increased SNR and spectral separation at 7 T allowed Mangia and coworkers to measure the changes in the concentrations of 17 metabolites in the human visual cortex during two short paradigms of visual stimulation lasting 5.3 and 10.6 min (Mangia et al., 2007). MRS was accompanied by anatomical MRI and was preceded by a functional MRI task that was used to localize the VOI. MRS provided dynamic functional data on the increase of not only lactate but also glutamate and the decrease of aspartate and glucose during visual activation; no changes were detected for glutamine, GABA, glutathione, NAA, NAAG, inositol, and choline; the study reported detectable changes in metabolite concentrations of 0.2 μmol/g = 0.2 mM, which is only a 3% change of Glu concentration. Functional ^{1}H MRS at ultrahigh magnetic field strength may be feasible in the study of higher cognitive functions, such as comparing SZ and healthy groups on the changes in Glu/Gln and GABA in the DLPFC during a working memory task. The metabolic and neurotransmitter response to a higher cognitive task, however, will not be as robust as the changes during visual stimulation, since the difference in BOLD signal increase during a simple sensory task and higher cognitive task may be about 30:1 (Huettel et al., 2004). Another interesting recent study that was performed at a standard field strength of 3 T illustrates the potential of a multimodal approach to determine relationships between functional MRI BOLD phenomena and metabolite concentrations in a region of interest (Northoff et al., 2007). The authors found that in healthy volunteers a negative fMRI BOLD response in the anterior cingulate cortex during emotional processing was correlated with MRS GABA concentration at rest, obtained from the same region, but not with Glx concentration.

The low sensitivity of MRS results from the low magnetic energy of nuclear spins compared with the thermal energy at room temperature, leading to very low spin polarization. Even at the limits of increase of the external magnetic field, human MRS will still operate in the high micromolar range and functional MRS will have a temporal resolution in the minute range, whereas important processes in the brain related to neurotransmission and neuroenergetics occur in the nanomolar range of concentration changes and in a time range of seconds. A number of methods have been proposed to enhance the nuclear spin polarization and these "hyperpolarization" methods have begun to show potential for in vivo research. Two novel methods of hyperpolarization of the ^{13}C nucleus, dynamic nuclear polarization (DNP) (Ardenkjaer-Larsen et al., 2003; Golman et al., 2003) and "parahydrogen and synthesis allow dramatically enhanced nuclear alignment" (PASADENA) (Bowers and Weitekamp, 1986, 1987) provide a ^{13}C NMR signal enhancement in excess of 10,000-fold compared with natural polarization and offer the potential for in vivo measurement of nanomolar quantities of metabolites and metabolic reaction rates in seconds; a number of animal studies have been published and there are no significant human safety concerns that would preclude these methods from being used in human research and clinical applications (Bhattacharya et al., 2005, 2007; Golman et al., 2006a,b; Olsson et al., 2006).

5.2 MR Spectroscopy in Schizophrenia Diagnosis and Drug Discovery

MRS is used in the clinical setting in neurology and oncology, but in psychiatry and schizophrenia research it remains a research method. This is due to limitations inherent in psychiatric diagnosis: schizophrenia is diagnosed on purely clinical grounds, yet none of its clinical features is pathognomonic (Carpenter and Buchanan, 1994). The diagnostic criteria in DSM IV-TR and other diagnostic systems require a combination of psychotic, disorganized, and negative symptoms, and deterioration of occupational and social functioning continuously for a period of time (American Psychiatric Association, 2000). In addition to the clinical hetrerogeneity, the pathogenesis of SZ is not clear and there may be different subtypes with different mechanisms and different MR imaging and spectroscopy correlates. In spite of some promising findings discussed in this chapter, neither MRS nor any other brain imaging modalities are currently used as a diagnostic or prognostic tool.

MRS studies of the glutamate system in schizophrenia are consistent with the NMDA receptor hypofunction hypothesis, suggesting that the enhancement of NMDA receptor function could be a target for new antipsychotic compounds. However, drugs that act through agonism at the receptor's glycine binding site, such as glycine and D-serine, lead to only a modest improvement in negative symptoms when used as add-on agents to conventional antipsychotic drugs (Tuominen et al., 2005; Shim et al., 2008) (Also see Javitt in this volume). Lamotrigine was developed as an anticonvulsant drug that acts on glutamate neurotransmission and it also reduced ketamine-induced positive schizophreniform symptoms in healthy subjects. This generated interest in testing lamotrigine in treatment-resistant SZ patients who were already taking clozapine, and the results are encouraging, but mixed. A randomized controlled trial showed improvement in both positive and negative symptoms (Tiihonen et al., 2003); this was replicated by another trial, but only for study completers (Kremer et al., 2004). The most recent randomized controlled trial (Goff et al., 2007) found improvement of cognition with lamotrigine, but no significant effect on positive or negative symptoms. Extensive work is currently conducted on developing novel SZ drugs that modulate glutamate neurotransmission and are based on neurochemical knowledge specific to schizophrenia. Recently, a phase 2 proof-of-concept clinical trial was reported that tested an amino acid analog that is an agonist at the metabotropic receptors mGlu2/3 (Patil et al., 2007), and found it to be safe, and equally effective as olanzapine. A number of other glutamatergic and GABAergic SZ agents are in development, and ^{1}H MRS can play a role as a platform to assess the effect of glutamatergic and/or GABAergic candidate drugs on the regional concentrations of Glu, Gln, and GABA in the brains of healthy individuals during the early stages of drug development.

Acknowledgments

This work was supported by a NARSAD Young Investigator Award to Peter Milev and by a K12 Clinical Research Career Development Award from the National Institutes of Health, National Institute of Child Health and Human Development (NIH, NICHHD), Grant number 5K12RR023247–03.

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4.2 Alterations of Neurotransmitter Receptors in Schizophrenia: Evidence from Postmortem Studies

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_4.2,

List of Abbreviations: α-Bgtx, α-bungarotoxin; 5HT, serotonin; 7TM, seven-transmembrane; AC, adelylate cyclase; ACC, anterior cingulate cortex; AChR, Acetylcholine receptor; ADRA1A, α1A-adrenergic receptor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; αAR, α-adrenoreceptor; βAR, β-adrenoreceptor; BZ, benzodiazepines; cAMP, cyclic adenosine monophosphate; CB, cannabinoid receptor; CNS, central nervous system; DA, dopamine; DAG, diacylglycerol; DLPFC, dorsolateral prefrontal cortex; DSE/DSI, depolarization-induced suppression of activation/inhibition; EAAT, excitatory amino acid transporter; ER, endoplasmic reticulum; GABA, γ-aminobutyric acid; GPCR, G-protein coupled receptor; IAR, immunoautoradiography; iGluR, ionotropic glutamate receptor; IP3, inositol 1,4,5 triphosphate; LSD, D-lysergic acid diethylamide; mAChR, muscarinic acetylcholine receptor; mgluR, metabotropic glutamate receptor; NA, neurokinin A; nAChR, nicotinic acetylcholine receptor; NB, neurokinin B; NF-L, neurofilament light chain; nM, nanomolar; NMDA, N-methyl-D-aspartate; NSF, N-ethylmaeimide-sensitive fusion protein; OC, occipital cortex; PCC, posterior cingulate cortex; PDZ, postsynaptic density protein, Drosophila disc large tumor suppressor, Zonula occludens 1 protein; PFC, prefrontal cortex; PI, phosphatidyl-inositol; PKC, protein kinase C; PLC, phospholipase C; PSD, postsynaptic density; RGS, Regulators of G protein signaling; SN, substantia nigra; SP, substance P; Stg, stargazin; TG, temporal gyrus; VTA, ventral tegmental area

1 Introduction

Humans have long searched for the origins of distinctive traits including character and emotion. Central to these investigations have been studies of the human brain. Following the first clinical characterization of schizophrenia by Kraepelin (1887) as a unique psychiatric illness and the realization that mental illness resides in select areas of the brain (Crichton-Browne, 1879), the use of postmortem brain to study the pathophysiology of psychiatric illness has significantly advanced our understanding of these disorders.

The absence of animal models to reproduce all aspects of schizophrenia makes the study of human brains from patients suffering from this psychiatric illness essential to advance our knowledge of its underlying pathology. Studies using neuroimaging of patients with schizophrenia, although extremely helpful and informative, do not inform molecular and cellular aspects of this illness. Thus, postmortem studies are central to the investigation of the neurochemical basis of schizophrenia, as well as to the identification of cell-level alterations in gene expression associated with this illness.

Schizophrenia likely results from a combination of environmental factors and genetic susceptibility (Tamminga and Holcomb, 2005). In addition, changes in biochemical markers for multiple neurotransmitter systems have been implicated in schizophrenia, thus linking genetic and environmental risk factors with altered neurotransmission. Studies of altered neurotransmission are essential to better understand the underlying pathophysiological mechanisms of schizophrenia. In addition to revealing the abnormalities of cellular function and circuitry, such studies contribute to identify new pharmaceutical targets for improved treatment of patients with schizophrenia.

In this chapter, we critically review evidence from postmortem human brain implicating molecular alterations of neurotransmission in schizophrenia, focusing on expression abnormalities of receptors and their associated intracellular signaling complexes for the principal CNS neurotransmitters.

1.1 Dopamine Receptors

1.1.1 The Dopamine System

Based on early observations that therapeutic effects of neuroleptic drugs were principally mediated by antagonizing dopamine function in the CNS, dopamine (DA) has received much attention in

schizophrenia research, leading to the formulation of the "dopamine hypothesis of schizophrenia" (Seeman, 1987; Davis et al., 1991; Baumeister and Francis, 2002). Additional support for this hypothesis has been provided by observations that the administration of substances such as amphetamine and cocaine that cause a marked increase in synaptic dopamine can cause positive psychotic symptoms indistinguishable from paranoid schizophrenia in healthy subjects (Berger, 1981; Ellison and Eison, 1983). These observations led to the hypothesis that schizophrenia was an illness of excessive DA. However, this view has been refined to a model of selective increased and decreased DA in distinct areas of the CNS, causing an imbalance in dopaminergic activity between different brain regions (Abi-Dargham and Laruelle, 2005).

DA projections primarily originate from the substantia nigra (SN) and ventral tegmental area (VTA), both small nuclei located in the midbrain. Neurons of the SN project through the nigrostriatal pathway, conveying dopaminergic input to sensorimotor and the associative striatum that are involved in control of voluntary movement, reflexes, and modulation of the reward system. VTA neurons project more diffusely through the mesolimbic and mesocortical pathways to subcortical and cortical structures, including the amygdala, the hippocampus, the nucleus accumbens, and the prefrontal cortex (PFC) (Greene, 2006). Dopaminergic VTA projections are involved in limbic functions such as control of higher order motor execution, emotions, reward, attention, motivation, and cognition (Sesack and Carr, 2002; Greene, 2006). Based on these functional distinctions, it is not surprising that abnormalities of VTA-mediated DA neurotransmission, especially of the mesocortical pathway, are often implicated in neuropsychiatric disorders including schizophrenia (Yang et al., 1999; Seamans and Yang, 2004).

Depending on the local neurochemical signaling environment and cellular receptor composition, DA likely has differential modulatory effects on distinct neurons, and cellular functions mediated by DA, therefore, might work in opposite directions (Lapish et al., 2007). The seven-transmembrane (7TM), G-protein coupled receptors for DA constitute five different receptors (D_1, D_2, D_3, D_4, and D_5), that, based on their pharmacology, G-protein coupling, and structural characteristics, are divided into two major groups: the D_1-type (D_1 and D_5) and D_2-type (D_2-D_4) of receptors. In addition to distinct pharmacological profiles, D_1 and D_2 type DA receptors differ in the way they couple to the $G_S\alpha/G_{olf}\alpha$- (D_1) or $G_{i/o}$- (D_2) intracellular G-proteins, which consequently leads to either an activation or an inhibition of adenylyl cyclase activity (Jaber et al., 1996; Sidhu and Niznik, 2000; Neve et al., 2004).

The expression of DA receptors varies greatly in different brain regions. D_2-type receptors are expressed in presynaptic neurons and function as autoreceptors, while both D_1 and D_2 subtypes are located postsynaptically (Filloux et al., 1988; Geldwert et al., 2006). In the brain, the D_1 receptor is expressed at higher levels than any other DA receptor, with high expression of both transcript and protein in the nucleus accumbens, the striatum, the neocortex as well as the hippocampus, with lower expression in the thalamus and the amygdala (Fremeau et al., 1991; Gingrich et al., 1992; Ariano and Sibley, 1994; Meador-Woodruff et al., 1994; Meador-Woodruff et al., 1996; Ariano et al., 1997). The D_5 receptor is expressed at lower levels than the D_1 receptor, with expression in the cortex, the hippocampus, and the thalamus, and a very low expression in the striatum (Huntley et al., 1992; Meador-Woodruff et al., 1992; Choi et al., 1995). Despite their anatomically overlapping expression, D_1 and D_5 are differentially expressed at the ultrastructural level, likely reflecting differences in their cellular functions (Bergson et al., 1995).

Highest D_2 receptor expression is found in the striatum and SN, with lower levels in the nucleus accumbens, the cortex, the amygdala, the thalamus, the hippocampus, the hypothalamus, and in the VTA (Ariano et al., 1992; Ariano et al., 1993; Fisher et al., 1994). The D_3 receptor is more restricted in its expression, with highest levels within the mesolimbic system including the nucleus accumbens, SN, VTA, and lower level expression in the hippocampus and the globus pallidus (Joyce et al., 1997). Finally, D_4 receptors are expressed in limbic regions at relatively high levels, including the frontal cortex and the amygdala and lower levels in the thalamus, the globus pallidus, the hippocampus; it is debatable whether D_4 is expressed at all in the striatum (Joyce and Meador-Woodruff, 1997; Missale et al., 1998). In summary, the anatomical distribution of DA receptors is heterogeneous in the brain, with overlapping expression of most receptors in many brain regions. However, a tendency for higher D_2 expression in the striatum, combined with relatively higher expression of D_3 and D_4 type receptors in mesolimbic and mesocortical pathways, has

received attention due to the potential to separate therapeutic effects of antipsychotic treatment from drug-induced extrapyramidal side-effects (Hartman and Civelli, 1996).

1.1.2 Dopamine Receptor Expression in Schizophrenia

As is also true for other neurotransmitters, comparisons of transcript versus protein expression of the different DA receptors can be discordant due to the complex projection pattern of the different DA pathways and differences in pre- and postsynaptic receptor expression. Studies of expression at the transcriptional level in schizophrenia can reflect protein expression in that particular structure as well as in other regions of the brain with efferent innervation from the region analyzed. Interpretation of these results should therefore be considered in this context.

1.1.2.1 Cortex Postmortem studies have, in addition to striatal expression, also focused on DA receptor expression in frontal and temporal cortices, with a few studies in the hippocampus and the thalamus (*Table 4.2-1*). In cortex, abnormalities of transcript expression for D_1, D_2, and D_5 have not been found in schizophrenia (Roberts et al., 1994; Meador-Woodruff et al., 1997). Two studies have found a decreased cortical expression of the D_3 receptor transcript (Schmauss et al., 1993; Meador-Woodruff et al., 1997), while postmortem D_4 receptor transcript expression was reported either decreased (Meador-Woodruff et al., 1997), increased (Stefanis et al., 1998), or unchanged (Mulcrone and Kerwin, 1996; Roberts et al., 1996).

Studies of cortical transcript expression and receptor autoradiography are not consistent. Thus, one study reported an imbalance of D_2/D_1 receptor binding in the cortex with a concomitantly increased binding to D_2-type receptors and a decreased D_1 binding (Hess et al., 1987). However, other studies have reported no change in D_2, D_3, and D_4 cortical binding but an increased [^{3}H]SCH23390 binding to D_1 receptors in schizophrenia (Ruiz et al., 1992; Knable et al., 1996; Lahti et al., 1996). Finally, an increased D_2 binding in the granular layers, and a decreased binding in supragranular layers of the temporal cortex may reflect altered cortical neuronal organization in schizophrenia (Goldsmith et al., 1997).

1.1.2.2 Striatum Many postmortem studies have focused on DA receptor expression in the striatum, due to its high expression of D_1 and D_2 receptors, the importance of D_2 receptors in the treatment of schizophrenia, and the involvement of the striatum in generating extrapyramidal side effects. Several studies of striatal expression of both D_1- and D_2-type receptors in postmortem brain did not find altered expression of transcripts for any of these receptors in schizophrenia (*Table 4.2-1*) (Roberts et al., 1994, 1996; Harrington et al., 1995; Meador-Woodruff et al., 1997; Stefanis et al., 1998). In addition, one study measured D_2 protein expression, reporting unaltered levels of this receptor in the caudate and putamen (Dean et al., 2004).

Contrary to the analysis of transcript and protein expression, measurement of DA receptor expression by ligand-autoradiography in postmortem brain has generally found an increased D_2 or D_2-type receptor binding (Owen et al., 1978; Mackay et al., 1982; Seeman et al., 1987; Ruiz et al., 1992; Seeman et al., 1993; Reynolds and Mason, 1994), once thought to be due, in particular, to an increase in the D_4 receptor subtype (Seeman et al., 1993; Murray et al., 1995; Sumiyoshi et al., 1995). Only one study has specifically reported on D_3 expression in the striatum in postmortem brain, finding an increased [^{125}I]ltrans-7OH-PIPAT binding, which may have been caused by an antemortem antipsychotic treatment (Gurevich et al., 1997). Finally, several studies have failed to find significantly altered D_2-type receptor abnormalities in the striatum (Knable et al., 1994; Dean et al., 2004). Among the D_1 type receptors, only the D_1 receptor has been measured using ligand binding autoradiography, with two studies reporting unaltered striatal expression (Seeman et al., 1987; Knable et al., 1994).

1.1.2.3 Thalamus, Hippocampus Few postmortem studies have focused on the expression of DA receptors in the thalamus and hippocampal formation (*Table 4.2-1*). One study reported a decreased number of cells positive for D_1 transcripts in the CA3 hippocampal subfield, but with no change in D_1

Table 4.2-1
Dopamine D_1-(D_1/D_5) and D_2-type (D_2/D_4) receptor abnormalities in schizophrenia

D_1/D_5 receptors	D_2/D_3/D_4 receptors	References
Cortex		
Transcripts		
	↓ D_3 + D_4	Meador-Woodruff et al. (1997)
– D_1, D_5	– D_2	
	↑ D_4	Stefanis et al. (1998)
	↓ D_3	Schmauss et al. (1993)
	– D_4	Roberts et al. (1996)
	– D_2	Roberts et al. (1994)
	– D_4	Mulcrone and Kerwin (1996)
Autoradiography		
	↓ [^{125}I]epidepride (D_2) TG, supragranular layer ↑ [^{125}I]epidepride (D_2) TG, granular layer	Goldsmith et al. (1997)
↑ [^{3}H]SCH23390 (D_1)		Knable et al. (1996)
	– [^{3}H]YM-09151–2 minus [^{3}H]raclopride (D_3, D_4)	Lahti et al. (1996)
	– [^{3}H]raclopride (D_2, D_3)	Ruiz et al. (1992)
↓ [^{3}H]SCH23390 (D_1)	↑ [^{3}H]spiperone (D_2)	Hess et al. (1987)
Striatum		
Transcripts		
– D_1, D_5	– D_2, D_3, D_4	Meador-Woodruff et al. (1997)
	– D_4	Stefanis et al. (1998)
– D_1	– D_2	Harrington et al. (1995)
	– D_4	Roberts et al. (1996)
	– D_2	Roberts et al. (1994)
Protein analysis		
	– D_2	Dean et al. (2004)
Autoradiography		
	↑ [^{3}H]YM-09151–2 minus [^{3}H]raclopride (D_4) likely abnormal G-protein coupling	Sumiyoshi et al. (1995)
	↑ [^{3}H]YM-09151–2 minus [^{3}H]raclopride (D_4)	Murray et al. (1995)
– [^{3}H]SCH23390 (D_1)	– [^{3}H]raclopride (D_2, D_3)	Knable et al. (1994)
	↑ [^{3}H]emonapride (D_2, D_3) – [^{3}H]emonapride minus raclopride (D_4)	Reynolds and Mason (1994)
	↑ [^{3}H]raclopride (D_2, D_3) ↑ [^{3}H]emonapride minus [^{3}H]raclopride (D_4)	Seeman et al. (1993)
	↑ [^{3}H]raclopride (D_2, D_3)	Ruiz et al. (1992)
	↑ [^{3}H]spiperone (D_2)	Mackay et al. (1982)
	↑ [^{125}I]iodosulpride minus haloperidol (D_2) – [^{125}I]iodosulpride (D_2, D_3) ↓ [^{3}H]spiperone (D_2-like receptors)	Dean et al. (2004)
– D_1	↑ D_2	Seeman et al. (1987)
	↑ [^{125}Itrans-7-OH-PIPAT (D_3) – drug effect	Gurevich et al. (1997)
	↑ [^{3}H]Spiroperidol (DA receptors)	Owen et al. (1978)

continued

Table 4.2-1 (continued)

Hippocampus		
Transcripts		
↓ D_1 (no change in grains/cell – CA3) ↓ fewer D_1 positive cells (CA3)		Pantazopoulos et al. (2004)
Autoradiography		
	– [^{125}I]epidepride (D_2) altered laminar pattern	Goldsmith et al. (1997)
Thalamus		
Transcripts		
– D_1, D_5	– D_2, D_3, D_4	Clinton et al. (2005)
Autoradiography		
– [^{3}H]SCH23390 (D_1)	– [^{3}H]raclopride, 7-OH-DPAT (D_2) – [^{3}H]PD$_1$28907 (D_3)	Clinton et al. (2005)

Data for each anatomical region are subdivided into transcript, protein, and autoradiography findings, when available. Arrows (↑↓) and line (–) indicate changes (increase, decrease, and no changes, respectively). For autoradiography studies, ligand and isotope used are indicated, followed by receptor subtypes labeled under study conditions, as reported by authors. TG temporal gyrus, trans-7-OH-PIPAT trans-7-hydroxy-2-(*N*-n-propyl-*N*-3′-iodo-2′-propenyl) aminotetralin, 7-OH-DPAT 7-hydroxy-dipropylaminotetralin

transcript per cell (Pantazopoulos et al., 2004). In a different study, [^{125}I]epidepride (D_2-type) binding in the hippocampus was measured in postmortem brain, finding no changes in binding but an altered laminar pattern, possibly reflecting morphological changes in schizophrenia (Goldsmith et al., 1997). In addition, no changes in the thalamic expression of transcripts for all DA receptors and D_1, D_2, D_3 binding were detected in a recent study (Clinton et al., 2005). These studies of hippocampal and thalamic DA receptor expression are in agreement with the hypothesis of altered neurotransmitter circuitry in cortical-temporal areas, which cause imbalanced neurotransmission between the cortical and subcortical areas.

Interestingly, several recent postmortem studies have presented evidence for the abnormal expression of DA receptor interacting proteins in schizophrenia, suggesting complex altered functionality of the DA system. These studies have reported an increased cortical expression of calcyon and NCS-1, both proteins that are involved in regulating Ca^{2+} responses following DA receptor signaling (Koh et al., 2003; Bai et al., 2004; Clinton et al., 2005; Baracskay et al., 2006), and a decreased expression of the NGF1-B and Nurr1 transcription factors that have been implicated in specific DA mediated regulation (Xing et al., 2006). Also, cortical expression of DA receptor interacting G-protein regulatory proteins, including RGS4 and RGS9–2, has recently been reported to be decreased in schizophrenia (Erdely et al., 2006; Seeman et al., 2007). These data add to the body of literature arguing for altered function of the cortical DA system in schizophrenia, suggesting multiple levels of dysfunction at the cellular level.

In summary, alterations of DA receptor expression and binding are relatively consistent for studies reporting D_2-type changes in the striatum, while studies of other regions, including cortex, have been equivocal.

1.2 Glutamate Receptors

Altered glutamate function has been implicated in the pathophysiology of schizophrenia by a number of clinical observations and research findings (See Javitt this Volume) (Coyle, 2006). The administration of ketamine, a potent inhibitor of glutamate signaling, to healthy individuals induces a schizophreniform psychosis that can be indistinguishable from schizophrenia, and in patients with schizophrenia, NMDA

receptor antagonism can worsen both positive and negative symptoms (Lahti et al., 2001; Tamminga et al., 2003). Based on these observations, it is not surprising that molecular alterations of glutamate signaling have been studied in great detail in postmortem brain in schizophrenia. Such studies have led to the formulation of a hypothesis for glutamatergic hypofunction in schizophrenia (Tamminga, 1998), which in recent years has been substantiated by observations in postmortem brain of compromised expression with implications for cellular function of receptors and intracellular mediators of glutamate signaling. The importance of glutamate-mediated excitatory neurotransmission for brain function is illustrated by the ubiquitous expression of receptors for this neurotransmitter in the brain (Henneberry, 1992; Breese and Leonard, 1993; Brose et al., 1993). Glutamate-mediated signaling constitutes an estimated 70–80% of all resting brain activity, and the majority of neurons in the CNS either release or are receptive to glutamate (Erecinska and Silver, 1990; Gasic and Heinemann, 1991).

1.2.1 The Glutamate System

Based on their primary signal transduction mechanisms, receptors for glutamate are divided into two major groups: the ionotropic glutamate receptors (iGluR), which function as selective membrane ion channels, and the metabotropic glutamate receptors (mGluR), which signal through G-protein-activated second messenger pathways (Simeone et al., 2004; Kew and Kemp, 2005). Differences in their affinity for artificially derived glutamate analogs (iGluRs) or structural homology, pharmacological properties, and signal transduction pathways (mGluRs) have been used to further classify each receptor type into subcategories (Masu et al., 1993). For iGluRs, these groups are NMDA (*N*-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid), and kainate types of receptors (Bochet and Rossier, 1993; Mayer, 2005), and mGluRs are subdivided into group I, group II, and group III mGluR subtypes (Michaelis, 1998; Trist, 2000; Pellicciari et al., 2001; Coutinho and Knopfel, 2002; Kew and Kemp, 2005).

The AMPA and NMDA iGluRs are principally expressed on dendritic spines of postsynaptic neurons in the CNS, often within the same signaling complex (Kharazia et al., 1996; Conti et al., 1999; Takumi et al., 1999; Suzuki et al., 2001; Luo et al., 2002; Huang et al., 2003). Coexpression of AMPA and NMDA receptors in the postsynaptic membrane reflects their coupled signaling functions, such as during induction of synaptic plasticity, including long-term modulation of synaptic strength (Asztely and Gustafsson, 1996; Shapiro, 2001; Malenka and Bear, 2004; Simeone et al., 2004).

Besides AMPA and NMDA receptors, kainate receptors and mGluRs are also coexpressed in the postsynaptic membrane where they function to modulate an integrated synaptic response to glutamate (Van den Pol, 1994; Haak, 1999; Krieger et al., 2000; Mannaioni et al., 2001; Pisani et al., 2001; Skeberdis et al., 2001; Huettner, 2003; Coussen et al., 2005; Ruiz et al., 2005). Additionally, kainate and mGluRs expressed by the surrounding glia and in presynaptic neurons modulate cellular function, including glutamate reuptake, presynaptic excitability, and release of glutamate (Cartmell and Schoepp, 2000; Huettner, 2003; Grueter and Winder, 2005; Rodriguez-Moreno, 2006; Zhou et al., 2006).

In the postsynaptic terminal, receptors for glutamate are mostly expressed in a region termed the postsynaptic density (PSD) due to its dense structural appearance from electron microscopy (Collins et al., 2006). An essential role of this intricate molecular network is the establishment of a scaffold that functions to stabilize receptors in the cell membrane and bring different types of receptors together in organized postsynaptic signaling complexes, thereby linking and coordinating signaling through different postsynaptically expressed iGluRs and mGluRs (Coutinho and Knopfel, 2002; Mao and Wang, 2002; Huettner, 2003). Several of these PSD associated proteins have been extensively studied in schizophrenia.

1.2.1.1 NMDA Receptors The NMDA receptor consists of a multimeric assembly of different subunits, with the obligatory NR1 subunit in combination with one or several of the NR2A-D or NR3 subunits (Monyer et al., 1992), each subunit combination conferring distinct functional properties to the receptor (Wafford et al., 1993; Cull-Candy et al., 2001; Loftis and Janowsky, 2003). In addition, alternative splicing of the NR1 gene gives rise to eight spliceforms, contributing additional functional complexity to this receptor system (Stephenson, 2001). The activation of the NMDA receptor involves concomitant agonist binding to its

glycine and glutamate sites in addition to depolarization-dependent removal of its intrachannel Mg^{2+} block. In addition to flow of ions through the receptor, activation of the NMDA receptor involves, signaling mediated by its associated PSD proteins that function as kinases as well as binding partners for multiple other intracellular signaling molecules.

1.2.1.2 AMPA – Kainate Receptors The AMPA and kainate glutamate receptors consist of assemblies of closely related subunits (AMPA: GluR1–4; Kainate: GluR5–7, KA1,2) that together form membrane-spanning cation channels with selective permeability. The regulation of AMPA receptor expression at the PSD is controlled by two distinct mechanisms termed the constitutive and regulated pathways, the latter of which is regulated by the phosphorylation and selective binding of intracellular PDZ proteins (Post synaptic density protein, *Drosophila* disc large tumor suppressor, Zonula occludens 1 protein) (Srivastava and Ziff, 1999; Braithwaite et al., 2002; Lee et al., 2004; Park et al., 2004; Lu and Ziff, 2005; Zhao et al., 2007). Emerging evidence suggests that the trafficking of kainate receptors is also involved in controlling synaptic strength through phosphorylation and binding to PDZ proteins (Hirbec et al., 2003; Coussen and Mulle, 2006; Mellor, 2006).

1.2.1.3 Metabotropic Glutamate Receptors Metabotropic glutamate receptors are members of a large 7-transmembrane G-protein coupled receptor family that also include the $GABA_B$, pheromone, and Ca^{2+} sensing receptors (Pin et al., 2003). Group I mGluRs, which consist of mGluR1 and mGluR5, primarily couple to phospholipase C (PLC) via G-protein activation with subsequent inositol 1,4,5 triphosphate (IP_3) formation and Ca^{2+} release from intracellular stores (Hermans and Challiss, 2001). Dissimilar to group I, group II (mGluR2, mGluR3), and group III (mGluR4, 6, 7, 8) mGluRs do not associate with the IP_3 pathway, but couple to inhibitory G-proteins, which leads to the inhibition of adenylyl cyclase activity and a reduced cAMP formation (Conn and Pin, 1997). Several alternatively spliced isoforms have been identified for the mGluRs (Pin et al., 2003), which determine receptor functions including surface expression, G-protein coupling, agonist potency, receptor targeting, and interaction with postsynaptic scaffolding proteins that connect them to other receptor complexes (Stowell and Craig, 1999; Tu et al., 1999; Hermans and Challiss, 2001).

1.2.2 Glutamate Receptor Expression in Schizophrenia

Postmortem studies of molecules involved in glutamate transmission have found abnormal expression at the transcript and protein levels in schizophrenia. However, these changes appear to be regional in nature with most changes localized to areas of the frontal cortex, the thalamus, and the limbic structures, with few findings of altered expression in the basal ganglia.

1.2.2.1 Cortex The expression of the predominant cortical NR2 NMDA receptor subunits, NR2A and NR2B, and the less abundantly expressed NR2C and NR2D subunits has, with few exceptions, been reported unchanged in the cortex in schizophrenia (*Table 4.2-2*) (Akbarian et al., 1996; Meador-Woodruff and Healy, 2000; Dracheva et al., 2001; Toro and Deakin, 2005; Kristiansen et al., 2006; Beneyto et al., 2007; Kristiansen et al., 2007). A small increase in transcripts for the NR2A subunit in the occipital cortex (OCC) (Dracheva et al., 2001), and a decreased transcript expression for the less abundantly expressed NR2C subunit in the frontal pole, which was additionally associated with an increase in the proportion of the NR2D subunit, have been reported (Akbarian et al., 1996). Additionally, one study reported a decreased expression of NR2A subunits in GABAergic cells in cortical layers II and V in the anterior cingulate cortex (ACC) (Woo et al., 2004).

Several studies have analyzed the cortical expression of the NR1 subunit in postmortem brain (*Table 4.2-2*). These studies report conflicting results, including both an increased and a decreased NR1 transcript expression in temporal cortex, OCC, and Dorsolateral prefrontal cortex, DLPFC (Humphries et al., 1996; Sokolov, 1998; Le Corre et al., 2000; Dracheva et al., 2001). However, other studies have reported unaltered NR1 transcript levels in PFC and temporal cortices (Akbarian et al., 1996; Beneyto et al., 2007). In addition, the expression of total NR1 protein in both frontal and temporal cortices was not significantly

Table 4.2-2
Glutamate receptor expression in cortex in schizophrenia

Cortex				
NMDA	**AMPA**	**Kainate**	**mGluRs**	**References**
Transcripts				
– NR1, NR2A-D	– GluR1–4	↓ GluR5 – GluR6/7, KA1/2		Beneyto et al. (2007)
	– GluR1–4 (flip/flop isoforms)			O'Connor et al. (2007)
			↓ RGS4	Bowden et al. (2007)
			– RGS4	Lipska et al. (2006)
↑ PSD95, PSD93 (ACC), NF-L (DLPFC)				Kristiansen et al. (2006)
– PSD95, PSD93 (ACC), NF-L (DLPFC), SAP102 (ACC, DLPFC)				
	↓ GluR2, GluR4, PICK1			
		– GluR1, GluR3, NSF, syntenin		Beneyto and Meador-Woodruff (2006)
	↑ Stargazin			
		↓ GluR5 – GluR6/7, KA1/2		Scarr et al. (2005)
	↑ GluR1 (DLPFC), ABP (OC), GluR4, GRIP (OC, DLPFC) – GluR1(OC), ABP (DLPFC), GluR2, GluR3, SAP97, PICK1 (DLPFC, OC)			Dracheva et al. (2005)
– NR2A, PSD95	– GluR1, GluR2, GluR4	– GluR5	– mGluR1	Hemby et al. (2002)
↓ NR1	↓ GluR3			
↑ NR1 (DLPFC/OC), NR2A, PSD95 (OC) – NR2B (DLPFC/OC)				Dracheva et al. (2001)
			↓ RGS4	Mirnics et al. (2001)
	– NSF			Imai et al. (2001)
		– GluR5, GluR6, KA1		
		↑ GluR7		Meador-Woodruff et al. (2001)
		↓ KA2		
	↓ NSF			Mirnics et al. (2000)

continued

Table 4.2-2 (continued)

Cortex				
↑ NR1^{C1} spliceform				Le Corre et al. (2000)
↓ PSD95				Ohnuma et al. (2000a)
↓ NR1	↓ GluR1	↓ GluR7, KA1		Sokolov (1998)
	– GluR1–4			Healy et al. (1998)
			↑ mGluR5	Ohnuma et al. (1998)
			– mGluR3	
↑ Proportion of NR2D				
↓ NR2C				Akbarian et al. (1996)
– NR1, NR2A, NR2B, NR2D				
↓ NR1				Humphries et al. (1996)
Protein analysis				
		↓ GluR5–7 density		Garey et al. (2006)
↑ NR1$^{C2'}$ isoforms (ACC)				
– NR1^{C2}, NR2A-D SAP-102 (ACC, DLPFC), NR1$^{C2'}$, PSD93, PSD95 (DLPFC) NF-L (ACC)				Kristiansen et al. (2006)
↓ PSD93, PSD95 (ACC), NF-L (DLPFC)				
			↓ RGS4	Erdely et al. (2006)
			↑ mGluR1a, mGluR2/3	Gupta et al. (2005)
			– mGluR4, mGluR5	
↓ Phospho NR1-S897				Emamian et al. (2004)
– NR1				Nudmamud-Thanoi and Reynolds (2004)
	↓ SAP-97, GluR1			Toyooka et al. (2002)
– SAP-102, PSD95, PSD93	– GRIP			
	– NSF			Imai et al. (2001)
	– GluR2, GluR3			Breese et al. (1995)
Autoradiography				
– [^{3}H] binding	– [^{3}H]AMPA	– [^{3}H]kainate		Beneyto et al. (2007)
	– [^{3}H]AMPA			Beneyto and Meador-Woodruff (2006)
– [^{3}H]MK801, [^{3}H]CGP39653	– [^{3}H]AMPA	↓ [^{3}H]kainate		Scarr et al. (2005)

Table 4.2-2 (continued)

Cortex			
– NR1, NR2A-D, PSD-95 (IAR)			Toro and Deakin (2005)
↑ [^{3}H]MK801	– [^{3}H]AMPA	– [^{3}H]kainate	Newell et al. (2005)
– [^{3}H]L-689,560			Nudmamud-Thanoi and Reynolds (2004)
↑ [^{3}H]MK801	↑ [^{3}H]AMPA	– [^{3}H]kainate	Zavitsanou et al. (2002)
		↓ [^{3}H]kainate	Meador-Woodruff et al. (2001)
– [^{3}H]MK801	↑ [^{3}H]CNQX	– [^{3}H]kainate	Noga et al. (2001)
↑ [^{3}H]MK801, [^{3}H]Glycine		↑ [^{3}H]kainate	Toru (1988)
	– [^{3}H]AMPA		Healy et al. (1998)
	– [^{3}H]AMPA		Kurumaji et al. (1992)
↑ [^{3}H]TCP			Simpson et al. (1991)
		↑ [^{3}H]kainate	Deakin et al. (1989)
↑ [^{3}H]MK801			Kornhuber et al. (1989)

Data for NMDA-, AMPA-, kainate-, and mGluR-type receptor expression are subdivided into transcript, protein, and autoradiography findings. Arrows (↑↓) and line (–) indicate changes (increase, decrease, and no changes, respectively). For autoradiography studies, ligand and isotope used are indicated. RGS regulator of G-protein signaling, ACC anterior cingulate cortex, DLPFC dorsolateral prefrontal cortex, OC occipital cortex, IAR immunoautoradiography, CNQX 6-cyano-7-nitroquinoxaline-2,3-dione, TCP thienyl cyclohexylpiperidine, CGP39653 D,L-(E)-2-amino-4-[3H]-propyl-5-phosphono-3-pentenoic acid, L-689,560 (+/−)-4-(trans)-2-carboxy-5,7-dichloro-4-phenylaminocarbonylamino-1,2,3,4-tetrahydroquinoline

altered in schizophrenia (Nudmamud-Thanoi and Reynolds, 2004; Scarr et al., 2005; Toro and Deakin, 2005; Beneyto et al., 2007), although a few studies have reported an increased [^{3}H]MK801 and [^{3}H]TCP binding to their intrachannel sites in cingulate cortex suggesting an increased NMDA receptor density (Simpson et al., 1991; Zavitsanou et al., 2002; Newell et al., 2005). In summary, small changes in cortical transcript and protein expression of NMDA receptor subunits in schizophrenia, combined with some indication of increased binding to specific sites in assembled NMDA receptors, suggest regional alterations of receptor stoichiometry and/or altered cellular receptor processing.

Interestingly, two studies have addressed the question of whether alternative splicing of the NR1 subunit is altered in schizophrenia, identifying disease-specific changes of alternative splicing of the intracellular C1 and C2 cassettes (Le Corre et al., 2000; Kristiansen et al., 2006). These findings might have functional significance as alternative splicing of the NR1 C-terminus regulates cellular processing of the receptor including transcription, phosphorylation, and trafficking of the receptor (Tingley et al., 1993; Ehlers et al., 1998; Standley et al., 2000; Scott et al., 2001; Mu et al., 2003; Scott et al., 2003; Bradley et al., 2006).

The increased expression of the C2′ cassette in schizophrenia (Kristiansen et al., 2006) might increase the ratio of $NR1^{C2'}$/ $NR1^{C2}$ subunits in the ER, and consequently increase the percentage of NMDA receptors assembled from this subunit. According to recent studies of the functional relevance of these forms, this suggests an increased forward trafficking of NMDA receptors in schizophrenia, possibly as a

compensatory response to a decreased signaling at the synapse, which is consistent with a hypothesis of NMDA receptor hypofunction (Coyle et al., 2003).

In agreement with an increase in the expression of the NR1 subunit lacking the C1-exon (Le Corre et al., 2000), the phosphorylation of the NR1S897 site present in this exon, was recently reported to be decreased in schizophrenia (Emamian et al., 2004). The inclusion of the C1-exon is regulated by neuronal activity and is proposed to serve as a primary protective regulatory element against NMDA receptor hyperactivity of the receptor (Kraus et al., 1996; Jaekel et al., 2006). These data are therefore in agreement with a cellular response to NMDA receptor hypoactivity in schizophrenia.

The expression of molecules involved in AMPA receptor signaling has been analyzed in cortical areas. Generally, these studies have measured the receptor and transcript expression by binding autoradiography and *in situ* hybridization, and have not consistently revealed a significantly altered AMPA receptor expression (*Table 4.2-2*) (Kurumaji et al., 1992; Breese et al., 1995; Healy et al., 1998; Dracheva et al., 2005; Newell et al., 2005; Scarr et al., 2005; Beneyto et al., 2007). However, an increased receptor density in ACC (Noga et al., 2001; Zavitsanou et al., 2002), and a regionally abnormal transcript expression for different AMPA receptor subunits in OCC, entorhinal cortex, and DLPFC have been reported (Sokolov, 1998; Hemby et al., 2002; Dracheva et al., 2005; Beneyto and Meador-Woodruff, 2006). Based on these studies, the expression and/or stoichiometry of the AMPA receptor might be affected regionally in schizophrenia. Only a few studies have measured the expression of AMPA subunits at the protein level, indicating a decreased expression of the GluR1 subunit and an unaltered expression of the GluR2 and GluR3 subunits in the PFC (Breese et al., 1995; Toyooka et al., 2002).

Similar to NR1, alternative splicing of the AMPA receptor, which determines receptor desensitization, may also be altered in schizophrenia (Stine et al., 2001). However, in a recent study by O'Connor and colleagues (2007), transcripts for the flip/flop variants of all four AMPA receptor subunits were measured in DLPFC from patients with schizophrenia by quantitative PCR, and no changes in these isoforms were identified in two different patient samples.

Alterations in the cortical expression of the kainate receptor subunits in schizophrenia have been implicated by reports of decreased [^{3}H]kainate binding in schizophrenia in frontal cortex (*Table 4.2-2*) (Meador-Woodruff et al., 2001; Scarr et al., 2005). However, other studies have not replicated these findings (Zavitsanou et al., 2002; Newell et al., 2005; Beneyto et al., 2007), and earlier studies reported an increased [^{3}H]kainate binding in cortex (Deakin et al., 1989; Toru, 1998). Relatively small changes in transcript expression for the kainate receptor subunits have been reported, with increases in GluR7 and decreases in GluR5, GluR7, KA1, and KA2 in frontal cortex (Sokolov, 1998; Meador-Woodruff et al., 2001; Scarr et al., 2005). However, in a recent study, the expression of all subunits except GluR5 in temporal cortex was found at levels equivalent to comparison subjects (Beneyto et al., 2007). Generally, studies have found most kainate receptor subunits either unchanged or decreased, the most consistent change at the transcriptional level being a decrease of the GluR5 subunit (Meador-Woodruff et al., 2001; Scarr et al., 2005). Using a different approach to assay protein expression, Garey et al. (2006) used cresyl violet and immunostaining of GluR5/6/7 kainate receptor subunits to quantify kainate receptor positive cells in orbitofrontal cortex, and reported a significant reduction in kainate receptor-positive neurons without a reduction in total neuronal density. Based on these studies, it appears that in areas of the frontal lobe, kainate receptors are probably less abundantly expressed in schizophrenia.

Despite their importance as modulators of glutamatergic synaptic function and potential as pharmacological targets (Moghaddam, 2004), relatively few studies have analyzed the cortical expression of mGluRs in postmortem brain in schizophrenia (*Table 4.2-2*). One study reported an increased expression of the mGluR5 transcript across pyramidal cell layers in DLPFC with no change in mGluR3 expression (Ohnuma et al., 1998). However, this is inconsistent with a recent study in which no change in mGluR4 and mGluR5, and an increased mGluR1 and mGluR2/3 protein expression were reported in PFC (Gupta et al., 2005). Based on these few studies, it is difficult to conclude whether changes in mGluR expression are involved in the cortical pathophysiology of schizophrenia. However, increases of the group I type receptors (mGluR1/5) are suggested by these studies.

Recent evidence suggests decreased transcript and protein expression levels in the cortex of the regulator of G-protein signaling 4 (RGS4), which is a predominantly neuronally expressed protein

(Erdely et al., 2004) that regulates the activity of the G-proteins that couple to metabotropic-type receptors, including the mGluRs and dopamine receptors (Mirnics et al., 2001; Erdely et al., 2006; Bowden et al., 2007). RGS4 is thought to inhibit group I mGluRs (mGluR1, mGluR5) through GTPase activation and subsequent block of Gα activation (Saugstad et al., 1998). We speculate that a decreased expression of RGS4 in the face of an increased expression of group I mGluRs in schizophrenia leads to an increased activity of mGluR1/5 signaling, and therefore possibly represents a regulatory response to hypofunction of the glutamatergic synapse.

Based on what is known about cortical expression abnormalities of glutamate receptors in schizophrenia from postmortem tissue, it is evident that these studies have provided equivocal support for the hypothesis of abnormal glutamate signaling in schizophrenia. However, analogous to the recent findings of abnormal expression of proteins that interact with and regulate mGluR function in the PSD, altered expression of PSD proteins that interact with AMPA and NMDA receptors has more consistently been found in postmortem brain in schizophrenia. Thus, rather than glutamate receptors per se, the pathophysiology of schizophrenia might include abnormalities of the entire synaptic cellular machinery including the PSD, with functional consequences for receptor trafficking, expression, and integrated signaling function (*Table 4.2-2*).

The expression of the AMPA receptor interacting proteins PICK1, *N*-ethylmaleimide-sensitive fusion protein (NSF), syntenin, and stargazin (Stg) was recently analyzed in schizophrenia. Decreased PICK1 and increased Stg transcript expression was associated with large cells of cortical layer III in DLPFC (Beneyto and Meador-Woodruff, 2006). This is interesting since PICK1 promotes activity-dependent phosphorylation of the GluR2 subunit, causing AMPA receptor membrane targeting in the PSD (Lu and Ziff, 2005; Sossa et al., 2006). Thus, decreased PICK1 suggests reduced cellular capacity in the frontal cortex to correctly respond to altered signaling requirements in schizophrenia. Previous studies have measured the expression of these and other PSD-associated AMPA receptor interacting proteins, and found a significantly increased cortical expression of transcripts for GRIP and ABP, and unaltered PICK1 and SAP97 (Dracheva et al., 2005) as well as a decreased SAP97 protein expression (Toyooka et al., 2002), which is another PDZ protein involved in AMPA receptor trafficking to the cell membrane (Rumbaugh et al., 2003; Cai et al., 2006; Schluter et al., 2006).

Taken together, combinations of a reduced expression of PICK1 and SAP97 with an increased ABP/GRIP in schizophrenia likely have consequences for PSD trafficking of the AMPA receptor. PICK1 and SAP97 generally facilitate AMPA receptor trafficking to the cell membrane during activity, whereas ABP/GRIP are involved in retaining the intracellular storage of AMPA receptors associated with the PSD (Lu and Ziff, 2005; Cai et al., 2006; Sossa et al., 2006). These changes would therefore cause inhibition of AMPA receptor-dependent neuronal responses to activity. Increased stargazin expression (Beneyto and Meador-Woodruff, 2006) might be part of a cellular compensatory response to these changes, as it promotes the lateral mobility of the AMPA receptor in the cell membrane, linking it to the NMDA receptor complex (Mi et al., 2004; Tomita et al., 2005).

In addition to AMPA receptor interacting PSD proteins, other molecules of the PSD that associate primarily with the NMDA receptor complex are abnormally expressed in the cortex in schizophrenia. Prominently, region-specific changes in transcript and protein expression of the PSD proteins PSD95 and PSD93 have been reported in ACC with few changes in other cortical areas (Ohnuma et al., 2000a; Dracheva et al., 2001; Toyooka et al., 2002; Toro and Deakin, 2005; Kristiansen et al., 2006). Also, the expression of NF-L, a PSD molecule that selectively interacts with C1-exon containing NR1 spliceforms (Ehlers et al., 1998), was altered in a region-specific manner in DLPFC (Kristiansen et al., 2006). Interestingly, contrary to regionally increased expression of transcripts for NF-L, PSD95, and PSD93 (*Table 4.2-2*), protein expression of these molecules in the same subjects and anatomical regions was downregulated (Kristiansen et al., 2006). Such opposite changes of the molecular constituents for transcription and translation might provide insight into important aspects of functional abnormalities central to the pathophysiology of schizophrenia. Thus, discordant data from studies of transcription and translation in schizophrenia might reflect a primary functional deficit of these molecules at the protein level, which leads to an increased transcription as a cellular compensatory response.

1.2.2.2 Hippocampus Based on the likely involvement of excitatory signaling abnormalities in the hippocampus in schizophrenia, a number of studies have analyzed the expression of NMDA receptor subunits in this brain region in postmortem tissue (*Table 4.2-3*) (See Heckers this Volume). With the

Table 4.2-3
Glutamate receptor abnormalities in hippocampus in schizophrenia

Hippocampus				
NMDA	**AMPA**	**Kainate**	**mGluRs**	**References**
Transcripts				
– NR1, NR2A-D	– GluR1–4	– GluR5–7, KA1/2		Beneyto et al. (2007)
– NR1, NR2A-D, NF-L, PSD95, PSD93, SAP-102				McCullumsmith et al. (2007)
↓ NR1				Law and Deakin (2001)
			– mGluR5	Ohnuma et al. (2000b)
↓ NR1				Gao et al. (2000)
↑ NR2B				
– PSD95				Ohnuma et al. (2000a)
	↓ GluR2 (flop isoform)			Eastwood et al. (1997a)
	– GluR2 (flip isoforms)			
		↓ GluR6, KA2		Porter et al. (1997)
	↓ GluR1, GluR2			Eastwood et al. (1995)
Protein analysis				
– SAP-102, PSD95, PSD93	– GRIP, SAP97			Toyooka et al. (2002)
	– GluR1, GluR2, GluR3	– GluR5		Breese et al. (1995)
Autoradiography				
↓ [^{3}H]MK801	– [^{3}H]AMPA	– [^{3}H]kainate		Beneyto et al. (2007)
– [^{3}H]MDL105519, [^{3}H]Ifenprodil, [^{3}H]CGP39653				
– [^{3}H]MK801, [^{3}H]CGP39653				McCullumsmith et al. (2007)
– NR1, PSD-95 (IAR)				Toro and Deakin (2005)
	– [^{3}H]CNQX	– [^{3}H]kainate		Noga and Wang (2002)
– [^{3}H]NMDA	↓ [^{3}H]AMPA	– [^{3}H]Kainate		Gao et al. (2000)
↓ [^{3}H]TCP				Dean et al. (1999b)
	↓ GluR1, GluR2/3 (IAR)			Eastwood et al. (1997b)
– [^{3}H]NMDA		↓ [^{3}H]kainate		Kerwin et al. (1990)
↑ [^{3}H]MK801				Kornhuber et al. (1989)

Data for receptor expression are subdivided into transcript, protein, and autoradiography findings. Arrows (↑↓) and line (–) indicate changes (increase, decrease, and no changes, respectively). For autoradiography studies, ligand and isotope used are indicated. IAR: immunoautoradiography; CNQX: (6-cyano-7-nitroquinoxaline-2,3-dione); TCP: (thienyl cyclohexylpiperidine); CGP39653: (D,L-(E)-2-amino-4-[3H]-propyl-5-phosphono-3-pentenoic acid); MDL: ((E)-3-(2-phenyl-2-carboxyethenyl)-4,6-dichloro-1H-indole-2-carboxylic acid)

exception of three studies showing either increased or decreased [^{3}H]MK801 and decreased [^{3}H]TCP NMDA receptor binding in schizophrenia (Kornhuber et al., 1989; Dean et al., 1999b; Beneyto et al., 2007), studies have found no change in [^{3}H]CGP39653 and [^{3}H]L-689560 binding to the glutamate and glycine sites of the NMDA receptor respectively (Kerwin et al., 1990; Gao et al., 2000; Beneyto et al., 2007;

McCullumsmith et al., 2007). Two studies have reported an altered transcript expression including a decreased NR1 and an increased NR2B in the CA3 and dentate gyrus regions (Gao et al., 2000; Law and Deakin, 2001), although later studies have not replicated these findings in different patient samples (Beneyto et al., 2007; McCullumsmith et al., 2007). Additionally, in a recent study, the hippocampal protein expression of the obligatory NR1 subunit was reported unchanged in schizophrenia (Toro and Deakin, 2005).

The hippocampal expression of several PSD proteins was not significantly altered in schizophrenia, with the exception of a decreased PSD95 transcript expression in the molecular layer of the dentate gyrus, albeit only when compared with patients with major depression (Ohnuma et al., 2000a; Toyooka et al., 2002; Toro and Deakin, 2005; McCullumsmith et al., 2007). Based on these studies, the expression in the hippocampus of the NMDA receptor complex does not appear profoundly affected in schizophrenia, although a few studies have reported modest regional changes of subunit expression (Harrison et al., 2003; Kristiansen et al., 2007).

Only one study has examined the expression of mGluRs in the hippocampus in schizophrenia. No changes in the expression of the group I mGluR5 receptors were found, while a significantly increased mGluR5/EAAT2 ratio was noted within the parahippocampal gyrus due to a decrease in the transcript for the excitatory amino acid transporter 2 (EAAT2) (Ohnuma et al., 2000b). This finding might be important as the glial expression of EAAT1 and EAAT2 is regulated by mGluR5, and thus suggests that mGluR function in the hippocampus is compromised at a posttranscriptional level in schizophrenia (Gegelashvili et al., 2000; Aronica et al., 2003).

Some lines of evidence suggest that both AMPA and kainate receptor expressions in the hippocampus are affected in schizophrenia. First, the expressions of transcripts for the GluR1 and GluR2 AMPA receptor subunits, and the GluR6 and KA2 kainate subunits have been reported significantly decreased in several hippocampal subregions in schizophrenia (Collinge and Curtis, 1991; Eastwood et al., 1995; Eastwood et al., 1997a; Porter et al., 1997; Meador-Woodruff and Healy, 2000; Harrison et al., 2003), although a recent study found no changes in the transcript expression for any of the glutamate receptor subunits including AMPA and kainate receptors (Beneyto et al., 2007). Second, the direct measurement of AMPA and kainate subunit protein expression and several receptor binding studies have converged in reporting a decreased [^{3}H]AMPA and [^{3}H]kainate binding (Kerwin et al., 1990; Eastwood et al., 1997b; Gao et al., 2000; Benes et al., 2001), although other studies did not report any changes in AMPA and kainate receptor binding in the hippocampus (Gao et al., 2000; Noga and Wang, 2002; Beneyto et al., 2007). While the majority of these earlier positive findings were reported in studies using the same cohort of subjects, the latter conflicting data were obtained using different groups of patients. Such differences in subject characteristics between studies may be a critical source of variance in studies involving postmortem human brain.

The expression of AMPA receptor interacting proteins has presently only been measured in three studies in the hippocampus. These studies find no changes in the expression of SAP-97, GRIP, NF-L, SAP-102, PSD95, and PSD93 in the hippocampus in schizophrenia (Toyooka et al., 2002; Toro and Deakin, 2005; McCullumsmith et al., 2007).

1.2.2.3 Thalamus Altered thalamic expression of transcripts for the different iGluR subunits include a decreased expression of the NR1, NR2B, and NR2C transcripts in several nuclei, including the dorsomedial and central medial nuclei, with no alterations reported for NR2A and NR2D expression (*Table 4.2-4*) (Ibrahim et al., 2000; Popken et al., 2002). In addition, the AMPA GluR1 and GluR3, and the kainate KA2 subunits were reported to be decreased in the central medial nucleus with all other subunits expressed normally (Ibrahim et al., 2000; Popken et al., 2002). However, a subsequent study using tissue from a different group of patients reported an increase in NR2B transcript expression with no changes in the expression of the NR1, NR2A, NR2C, and NR2D subunits (Clinton and Meador-Woodruff, 2004a). Interestingly, transcripts for NR1 exon 22 containing splice variants were decreased in schizophrenia with no changes in other spliceforms (Clinton et al., 2003). As the alternative splicing of exon 22 introduces two additional sites for serine phosphorylation in the NMDA receptor, this change suggests an altered trafficking of the receptor in thalamic neurons in a similar manner as reported for the NR1 subunit in ACC (Kristiansen et al., 2006). In a recent study, Clinton et al. (2006) analyzed the expression of the NR1, NR2A, and NR2B proteins in the dorsomedial and ventral subregions of the thalamus. An increased

Table 4.2-4
Glutamate receptor abnormalities in thalamus in schizophrenia

Thalamus				
NMDA	**AMPA**	**Kainate**	**mGluRs**	**References**
Transcripts				
↑ NR2B				
– NR1, NR2A, NR2C, NR2D				Clinton and Meador-Woodruff (2004a)
↓ NF-L, PSD95, SAP-102				
↑ NF-L				
↓ NR1 exon 22				
– NR1 exon 5, 21, PSD-93				Clinton et al. (2003)
↑ NF-L, PSD95, SAP102				
– NR1, NR2A	– GluR2/4	– GluR5/6		Popken et al. (2002)
↓ NR1, NR2B, NR2C	↓ GluR1, GluR3	↓ KA2		Ibrahim et al. (2000)
– NR2A, NR2D	– GluR2, GluR4	– GluR5–7, KA1		
			– mGluR1–8	Richardson-Burns et al. (2000)
Protein analysis				
↑ NR2B, PSD95 (DM)				Clinton et al. (2006)
– NR1, NR2A, SAP102, NF-L (DM, V)				
Autoradiography				
↓ [^{3}H]Ifenprodil, [^{3}H] MDL105519	– [^{3}H]AMPA	– [^{3}H]kainite		Ibrahim et al. (2000)
– [^{3}H] CGP39653, [^{3}H] MK801				

Data are subdivided into transcript, protein, and autoradiography findings. Arrows (↑↓) and line (–) indicate changes (increase, decrease, and no changes, respectively). For autoradiography studies, ligand and isotope used are indicated. DM: dorsomedial nucleus; V: ventral nuclei

expression of NR2B protein was reported in the dorsomedial thalamus with no changes for NR2B in ventral thalamus; NR1 and NR2A were not changed in any thalamic areas. In addition, the expression of transcripts for the mGluRs was not altered in schizophrenia (Richardson-Burns et al., 2000).

Studies of receptor binding using autoradiography in the thalamus have indicated a decreased binding of [^{3}H]Ifenprodil and [^{3}H]MDL105519 to the polyamine and glycine sites respectively, with no changes in binding to the glutamate and intrachannel (MK-801) sites, suggesting an altered stoichiometry of the NMDA receptor (Ibrahim et al., 2000). This study also analyzed [^{3}H]AMPA and [^{3}H]kainate binding and did not report any changes in binding to these receptors in schizophrenia. Therefore, contrary to the hippocampus, thalamic changes in glutamate receptor expression primarily involve altered expression and/or assembly of the NMDA receptor, with few changes to other glutamate receptor types (Clinton and Meador-Woodruff, 2004b).

Few studies have studied thalamic expression of the PSD proteins that associate with and regulate AMPA and NMDA receptor signaling. These studies have reported an increased expression of transcripts for NF-L, PSD-95, and SAP102 with no changes in PSD93 expression (Clinton et al., 2003; Clinton et al., 2004). The expression of proteins for these PSD molecules included an increased PSD95 in the dorsomedial nucleus with no change in PSD95 expression in the ventral thalamus, and no changes of NF-L or SAP102 protein expression in any of these areas (Clinton et al., 2006). However, due to the complex afferent and efferent projection pattern of the thalamic circuitry, the uncoupling of transcript and protein expression

might not reflect local cellular changes. PSD95 specifically binds to NR2B subunit-containing NMDA receptors and is involved in their functional regulation and trafficking. Thus, an increased expression of PSD95 and NR2B in the same thalamic nucleus (Clinton et al., 2006) might suggest abnormal trafficking of a subset of NMDA receptors in the thalamus in schizophrenia.

In summary, studies of the expression of receptors for glutamate in schizophrenia have been inconsistent. Glutamate receptor expression, based on postmortem findings, in the hippocampus appears less affected, whereas cortical and thalamic expression of these receptors, and in particular their associated PSD proteins, provide evidence for altered expression with likely changes in the composition of the PSD in schizophrenia. Recent evidence provides some insight into the molecular consequences of expression changes seen in schizophrenia, suggesting that these molecular alterations could suggest an altered targeting of receptors to the PSD.

1.3 GABA Receptors

1.3.1 The GABAergic System

The involvement of γ-aminobutyric acid (GABA) in the pathogenesis of schizophrenia was suggested in part when it was discovered that GABA neurotransmission can modulate DA signaling (Van Kammen, 1977), followed by an early report of GABAergic interneurons being affected in schizophrenia (Benes et al., 1991). Since these early observations, considerable evidence in support of altered GABA synthesis, release, and uptake have been identified in schizophrenia (Lewis et al., 1999) and that reduced functioning is most pronounced in fast-spiking, parvalbumin-containing, GABA interneurons (Lewis et al., 1999; Reynolds et al., 2001). These neurons receive dopaminergic input from the midbrain and express D_1, D_2, and D_4 DA receptors (Le Moine and Gaspar, 1998; Wedzony et al., 2000). Behaviorally, dysfunction of this neuronal subtype affects the synchronization of DLPFC networks that facilitate working memory (Lewis et al., 2004). Ultrastructurally, there are reduced numbers of synaptic "cartridges" of parvalbumin-positive GABAergic interneurons that make synaptic contact with the axon initial segments of cortical pyramidal cells (Pierri et al., 1999).

GABA is the main inhibitory neurotransmitter in the brain, with extensive representation in most brain regions, including cortex, striatum, thalamus and the hippocampus. Interneurons that release GABA have a major modulatory influence on cortical glutamatergic output through inhibitory synapses located on dendrites or axon initial segments of large glutamatergic projection neurons in the cortex.

There are three types of receptors responsive to GABA in the CNS: $GABA_A$, $GABA_B$, and $GABA_C$ (Martin and Dunn, 2002). While $GABA_A$ and $GABA_C$ both are ionotropic receptors, the $GABA_B$ receptor is a G_i-protein-coupled metabotropic receptor with high sequence homology to the metabotropic glutamate receptors (Kaupmann et al., 1997). The activation of $GABA_A$ and $GABA_C$ receptors allows passage of chloride ions into the neuron, leading to hyperpolarization (inhibition) of the cell membrane. $GABA_B$ receptors, on the other hand, negatively couple to adenylyl cyclase and inactivate voltage-dependent Ca^{2+} channels and decrease IP_3 production (Kaupmann et al., 1997). Even though the $GABA_A$ and $GABA_C$ receptors have important differences with respect to subunit composition, ligand binding, and activation kinetics, given the primary expression of $GABA_C$ receptors in the retina (Enz and Cutting, 1998), we focus here on the ubiquitously expressed $GABA_A$ and $GABA_B$ receptors in schizophrenia.

1.3.1.1 $GABA_A$ Receptors $GABA_A$ receptors mediate fast synaptic inhibition and are primarily concentrated postsynaptically (Michels and Moss, 2007). Most studies on GABA receptors in schizophrenia have focused on the $GABA_A$ receptor, which is a pentameric assembly of various subunits: α (1–6), β (1–4), γ (1–3) and δ (1–2), ε, π, ρ (1–3) and θ (Whiting et al., 1999; Michels and Moss, 2007). Typically, receptors are made up of 2 α subunits, 2 β subunits, and 1 γ subunit (Chang et al., 1996), the most common configurations being α1β2γ2 and α2β3γ2 (McKernan and Whiting, 1996; Whiting et al., 1999).

While $GABA_A$ receptors are distributed widely throughout the brain, cell-specific effects of receptor activation are dictated by the configuration of receptor subunits and the cellular location of the receptors (synaptic versus extrasynaptic). α1 subunits, for example, undergo rapid deactivation and are often found

in synapses involving parvalbumin-expressing "fast-spiking" GABAergic neurons (Klausberger et al., 2002; Mohler, 2006). The α1β2γ2 assembly (60% of $GABA_A$ receptors) is expressed in most neurons, both synaptically and extrasynaptically, in areas such as the cortex (in both principal neurons and selected interneurons) and the hippocampus, while the α2β3γ2 assembly (15–20% of $GABA_A$ receptors) is concentrated in the axon initial segments of hippocampal and cortical pyramidal neurons (Mohler, 2006). Functionally, the configuration of receptor subunits confers specificity for certain ligands; for instance, receptors containing α1, α2, α3, or α5 subunits have binding sites for benzodiazepines (BZs) and BZ antagonists (Rudolph et al., 1999), and receptors containing α/β interfaces flanked by γ and β subunits bind the $GABA_A$ agonist muscimol (Squires and Saederup, 2000).

1.3.1.2 $GABA_B$ Receptors $GABA_B$ receptors can be located either pre- or postsynaptically (Kratskin et al., 2006). The activation of $GABA_B$ autoreceptors negatively modulates neurotransmitter release, whereas postsynaptic receptor activation leads to membrane hyperpolarization (Martin and Dunn, 2002). $GABA_B$ receptors require heteromeric assembly of two different proteins, $GABA_{B1}$ and $GABA_{B2}$, to form a functional receptor (Kuner et al., 1999). Additionally, the alternative splicing of the $GABA_{B1}$ receptor transcript, which gives rise to two different $GABA_{B1}$ spliceforms (Kaupmann et al., 1998), was recently shown to confer different subcellular expression pattern and distinct functional properties to the receptor in cortical neurons (Perez-Garci et al., 2006; Vigot et al., 2006).

1.3.2 GABA Receptor Expression in Schizophrenia

1.3.2.1 Cortex Most of the alterations in $GABA_A$ receptor expression in schizophrenia have been found in the cortex (*Table 4.2-5*). An increased $GABA_A$ receptor expression was found in layers I-III in the ACC in schizophrenia using bicuculline-sensitive [^{3}H]muscimol binding (Benes et al., 1992). Subsequently, an increased [^{3}H]muscimol binding in layer II of the PFC was reported (Benes et al., 1996a), and later studies reported an increased [^{3}H]muscimol binding in the cortex in this illness (Dean et al., 1999a; Deng and Huang, 2006; Newell et al., 2007). However, a study using another $GABA_A$ receptor ligand found conflicting results: a decreased [^{3}H]flunitrazepam binding was found in the ACC and somatomotor cortices (Squires et al., 1993). These divergent results may be due to an "uncoupling" of $GABA_A$ receptor binding and BZ binding sites, particularly since specific receptor subunit configurations are required for BZ binding (Benes et al., 1997; Rudolph et al., 1999).

Similar to divergent reports of altered cortical receptor binding, several groups have found an increased transcript expression for the α1 and δ $GABA_A$ receptor subunits in PFC (Ohnuma et al., 1999; Hakak et al., 2001), while others have found a decreased expression of δ, α1, and γ2 $GABA_A$ receptor subunits (Huntsman et al., 1998; Vawter et al., 2002; Hashimoto et al., 2007). Adding to the complexity, some $GABA_A$ receptor subunits have several splice variants that change the signaling properties of the receptor. For example, both the β2 and γ2 subunits have "short" and "long" splice variants; Zhao et al (2006) found a decreased expression of β2 long and short forms in DLPFC in schizophrenia, with a change in the ratio of long to short variants, suggesting altered splicing (Zhao et al., 2006). Also, a reduction in the expression of the short variant of the γ2 subunit with respect to the long variant has also been reported in PFC (Huntsman et al., 1998).

GABA receptor protein expression has also been studied in schizophrenia. Immunoreactivity for the α1 and β2/3 $GABA_A$ receptor subunits was increased in the PFC in cell bodies of pyramidal neurons (Ishikawa et al., 2004b), while γ1/3 subunit expression was unchanged (Ishikawa et al., 2004a). In another study, fewer presynaptic cartridges of parvalbumin-positive neurons associated with pyramidal neurons were demonstrated in parallel with an increased α2 subunit expression (Volk et al., 2002). These findings are significant in that this particular receptor subunit is concentrated in the axon initial segment (Fritschy and Mohler, 1995), where synaptic input has the greatest effect on action potential initiation. It is thought that the increase in receptor expression reflects a compensation for reduced presynaptic release (Benes and Berretta, 2001; Lewis et al., 2005), and that disturbances in GABAergic signaling at the axon initial segment contribute to the deficits in working memory observed in patients with schizophrenia (Volk and Lewis, 2002; Lewis et al., 2005).

Only one study has examined cortical $GABA_B$ receptor expression in schizophrenia, finding decreased protein levels in the temporal cortex in this illness (*Table 4.2-5*) (Mizukami et al., 2002).

Table 4.2-5
$GABA_A$- and $GABA_B$-type receptors abnormalities in thalamus in schizophrenia

Cortex		
$GABA_A$	**$GABA_B$**	**References**
Transcripts		
↓ α1		Hakak et al. (2001)
↑ α1		Ohnuma et al. (1999)
↑ α1		Impagnatiello et al. (1998)
↑ α5		Impagnatiello et al. (1998)
↓ α1		
↓ α4		
↓ γ2		Hashimoto et al. (2007)
↓ δ		
↓ β3		
↓ β2 (change in long/short isoform ratio)		Zhao et al. (2006)
– β1		
– β2		
– β5		Akbarian et al. (1995)
– γ2		
– α1		
– α2		
↓ γ2 (decrease in short isoform)		Huntsman et al. (1998)
↓ δ		Vawter et al. (2002)
↑ δ		Paz et al. (2006)
Autoradiggraphy		
↑ [^{3}H]Muscimol		Benes et al. (1992)
↑ [^{3}H]Muscimol		Benes et al. (1996b)
↑ [^{3}H]Muscimol		Deng and Huang (2006)
↑ [^{3}H]Muscimol		Dean et al. (1999a)
↑ [^{3}H]Muscimol		Newell et al. (2007)
↓ [^{3}H]flunitrazepam (benzodiazepine)		Squires et al. (1993)
Immunohistochemistry		
↑ α1		
↑ β2/3		Ishikawa et al. (2004a)
– γ1/3		
↑ α2		Volk et al. (2002)
	↓ Entorhinal and inferior temporal cortex	Mizukami et al. (2002)
Hippocampus		
Autoradiography		
↑ [^{3}H]Muscimol		Benes et al. (1996a)
↓ [^{3}H]Muscimol, flumazenil		Dean et al. (2005)
↓ [^{3}H]flunitrazepam (benzodiazepine)		Squires et al. (1993)
Immunohistochemistry		
	↓ Pyramidal cells, granule cells	Mizukami et al. (2000)

continued

Table 4.2-5 (continued)

Thalamus	
Transcripts	
– α1	
– β2	Popken et al. (2002)
– γ2	

Data for each region subdivided into transcript, autoradiography and immunohistochemistry findings, when available. Arrows (↑↓) and line (–) indicate changes (increase, decrease, and no changes, respectively). For autoradiography studies, ligand and isotope used are indicated

1.3.2.2 Hippocampus, Cerebellum and Basal Ganglia [^{3}H]muscimol binding was increased in both pyramidal and nonpyramidal cells of the hippocampus (Benes et al., 1996b), while a decreased [^{3}H] flunitrazepam binding was reported in the hippocampus and the globus pallidus (Squires et al., 1993). An increased mRNA expression for the α1 and δ $GABA_A$ receptor subunits has been reported in the cerebellum in schizophrenia (Paz et al., 2006).

Only one study has examined hippocampal $GABA_B$ receptors in schizophrenia, noting decreased protein levels in this illness (*Table 4.2-5*) (Mizukami et al., 2000).

Interpretation of these results is complicated by the differential firing properties that each receptor subunit confers on the assembled receptor complex. For example, α1 subunit-containing receptors are associated with long-duration openings with multiopening bursts and faster deactivation, while α6 subunits are associated with short-duration openings with isolated single openings and slower deactivation kinetics (Fisher, 2004). In addition, GABA receptor subunits can be differentially distributed in the postsynaptic membrane; α2 and α3 are postsynaptic and directly apposed to presynaptic GABAergic neuron terminals, while α5 subunit-containing receptors are extrasynaptic, closer to sites of NMDA receptor activation (Fritschy and Brunig, 2003; Mohler et al., 2004). Therefore, it has been proposed that α5 subunit-containing receptors have a stronger inhibitory effect on pyramidal neurons (Mohler et al., 2004).

In summary, studies of GABA receptor expression in schizophrenia suggest an increase in receptor binding sites in the cortex, coupled with changes in receptor subunit stoichiometry. Abnormalities have also been found in the machinery involved in GABA transport into presynaptic nerve terminals (Ohnuma et al., 1999; Volk and Lewis, 2002), and the release of synaptogenesis-promoting proteins from GABAergic neurons (Impagnatiello et al., 1998). These data support a prevailing hypothesis that presynaptic deficits in GABAergic signaling, in conjunction with compensatory postsynaptic effects, contribute to the pathophysiology of schizophrenia (Lewis et al., 2005).

1.4 Serotonin Receptors

1.4.1 The Serotonergic System

The involvement of the serotonin (5HT) neurotransmitter system in the pathophysiology of schizophrenia was originally based on the observation that some hallucinogens, such as D-lysergic acid diethylamide (LSD), act in the central nervous system through the modulation of 5HT receptors (Aghajanian and Marek, 2000). LSD and similar compounds have been characterized as partial agonists of $5HT_2$ receptors, suggesting a role for the 5HT system in disorders of sensory perception including schizophrenia (Egan et al., 1998). The 5HT system has also been linked to schizophrenia by pharmacological studies that suggest that the effects of NMDA receptor antagonists may be, in part, due to activation of AMPA and kainate receptors following modulation of $5HT_{2A}$ receptor activity (Egan et al., 1998; Aghajanian and Marek, 2000).

The serotonergic system includes a group of nuclei containing 5HT neurons in the dorsal and median raphe that project throughout the brain. The release of 5HT from serotonergic projections activates a diverse family of ionotropic ($5HT_3$) and metabotropic ($5HT_1$, $5HT_2$, $5HT_4$, $5HT_5$, $5HT_6$, $5HT_7$) receptors

variably expressed both pre- and postsynaptically in many brain regions (Filip et al., 2005). 5HT-mediated neurotransmission can, depending on the receptor type, cause either depolarization or hyperpolarization of target neurons. The activation of the $5HT_3$ receptor, which is member of a large family of ligand gated cation channels with specificity for Na^+, Ca^{2+}, K^+ ions, results in fast depolarization kinetics. Unlike the $5HT_3$ receptor, the $5HT_2$, $5HT_4$, and $5HT_7$ receptors are metabotropic receptors that activate intracellular signaling through differential coupling to G_S- ($5HT_4$, and, $5HT_7$) or G_q/G_{11}-proteins ($5HT_2$), followed by the activation of adenylate cyclase or PLC pathways (Filip et al., 2005). Unlike the majority of 5HT receptors that, through different mechanisms, mediate increased intracellular signaling, the $5HT_1$ and $5HT_5$ receptors couple to inhibitory G-proteins, which results in decreased cellular levels of cAMP. The net effects of 5HT, in addition to the type of neuron and receptor location on the neuron, are therefore highly dependent on the specific receptor type that is activated (Filip et al., 2005).

$5HT_1$ receptors are expressed in the basal ganglia, the hippocampus, the raphe nuclei, and other regions, where they act as autoreceptors or heteroreceptors (Filip et al., 2005). $5HT_{2A}$ and $_{2C}$ are expressed in the neocortex, the hippocampus, and the basal ganglia, while $5HT_{2B}$ receptors are significantly expressed in the cerebellum and hypothalamus (Filip et al., 2005). $5HT_3$ receptors are expressed in the hippocampus, the basal ganglia, and the cortex (Filip et al., 2005). $5HT_{4A\text{-}C}$ receptors are expressed as heteroreceptors in the basal ganglia, the hippocampus, and the cortex (Filip et al., 2005). The function of $5HT_5$ receptors, which are mainly expressed in the hippocampus, the habenula, and the cortex, has not been well characterized (Filip et al., 2005). $5HT_6$ and $5HT_7$ receptors are expressed in the hippocampus, the basal ganglia, the cortex, and the amygdala (Filip et al., 2005). Most of the 5HT receptors are expressed on glutamatergic, cholinergic or GABAergic neurons, while $5HT_{1A}$, $5HT_{1B}$, and $5HT_{2A}$ receptors are also expressed on dopaminergic neurons (Filip et al., 2005).

Modulation of 5HT receptors is of particular interest in schizophrenia due to the potent $5HT_2$, $5HT_6$, and $5HT_7$ receptor antagonist properties of atypical antipsychotic medications (Meltzer et al., 2003; Roth et al., 2004). The 5HT system exerts potent modulatory effects on dopamine, GABA, and glutamate transmission, highlighting its importance in the pathophysiology of schizophrenia.

1.4.2 Serotonergic Receptor Expression in Schizophrenia

1.4.2.1 Cortex Binding studies in cortex have generally found a decreased $5HT_2$ and an increased $5HT_{1A}$ receptor binding in schizophrenia, with a few exceptions (*Table 4.2-6*). In addition, no changes have been reported for $5HT_{1F}$, $5HT_3$, $5HT_4$, or $5HT_6$ receptor binding in this illness, although one group found a decreased $5HT_7$ binding in the PFC (Dean et al., 2006).

5HT transcript expression has not been as extensively studied as receptor binding in schizophrenia. Two studies found no changes in $5HT_1$ receptor transcript isoforms (Burnet et al., 1996; Lopez-Figueroa et al., 2004). In contrast, $5HT_2$ transcript expression was decreased in multiple cortical regions, but unchanged in the cerebellum (Burnet et al., 1996; Hernandez and Sokolov, 2000; Polesskaya and Sokolov, 2002; Castensson et al., 2003). Only one study has examined 5HT receptor protein in the cortex in schizophrenia, finding no changes in $5HT_{1A}$ expression (Cruz et al., 2004).

1.4.2.2 Hippocampus, Amygdala, Striatum Similar to other neurotransmitters, fewer studies have studied the expression of 5HT outside the cortex in postmortem tissue (*Table 4.2-6*). These studies have reported relatively few changes in serotonergic receptor expression, including a study that found an increased $5HT_{1A}$ binding in the hippocampus (Joyce et al., 1993), while other studies of this receptor, measured by ligand binding or transcript expression, found no changes in the hippocampus, the amygdala, or the striatum (Hashimoto et al., 1991; Joyce et al., 1993; Burnet et al., 1996; Lopez-Figueroa et al., 2004; Scarr et al., 2004).

Binding to the $5HT_2$-type receptor in the hippocampus and the striatum has been reported to be either increased (Joyce et al., 1993), decreased (Burnet et al., 1996; Scarr et al., 2004) or unchanged (Matsumoto et al., 2005), while the expression at the transcript level for this receptor was not changed in the hippocampus (Burnet et al., 1996; Lopez-Figueroa et al., 2004). Additionally, decreased expression of $5HT_6$ and $5HT_7$ transcripts in the hippocampus have been reported (East et al., 2002).

Table 4.2-6
Serotonin receptor abnormalities in schizophrenia

Cortex			
$5HT_1$	**$5HT_2$**	**$5HT_{3/4/5/6/7}$**	**References**
Transcripts			
– $5HT_{1A}$	↓ $5HT_{2A}$		Burnet et al. (1996)
	↓ $5HT_{2A}$		Hernandez and Sokolov (2000)
	↓ $5HT_{2C}$		Castensson et al. (2003)
– $5HT_{1A}$, $5HT_{1B}$	– $5HT_{2A}$		Lopez-Figueroa et al. (2004)
	↓ $5HT_{2A}$		Polesskaya and Sokolov (2002)
	↑ $5HT_{2C\text{-}ini}$		
	↓ $5HT_{2C\text{-}vsv}$		Sodhi et al. (2001)
	↓ $5HT_{2C\text{-}vnv}$		
	– $5HT_{2C}$		Dracheva et al. (2003)
	– $5HT_{2C}$ site A and site B		Iwamoto and Kato (2003)
Protein			
– $5HT_{1A}$			Cruz et al. (2004)
Autoradiography			
	↓ [^{3}H]LSD ($5HT_2$)		Bennett et al. (1979)
	– [^{3}H]LSD ($5HT_2$)		Whitaker et al. (1981)
	– [^{3}H]ketaserin ($5HT_2$)		Reynolds et al. (1983)
	↓ [^{3}H]spiperone ($5HT_2$)		Arora and Meltzer (1991)
	↓ [^{3}H]ketaserin ($5HT_2$)		Mita et al. (1986)
↑ [^{3}H]8-OH-DPAT ($5HT_{1A}$)			Hashimoto et al. (1991)
↑ [^{3}H]8-OH-DPAT ($5HT_{1A}$)	↓ [^{3}H]ketaserin ($5HT_2$)		Hashimoto et al. (1993)
	↑ [^{3}H]ketaserin ($5HT_2$)		Laruelle et al. (1993)
↑ [^{3}H]8-OH-DPAT ($5HT_{1A}$)	↑ [^{3}H]ketaserin, [^{125}I]LSD ($5HT_2$)		Joyce et al. (1993)
↑ [^{3}H]8-OH-DPAT ($5HT_{1A}$)			Simpson et al. (1996)
↑ [^{3}H]8-OH-DPAT ($5HT_{1A}$)	↓ [^{3}H]ketaserin ($5HT_2$)		Burnet et al. (1996)
↑ [^{3}H]WAY-100635 ($5HT_{1A}$)			Burnet et al. (1997)
↑ [^{3}H]8-OH-DPAT ($5HT_{1A}$)	↓ [^{3}H]LSD ($5HT_{2A\text{-}C}$)		Gurevich and Joyce (1997)
	↓ [^{3}H]ketaserin ($5HT_2$)		Dean et al. (1998)
– [^{3}H]8-OH-DPAT ($5HT_{1A}$)		– [^{3}H]GR113808 ($5HT_4$)	Dean et al. (1999c)
	↓ [^{3}H]ketaserin ($5HT_{2A}$)		Dean et al. (1999a)

Table 4.2-6 (continued)

Cortex			
	↓ [^{3}H]ketaserin ($5HT_{2A}$)		Pralong et al. (2000)
		– [^{125}I]SB-258585 ($5HT_6$)	East et al. (2002)
	↓ [^{3}H]ketaserin ($5HT_{2A}$)		Matsumoto et al. (2005)
– [^{3}H]8-OH-DPAT ($5HT_{1A}$)			Gray et al. (2006)
– [^{3}H]sumatriptin ($5HT_{1D}$)		↓ [^{3}H]SB269970 ($5HT_7$)	Dean et al. (2006)
– [^{3}H]sumatriptin ($5HT_{1F}$)			
Hippocampus			
Transcripts			
– $5HT_{1A}$	– $5HT_{2A}$		Burnet et al. (1996)
		↓ $5HT_6$	East et al. (2002)
		↓ $5HT_7$	
– $5HT_{1A}$, $5HT_{1B}$	– $5HT_{2A}$		Lopez-Figueroa et al. (2004)
Autoradiography			
– [^{3}H]8-OH-DPAT ($5HT_{1A}$)			Hashimoto et al. (1991)
↑ [^{3}H]8-OH-DPAT ($5HT_{1A}$)	↑ [^{3}H]ketaserin, [^{125}I]LSD ($5HT_2$)		Joyce et al. (1993)
– [^{3}H]8-OH-DPAT ($5HT_{1A}$)	↓ [^{3}H]ketaserin ($5HT_2$)		Burnet et al. (1996)
– [^{3}H]8-OH-DPAT ($5HT_{1A}$)	↓ [^{3}H]ketaserin ($5HT_{2A}$)	– [^{3}H]GR113808 ($5HT_4$)	
↓ [^{3}H]sumatriptin ($5HT_{1D}$)			Scarr et al. (2004)
– [^{3}H]sumatriptin ($5HT_{1F}$)			
		– [^{3}H]ketaserin ($5HT_{2A}$)	Matsumoto et al. (2005)
Amygdala			
Autoradiography			
– [^{3}H]8-OH-DPAT ($5HT_{1A}$)			Hashimoto et al. (1991)
		– [^{3}H]LY278584 ($5HT_3$)	Abi-Dargham et al. (1993)
Striatum			
Autoradiography			
– [^{3}H]8-OH-DPAT ($5HT_{1A}$)	↑ [^{3}H]ketaserin, [^{125}I]LSD ($5HT_2$)		Joyce et al. (1993)
		– [^{3}H]ketaserin ($5HT_{2A}$)	Matsumoto et al. (2005)

Data for each region are subdivided into transcript, protein, and autoradiography findings, when available. Arrows (↑↓) and line (–) indicate changes (increase, decrease, and no changes, respectively). For autoradiography studies, ligand and isotope used are indicated, followed by receptor subtypes labeled under study conditions, as reported by authors. LSD: lysergic acid diethylamide; 8-OH-DPAT: (8-hydroxy-2-(di-n-propylamino) tetralin)

The findings of decreased $5HT_2$ and increased $5HT_{1A}$ receptor binding in the cortex in schizophrenia are particularly interesting when considered in the context of the anatomical distribution and function of these receptors. For example, $5HT_{2A}$ and $5HT_{1A}$ receptors are highly coexpressed on pyramidal neurons in the PFC, and to a lesser extent, on GABAergic interneurons (Santana et al., 2004). $5HT_{2A}$ and $5HT_{1A}$ receptors promote and inhibit pyramidal neuron activation respectively, measured using electrophysiological functional endpoints (Carli et al., 2006). Other studies have found "dissociable contributions" of these receptors in the medial PFC for attentional and executive control in rodent behavioral models (Carli et al., 2006). In addition, selective activation of $5HT_{2A/2C}$ receptors in the rodent medial PFC stimulates dopamine release in the VTA and medial PFC (Bortolozzi et al., 2005). Taken together, these preclinical findings suggest that even small alterations in the relative proportion of the cellular expression of 5HT receptors may have potent effects on behavior and cognitive functioning. Decreased $5HT_2$ and increased $5HT_{1A}$ receptor density (Burnet et al., 1996) in the PFC in schizophrenia might reflect diminished pyramidal neuron output, contributing to psychopathology in this illness.

1.5 Acetylcholine Receptors

1.5.1 The Acetylcholine System

Acetylcholine was one of the first neurotransmitters to be identified (See Freedman this Volume). In addition to its role in peripheral skeletal and smooth muscle activation, cholinergic transmission was later recognized as an important neurotransmitter in the brain. The major cholinergic pathways originate from diffusely organized neurons principally located in the basal forebrain (nucleus basalis) and in the pontomesencephalic nucleus in the brain stem (Mesulam, 1990; Jones, 1993). Generally, basal forebrain cholinergic neurons innervate the cerebral cortex, the amygdala and the hippocampus, while brainstem cholinergic neurons innervate the thalamus, the cerebellum, and midbrain dopaminergic areas. In addition, intrinsic cholinergic interneurons are present locally in the nucleus accumbens and in the basal ganglia (Haber, 1986; Kasa, 1986; Zhou et al., 2002). Interestingly, enzymes for the synthesis of acetylcholine have also been found in oligodendrocytes (Lan et al., 1996). Central cholinergic neurotransmission primarily causes excitatory activation, and generally functions to modulate other neurotransmitter systems, including those of DA and glutamate (Apicella, 2002; Zhou et al., 2002; Zhou et al., 2003; Tepper and Bolam, 2004).

Two groups of acetylcholine receptors have been identified in humans: the muscarinic (mAChR) and nicotinic (nAChR) receptors, named after their artificial activating ligands, muscarine and nicotine. These receptors function through entirely different mechanisms, either as a slow acting metabotropic G-protein-coupled receptor (mAChR) or fast acting ion-channel type receptor (nAChR).

1.5.1.1 Nicotinic Receptors Based on structural and functional similarities, the nAChRs, with $GABA_A$, glycine, and $5\text{-}HT_3$ receptors, are members of one large superfamily of ligand-gated ion-channels (Smith and Olsen, 1995). nAChRs are a heterogeneous group of receptors that consist of pentameric assemblies of 12 different subunits (α2–10; β2–4). Based on their sensitivity to the natural antagonist, α-bungarotoxin (α-Bgtx), nAChRs can be subclassified into two groups: α-Bgtx sensitive (α7–10 containing receptors; nM affinity) and α-Bgtx insensitive (α2–6 and β2–4 containing receptors; μM affinity) (Gotti and Clementi, 2004; Nashmi and Lester, 2006). In the brain, both heteromeric α-Bgtx insensitive αβ containing receptors with high agonist affinity (α2–6; β2–4) or homomergic, α-Bgtx sensitive, low agonist affinity receptors (α7–10) are expressed. The majority of nAChRs are made up from 2 α- and 3 β-subunits, β2/α4 being the most abundantly receptor type expressed (Romanelli et al., 2007), and the homomeric α7 subunit containing receptors the second most prevalent nAChR in brain (Nashmi and Lester, 2006; Dani and Bertrand, 2007).

nAChRs are widely distributed throughout the brain with the β2/α4 type ubiquitously expressed in most regions (Gotti et al., 2006; Gaimarri et al., 2007). In humans and primates, nAChR distribution differs somewhat from that in rodents, with highest expression associated with the hippocampus, thalamus,

cortex, and striatum (Quik et al., 2000; Gotti and Clementi, 2004). Interestingly, α7-type receptors are highly expressed in the cortex, the hippocampus, and other limbic regions, whereas α6 and β3 subunit containing receptors especially localize to discrete structures including the medial habenula, SN, VTA, and striatum (Quik et al., 2000; Gotti and Clementi, 2004). In particular, in the hippocampus, the expression of different nAChR forms is heterogeneous, likely reflecting the complex requirements for regulated signaling between numerous neurotransmitters in learning and memory formation (Albuquerque et al., 1996; Quik et al., 2000; Levin et al., 2002; McGehee, 2002; Dani and Bertrand, 2007).

Although nAChR expression is found both at postsynaptic and extrasynaptic sites, as well as in nonneuronal tissue (Brumwell et al., 2002; Gotti and Clementi, 2004), nAChRs are mainly located presynaptically where they regulate the release of other neurotransmitters, including norepinephrine, DA, glutamate, and GABA (Clarke and Pert, 1985; Lambe et al., 2003; Gotti and Clementi, 2004; Sher et al., 2004; Gaimarri et al., 2007). Signaling through the nAChR, which differs between receptor subtypes in terms of pharmacology and kinetics, generally involves currents of Na^+, K^+, and Ca^{2+} through the receptor and activation of voltage-gated Ca^{2+} channels (Dani and Bertrand, 2007; Gotti et al., 2007).

1.5.1.2 Muscarinic Receptors Based on their affinity to the muscarinic ligands pirenzepine and AF-DX116, mAChRs were initially classified into M_1 and M_2 subtypes (McCormick and Prince, 1985; van der Zee and Luiten, 1999). Currently, 5 different receptors (M1-M5) are identified, of which M1, M2, and M4 are the main ones expressed in brain, with little or no expression of the M3 and M5 receptors (Tice et al., 1996; Volpicelli and Levey, 2004). mAChRs are expressed throughout the brain, with most abundant expression in the cortex (M1, M2, M4), the cerebellum (M2), the hippocampus (M1, M2, M4), the thalamus (M2), and the striatum (M1, M4) (Levey et al., 1991; Tice et al., 1996). Ultrastructural analysis of mAChR expression suggests that M1 principally associates with postsynaptic sites, where it is involved in modulating neurotransmitter signaling. M2 and M4 receptors likely are expressed in both postsynaptic sites and presynaptic sites, where they function as autoreceptors (Levey et al., 1991; Rouse and Levey, 1997; Bymaster et al., 2003).

mAChRs are metabotropic, G-protein-coupled receptors. Through G_q-protein coupling, M1 type receptors activate PLC and phosphatidyl-inositol (PI) turnover, whereas M2 and M4 receptors, through G_i-protein binding inhibit the activity of adenylate cyclase (AC) and consequently the production of cAMP (Brann et al., 1993; McKinney, 1993).

1.5.2 Acetylcholine Receptor Expression in Schizophrenia

1.5.2.1 Cortex A confounding factor for analyzing nAChR expression in postmortem brain is the high prevalence of smokers among patients with schizophrenia (Dursun and Kutcher, 1999). However, despite this difficulty, considerable progress has been made in the understanding of nicotine dependency in schizophrenia and its relevance to altered receptor expression (Dalack et al., 1998; Dalack et al., 1999). For the heteromeric receptors, binding studies have reported a decreased binding of [^{3}H]nicotine and [^{3}H] epibatidine in the frontal cortex in postmortem brain (Breese et al., 2000) (*Table 4.2-7*). However, contrary to these studies, an increased [^{3}H]cytosine and [^{3}H]epibatidine binding in the cortex has been reported by other groups (Marutle et al., 2001; Martin-Ruiz et al., 2003). Binding studies for the α7 subunit have found a decreased [^{125}I]α-bungarotoxin and unaltered [^{3}H]methyllycaconitine binding in the cortex for this homomeric nAChR (Breese et al., 2000; Marutle et al., 2001). In support of the reduced α7 binding in schizophrenia, a reduced protein expression for this subunit has been reported in the frontal cortex using both western blotting and immunohistochemistry (Guan et al., 1999; Martin-Ruiz et al., 2003). However, similar to transcripts for other nAChR subunits, no changes have been reported for α7 transcript expression (Martin-Ruiz et al., 2003; De Luca et al., 2006). Based on these studies, the expression of the α7 subunit might therefore be down-regulated, likely due to posttranslational mechanisms.

Contrary to the opposing findings for nAChRs, the M1, M2, and M4 mAChR in the cortex have, with few exceptions, been reported decreased in schizophrenia (*Table 4.2-7*) (Crook et al., 2001; Dean et al., 2002; Zavitsanou et al., 2004b; Deng and Huang, 2005; Zavitsanou et al., 2005; Scarr et al., 2006). These changes have been extended to include analysis at both transcript and protein levels, where decreases

Table 4.2-7
Acetylcholine receptor abnormalities in schizophrenia

Cortex		
Nicotinic receptors	**Muscarinic receptors**	**References**
Transcripts		
	↓ M1, M4	Dean et al. (2002)
	↓ M1	Mancama et al. (2003)
	– α7 (DLPFC)	Martin-Ruiz et al. (2003)
	– M3	Scarr et al. (2006)
Protein analysis		
	↓ M1 (DLPFC; no change in parietal cortex)	Dean et al. (2002)
	– M4	
↓ α7 (DLPFC)		Martin-Ruiz et al. (2003)
– α4, α3, β2 (DLPFC)		
↓ α7 (DLPFC)		Guan et al. (1999)
– α7 (parietal cortex)		
	– M2, M3	Scarr et al. (2006)
Autoradiography		
↑ [^{3}H]epibatidine (α3 α4) (DLPFC)		Martin-Ruiz et al. (2003)
↓ [^{125}I]α-bungarotoxin (α7; cingulate cortex)		Marutle et al. (2001)
↑ [^{3}H]cytosine (α4β2; cingulate cortex)		
↑ [^{3}H]epibatidine (α3 α4; TG)		
	↓ [^{3}H]pirenzepine (M1, M4; STG)	Deng and Huang (2005)
	↓ ^{3}H]AF-DX 384 (M2, M4; STG)	
	↓ [^{3}H]pirenzepine (M1, M4; ACC)	Zavitsanou et al. (2004b)
	– [^{3}H]AF-DX 384 (M2, M4; ACC)	Zavitsanou et al. (2005)
	– [^{35}S]GTPgammaS (M2, M3; DLPFC, par. Ctx)	Scarr et al. (2006)
	– [^{3}H]pirenzepine (M1, M4; parital ctx)	Dean et al. (2002)
	↓ [^{3}H]pirenzepine (M1, M4; DLPFC)	
	↓ [^{3}H]pirenzepine (M1, M4)	Crook et al. (2001)
	↓ [^{3}H]QNB (due to medication)	Toru (1988)
	↓ [^{3}H]QNB (due to medication)	Watanabe et al. (1983)
	↓ [^{3}H]QNB	Bennett et al. (1979)
↓ [^{3}H]nicotine (α4β2; in group of smokers)		Breese et al. (2000)
↓ [^{3}H]epibatidine (α3 α4; in group of smokers)		
– [^{3}H]methyllycaconitine (α7)		
Striatum		
Transcripts		
	– M1	Dean et al. (2000)
Protein analysis		
↓ α7		Leonard et al. (1998)
Autoradiography		
↓ [^{3}H]epibatidine (α3 α4; in group of smokers)		Breese et al. (2000)
	↓ [^{3}H]pirenzepine (M1, M4)	Dean et al. (1996)
	↓ [^{3}H]AF-DX384 (M2, M4)	Crook et al. (1999)

Table 4.2-7 (continued)

Striatum		
↑ [^{3}H]nicotine (α4β2)		Court et al. (2000)
↓ [^{3}H]cytosine (α4β2)		Durany et al. (2000)
↓ [^{3}H]nicotine (α4β2)		Leonard et al. (1998)
Hippocampus		
Transcripts		
	– M1, M4	Scarr et al. (2007)
Autoradiography		
↓ [^{3}H]nicotine (in group of smokers)		Breese et al. (2000)
– [^{3}H]methyllycaconitine (α7)		
	↓ [^{3}H]pirenzepine (M1, M4)	Crook et al. (2000)
	↓ [^{3}H]pirenzepine (M1, M4)	Scarr et al. (2007)
↓ [^{125}I]α-bungarotoxin (α7)		Freedman et al. (1995)
↓ [^{3}H]cytosine (α4β2)		
Thalamus		
Autoradiography		
– [^{3}H]nicotine (in group of smokers)		Breese et al. (2000)
↓ [^{125}I]α-bungarotoxin (α7)		Court et al. (1999)
– [^{3}H]nicotine		

Arrows (↑↓) and line (–) indicate changes (increase, decrease, and no changes, respectively). For autoradiography studies, ligand and isotope used are indicated, followed by receptor subtypes labeled under study conditions, as reported by authors. DLPFC: Dorsolateral prefrontal cortex; TG: Temporal gyrus; AF-DX384: (2,3-Dipropylamino); GTPgammaS: (guanosine 5′-3-O-(thio) triphosphate); QNB: (Quinuclidinyl benzilate)

have also been reported for M1 and M4 (Dean et al., 2002; Mancama et al., 2003), and unaltered levels of M2 and M3 expression have been noted (Scarr et al., 2006). These findings of specific mAChRs have led to hypotheses including primary abnormal muscarinic-based signaling in schizophrenia (Raedler et al., 2007).

1.5.2.2 Striatum, Hippocampus, Thalamus Relatively few studies have investigated the expression of AChRs outside the cortex in schizophrenia (*Table 4.2-7*). However, studies done in the striatum, the hippocampus, and the thalamus have identified changes similar to those in the cortex including increased (striatum), unaltered (thalamus), or decreased (striatum, hippocampus) binding of [^{3}H] nicotine to α4β2 type nAChRs in schizophrenia (Leonard et al., 1998; Court et al., 1999; Breese et al., 2000; Court et al., 2000). Supporting decreased expression of nicotinic type receptor in the striatum and the hippocampus, [^{3}H]cytosine and [^{3}H]epibatidine were similarly reported decreased in schizophrenia (Freedman et al., 1995; Breese et al., 2000; Durany et al., 2000). Additionally, α7 receptor binding in the hippocampus and the thalamus, as well as α7 protein expression in the striatum, was reported to be decreased in this illness (Freedman et al., 1995; Leonard et al., 1998; Court et al., 1999).

Consistent with changes observed in cortex, studies of mAChRs in the striatum and the hippocampus have found a decreased binding to M1/M4 ([^{3}H]pirenzepine) and M2/M4 ([^{3}H]AF-DX384) type receptors in postmortem tissue (*Table 4.2-7*) (Dean et al., 1996; Crook et al., 1999; Crook et al., 2000; Scarr et al., 2007). However, the expression of transcripts for the M1 and M4 receptors was not altered. Binding to mAChRs has not been determined in the thalamus.

In summary, most prominent changes associated with AChRs in schizophrenia involve a decreased binding and expression of the α7 homomeric and the M1/M4 muscarinic receptors (*Table 4.2-7*). Due to its high expression in limbic regions, decreased levels of the α7 homomeric receptor could be central to the pathology of schizophrenia and is therefore thought of as an interesting pharmaceutical target

(Hurst et al., 2005; Martin and Freedman, 2007; Romanelli et al., 2007). However, more studies are required to determine the mechanisms and whether other changes in nAChR subunit expression in schizophrenia are involved. Additionally, in light of the subtype-specific decreases in muscarinic receptor expression, investigators have suggested that it is likely that schizophrenia does not involve abnormally high levels of presynaptic acetylcholine release, which might be expected to cause a compensatory downregulation of all mAChR subtypes (Raedler et al., 2007). Therefore, the targeting of the M1 subtype has been proposed for treatment. The M1 receptor agonist xanomeline was initially believed to be an attractive agent for increasing cholinergic neurotransmission in schizophrenia; however, there is a high incidence of gastrointestinal side effects with treatment, leading to discontinuation of the drug (in trials for Alzheimer's disease) (Mirza et al., 2003). Further studies are needed to determine ways to selectively target and activate mAChR subtypes for the treatment of schizophrenia.

1.6 Catecholamine Receptors

1.6.1 Adrenoreceptors

Adrenergic transmission is well known to be involved in the regulation of homeostatic control through its functions in the autonomic nervous system. Additionally, adrenergic projections in the brain have been identified with important roles in neurocognition. Adrenoreceptors are seven transmembrane G-protein-coupled receptors that mediate the physiological responses of epinephrine and norepinephrine. The first classification of these receptors resolved α (alpha)-adrenoreceptors (αARs) from β (beta)-adrenoreceptors (βARs) (Ahlquist, 1948). Since then, additional subtypes and variants have been described.

Once activated, adrenoreceptors can mediate either excitatory or inhibitory intracellular signaling through differential coupling to G_q, G_s, and G_i, G-proteins (Raymond, 1995; Hall, 2004), with downstream modulation of cAMP levels and of protein kinase A and protein kinase C (PKC) activity (Duman et al., 1994; Hall, 2004). Additional downstream activation mechanisms have been described for adrenergic receptors, including facilitation of L-type Ca^{2+} channels (Hoogland and Saggau, 2004), control of K^+ channels (Yuan et al., 2002), MAP kinase regulation (Cao et al., 2000), and phosphorylation of glutamate receptors (Vanhoose and Winder, 2003). In addition to the activation of signaling cascades, a dense intracellular network of proteins has been demonstrated to interact with adrenoreceptors similar to those discussed earlier for the glutamate receptors, and include endophilins, PSD-95, GIPC, MAGI-2, AKAPs, and Grb2 (Hadcock et al., 1992; Hu et al., 2000; Premont and Hall, 2002; Hall, 2004). Moreover, homodimerization of adrenergic receptors or heterodimerization with other receptors (αARs plus βARs, βARs plus δ-opioid or GPCRs receptors) have been shown to have functional implications (McVey et al., 2001; He et al., 2002; Hall, 2004).

Most of catecholaminergic excitatory functions are mediated by alpha-adrenoreceptors, which are abundantly expressed in the hippocampus, the amygdala, the locus coeruleus and the PFC (Arnsten and Goldman-Rakic, 1985; Booze et al., 1993; Civantos Calzada and Aleixandre de Artinano, 2001; DeBock et al., 2003; Hillman et al., 2005), where they play key roles in cognitive processes such as attention (Aston-Jones et al., 1999), and memory formation (Harrison et al., 1991). In these regions, α2ARs are thought to be primarily autoreceptors, which play an important role in regulating the release of norepinephrine in the brain (Aoki et al., 1994). However, other studies suggest a primary postsynaptic expression, particularly in frontal cortex (Arnsten et al., 1996; Klimek et al., 1999). These different anatomical distributions of individual adrenoreceptor subtypes and variants suggest complexity of this system (Tanoue et al., 2002).

1.6.2 Adrenoreceptor Expression in Schizophrenia

Although pharmacological evidence suggests an important role for the adrenergic system in schizophrenia, few studies of receptor expression in postmortem brain in schizophrenia have been conducted. In the PFC, no relationship was found between polymorphisms in the regulatory region of the α1A-adrenergic receptor

(ADRA1A) and its expression in schizophrenia (Clark et al., 2006). A study using [^{3}H]RX 821002 binding at the α2 adrenoreceptor found no differences in schizophrenia in DLPFC (Dean, 2003). Similarly, no significant differences were found for [^{125}I]p-iodoclonidine binding to α2ARs in DLPFC (Klimek et al., 1999). In addition, this study reported unchanged β1 and β2 adrenoreceptor binding using [^{125}I]IPIN. On the other hand, another study reported hemisphere-dependent [^{125}I]IPIN binding to β2-adrenoreceptors in the hippocampus and an increased β1-adrenoreceptor binding in the striatum in schizophrenia (Joyce et al., 1992). A recent study found [^{125}I]IPIN binding to the β1-adrenoreceptor in the hippocampus significantly decreased, while β2-receptors were unchanged (Klimek et al., 1999).

1.7 Cannabinoid Receptors

1.7.1 Cannabinoid Receptor System

The endogenous cannabinoid system is proposed to consist of a unique class of retrograde messengers that presynaptically function to inhibit glutamatergic and GABAergic neurotransmission (Sullivan, 1999; Hoffman and Lupica, 2000; Maejima et al., 2001). In the 1970s, specific brain binding sites for cannabinoids were suggested. However, cannabinoid receptors were not identified until almost 20 years later when the central CB1 and peripheral CB2 receptors were described (Matsuda et al., 1990; Gerard et al., 1991; Munro et al., 1993). Alternative splicing of the CB1 gene gives rise to CB1a and CB1b spliceforms (Shire et al., 1995; Ryberg et al., 2005). In addition, the presence of additional endocannabinoid receptor subtypes are suspected (Jarai et al., 1999; Breivogel et al., 2001).

CB1 and CB2 are seven transmembrane G-protein-coupled receptors (Childers et al., 1993; Prather et al., 2000). CB1 is the primary receptor type in the brain, with highest expression levels in the cerebellum, the hippocampus, the basal ganglia, and cortical layers II and III (Herkenham et al., 1991; Westlake et al., 1994; Eggan and Lewis, 2007). CB1 receptors are principally expressed at presynaptic axon terminals and dendrites (Tsou et al., 1998; Irving et al., 2000), although electrophysiological studies suggest a postsynaptic localization as well (Ronesi et al., 2004). The CB2 cannabinoid receptor is primarily expressed in cells and tissues associated with the immune system (Lynn and Herkenham, 1994), although low CB2 expression in the brain has recently been reported (Gong et al., 2006; Onaivi et al., 2006).

Following cannabinoid binding, multiple signaling pathways can be activated: $G_{i,o,s}$-protein mediated modulation of adenylate cyclase and cAMP levels; Ca^{2+} and K^{+} ion channel activation; or activation of different intracellular enzymes/effectors (i.e., kinases, ceramide) in a non-G-protein dependent manner (Childers et al., 1993; Felder et al., 1995; Mackie et al., 1995; Prather et al., 2000; Sanchez et al., 2001).

1.7.2 Cannabinoid Receptor Expression in Schizophrenia

Several studies have investigated how cannabis use and/or the endocannabinoid system itself may be associated with schizophrenia. Although the endogenous cannabinoid system might be implicated in schizophrenia, relatively few studies have analyzed receptor expression or binding in postmortem brain.

The posterior cingulate cortex (PCC), which plays an important role in working memory (Vogt et al., 1992), has recently been implicated in the pathophysiology of schizophrenia. Using quantitative autoradiography, [^{3}H]CP55,940 CB1 binding was found to be increased in superficial layers (layer I, II) of the PCC in subjects with schizophrenia without recent cannabis exposure. No difference in CB1 binding was reported in deeper cortical layers (layers III-VI) (Newell et al., 2006).

Altered expression of the endocannabinoid system in the ACC has also been studied in postmortem brain. Using quantitative autoradiography, [^{3}H]SR-141716A binding to the CB1 receptor in ACC was found to be increased in schizophrenia. This result supports the hypothesis that changes in the endogenous cannabinoid system in the ACC may be involved in the pathology of schizophrenia, in particular in relation to negative symptoms (Zavitsanou et al., 2004a). However, another study did not find changes in the number of CB1 receptor immunopositive cells in the ACC (Koethe et al., 2007).

Using *in situ* radioligand binding, another study reported an increased CB1 density in DLPFC, an effect that was not dependent on previous cannabis use (Dean et al., 2001). In addition, an increased CB1 receptor density in the striatum has been reported, which may have been associated with recent cannabis intake (Dean et al., 2001). Postmortem studies of cannabinoid receptor expression, in particular for the CB1 receptor in PCC, ACC and DLPFC support the involvement of the cannabinoid system in the pathophysiology of schizophrenia.

Endogenous cannabinoids are retrograde messengers that presynaptically inhibit glutamatergic and GABAergic neurotransmission. This action elicits a corresponding reduction of GABA and glutamate receptor activity at the postsynaptic level (depolarization-induced suppression of activation and inhibition, DSE and DSI) (Sullivan, 1999; Hoffman and Lupica, 2000; Maejima et al., 2001). Considering the role of GABA and glutamate in schizophrenia, cannabinoid involvement may be secondary to its effects on these important neurotransmitter systems. In the PFC and cingulate cortex where CB1 may modulate GABA release from interneurons projecting to pyramidal neurons, CB1 abnormalities may disrupt presynaptic GABA and glutamate signaling (Bodor et al., 2005; Eggan and Lewis, 2007). In this way, altered CB1 receptor expression may disturb connections of auto-regulation of inhibitory input to pyramidal neurons. Endocannabinoids may act not only on the glutamatergic machinery of cortical neurons but also in the hippocampus. In fact, an endocannabinoid role on glutamate mediated long-term synaptic plasticity has been reported (Riedel and Davies, 2005).

1.8 Histamine Receptors

1.8.1 The Histamine System

Histamine projections originate from a small group of neurons in the posterior hypothalamus that through diffuse pathways innervate the H1, H2, and H3 histamine receptors in multiple brain regions including the PFC, the hippocampus, the thalamus, the striatum, and the basal ganglia (Brown et al., 2001). Functionally, the histamine system is associated with the regulation of physiological functions such as arousal, food intake, temperature regulation and cardiovascular control. In addition, higher order regulation of learning, reward, anxiety, and stress also involves histamine neurotransmission (Brown et al., 2001).

Histamine receptors are G-protein coupled receptors. The H1 receptor couples through $G_{q/11}$ to phospholipase C activation with subsequent generation of inositol -1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), resulting in Ca^{2+} release from intracellular stores and PKC activation. The H1 receptor is located principally on postsynaptic neurons and has been shown to enhance NMDA receptor-mediated currents in pyramidal neurons. This mechanism likely involves actions of PKC and intracellular Ca^{2+} on the NMDA receptor, possibly through altered sensitivity of NMDA receptor-associated magnesium block (Chen and Huang, 1992; Payne and Neuman, 1997). Like the H1 receptor, H2 receptors are expressed principally at postsynaptic sites where, in most regions, it is co-localized with the H1 receptor. H2 receptors couple through G_S to activate adenylyl cyclase and regulate cAMP production (Brown et al., 2001). In contrast to the H1/2 receptors, the H3 receptor is expressed presynaptically and function, through its coupling to $G_{i/o}$ and inhibition of adenylyl cyclase as an inhibitory autoreceptor (Brown et al., 2001). Interestingly, the H3 receptor does not exclusively inhibit histamine release, but also that of other aminergic neurotransmitter systems such as the adrenergic, serotonergic, and dopaminergic systems (Brown et al., 2001).

1.8.2 Histamine Receptor Expression in Schizophrenia

The histamine system has been implicated in the pathophysiology of schizophrenia by several observations, including evidence for increased CNS metabolism of histamine (Prell et al., 1995), lower levels of H1 receptor expression in the frontal cortex, the striatum, and the thalamus as evaluated by positron emission tomography (Iwabuchi et al., 2005; Yanai and Tashiro, 2007), and the therapeutic efficacy of H3 antagonists on cognitive symptoms in patients with schizophrenia (Vohora, 2004; Esbenshade et al., 2006). Functionally, this

latter effect may be mediated through disinhibition of corticostriatal glutamatergic neurotransmission, as H3 receptor activation has been shown to inhibit this pathway (Doreulee et al., 2001).

These observations suggest a relative decrease in histamine function in schizophrenia. Consistent with this hypothesis, one study that examined histamine receptor expression in postmortem brain found a decreased [^{3}H]mepyramine binding to the H1 receptor in the PFC (Nakai et al., 1991). Given the modulatory roles of the histamine receptors in the CNS, including direct effects on NMDA receptor activation and therapeutic effects in affected individuals, this receptor family warrants closer scrutiny in this illness (Brown et al., 2001).

1.9 Other Receptor Types

1.9.1 Tachykinin Receptors

The tachykinins are a family of neuropeptides that include substance P (SP), neurokinin A (NA), neurokinin B (NB), and the recently discovered hemokinin-1 and endokinin (Wijkhuisen et al., 1999; Page, 2004). The tachykinins bind to three different receptors, the NK1, NK2, and NK3 receptors (Maggi et al., 1987; Maggi et al., 1993; Wijkhuisen et al., 1999). These receptors belong to the 7-transmembrane, G-coupled receptor superfamily (Maggi et al., 1993). Signaling through these receptors involves inhibition of potassium conductance and the modulation of intracellular calcium levels (Akasu et al., 1996; Yamada et al., 1999; Jun et al., 2004).

The distribution of NK receptors is heterogeneous, and has been described in both peripheral tissues and in the brain. Several lines of evidence support a role in schizophrenia for NK receptors, including the observation that tachykinin-like compounds, especially antagonists, might have therapeutic effects in this illness (Kamali, 2001; Meltzer et al., 2004; Spooren et al., 2005; Chahl, 2006)

An early study found elevated levels of substance P in the hippocampus in schizophrenia (Roberts et al., 1983). Moreover, a reduction of the transcript for preprotachykinin A (a common precursor for substance P and neurokinin A) was reported in amygdaloid nuclei in schizophrenia (Carletti et al., 2005). An increased NK1 receptor density ([^{125}I] substance P) has been reported in the caudate and nucleus accumbens (Rioux et al., 1998). In addition, the NK1 receptor was reported altered in PFC in schizophrenia, with NK1 receptor immunopositive cells seen through all layers of PFC in schizophrenia, while in controls, expression was restricted to superficial cortical layers (Tooney et al., 2001). On the other hand, a different study did not find altered density of NK1 positive cell bodies in the amygdala in schizophrenia (Weidenhofer et al., 2006). Burnet and Harrison (2000) reported unchanged NK1 density in the cingulate cortex in schizophrenia. These studies suggest that dysfunction of the tachykinin system may be involved in schizophrenia.

1.9.2 Sigma Receptors

Identification and understanding of Sigma (σ)-receptors has been complicated (Guitart et al., 2004). First, these receptors were assumed to be opiate-like receptors (Martin et al., 1976), and were also hypothesized to bind phencyclidine (Zukin et al., 1984). However, more recent biochemical and pharmacological studies have reclassified them as an unique group of receptors, comprising the σ-1 and σ-2 subtypes (Quirion et al., 1992; Bowen, 2000). Several studies suggest that sigma receptors are coupled to a G-protein signaling mechanism (Soriani et al., 1999; Tokuyama et al., 1999; Maruo et al., 2000), and may modulate classical second messenger pathways such as PKC and PLC (Romero et al., 2000; Derbez et al., 2002), or regulate calcium and potassium ion channels (Church and Fletcher, 1995; Wilke et al., 1999).

Autoradiography, *in situ* hybridization, western blot analysis, and immunohistochemical studies have described the distribution of sigma receptors in the brain (Samovilova et al., 1985; Largent et al., 1986; Jansen et al., 1991; Bouchard and Quirion, 1997; Alonso et al., 2000; Zamanillo et al., 2000). The σ1 receptor is expressed in septum, olfactory bulb, hypothalamus, anterodorsal thalamic nucleus, dorsal raphe, locus coeruleus, substantia nigra, and the cerebellum.

In postmortem studies, a reduction in σ binding in temporal cerebral cortex has been reported in schizophrenia (Weissman et al., 1991). In addition, [^{3}H]nemonapride and [^{3}H](+)3-PPP binding associated with the σ receptor was reported to be decreased in the striatum (Simpson et al., 1991; Helmeste et al., 1996). However, an increased binding was found in the superior parietal cortex using the sigma receptor ligand [^{3}H]1, 3, di-o-tolylguanidine (DTG) in schizophrenia (Shibuya et al., 1992).

1.9.3 Opioid Receptors

The opioid receptors are divided into the mu (μ)-, kappa (κ)-, and delta (δ)-opioid receptors (Martin et al., 1976; Lord et al., 1977). The opioid receptors have been cloned (Evans et al., 1992; Wang et al., 1993; Yasuda et al., 1993), and have been found to exist in distinct spliceforms: μ (μ1, μ2, μ3) (Pasternak and Wood, 1986; Cadet et al., 2003), δ(δ1, δ2) (Fang et al., 1994), and κ(κ1, κ2, κ3) (Clark et al., 1989; Rothman et al., 1990; Ni et al., 1995). Moreover, other opiate-like receptors with a similar pharmacological profile have been described, including the epsilon (ε) (Wuster et al., 1979), zeta (ζ) (Zagon et al., 1991), and lambda (λ) receptors (Grevel et al., 1985).

Opioid receptors are seven transmembrane G-protein coupled inhibitory receptors (Bockaert, 1991), although an association to excitatory G-proteins has also been reported (Varga et al., 2003). Intracellular signaling by these receptors involves the inhibition of adenylate cyclase with a subsequent decrease in cAMP levels (Surprenant et al., 1990), regulation of intracellular calcium levels, modulation of potassium channels (Williams et al., 2001), and control of MAP-kinase and ERK activity (Eitan et al., 2003; Varga et al., 2003).

An altered pattern of cortical [^{3}H]U 69593 binding to the kappa receptor has been reported in schizophrenia (Royston et al., 1991). However, by *in situ* hybridization, transcripts for the κ-receptor, as well as its endogenous ligand prodynorphin, were reported unchanged in dorsolateral PFC and ACC in schizophrenia (Peckys and Hurd, 2001). This is consistent with a previous study in which no differences in [^{3}H]etorphine and [^{3}H]naloxone binding to opioid receptors were reported in striatum in schizophrenia (Owen et al., 1985). Additional postmortem studies are warranted to further clarify the role of the endogenous opioid system in schizophrenia.

2 Summary

Based on these studies providing evidence for abnormal neurotransmitter receptor expression in schizophrenia, it is clear that neurotransmission is broadly affected in this illness both in terms of neuroanatomical region and neurotransmitter systems affected. Likely, some of these changes occur as primary deficits, perhaps as a consequence of altered CNS development, while others are secondary and occur as a response to primary changes. This is illustrated by the complex interactions between GABA, glutamate, and dopamine in autoregulatory control of cortical dopamine levels through excitatory efferent VTA innervation from the frontal cortex, which in turn modulates mesocortical activity. Detailed understanding of these circuits, which additionally involve modulation by endocannabinoids, histamine, 5HT, and other transmitter systems, is essential for advancing our knowledge of the pathophysiology of schizophrenia, and to improve treatment. Future studies in postmortem brain should focus on cellular and subcellular alterations of this complex circuitry through cell specific studies of transmitter abnormalities and analysis of markers for cellular functions implicated in this illness.

Acknowledgements

The authors are supported by MH53327 and MH70895 from NIMH (JMW), MH74016 from NIMH (REM), NARSAD Young Investigator Award (LVK), and the Health Services Foundation of the University of Alabama (RMC).

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4.3 An Overview and Current Perspective on Family Studies of Schizophrenia

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_4.3,

Abstract: The family as a unit for support and care of chronically ill individuals with schizophrenia, as well as a source for valuable research information, needs emphasis. However, over the years, studies of families have been misinterpreted in a variety of political, social, and psychodynamic ways that have only hindered research progress. Currently, families are important resources for genetic research. Nevertheless, the new accumulated data on gene variation and expression in schizophrenia and families based on this research need to be better understood. The following is a review of the influence of the family on schizophrenia over the past century since "Dementia Praecox" was first defined as a separate entity by Kraepelin.

List of Abbreviation: SNP, single nucleotide polymorphism

1 Introduction

Schizophrenia is an inherited illness for which there is no known cause and no specific and sensitive biological evidence of illness for diagnosis and treatment. It was first distinguished as a unique entity, Dementia Praecox, by Kraepelin in the late 1800s in his series of textbooks on psychiatry (Kraepelin, 1896/1899), and then later defined by its symptomatology and renamed "schizophrenia" by E. Bleuler in later years (Bleuler, 1911). Debate continues into the present about the nature of its heterogeneity, and how many illnesses this category truly represents. Regardless, the family of an individual with schizophrenia constitutes an important entity that in some years has been overlooked. It was a family member who, until recently, was able to put away the unwanted deviant member with this illness by "signing" the individual into a psychiatric hospital against his/her own wishes as a confined resident for years. It was the family that was also able to come and retrieve their ill member, if warned by the treating psychiatrists of imminent danger of annihilation by Nazi procedures during World War II. However, it was the family that as a unit was often blamed for causing the illness in the post-World War II era. Presently, it is the family from which researchers now need cooperation for participation in studies that may produce answers about many biologic vulnerability factors that lead to the illness, schizophrenia. Finally, it is the overwhelming lifelong burden placed on families with an affected member (Brady and McCain, 2005; Willis, 1982) that makes it imperative that we attempt to eradicate this disorder by finding methods for early detection, prevention, and cure.

2 A Brief History of Family Studies in Schizophrenia

2.1 Focus on Families During the First Quarter of the Twentieth Century

Kraepelin in his textbooks (1896/1899) clearly characterized this disorder as one where inheritance played a major role in as much as 70% of cases. In fact, throughout the 1900s, large cohorts of families were found to have an excess of schizophrenia within families compared with the population prevalence although the amount of risk to other family members varied, with more recent estimates based on present-day diagnostic criteria somewhat lower (reviewed in Gottesman and Shields, 1982; Gershon et al., 1988). Gottesman reviewed these studies and summarized the risks as somewhat peculiar in that they did not follow Mendelian patterns of inheritance (Gottesman, 1994; see *Table 4.3-1*) and in fact fell quite short of the rates one would expect if only transmission of an illness-associated gene variation was involved. They suggested a "stress-diathesis" additive and multifactorial polygenic model of how schizophrenia develops, with multiple environmental as well as genetic factors coming together to cause illness (e.g., Gottesman and Shields, 1982; McGue et al., 1986). Yet, it should be noted that none of the several known environmental factors claimed to be associated with risk confer any more that 1–5% relative risk for developing illness, far less than that of having a family history (8–10% for siblings and 50% on average to monozygotic twins of affected individuals; Moises and Gottesman, 2001).

Table 4.3-1
Relative risk for schizophrenia among relatives of people with schizophrenia based on the sum of several large family studies (Gottesman, 1994)

Relationship to person with schizophrenia	Relative risk (%)	Percent (%) genes shared on average
General population	<1	–
First cousins	2	12.5
Uncles/aunts	2	25
Nephews/nieces	4	25
Grandchildren	5	25
Half siblings	6	25
Parents	6	50
Siblings	9	50
Children	13	50
Fraternal twins	17	50
Identical twins	48	100

2.2 The Family of Patients with Schizophrenia During World War II

Despite the complex nature to the inheritance of schizophrenia, the eugenics movement of the earlier twentieth century (based on Mendelian inheritance of undesirable traits) took a strong position on individuals with psychiatric disabilities and mental retardation, and the principles of eugenics were worked into the regulations established by Hitler and his psychiatrist advisors during the Nazi Regime (Gottesman and Bertelsen, 1996). The prevailing thought was that if people with these disorders were not allowed to reproduce, the disorders would be eventually eliminated from humanity. This then led to extermination of such designated people if they were unable to contribute to the economic work force and yet were consuming its products. Psychiatrists were asked to rate their patients according to these guidelines. Those psychiatrists who were not sympathetic to the Nazi cause would then warn families to demand discharge of their affected family members from the hospital quickly in order to prevent their extermination. The role of the family in providing strong active support to the ill family member was essential and unfortunately, those without closely maintained family connections perished during those times. An accounting for this time in psychiatric and German history can be found in *By Trust Betrayed: Patients, Physicians and the License to Kill in the Third Reich* (Gallagher, 1995).

2.3 The Post-War Era and the Family (Late 1940s–1970s)

Shortly after World War II, psychoanalytic treatment of schizophrenia began to take hold. Frieda Fromm Reichmann was known for her psychoanalytic Freudian-based theories that provided methods to regress patients back to infancy in order to get them rid of the deviant patterns of nurturing previously received (Stanton, 1982). For many years, this form of therapy existed in private psychiatric hospitals, such as Chestnut Lodge, where Reichmann practiced, near Washington, DC. Even when neuroleptic treatment was introduced, these hospitals continued well into the 1980s to maintain patients medication-free and submitted to this form of therapy. Along with psychoanalytic treatment was the notion that the family, particularly the mother, was responsible for the development of schizophrenic behavior in her offspring. Theodore Lidz was known for his theories about the "schizophrenogenic mother" that often held in many psychiatrists minds long after this notion was abandoned (Lidz, 1972; Parker, 1982; Neill, 1990; Hartwell, 1996). Similarly, Margaret Singer and Lyman Wynne developed methods to assess deviant communication among family members leading to the popularity of family-based treatments for schizophrenia peaking in the 1970s (Wynne and Singer, 1963; Singer and Wynne, 1965, 1966; Wynne 1970; Perlberg, 1979; Wynne et al., 1992),

and the well-known psychoanalyst, Arieti espoused many similar psychodynamic approaches during that time (Arieti, 1968, 1974, 1980). The main concept was that the "patient" was the family and by treating the deviant patterns of communication, such as double binds, schisms, and skews among family members, the designated individual with schizophrenia would be cured (e.g., Esterson et al., 1965; Laing and Esterson, 1967; Doane et al., 1982; Wichstrom et al., 1996). Another set of reports, popular particularly in the UK, labeled family interactions according to the degree of "expressed emotion" observed. Supportive behavior of families in which there was reduced expressed emotion was shown to lead to better outcome among the patients with schizophrenia, while increased expressed emotion led to relapse and poorer outcome (Leff and Vaughn, 1981; Leff, 1994; Leff, 2000; Vaughn and Leff 1976). The consequence of this was the rise of family treatment specifically aimed at reducing expressed emotion. Unfortunately, implicating the family in such ways caused resentment and distrust of psychiatrists among families during that period of time and thus a lack of cooperation with recommended pharmacotherapy for the affected individual. It was only after family therapy as a form of treatment for schizophrenia was deemphasized and the importance of biological psychiatry began to be recognized in the early 1980s that support groups for families, such as The National Alliance for the Mentally Ill (NAMI in the USA) and Schizophrenia A National Emergency, (SANE in the UK) rose as powerful groups in support of psychopharmacology and the quest for more biologic research. Nevertheless, involvement of the family in treatment today certainly is important for contributing to the outcome of an acute schizophrenic-like episode. Family meetings with psychiatrists and other professionals now focusing on psychoeducation of the family has enormous beneficial effects on treatment compliance among the patients and thus better outcome, but does not take the place of medication (Hogarty et al., 1986, Perkins 2002; Pharoah et al., 2000/2003; Sherman et al., 2006, 1988, 1991; Penn and Mueser, 1996; Barrowclough et al., 2001; McFarlane et al., 2003; Addington et al., 2005; Penn et al., 2005). There is no doubt that the burden on families with a member having schizophrenia is tremendous and that family interventions should be made (Magliano et al., 1998; Glynn et al., 2006). However, it is now clearly understood that poor or deviant family relations do not in any way cause schizophrenia, and may rather be the result of having the burden of a chronically ill family member to care for (King, 2000); nor is family therapy or psychoanalysis, a current recommended treatment for this disorder, and certainly not a replacement for pharmacotherapy. In fact, severity of the illness of the patient may have an effect on the emotions expressed by other family members (King, 2000). If anything, this era set back treatment and biological research on schizophrenia for several years.

3 Early Genetic Studies

3.1 The Adoption Studies (1960s–1970s)

Partly due to the immoral misuse of genetic information during the Nazi Era leading to sterilization and extermination of psychiatric patients, genetics was not a research consideration until a group of adoption studies was highly publicized. The first was performed by Heston (1966) and studied offspring of schizophrenic mothers. This methodology was refined and expanded by Rosenthal and Kety who published the results of their Denmark series of adoption studies beginning in the late 1960s (the influence of SS Kety reviewed in DeLisi, 2000). Both adopted-away at birth children whose biologic parents had schizophrenia and adoptees who had schizophrenia were studied in several designs. The collective series of these studies of biologic and adoptive families implicated inherited biological factors rather than environmental influences on the development of illness (Rosenthal et al., 1968; Kety et al., 1968, 1978, 1994). However, some criticism has also been published about the interpretation of the Danish adoption study results. Although, biased by the preconceived views of the author (Lidz et al., 1981), they point out weaknesses in the Kety and Rosenthal data that have never been resolved, despite the openness of Seymour Kety to share his raw dataset for reanalyses in his later years (personal communication with SS Kety in the 1980s). In addition, Tienari et al. (2004) later performed an independent adoption study in Finland that, while confirming a substantial biological effect for risk, suggests that also pathology in the adoptive families (environmental influence) may contribute to some risk.

3.2 Molecular Genetics and the Family

There was no clear way forward to investigate genetic change as the underlying basis for schizophrenia until the 1980s when the field of molecular genetics burgeoned with new technology, all of which continued to escalate to the present day. Subsequent to the announcement that the total human genome had been sequenced in 2001 (Lander et al., 2001; Venter et al., 2001) and then the complete hapmap results of human variation published in 2005 (International Hapmap Consortium, 2005), these new tools have become facilitators of searches to find several major disease genes.

Paralleling the rise in new exciting technology over the past two decades was its application to psychiatry. Unfortunately, early optimism was quelled, and continues to be, by several highly publicized, but false positive studies (e.g., Egeland et al., 1987; Sherrington et al., 1988; Novak et al., 2002; Gerber et al., 2003) and no one gene can be said yet to be definitely a risk factor for schizophrenia. The following is a summary of the data that exist to date.

In the mid-1980s, genetic studies began with investigators searching for specific families that were considered informative for linkage. Ignoring the complex nature to the inheritance of schizophrenia and that a polygenic model was likely (Gottesman and Shields, 1967), the rules of Mendelian genetics, the only that existed for pursuit of single major genes, were applied to the search for genes for psychiatric disorders. Thus, families were considered informative when a proband had schizophrenia and at least one other sibling and, preferably, another branch of the family had one or more members with illness. In addition, it was essential that illness was transmitted from either paternal or maternal lineages, but not both. These rules were based on simulated calculations of how likely it would be that genes transmitted within such a family structure would cosegregate with illness (the LOD score; see Ott, 1999) and for Mendelian traits, a LOD score of 3.0 (p of 0.001) was widely considered evidence of linkage. Lander and Kruglyak (1995) further calculated rules that substantiated what might be significant linkage given multiple testing in a genome-wide screen. Yet, it is likely that these rules no longer apply given the now available dense single nucleotide polymorphism (SNP) maps of as many as 1,000,000 markers, even if the disorder was transmitted in a Mendelian fashion. Given that schizophrenia is a complex genetic disorder with no clear mode of transmission, Risch (1990) proposed the more preferable sib-pair analysis approach. Subsequently, beginning in the mid-1990s, large numbers of sibling pair collections were established internationally and analyzed using new computer mathematical approaches that were being developed. The reports that emerged showed a confusing picture of claimed linkage on almost every chromosome arm in the genome (DeLisi et al., 2000a; DeLisi et al., 2002; DeLisi, 2003; Lewis et al., 2003; Crow, 2007) with many inconsistencies across studies.

The probable genetic heterogeneity of the disorder led to the further focus on obtaining genetic isolates as groups of families that would be more informative for linkage studies than heterogeneous populations, such as USA and European cohorts. The principle behind the search for genetic isolates is that groups of individuals founded only a few centuries ago by a small number of individuals would have larger chromosome regions of linkage disequilibrium because of less previous generations for recombination to occur more than in ancient populations. In addition, if only a few individuals married into the population from the outside over generations, the population would form what is known as a genetic isolate. Such isolates are known to exist in such region as Northern Finland, Northern Sweden, and the Central Valley of Costa Rica (Peltonen, 2000; Peltonen et al., 2000; DeLisi et al., 2001; Varilo and Peltonen, 2004; DeYoung et al., 2006; Mathews et al., 2004), although modern worldwide travel is lessening their isolated character for future generations. Successful identification of rare disease mutations have been thus found in such isolates for Mendelian inherited illnesses, although this has not yet been the case for schizophrenia or other complex disorders (e.g., DeLisi et al., 2002b).

Lewis et al. (2003) performed a meta-analysis of the accumulated linkage data on schizophrenia using both heterogeneous and isolated population cohorts until approximately 2001, but they did not clearly find definitive linkages. Since meta-analyses are only as good as the data they use, it is not surprising that nothing definitive emerged. Despite this, some laboratories have promoted specific candidate genes within the putative linked regions, such as dysbindin on chromosome 6p (Straub et al., 2002; Williams et al., 2005), neuregulin on 8p (Munafo et al., 2006), and G72 on 13q (Detera-Wadleigh and McMahon, 2006) stimulating many follow-up association studies. Most investigators feel that the first generation of linkage studies confused the field because the number of families in each cohort was small (ranging from 25 to close

to 400) and that the markers were too widely spaced with gaps, thus false negatives from individual studies might have been as prevalent as false positives. In addition, the literature is clouded with findings that have been claimed as replications, but may not be. What constitutes a replication has been recently debated and rigorous rules outlined (see DeLisi and Faraone, 2006). The troubling inconsistencies in risk haplotypes transmitted to different populations for associations with these genes and the lack of consistency in study methodology are particular problems in deciding whether a finding has been replicated. Yet neuregulin, dysbindin, G72, and others continue to be popular candidates pursued in further studies. Perhaps, these will prove to be true contributors to risk for schizophrenia, despite researchers currently having little understanding of how they could contribute to its development and symptoms and despite the weakness of current claims.

4 Recent Advances

4.1 Whole Genome-Wide Studies

Nevertheless, the field has now risen beyond the linkage and candidate gene approach to whole genome-wide association studies currently with 500,000 or more markers on one chip used in large numbers of affected individuals and controls (<2,000 per cohort). More importantly, the genomics field has moved to pathway analysis using bioinformatics (e.g., Ingenuity systems) for each of these findings. Understanding functional pathways and mechanisms, in turn, provide hope to the pharmaceutical industry for new compound targets for cures and prevention at an early stage of illness. However, will these studies provide much needed confirmation for the first-generation candidates, or will they only put forth new candidates with new puzzles to solve? Results will be accumulating over the next couple of years.

It is rather hoped that true genotype variants will emerge in the near future that may eventually be valuable predictive measures of both who gets illness, and who responds to specific medications. However, one caveat remains. That is, there may not be a true sequence variation in a gene that causes illness, the premise that all the above linkage and association studies are based on. Rather, variable expression through epigenetic modification of gene activation may be the key (Petronis et al., 2000; Wong et al., 2005). The problem is that at the time of this writing, end of 2006, the methodology is not yet in place to explore epigenetic events that cause a complex genetic disorder such as schizophrenia. Nevertheless, several pieces of indirect evidence suggest an underlying epigenetic explanation, such as the lack of 100% concordance among the monozygotic twins, the difference in risk for disease between dizygotic twins and siblings both of on an average share the same percentage of parental genes, the considerable fall off in elevated risk for illness from first-degree to second-degree relatives, as well as the multiple environmental factors of modest, but elevated risk.

4.2 Imprinting and Anticipation

The imprinting and anticipation effects that are sometimes observed in family cohorts of people with schizophrenia could also change risk rates (Chotai et al., 1995; Husted et al., 1998; Fortune et al., 2003) and are forms of epigenetic modification. Imprinting of a parental gene preferentially will skew the appearance of inheritance of disease if a gene that is transmitted from the imprinted parental DNA is not expressed and the gene expression is entirely based on the nonimprinted other parental allele. Patterns of inheritance consistent with imprinting have been suggested by some, but not all studies having looked for it (e.g., DeLisi et al., 2000b).

Anticipation is another clinical familial phenomenon that shows increasing severity of illness from earlier to later generations. This is usually detected by age of onset becoming progressively earlier from generation to generation, or form of illness progressively changing. At least with other degenerative central nervous system disorders, such as Huntington's disease, the underlying basis for anticipation appears to be due to the expansion of pathological unstable repeat sequences and this could have relevance to schizophrenia as well (e.g., Ranen et al., 1995; Margolis et al., 1999).

5 Defining the Endophenotype Using Families

5.1 What is an Endophenotype?

Although genetic studies are being pursued in schizophrenia research because of the high heritability to the diagnosis of schizophrenia (70–80%), nevertheless, the concept that a biological, physiological, or cognitive measure might constitute an intermediate phenotype between the gene and the diagnosis, currently termed the "endophenotype" (formerly "genetic or biological marker", Rieder and Gershon 1978) has gained popularity (Gottesman and Shields, 1972; Gottesman and Gould, 2003; Gould and Gottesman, 2006). Genes, obviously, do not directly cause clinical symptoms; these are mediated at the protein, biochemical pathway, cellular systems, and regional brain level prior to symptom formation. Thus, if genes contribute at all of these levels, finding a biological marker (endophenotype) for deviance that makes one more vulnerable to illness could lead to precise tools for early detection and treatment targets. The endophenotype would need to be demonstrated as significantly different in people who have the disease, as highly heritable as the clinical disorder itself in twin studies, and shown to cosegregate with illness within families. The later criterion has been under some dispute by investigators who feel vulnerability factors are neither necessary nor sufficient to cause disease, and so does not need to cosegregate with illness, but need to be significantly more present in unaffected family members. This author disagrees with that notion, as anything that is neither necessary nor sufficient is secondary and thus unlikely to be crucial to the underlying basis for the disease.

5.2 Early Endophenotype Investigations

Although endophenotype studies have risen in popularity recently, several early ones under the guise of "biological markers" from over a decade ago are worth mentioning. The first twin brain imaging studies showed that ventricular size was heritable (more strongly correlated among monozygotic than dizygotic twins; Reveley et al., 1984) and followed-up by a family study showing heritability of ventricular size that cosegregated with illness within families (DeLisi et al., 1986). However, well relatives appeared to have no difference in ventricular size from controls in the latter study and thus, enlarged ventricles could be considered as secondary to the brain process underlying the illness itself and not a vulnerability factor. For example, in a more recent study, Seidman et al. (2003) showed in an initial group of families that hippocampal volume may be a more sensitive indicator of vulnerability for illness. Nevertheless, using another twin design (studying only discordant monozygotic twins), Weinberger and coworkers showed several regional brain structural differences to be present in unaffected co-twins of people with schizophrenia relative to controls (Suddath et al., 1990). Thus, overall, structural brain deficits may prove, if the methods are sensitive enough, to be useful as vulnerability markers for early detection of whom among those at genetic risk for schizophrenia develop illness, as some preliminary evidence from a few independent high-risk studies suggest (Lawrie et al., 2002; Pantelis et al., 2003; Seidman et al., 2003; DeLisi et al., 2006).

5.3 Potential Cognitive Endophenotype Investigations

Cognitive deficits have also been examined closely as possible endophenotypes and for evidence of heritability and clustering in families with schizophrenia (reviewed in Heydebrand, 2006). Most studies have examined individuals originating from families with only one ill member and thus have focused on whether any of the unaffected members have significantly more deficits than controls (e.g., Egan and Goldberg, 2003). However, this strategy fails to determine whether the cognitive deficits are associated with the familial cause of schizophrenia, itself. They only indirectly imply that this might be the case because of their presence in family members of people with schizophrenia. The more powerful strategy of selecting families with more than one ill member to detect whether the deficit cosegregates with illness provides

evidence that it is related to its inheritance and may be a vulnerability factor as well. A few of these studies have been reported (e.g., Shedlack et al., 1997; Hoff et al., 2005; Braff et al., 2007) and show that well relatives demonstrate deficits across a wide range of tasks, particularly measures of attention, verbal learning, and some expressive and receptive language. However, other measures of language and memory that distinguish schizophrenia from normal controls do not appear to cosegregate with illness within families. Thus, at present, it is concluded that the literature on cognitive endophenotypes is thus far inconsistent and no known cognitive test clearly qualifies as an endophenotype and likely to be useful to predict with a high degree of sensitivity and specificity who will develop schizophrenia among individuals at high risk, nor are useful yet for determining molecular genetic relationships. Several research groups actively continue the search for endophenotypes.

6 The Family and Stigma

The social consequences of schizophrenia have been enormous for decades. There is now new awareness of the stigma attached to having a family member with schizophrenia (Phelan et al., 1998; Thompson 2003), and how such stigma must be eradicated in order to obtain better treatment for people afflicted with this disorder, and to obtain public support for the much needed research to find preventive measures and new pharmaceutical agents for treatment (Corrigan et al., 2006). It has only been in recent years that family groups have organized and become powerful lobbyists for better care for their family members. Some of the issues raised above, such as blaming families for causing the illness and suggesting that the "family unit" is the "patient" only perpetuated the attached stigma. The World Health Organization has mounted a campaign against the stigma of schizophrenia, but it still exists in most countries, particularly in developing countries where family members with schizophrenia are often chained to walls of homes, isolated in basements or closets, or delivered to psychiatric institutions, and abandoned by their families (Warner, 2005). With earlier detection and new treatments without visual side effects, individuals who have schizophrenia can now live in the community unnoticed and thus not stigmatized as they had been in the past. Finding the familial basis for the illness will also aid in lifting the associated stigma. However, one cautionary note: gene defects may also stigmatize individuals if legislature anticipating the rise in genetic detection of a variety of illnesses is not in place to prevent insurance company and job discrimination of people having DNA screened for supposed vulnerability genes for illness.

7 Conclusions

Schizophrenia is a complex heritable disorder, but its underlying basis is likely to remain unknown until the genetic mechanism for normal brain and cognitive development is better understood. It is likely that many of the current positive genetic findings in the field will be shown not to be major contributors to the cause of this complex disorder either because they are simply false positives or epiphenomena. Time will tell how all the above observations will come together. But, what is clear is that the field of genetics, and thus ascertaining families as a unit for study, currently forms the center of all research in medicine and thus psychiatry and is likely to continue to do so.

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Section 5

Behavior

5.1 The Neurobiology of Negative Symptoms and the Deficit Syndrome

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_5.1,

Abstract: Primary negative symptoms were a fundamental aspect of the initial description of schizophrenia. The advent of antipsychotic medication in the 1950s shifted the focus of schizophrenia research and treatment towards reality distortion symptoms even though these positive symptoms are less discriminating in diagnosis and are less correlated with overall outcome. Although research in deficit symptoms is sparse, work done has demonstrated consistent patterns of associated brain regions and neurochemistry. This includes dysfunction of the dorsolateral prefrontal – basal ganglia – thalamocortical circuit and abnormalities in the dopaminergic, glutamatergic, and cholinergic neurotransmitter systems. Future research in this area faces a number of challenges including distinguishing deficit from secondary negative symptoms and development of adequate measures of social and occupational function for use in intervention studies.

List of Abbreviations: Ach, acetylcholine; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CNS, central nervous system; COMT, catechol-*O*-methyltransferase; DA, dopamine; DRP-2, dihydropyrimidinase-related protein 2; DSM, diagnostic and statistical manual of mental disorders; GNAS1, guanine nucleotide-binding protein (G-protein) alpha stimulating activity polypeptide 1; 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin (5-hydroxytryptamine); MAO, monoamine oxidase; MHPG, 3-methoxy-4-hydroxyphenylglycol; NE, norepinephrine; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; SSRI, selective serotonin reuptake inhibitor; SDS, schedule for the deficit syndrome

1 A Brief History of Negative Symptoms and the Deficit Syndrome

The concept of nuclear schizophrenia defined by highly discriminating symptoms has led to the current diagnostic criteria, which are skewed toward hallucinations and delusions, and away from the pathologies first put forward as defining the disease category. Kraepelin wrote of two distinct dimensions of psychopathology in what he called dementia praecox: The first, a "dissociative" pathology, encompassed disorganization of thought. The second, an "avolitional" pathology, was described as "weakening of those emotional activities which permanently form the wellspring of volition" (Kraepelin, 1971/1919). Blueler (1950/1911) gave emphasis to the "split" within cognition and between cognition and volition and emotion. He saw this "avolition" as one of the primary defects of the illness, with other symptoms (like hallucinations and delusions) in an accessory role. The psychoanalyst Rado made "avolition" a topic of investigation and wrote extensively on the "integrative pleasure deficiency" of schizophrenia (Rado et al., 1962). According to him this deficiency prevented both the development of a sound sense of self and the incorporation of the self into a social environment. It was this anhedonia that led to the most profound personal and social impairments of the disorder.

In 1950s, the discovery of chlorpromazine's ability to decrease psychotic symptoms gave birth to the modern era of neuropsychopharmacology. Chlorpromazine's method of action led directly to the dopamine theory of schizophrenia (i.e., that a dysregulation of dopamine, in particular dopamine excess, may be responsible for the psychotic symptoms of the illness). In the excitement over an effective treatment for psychotic symptoms, attention was diverted from the avolitional pathology. This is illustrated by efforts to describe pathognomonic symptoms for schizophrenia, best represented by the symptoms of first rank described by Schneider (1959). Schneiderian first rank symptoms can be viewed as ego boundary disturbances and reflect the reality distortion component of schizophrenia that has become dominant in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) and *International Classification of Diseases* (ICD) criteria for schizophrenia. This emphasis on reality distortion continues despite refutation of the nuclear schizophrenia concept (Carpenter, 2006), and evidence suggesting cognitive impairments and avolitional pathology are most associated with poor functional outcomes in schizophrenia (see Hyman and Fenton, 2003 for a brief review).

Interest was revived in the "avolition" dimension, in part, by the 1974 publication of "An Approach to the Diagnosis and Understanding of Schizophrenia" by Strauss et al. (1974). Strauss et al. examined patients with schizophrenia to determine which characteristics would be most reliable in establishing the diagnosis. They found a group of symptoms that were extremely statistically reliable in producing agreement among diagnosticians. Citing terminology used by the nineteenth century neurologist J. Hughlings Jackson, they organized

these symptoms into positive and negative symptom domains and a third domain related to psychopathology observed in disordered relationships. Positive symptoms "appear active," as exaggerations of normal processes (e.g., hallucinations and delusions). On the other hand, negative symptoms represent diminished normal function (e.g., blunted affect and thought blocking [see *Table 5.1-1*]). The positive symptom domain proposed by Strauss et al.(1974) was later modified. Andreason and Olsen (1982) found little relationship between disorganization of thought and reality distortion. Interpersonal pathology received little systematic attention. The three symptom domains presently emphasized are reality distortion, disorganization of thought and behavior, and negative symptoms (Buchanan and Carpenter, 1994). In addition, impairments in cognition detected through neuropsychological testing are viewed as a pathologic feature of core importance to schizophrenia, but relatively unrelated to the three symptom domains.

It is worth noting that Jackson's use of the terms "positive" and "negative" implied an etiology, i.e., negative symptoms were caused by a specific defect or lesion, while positive symptoms were caused by a lack of inhibitory control secondary to the lesion (Jackson, 1887). Therefore, positive and negative symptoms were related and linked in the individual. Because Strauss et al. specifically stated they did not mean to imply etiology with the terminology, Berrios (1985) has suggested the credit for the more descriptive use of the terms be given to the neurologist John Reynolds (whose use predates Jackson's as well).

After the publication of the Strauss et al. article, there was considerable interest in whether a subtype of schizophrenia could be defined by positive or negative symptoms. Crow (1980) postulated the existence of two distinct syndromes within schizophrenia, Type I and Type II. Type I was characterized as having more positive symptoms, more acute, responding well to neuroleptics, and with limited intellectual impairment. Type II was defined by irreversible flat affect, insidious onset and chronic course, responding poorly to neuroleptics, and more often associated with gross brain abnormalities. Later, Crow clarified the two types could coexist within the same individual, thus bringing his ideas more in line with the domain concepts of positive and negative symptoms (Crow, 1985).

Table 5.1-1
List of negative symptoms[a]

Affective flattening (manifested by)
Unchanging facial expression
Decreased spontaneous movements
Decreased expressive gestures
Poor eye contact
Affective nonresponsivity
Lack of vocal inflections
Alogia (manifested by)
Poverty of speech
Thought blocking
Increased latency of response
Apathy (manifested by)
Poor grooming and hygiene
Problems with social/vocational role (manifested by)
Failure of appropriate role responsibilities
Anergy
Asociality/anhedonia (manifested by)
Failure to engage with peers socially
Loss of interest in stimulating activities
Decreased interest in sex
Decreased intimacy with others

[a]Modified from Andreason (1982)

Andreason and Olsen (1982) proposed distinguishing subgroups of patients by whether positive symptoms were prominent (positive schizophrenia), negative symptoms were prominent (negative schizophrenia), or both/neither were prominent (mixed schizophrenia). They initially postulated that positive and negative symptoms were inversely related, with negative symptoms becoming more prominent after successful treatment of positive symptoms or as the illness progressed (Andreason and Olsen, 1982; Andreason, 1985).

As more was written on the subject of negative symptoms the literature became blurred as to whether the symptoms discussed were related to state or trait (Sommers, 1985). To refine the concept, negative symptoms resulting from treatment (e.g., Parkinsonism related to neuroleptics), psychosis (e.g., elective mutism related to paranoia), institutionalization/monotonous routine, and/or related to depression were referred to as "secondary" negative symptoms (Carpenter et al., 1985). In contrast, "primary" negative symptoms were due to the illness itself. These enduring traits were labeled "deficit symptoms" (Carpenter et al., 1985).

Armed with this refined concept, Carpenter et al. (1988) proposed classifying patients with prominent deficit symptoms into a "deficit syndrome" subtype of schizophrenia (see *Table 5.1-2*). Organization into this subtype was independent of positive symptoms. Therefore, people diagnosed with the deficit syndrome could still be quite psychotic. In fact, studies have consistently found little to no relationship between the categorization of deficit/nondeficit and the severity of positive symptoms (Kirkpatrick and Buchanan, 1990; Kirkpatrick et al., 1992; Kirkpatrick et al., 2000a). Several lines of evidence support the distinction of a separate deficit syndrome within schizophrenia. Deficit patients have greater anhedonia, less depression and suicide, and abuse substances less compared with nondeficit patients (Kirkpatrick and Buchanan, 1990; Fenton and McGlashan, 1994; Kirkpatrick et al., 1994; Kirkpatrick et al., 1996).

Further reinforcing the concept of a discrete syndrome is the excess number of summer births seen in deficit patients in several countries (Kirkpatrick et al., 1998; Kirkpatrick et al., 2000a; Messias and Kirkpatrick, 2001; Tek et al., 2001; Kirkpatrick et al., 2002a; Kirkpatrick et al., 2002b; Messias et al.,

Table 5.1-2
Diagnostic criteria for the deficit syndrome of schizophrenia[a]

At least two of the following six negative symptoms must be present:
Restricted affect
Diminished emotional range
Poverty of speech
Curbing of interests
Diminished sense of purpose
Diminished social drive
Some combination of two or more of the negative symptoms listed above have been present for the preceding 12 months and always were present during periods of clinical stability (including chronic psychotic states). These symptoms may or may not be detectable during transient episodes of acute psychotic disorganization or decompensation
The negative symptoms above are primary, i.e., not secondary to factors other than the disease process. Such factors include:
Anxiety
Drug effect
Suspiciousness (and other psychotic symptoms)
Formal thought disorder
Hallucinations or delusions
Mental retardation
Depression
The patient meets DSM-IV-TR criteria for schizophrenia

[a]Modified from Kirkpatrick et al. (1989)

2004). This is in contrast to winter birth as a risk factor for schizophrenia in general and for affective disorders. Familial concordance studies also support a distinctive pathophysiology in deficit versus nondeficit schizophrenia. Schizophrenia is more likely in relatives when the proband patient has the deficit rather than the nondeficit form of the illness (Dollfus et al., 1996; Dollfus et al., 1998; Kirkpatrick et al., 2000a). Within this greater risk for schizophrenia, there is a threefold risk that relatives will have deficit schizophrenia (Ross et al., 2000). In fact, the concordance rate between deficit/nondeficit categorization in siblings with schizophrenia has been found to be 74% (Kirkpatrick et al., 2007). Also, among relatives of deficit patients who do not have schizophrenia, there are significantly more negative-like symptoms such as social avoidance compared with relatives of nondeficit patients (Kirkpatrick et al., 2000b).

This chapter will focus largely on the biology and neurochemistry of primary negative symptoms and the deficit syndrome.

2 Brain Regions of Interest

2.1 Neuroimaging/Gross Postmortem Studies

One of the most consistent findings in schizophrenia is increased lateral ventricle size compared with healthy controls or individuals with affective disorder (Johnstone et al., 1976; Andreason et al., 1982a,b; Brown et al., 1986; Crow et al., 1989; Bruton et al., 1990). Some authors have noted individuals with negative symptoms and the chronically institutionalized may have been oversampled in these studies (Owens et al., 1985; Williams et al., 1985). Others have made negative symptoms a variable and have reported a correlation between increased ventricle size and negative symptoms (Andreason et al., 1982a, b; Pearlson et al., 1984; Kemali et al., 1987; van Kammen et al., 1987; Andreason et al., 1990; Johnstone et al., 1994). Conversely, there have been a number of studies that have failed to find a correlation between enlarged ventricles and negative symptoms (Losonczy et al., 1986; Pandurangi et al., 1986; Keilp et al., 1988; Pfefferbaum et al., 1988), some even found an inverse correlation between negative symptoms and ventricle size (Bishop et al., 1983; Luchins et al., 1984).

Discrepancies may be related to a number of factors, including varying technique. However, none of the aforementioned studies used the deficit/nondeficit dimension to categorize patients. Thus, primary versus secondary negative symptoms may also have complicated the results. In studies using the deficit/nondeficit categorizations, there have either been no associations or the nondeficit patients have had larger ventricles (Buchanan et al., 1993; Quarantelli et al., 2002).

Measures of regional cerebral blood flow have demonstrated specific patterns in deficit patients compared with nondeficit patients and healthy controls. Individuals with the "psychomotor poverty syndrome" (analogous to the deficit syndrome) were shown to have decreased cerebral blood flow in the dorsolateral prefrontal cortex (left > right) and left parietal lobe (Liddle et al., 1992). These patients also had increased blood flow in the caudate. Imaging of patients with the deficit syndrome has replicated the finding of decreased frontal blood flow, especially during tasks of memory (Heckers et al., 1999). During tasks of auditory recognition, deficit patients had decreased cerebral blood flow to the frontal lobes during a control condition, and decreased blood flow to right frontal/right inferior parietal lobes during the task (Lahti et al., 2001). A more recent study has found decreased blood flow in the frontal and parietal cortices correlated with negative symptoms both in the deficit and nondeficit groups (Lahti et al., 2006). This does not necessarily indicate that the distinctions between deficit and nondeficit are artificial. Rather, it may imply that whether primary or secondary, negative symptoms have a common pattern of impairment in the brain.

Brain metabolism patterns have also differentiated individuals with the deficit syndrome. In deficit patients, the thalamus and frontal/parietal cortices utilize less glucose at rest than in nondeficit patients and healthy controls (Buchanan et al., 1989; Tamminga et al., 1992).

One magnetic resonance spectroscopy (MRS) study of 5 deficit patients versus 17 nondeficit patients and 21 controls noted lower ratios of *N*-acetylaspartate (NAA) to creatine and phosphocreatine in the medial prefrontal cortex of deficit patients (Delamillieure et al., 2000). NAA may reflect neuronal viability and so these results may indicate a poorly functioning prefrontal cortex.

2.2 Indirect Support for Brain Regions

Studies of individuals with the deficit syndrome have noted problems with tasks of sensory integration, e.g., stereognosis, graphesthesia, and right-left confusion (Buchanan et al., 1990; Arango et al., 2000). Somatosensory information is received in the thalamus before being redirected to the parietal cortex. These findings are consistent with impairment in the thalamus and/or parietal cortex in the deficit syndrome.

Studies have had mixed conclusions about whether negative symptoms are associated with specific visual attention/eye-tracking defects. However, when categorizing participants by the presence or absence of *deficit* symptoms, correlations are observed. Deficit patients have slow reaction times in tasks of visual attention and information processing (Buchanan et al., 1997; Bustillo et al., 1997). As the parietal lobes have been implicated in attention (Behrmann et al., 2004), this may reflect parietal impairment.

Deficit patients also have specific difficulties in eye-tracking (Ross et al., 1997; Hong et al., 2003). The eye-tracking paradigm has component parts. The predictive pursuit component is impaired in both deficit and nondeficit schizophrenia as well as in their biologic relatives. However, the initiation of smooth pursuit is abnormal only in the deficit syndrome subgroup and their relatives (Hong et al., 2003). Recall that deficit symptoms have been connected to decreased metabolism in the frontal lobes and increased metabolism/regional blood flow in the caudate. This agrees with the finding that individuals with eye-tracking difficulties, irrespective of diagnosis, also have decreased metabolism in the frontal lobes and increased metabolism in the caudate (Ross et al., 1994). This may indicate that the deficit pathology and certain eye-tracking difficulties have a common etiology and supports the use of eye-tracking as a potential endophenotypic marker for primary negative symptoms.

Further support for frontal and/or parietal lobe dysfunction in deficit patients has come from neuropsychological testing. An early study found that deficit and nondeficit patients perform less well on measures of frontal, parietal, and temporal lobe-related tasks compared with healthy controls, and deficit patients have further impairment on measures of frontal and parietal function (but not temporal lobe function) when compared with nondeficit patients (Buchanan et al., 1994b). However, subsequent studies have not confirmed the specificity of this pattern; rather, the deficit group appears to have very broad and anatomically nonspecific cognitive impairment compared to nondeficit patients (Cohen et al., 2007).

2.3 Histology

There have been very few studies of the histology of deficit patients. Akbarian and colleagues have published a series of articles describing a subset of people with schizophrenia with an excessive amount of neurons present in the white matter of the temporal and frontal lobes compared with controls (Akbarian et al., 1993a; Akbarian et al., 1993b; Akbarian et al., 1996). This subset was described as less of the paranoid type and as having less suicidality (Akbarian et al., 1996). Recognizing this subset may represent deficit patients, Kirkpatrick et al. examined these interstitial cells of the white matter (ICWM) in deficit, nondeficit, and control brains (Kirkpatrick et al., 1999; Kirkpatrick et al., 2003). Looking at the inferior parietal and dorsolateral prefrontal regions, they found an increased density of ICWM compared to nondeficit and control brains (Kirkpatrick et al., 1999; Kirkpatrick et al., 2003). The nondeficit brains did not significantly differ from the control brains in either area; having slightly less ICWM than control brains in the frontal lobe and slightly more in the inferior parietal (Kirkpatrick et al., 1999; Kirkpatrick et al., 2003).

While this offers further support for prefrontal and inferior parietal dysfunction in the deficit syndrome, other areas of the brain have not been examined. However, it prompts interesting theories of the deficit syndrome being related to failure of normal neuronal migration.

2.4 Summary

In summary, multiple lines of evidence have implicated the frontal and parietal lobes in the dysfunction of deficit symptoms. Most evidence implicates the dorsolateral prefrontal cortex and the inferior parietal cortex. Other brain areas of interest are the thalamus and caudate. There are indications that this

dysfunction is either caused by or manifested by impairments of blood flow and glucose metabolism and perhaps improper cell migration during development. The brain path implicated by these findings is the dorsolateral prefrontal–basal ganglia–thalamocortical circuit, which includes the dorsolateral prefrontal and inferior parietal cortices, the caudate, and aspects of the thalamus (Alexander et al., 1990).

3 Neurotransmitters and Chemicals of Interest

3.1 Dopamine

The most prominent neurotransmitter in schizophrenia research has historically been dopamine (DA). DA is a biogenic amine belonging to the catecholamine transmitters. It is synthesized from tyrosine, which is an essential amino acid in humans, and broken-down by monoamine oxidase (MAO). Ultimately, it is metabolized to homovanillic acid. DA is the major neurotransmitter in four brain signaling pathways: the nigrostriatal, tuberoinfundibular, mesolimbic, and mesocortical tracts. The nigrostriatal tract is involved in movement; the tuberoinfundibular tract regulates hormones in the pituitary. The mesolimbic and mesocortical tracts are thought to be important in the production of symptoms in schizophrenia. The mesolimbic path has been implicated in emotion and the production of positive symptoms, while the mesocortical path has been associated with sociality and motivation, the lack of which form negative symptoms.

There are five recognized types of DA receptors in humans: D_1, D_2, D_3, D_4, and D_5. D_1 and D_5 are similar in that their stimulation results in an increase of cyclic AMP rather than a decrease. Since approximately 80% of catecholamine neurotransmission is by DA (Vallone et al., 2000), the DA receptors are understandably widely distributed. D_1 is predominantly present in the cortex and hippocampus, and D_2 in the striatum, nucleus accumbens, amygdala, and less in the cortex and hippocampus.

There is some evidence that DA signaling is disturbed in the deficit syndrome. Plasma homovanillic acid has been found to be decreased in deficit patients compared with nondeficit patients (Ribeyre et al., 1994; Thibaut et al., 1998). Although one study found deficit patients had a higher plasma homovanillic acid level (Nibuya et al., 1995), the identification of deficit patients in the study has been questioned (Thibaut et al., 1998; Kirkpatrick et al., 2001). This finding is compatible with the possibility of decreased DA signaling, a hypodopaminergic state, in deficit patients. In particular, because of the contribution of the frontal lobes to the plasma level of homovanillic acid, Davis et al. (1991) have postulated a hypodopaminergic state particularly reflects the frontal lobes. Considering the distribution of DA receptors in the brain, this would, in turn, implicate the D_1 receptor specifically.

Hypodopaminergic frontal lobes in deficit patients are consistent with other signs of frontal lobe dysfunction as reviewed earlier. Consistent, documented, negative symptom response to DA agonists and amphetamines would lend support for this theory; however, results have been mixed (as reviewed by Buchanan et al., 1996). Most reports examining DA-enhancing strategies have been small, open-label studies or case reports. Two blind, placebo-controlled trials of MAO inhibitors have also had mixed results: selegiline seeming mildly effective for negative symptoms and mazindol not (Carpenter et al., 2000; Bodkin et al., 2005). Neither study differentiated secondary from deficit symptoms.

There have been several theories behind the brain circuits involved in the development of deficit symptoms. One, published in 1987 by Daniel Weinberger, attempted to explain the seemingly paradoxical concurrent hyperdopaminergic state of psychosis and hypodopaminergic state of negative symptoms. He postulated that an early developmental insult to mesocortical dopamine afferents would simultaneously deprive the dorsolateral prefrontal cortex of dopamine and lead to dysregulation/dopamine excess in mesolimbic projections. This is supported by what Pycock et al. (1980) had found in rats: a lesion of the dopaminergic afferents in the prefrontal cortex led to subcortical dopamine overactivity.

3.2 Norepinephrine

Norepinephrine (NE) is synthesized from DA by dopamine β-hydroxylase. As with DA, NE is broken-down by MAO. Ultimately, it is primarily metabolized to 3-methoxy-4-hydroxyphenylglycol (MHPG). In the brain,

NE afferents originate predominantly in the locus ceruleus. From there, neurons project widely throughout the cortex. In the frontal cortex particularly, NE interacts with and modulates DA afferents (van Kammen and Kelley, 1991; Tassin, 1992). Therefore, there has been some interest in NE and negative symptoms.

Negative symptoms have been variously correlated with levels of MHPG, during relapse with high, and during quiescence with low levels of MHPG (as reviewed by van Kammen and Kelly, 1991). The only study to examine MHPG in deficit patients reported higher plasma concentrations versus nondeficit patients (Thibaut et al., 1998). All patients in that study had been clinically stable for the preceding 12 months.

Recall that depression is a notable source of secondary negative symptoms, and NE is a neurotransmitter of interest in depression. This could potentially complicate investigation into NE and negative symptoms unless patients were specifically examined for deficit symptoms. This may help explain the mixed results of treating negative symptoms with NE blockers, reuptake inhibitors, and MAO inhibitors (as reviewed by Buchanan et al., 1996; Carpenter et al., 2000; Bodkin et al., 2005).

3.3 Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is synthesized from the essential amino acid tryptophan. It is a biogenic amine belonging to the indole family. 5-HT is broken-down by MAO and ultimately metabolized to 5-hydroxyindole acetic acid (5-HIAA). In the brain, the 5-HT afferents originate in the raphe nuclei and project to the striatum, thalamus, hippocampus, amygdala, and cortex.

There are at least 13 different 5-HT receptor types: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7. 5-HT_3 is associated with ion channels while the other receptors are associated with second messenger systems. 5-HT receptors have a very complicated relationship with central nervous system (CNS) DA (as reviewed by Lieberman et al., 1998). 5-HT_{2A} *antagonism* seems to increase DA release in the medial prefrontal cortex which, in turn, decreases subcortical DA levels (Lieberman et al., 1998). Alternatively, *stimulation* of the 5-HT_3 and 5-HT_{1B} and 1D receptors can also potentiate DA release- in the striatum, nucleus accumbens, and the medial prefrontal cortex (Lieberman et al., 1998).

There have been very few studies of 5-HT function and negative symptoms. Low 5-HIAA has been correlated with negative symptoms in one study (Pickar et al., 1986). During neuropsychological testing in people with chronic schizophrenia, low 5-HIAA was correlated with decreased regional cerebral blood flow in prefrontal areas compared with those of healthy controls (Weinberger et al., 1988). Recall that decreased activity in prefrontal regions has also been specifically noted in deficit patients. However, several studies have reported low 5-HIAA correlated with suicide in people with schizophrenia (as reviewed by Bleich et al., 1988), which is incompatible with the deficit syndrome.

Given the complexity of interactions between DA and 5-HT, any finding of abnormal levels of 5-HT in the brain is difficult to interpret. So, too, are the effects on negative symptoms of medications like selective serotonin reuptake inhibitors (SSRIs) that increase synaptic 5-HT irrespective of receptor type. Even studies with drugs that affect specific 5-HT receptors, such as atypical antipsychotics, become difficult to interpret given the role of 5-HT in depression. As of yet there are no studies of SSRIs in deficit patients and most studies purporting a benefit of atypical antipsychotics in deficit patients have been too inadequately controlled to make such a claim (Kirkpatrick et al., 2000c). However, the possibility that a specific 5-HT receptor interaction might preferentially increase DA in the prefrontal cortex is encouraging.

3.4 Glutamate

For decades researchers have looked at whether intoxication with certain drugs could mimic the signs and symptoms of schizophrenia. Amphetamines increase dopamine release and can induce some positive symptoms like paranoia. Lysergic acid diethylamide (LSD) affects 5-HT receptors but tends to induce visual perception changes and not frank hallucinations. Phencyclidine (PCP), however, induces a state in healthy controls that seems very similar to schizophrenia. This includes thought disorder and negative symptoms

like affective flattening, withdrawal from the environment, and poverty of speech (reviewed by Javitt and Zukin, 1991). A great deal of excitement was generated by the 1979 discovery of PCP's binding site and in the subsequent finding it was associated with a glutamate receptor (Javitt and Zukin, 1991).

Glutamate is a nonessential amino acid. There are two classes of glutamate receptors: excitatory (associated with ion channels) and modulatory (associated with metabotropic receptors). Indeed, glutamate is the primary excitatory neurotransmitter in the human CNS. After glutamate is released, it is deactivated by conversion to glutamine by astrocytes. Glutamine is then taken back into neurons where it is converted again to glutamate for release in neurotransmission.

The main types of glutamate ion channels are α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-stimulated, kainate-stimulated, and *N*-methyl-D-aspartate (NMDA)-stimulated. It is the NMDA receptor that has the PCP binding site. The glutamate and PCP binding sites are distinct and so there is no competition between these ligands. When PCP binds to the NMDA receptor, it blocks normal flow of ions ($Ca2^+$, K^+, Na^+) through the channel. Therefore, symptoms of schizophrenia, including primary negative symptoms, are thought to be related to hypofunction of NMDA receptors. This idea has been reinforced by the findings that ketamine (a PCP-type anesthetic) can induce hypofrontality, and kynurenic acid (an endogenous NMDA antagonist) is elevated in brains of people with schizophrenia (as reviewed by Coyle and Tsai, 2004).

Of note, there have also been links between glutamate receptor stimulation and pathology. Excessive stimulation of glutamate receptors can cause seizure and it is hypothesized that NMDA overstimulation can cause neuronal cell death from an excessive influx of calcium. This has led to the development of NMDA antagonists like memantine to treat Alzheimer's Disease.

In order to function, the NMDA receptor must bind the amino acid glycine in addition to glutamate. In healthy brains, the universal physiologic availability of glycine is usually not a rate-limiting step. However, several studies have noted a correlation between decreased plasma levels of glycine/its metabolites and negative symptoms (Sumiyoshi et al., 2004; Neeman et al., 2005). Considering this, there has been interest in exploiting the glycine "coagonist" site to enhance the function of NMDA receptors in schizophrenia. This includes using oral glycine as well as other agonists at its site (including D-serine and the partial agonist D-cycloserine). Several small trials have reported decreased negative symptoms with use of these agents; however, a thorough meta-analysis by Tuominen et al. (2005) found only modest benefit for negative symptoms. Furthermore, the Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST) – a large, multi-site, double-blind, placebo-controlled, randomized study – found no substantial benefit in using glycine agonists for negative symptoms (Buchanan et al., 2007). Despite these results, NMDA glycine-site agonists have not been studied in deficit patients, and not definitively in combination with a glycine transport inhibitor such as sarcosine.

One theory has that NMDA hypofunction leads to less inhibitory signaling and, thus, excitotoxicity via downstream AMPA glutamate receptors- especially in patients with chronic negative symptoms (Drapalski et al., 2001; Deutsch et al., 2002). The anticonvulsant topiramate potentiates inhibitory signaling and blocks AMPA glutamate receptors and was reported by Deutsch et al. (2003) to benefit patients with prominent negative symptoms. However, other studies and case reports (albeit in treatment-resistant patients versus those with prominent negative symptoms) have been unable to find a benefit, most of them finding topiramate worsened patients' symptoms (Dursun and Deakin, 2001; Millson et al., 2002; Hofer et al., 2003; Tiihonen et al., 2005).

3.5 Acetylcholine

Acetylcholine (ACh), although technically an amine, is not considered a member of the biogenic amine neurotransmitters because it is not synthesized from an amino acid. Rather, it is synthesized from acetyl CoA and choline. ACh is used for signaling throughout the CNS and at the neuromuscular junction. Ach is deactivated by acetylcholinestherase. In general, there are two classes of ACh receptors: muscarine-stimulated and nicotine-stimulated. Muscarinic receptors are metabotropic, and M_1, M_2, M_3, M_4, M_5 types are recognized. Nicotinic receptors are ion channels composed of several subunits given Greek letters. The most relevant to discussions of the brain and negative symptoms is α2β4.

A balance between ACh and DA in the brain has long been recognized; e.g., Parkinsonism caused by neuroleptic blockade of D_2 receptors can be relieved by anticholinergic medication. However, Tandon has suggested cholinergic dysregulation may be an intrinsic part of schizophrenia, and not just iatrogenic (Tandon and Greden, 1989). He has postulated an excess of DA in psychosis leads to a proportional increase in ACh to maintain the brain's balance, which, in turn, increases negative symptoms through muscarinic receptors (Tandon and Greden, 1989). This hypothesis is based, in part, on the observations that muscarinic receptor agonists can induce withdrawal from the environment, motor retardation, poverty of thoughts, and blunted affect (as reviewed by Tandon and Greden, 1989). In several trials, Tandon found anticholinergic medications improved negative symptoms; conversely, Tandon and others noted positive symptoms seemed to worsen (as reviewed by Buchanan et al., 1996; Tandon et al., 1990; Tandon et al., 1991). Given that negative symptoms in Tandon's hypothesis become evident after resolution of an acute psychotic phase, a time of increased risk of depression in individuals with schizophrenia, it is possible Tandon's negative symptoms are secondary and have limited relevance to the deficit syndrome.

Compared with the general population, a much larger proportion of individuals with schizophrenia use tobacco products. There has also been some indication that smoking is correlated with negative symptoms (Patkar et al., 2002). This has led to speculation regarding the role of nicotinic ACh receptors in negative symptoms. Although no study to date has tested negative symptom response to selective nicotinic drugs, evidence from animal studies make these receptors plausible targets. For example, studies in rats have shown that stimulating α4β2 receptors can increase glutamate release preferentially in the prefrontal cortex (Gioanni et al., 1999; Lambe et al., 2003). Additionally, Drew and colleagues have shown that α4β2 stimulation in rats increases amphetamine-provoked DA release, again, preferentially in the prefrontal cortex (Drew et al., 2000; Drew and Werling, 2001; Drew and Werling, 2003). Therefore, a selective α4β2 nicotinic receptor agonist may be one strategy to address a hypofunctioning prefrontal cortex in individuals with the deficit syndrome.

3.6 Folate

Goff et al. (2004) have postulated a link between folate and negative symptoms because of common enzymes involved in brain glutamate transmission and folate absorption. Indeed, in nonsmoker they have found significantly lower folate levels in deficit patients compared to nondeficit patients. They also found a much larger percentage of deficit patients met the criteria for hypofolatemia when compared with prior studies of a general population. Although the question of cause or effect is relevant here, folate functions in the biosynthesis of several neuroactive compounds including dopamine and glycine (Goff et al., 2004). Currently, Goff and colleagues are studying folate supplementation in individuals with schizophrenia and prominent negative symptoms.

3.7 Summary

In summary, negative symptoms have been associated with perturbations in multiple neuroactive chemicals. However, the deficit syndrome has only been investigated with regards to a few of these. The most promising areas of research currently seem to be in the area of decreased DA in the frontal lobes and NMDA receptor dysfunction.

4 Genes of Interest

4.1 COMT

Despite the surge of genetic studies in schizophrenia, few have examined genes in relation to the deficit syndrome. One gene that has received some attention is the catechol-*O*-methyltransferase *(COMT)* gene. COMT is an enzyme involved in the metabolism of catecholamine neurotransmitters. In the general

population there is a polymorphism within the gene for COMT at position 158 resulting in two forms of the enzyme – some containing valine, some methianine. The importance of this polymorphism is that the function of the enzyme differs depending on the amino acid substitution, with the valine substitution conferring a fourfold increase in activity. Theoretically, individuals homozygous for the valine variant would have much higher breakdown of dopamine in the frontal lobes. This, in turn, might lead to the hypofrontality associated with negative symptoms.

In a study of val/val, val/met, and met/met frequencies, one group found no association between alleles and categorization of deficit or nondeficit (Wonodi et al., 2005). However, the relationship between the *COMT* gene and the deficit syndrome may be more complex. Another study of the deficit/nondeficit categorization and the *COMT* allele found a significant relationship between motor deficits measured by the Neurological Evaluation Scale (NES; Buchanan and Heinrichs, 1989) and the val/val genotype only in a the deficit group (Galdirisi et al., 2005). These motor problems included coordination and sequencing of complex acts.

4.2 Other Genes

Two other genes have been linked to deficit patients: dihydropyrimidinase-related protein 2 (*DRP-2*) and guanine nucleotide-binding protein (G-protein) alpha stimulating activity polypeptide 1 (*GNAS1*).

DRP-2 is involved in neuronal development and axonal migration. It is located in a chromosomal area associated with schizophrenia with poor affect and outcome but less depression (8p21-p22; Kendler et al., 2000). The gene has many single nucleotide polymorphism(SNP); Hong et al. (2005) examined one, $T^{2236}C$, in relation to the deficit syndrome. They found a significant association between the C allele and schizophrenia and, in whites, found the relationship was much stronger in the deficit group versus the nondeficit group.

GNAS1 encodes a subunit of the G-proteins that are involved in cell signaling pathways. Of the dopamine receptors, D_1 and D_5 are G-protein coupled. One study of a SNP ($T^{393}C$) found a statistically significant higher frequency of the TT genotype in deficit patients versus nondeficit patients and controls (Minoretti et al., 2006). The TT genotype is associated with a higher expression of the G-protein subunit. Because this subunit is involved in the apoptotic pathway, the group hypothesized that some patients with the deficit syndrome may have inappropriate apoptosis due to overexpression of the TT gene product (Minoretti et al., 2006).

5 Why Do We Know so Little?

Even though we are just at the beginning of understanding the pathophysiology of schizophrenia, it is striking how little we know about negative symptoms and the deficit syndrome. Some of this is surely due to the focus on positive symptoms over the last half century. Most research focusing on treatments for schizophrenia have not focused on schizophrenia per se, but rather on one domain of symptoms common to several psychiatric disorders, i.e., positive symptoms. This is not to say that understanding and treatment of hallucinations and delusions are not important, but most studies have found that the real disability of schizophrenia often comes from negative symptoms and cognitive difficulties (Fenton and McGlashan, 1994).

Studies done on negative symptoms often have difficulties not encountered in studies of positive symptoms. Although there is general agreement in the field about what constitutes negative symptoms, there are difficulties in suitably measuring these symptoms. Most studies use general rating scales with only a few items specific to negative symptoms. Some studies use rating scales specific to negative symptoms, but even then there are problems. The rating scales specific to negative symptoms have some items that do not fit the concept of the deficit syndrome (e.g., inappropriate affect). Furthermore, the measurements of negative symptoms given by these ratings are snapshots and do not encompass the enduring quality of primary negative, deficit symptoms. Therefore, it is impossible to know whether two studies reaching

different conclusions about a treatment were testing it on participants with a different mix of deficit versus secondary negative symptoms Arango et al., 2004.

Not only do we know less than one might expect, given other developments in schizophrenia research, but much of what we think we know about negative symptoms may actually be misleading with regard to deficit schizophrenia. For instance, antipsychotics frequently improve negative symptoms in randomized clinical trials, but, as shown in our review earlier, there is little reason to believe they favorably affect the primary negative symptoms in the deficit syndrome.

One solution to this is a rating scale that includes only items agreed to represent the deficit syndrome and that takes into account a longitudinal period. The Schedule for the Deficit Syndrome (SDS) does this and has been determined to have retest and inter-rater reliability (Kirkpatrick et al., 1989; 1992). Furthermore, the SDS is not confounded by neuroleptic effects (Bustillo et al., 1995). However, the SDS requires instruction to be used correctly, and thus its employment can be limiting to researchers.

Another problem in studies of deficit symptoms is a failure to control for secondary negative symptom improvement. It is not incompatible for an individual with the deficit syndrome to have secondary negative symptoms as well (although they would be unlikely to be depression-based). Therefore, studies should include nondeficit patients as well as deficit patients in order to put results in context (Kirkpatrick et al., 2000c). This is in addition to controlling for confounders like depression, medication effects, psychosis, etc. However, even with the best designed trial involving deficit and nondeficit individuals and controlling for confounding sources of secondary negative symptoms there is a problem of measuring outcome. Although some ratings (like lack of vocal inflections) may improve immediately, other items (regarding relationships and school/work) may not have had sufficient time to improve by the end of a study (Kirkpatrick et al., 2000c).

Finally, while there are a number of satisfactory animal models for testing drugs for antipsychotic effect on positive symptoms, there are no comparably accepted models for negative symptoms. Although experimental lesions have induced asociality in otherwise social animals, these animals have not been widely used to test treatments for negative symptoms. Also, there is increasing indication that negative symptoms can be divided into two factors: asociality/anhedonia/avolition and blunted affect/poverty of speech, which may be independent (Kirkpatrick and Fischer, 2006). This may mean that testing animal models of asociality may not be relevant to blunted affect/poverty of speech.

6 In Conclusion

The primary negative symptoms of schizophrenia have been described from the beginning of recognition of the disorder. However, only relatively recently has medical research focused on investigating and treating them. From our limited understanding, it appears prominent deficit symptoms mark a subgroup of the illness. This subset of individuals has impairment traced to the dorsolateral prefrontal–basal ganglia–thalamocortical circuit. The group also seems to have dysfunction involving decreased DA and NMDA receptor dysfunction. Further research in this area will need to control for secondary negative symptoms, allow for improvement that will only be recognized in the long-term, and determine adequate animal models for drug development.

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5.2 Event-Related Potentials (ERPs) in the Study of Schizophrenia: How Preclinical ERP Studies have Contributed to our Understanding of Schizophrenia

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D. Javitt & J. Kantrowitz (eds.), *Schizophrenia,* DOI 10.1007/978-0-387-30410-6_5.2,

Abstract: Impairments in auditory event-related potentials (ERPs) have been studied extensively as endophenotypic markers of schizophrenia. Abnormalities in amplitude and latency of the ERPs as well as aberrations in gating of the auditory stimuli and difficulties in the detection of change in auditory stimulus characteristics are common in schizophrenic populations. This chapter introduces readers to ERPs and their role as an endophenotypic marker of schizophrenia. In addition to a review of the basic understanding of the role of ERPs in human research, this chapter also illustrates the congruence of mouse and human ERPs. The role of rodent-based preclinical models of ERP abnormalities, including pharmacological and genetic models, in schizophrenia research is reviewed. Pharmacologic models of treatment are also a promising area of research in the preclinical study of schizophrenia. The authors describe several well-studied mouse models and the effects of various antipsychotic treatments on ERPs in these model. More novel therapeutics and models, including abnormalities in gamma oscillations, also are discussed.

List of Abbreviations: ABRs, acoustic brainstem responses; cAMP, cyclic AMP; DHBE, dihydro-β-erythroidine; ERPs, event-related potentials; GBO, gamma band oscillation; ISI, interstimulus interval; MLA, methyllycaconitine; MMN, mismatch negativity; nAChR, nicotinic acetylcholinergic receptor; NMDA, *N*-methyl-D-aspartate

1 Introduction

This chapter will introduce readers to auditory event-related potentials (ERPs) and their role as an endophenotypic marker of schizophrenia. Impairments of auditory evoked potentials in schizophrenia have been extensively studied. This chapter will explore what we know about ERPs in schizophrenia and how they can be modeled in rodents. Several types of models and the effects of various pharmacological compounds will be discussed. Novel therapeutics and models are also included.

2 ERP Deficits in Schizophrenia – Background

2.1 Components of the Human ERP

Impairments of auditory evoked potentials have been extensively studied as an endophenotypic marker of schizophrenia. The human auditory evoked potential can be divided into three subsets of latency-defined components that correspond to progression of brain activity related to the auditory stimulus through the auditory pathway. Early components (wave I–VI) originate in the cochlea and auditory nuclei of the brainstem, while mid-latency components (including the P50 in humans) occurring between 8 and 50 ms are thought to correspond to the activation of auditory thalamus and auditory cortex (Picton et al., 1974). The longer latency components (including the N100 and P200 in humans) have been localized to the primary auditory cortex and cortical association areas (Gallinat et al., 2002). Mid-latency and longer latency components of auditory evoked potentials evaluated in many human studies are named for either position or latency. The first major positive deflection commonly studied is known as the P1 or P50, and occurs at approximately 50 ms poststimulus. The first major negative deflection is the N1 or N100, with a latency of approximately 100 ms. The second positive deflection is called the P2 or P200, and has a latency of approximately 200 ms. Relative topology of the P1, N1, and P2 is consistent across species, although the analogous components between humans and animals are debated.

2.2 Abnormalities in Obligate ERPs in Schizophrenia

The ERPs of individuals with schizophrenia are typified by alterations in both amplitude and latency of several key components relative to normal populations. The ERP amplitude reflects not only the amount of neural activity, but also the degree of synchrony in the firing of the many neurons involved (Frith and

Friston, 1996). Changes in component amplitude may reflect altered levels of arousal or alertness. Numerous studies have noted a decrease in the amplitude of the N100 (Roth et al., 1980a; Ogura et al., 1991; Ford et al., 1994; Boutros et al., 1997a; Laurent et al., 1999; Karoumi et al., 2000; Ford et al., 2001), either increased or decreased amplitude of the P200 (Roth et al., 1980a; Boutros et al., 1997a), and P300 (Roth et al., 1980a, b; Ogura et al., 1991; Ford et al., 1994; Boutros et al., 1997a; Laurent et al., 1999; Karoumi et al., 2000; Ford et al., 2001) components in patients with schizophrenia. The latency of select ERP components also appears to be changed in schizophrenic populations, although reports are mixed regarding which components are affected and in which direction. The latency to the peak or onset of a component is considered as the time at which certain processes or circuits in the brain are activated (Donchin et al., 1986; Kok, 1990, 1997) and may represent processing speed and efficiency of information processing. Both increases and decreases in N100 latency have been reported (Schlor et al., 1985; Adler et al., 1990; Adler and Gattaz, 1993); similar contradictory findings have been reported for the P200 component with studies describing P200 latency increases (Schlor et al., 1985; Adler et al., 1990; Adler and Gattaz, 1993; Ogura et al., 1991) and others reporting decreases (Roth et al., 1980a; Adler and Gattaz, 1993).

2.3 Gating of ERPs

Multiple studies have found impaired gating of auditory evoked potentials on a paired stimulus task in patients with schizophrenia and approximately half of their first-degree relatives (Adler et al., 1982; Siegel et al., 1984; Judd et al., 1992; Erwin et al., 1994; Stevens et al., 1997; Boutros et al., 1999). During the paired stimulus gating task, the amplitude of the response to the second stimulus (S2) is normally decreased or "gated" as compared with the amplitude of the first response (S1). This phenomenon is thought to reflect the brain's capacity to filter out repeated stimuli and modulate sensitivity to incoming sensory stimuli (Braff and Geyer, 1990). Gating deficits are commonly reported for the P50 component and have typically been framed as a failure to inhibit the S2 in a paired-click task; however, the original description of the phenotype and more recent studies have argued that the underlying gating deficit in unmedicated schizophrenia might be due to decreased S1 amplitude without further decrement of the S2 (Freedman et al., 1983; Jin and Potkin, 1996; Stevens and Wear, 1997; Clementz and Blumenfeld, 2001; Moxon et al., 2003). This profile of equal amplitude for S1 and S2 has been conceptualized as a loss of gating (Adler et al., 1998). Similarly, the amplitude of the N100 S1 is significantly reduced in schizophrenia patients, although S2 does not differ from nonaffected control subjects (Frangou et al., 1997; Boutros et al., 1997a, Clementz and Blumenfeld, 2001; Boutros et al., 2004). In addition, patients treated with antipsychotic medication have increased P50 amplitudes for both responses compared with unmedicated patients, maintaining the loss of gating relative to control subjects (Freedman et al., 1983; Clementz et al., 1998, Clementz et al., 2004).

2.4 Deviance and Novelty-Related ERPs

The ability to detect change in auditory stimulus characteristics is a basic neuronal function that can be measured in humans with ERPs. These include the mismatch negativity (MMN), the P3 or P300, and a novelty-elicited component occurring between 400 and 600 ms, termed the slow wave (Ford and Hillyard, 1981; Roth et al., 1981; Sams et al., 1983; Paavilainen et al., 1989; Ogura et al., 1991; Alho et al., 1998; Ceponiene et al., 1998; Escera et al., 1998; Schall et al., 1999; Spencer et al., 2001). MMN is an automatic electroencephalographic response to a change in stimulus characteristics such as tone or duration. It occurs between 100 and 225 ms in humans and is viewed as an indicant of early detection of novelty in the environment. Multiple studies have shown that MMN is reduced in people with schizophrenia, as well as their first-degree relatives, and is disrupted by NMDA antagonists including ketamine in humans and monkeys (Shelley et al., 1991; Javitt et al., 1993,1996; Umbricht et al., 2000; Jessen et al., 2001). The P300 is an auditory evoked potential with a latency of approximately 320 ms that is elicited by changes in stimulus

quality and has been shown to differ in people with schizophrenia in both amplitude and latency of the response (Schall et al., 1999; Mathalon et al., 2000; Grzella et al., 2001).

2.5 Gamma Frequency Abnormalities in Schizophrenia

In addition to traditional ERP-based measures of electrical brain activity, research has also focused on changes in slow-wave oscillatory patterns, specifically in the gamma range (30–60 Hz). Gamma activity is nonlocalized electrical activity in the brain and has been closely correlated with cognitive functions making it of particular interest to the study of schizophrenia and other neuropsychiatric disorders (Engel et al., 2001; Herrmann and Demiralp, 2005). Several different paradigms have been used to study gamma activity in schizophrenia including gating tasks, sustained and evoked gamma, oddball paradigm, and target detection tasks. Most studies have supported a general decrease in gamma activity across various tasks (Herrmann and Demiralp, 2005), although there is some indication that changes in gamma activity may be associated with specific symptom clusters of the disorder (Lee et al., 2003; Herrmann and Demiralp, 2005).

3 Human–Mouse ERP Homology

3.1 Comparing Mouse and Human Components

Animal models can be extremely useful tools for investigating mechanisms and treatments for a variety of human disorders including schizophrenia. A significant amount of research has focused on identifying rodent correlates for the established human ERP components and modeling schizophrenia-associated deficits in these animals. The majority of ERP animal models of schizophrenia have focused on mouse correlates of mid-latency ERPs in humans. These components, which reflect early stages of sensory processing, include the P50, N100, and P200. Mid-latency auditory-evoked components commonly examined in rodents include the P20 (positive deflection at 20–30 ms), the N40 (negative deflection at 40–50 ms), and the P80 (positive deflection at 80–100 ms). There is latency shift in mice such that the mouse components occur at approximately 40% of the latency of the human components. These components are also named for latency, P30, N50, and P80 in rats and P20, N40, and P80 in mice (Iwanami et al., 1994; Siegel et al., 2003). There is latency shift in mice such that the mouse components occur at approximately 40% of the latency of the human components. The analogous components between the rodent and human are debated (Adler et al., 1986; Ehlers et al., 1997; Miyazato et al., 1999) (*Figure 5.2-1*).

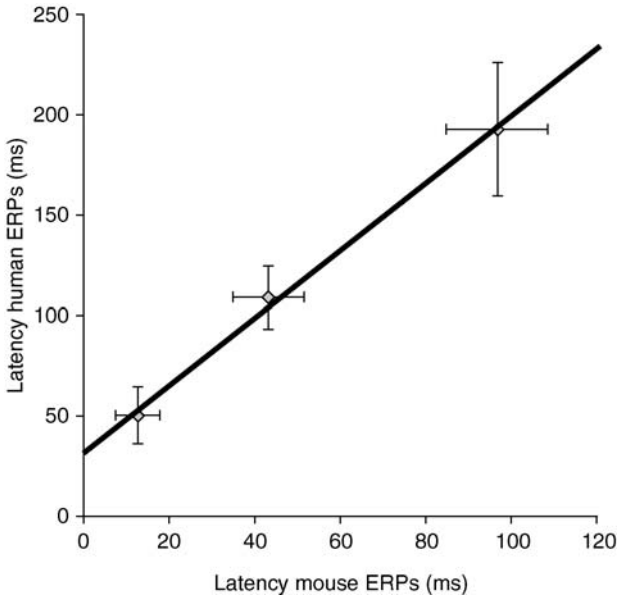
3.2 Acoustic Brainstem Responses

Acoustic brainstem responses (ABRs) are a series of evoked responses that occur within 8 ms of an auditory stimulus in humans, and are produced by activation of the brainstem auditory nuclei and pathways (Jewett and Williston, 1971). ABR abnormalities have been linked to auditory hallucinations in schizophrenic patients. A study by Lindstrom et al. (1987) found that one half of schizophrenic patients tested exhibited abnormal ABRs, and that nearly all of these patients admitted to having hallucinations. These data suggest that interference in brainstem function is involved in the psychopathology of schizophrenia. ABR in mice occurs within 6 ms of stimulus presentation, and is often used as an assessment of hearing sensitivity (Zheng et al., 1999; Connolly et al., 2003). Variations in ABR thresholds, amplitudes, and latencies between strains of mice can help to elucidate the underlying genetic differences in auditory pathology.

3.3 Mouse Correlates of the Human P50

The P50 represents preattentive sensory processing and is thought to originate in auditory thalamus and auditory cortex (Picton and Hillyard, 1974; Picton et al., 1974; Erwin et al., 1991). Previous studies have

Figure 5.2-1
Latency of mouse P1, N1 and P2 are shown in comparison with latencies of the corresponding human P1, N1 and P2 components (Reprinted from Siegel et al., 2005, with permission from Elsevier)



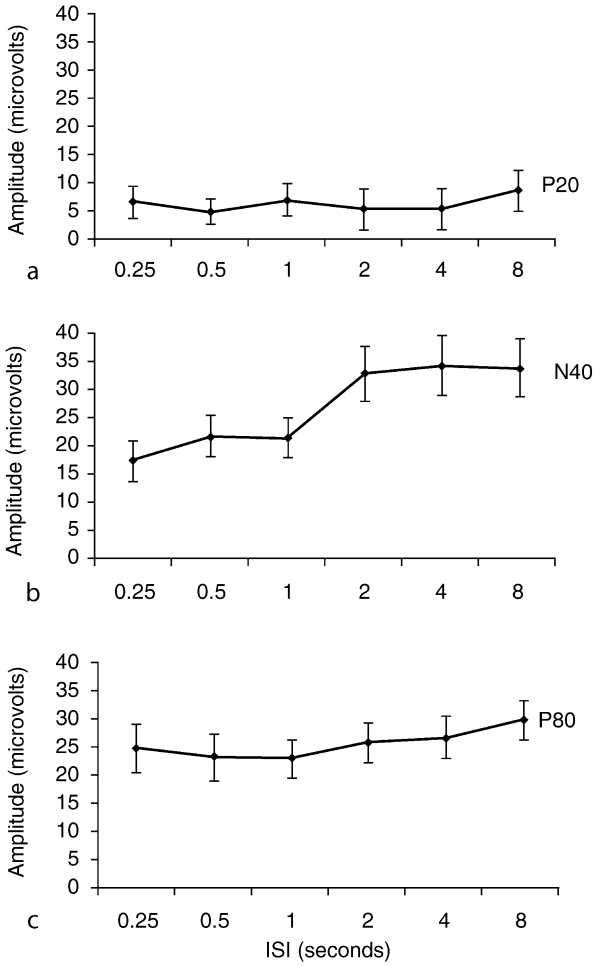
proposed a P20/N40 difference waveform in the mouse to be analogous to the human P50 based on stimulus properties and pharmacological treatments in the anesthetized mouse (Stevens and Wear, 1997; Simosky et al., 2003). However, several recent studies indicate that the P20 and N40 components behave independently in response to pharmacological and psychometric manipulation, obscuring the ability to interpret a difference waveform (Connolly et al., 2004; Umbricht et al., 2004; Maxwell et al., 2004a, b). The mouse P20, like the human P50, does not exhibit significant amplitude changes with varying stimulus intervals between 1 and 8 s (Onitsuka et al., 2000; Maxwell et al., 2004b). Additionally, many studies in rat compare the N40 to the human P50 (Adler et al., 1986; Boutros et al., 1997b; Koenig et al., 2005), while others consider the earlier P13 as the appropriate correlate of the human P50 (Miyazato et al., 1999; Mamiya et al., 2005).

Several studies recently have evaluated physiological response properties of various ERP components in mice in an effort to make evidence-based interspecies comparisons. These data, taken in their entirety now create a substantial body of evidence that indicate that the P20 is the appropriate ERP component in mouse to compare with the human P50.

3.4 Mouse Correlates of the Human N100 and P200

Similarly, the evoked components in rodents that are analogous to the N100 and P200 in humans are deliberated. These components represent attentive phases of informational processing, and originate in frontal cortex (Picton and Hillyard, 1974, Shaw, 1995). Maxwell et al. (2004b) observed that the mouse N40 displays an interstimulus interval (ISI) response relationship, intensity function, and pharmacological response properties similar to those found with the human N100. Several studies have also evaluated these psychometric and pharmacological responses for the P80 in mice and have determined that it shares a high degree of similarly with the human P200 (*Figures 5.2-2* and *5.2-3*).

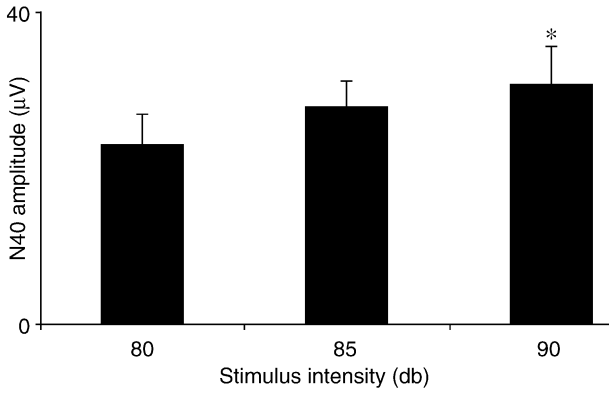
Figure 5.2-2
Amplitudes + SEM of the mouse P20, N40, and P80 components across all ISI in the control condition are shown with a main effect of ISI on the N40 component, but not P20 or P80 (Reprinted from Maxwell et al., 2004b, with permission from Macmillan)



3.5 Mouse Correlates of the Human Deviance-Related MMN and P300

People with schizophrenia misinterpret events in their environment. Such difficulties may manifest as inappropriate assignment of meaning to irrelevant stimuli leading to referential delusions. Alternatively, patients often do not recognize relevant social and occupational cues. The complex process of assigning meaning to stimuli is beyond our current understanding and can not be modeled in animals. However, the elementary ability to detect change in the environment can be reduced to a series of sequential operations including: (1) registration of a sensory input; (2) encoding of its qualitative features (pitch); (3) echoic memory of the stimulus; (4) comparison of sequential inputs; followed by (5) determination of deviance. These operations are abnormal in schizophrenia as measured with electrophysiological responses to auditory stimuli using auditory ERPs. For example, the P50 is an automatic response that registers the

Figure 5.2-3
Mean + SEM for the mouse N40 amplitude stimulus intensity response is shown (Reprinted from Maxwell et al., 2006a, with permission from Macmillan)

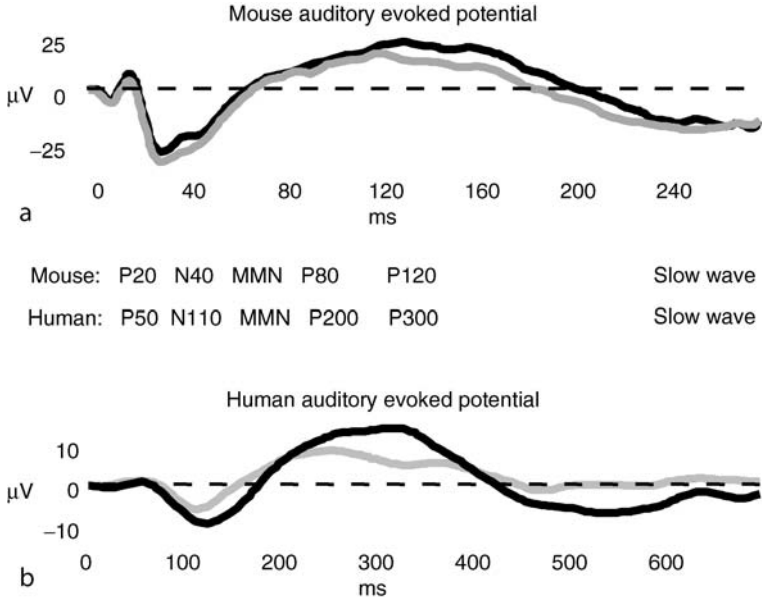


presence of an auditory stimulus, but does not vary substantially with its qualitative features. This is followed by the N100, which is sensitive to qualitative features such as pitch and intensity. The MMN follows the N100 temporally and is thought to reflect immediate, brief storage of stimuli in auditory cortex for comparison with subsequent inputs to detect a match or mismatch between serial stimuli. A later ERP component called the P3a reflects higher order processing of deviance that surpasses a threshold to elicit orienting to salient change in the environment. People with schizophrenia show reductions in all of these ERPs, demonstrating pervasive deficits in the neural responses to sensory stimuli that may lead to abnormal interpretation of events. Additional studies suggest that abnormalities in stimulus-related gamma and theta oscillatory rhythms may contribute to pervasive impairments and ERP abnormalities in schizophrenia. Previous mouse studies have focused on gating of early ERPs such as the P20 and N40. However, ERPs are also capable of modeling deficits in deviance detection. MMN also has an analogous component in the rodent ERP. As in the human, MMN in a rodent appears as a negative deflection between the N40 and P80 components. A recent study has shown that MMN can be elicited in mice through a deviance paradigm which varies duration of the stimulus (Umbricht and Krljes, 2005). Future studies may investigate MMN using deviations in other characteristics such as pitch or intensity. The human P300 is elicited by novel or task-relevant events, such as those presented in an oddball paradigm (Polich, 1987; Verleger, 1988). The mouse P120 has been shown to be augmented by novel stimuli (Siegel et al., 2003). Taken together, these data suggest that auditory ERP latencies in the mouse are approximately 40% of those seen in humans (*Figures 5.2-4*).

4 Pharmacological Models of Psychosis

The identification of rodent correlates for the various human ERP components has facilitated the modeling of various human conditions and disorders, including the ERP abnormalities associated with schizophrenia and psychosis. Like the mouse and human ERP correlates mentioned earlier, the human and mouse P1, N1, and P2 respond similarly to many pharmacological compounds. A variety of pharmacological models have been examined to help elucidate possible mechanisms underlying observed abnormalities in ERPs in schizophrenia. The most common pharmacological models of proposed mechanisms are reviewed later.

Figure 5.2-4
(a) Mouse ERP to novel (black) and standard (gray) stimuli are shown. (b) Human ERP responses to novel (black) and standard (gray). Note that the human P300 and mouse P120 display increased amplitude following novel stimuli (Reprinted from Siegel et al., 2006, with permission from Macmillan)



4.1 Dopamine – Amphetamine

There is evidence that dopamine may play a significant role in schizophrenia (Carlsson, 1977, 1978; Bennett, 1998). Additionally, the indirect dopamine agonist, amphetamine, produces a psychotic state in healthy individuals and exacerbates the symptoms of psychosis seen in patients with schizophrenia. As such, exposure to amphetamine is a well-characterized model of the auditory processing abnormalities common to schizophrenia and several studies have investigated the effects of amphetamine on rodent ERPs.

The dopamine agonist, d-amphetamine, has been administered to adult rats to validate an animal model for disturbances in ERP gating. Multiple labs have reported that amphetamine diminishes gating of the N40 and P80 ERP components (Stevens et al., 1991; Stevens et al., 1996b; de Bruin et al., 1999b; Maxwell et al., 2004a). One study reported no effects of d-amphetamine were found on the gating of the P20 component, suggesting the possibility that this component is affected by other neurotransmitter systems (de Bruin et al., 1999a). These findings are consistent with most theories of schizophrenia that suggest a multifactorial mechanism for the symptoms and dysfunction associated with the disorder.

4.2 NMDA Antagonists – Ketamine

One of the most popular hypotheses to explain schizophrenia, the glutamate hypothesis of schizophrenia, is based on the observation of psychotomimetic effects of noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists, such as phencyclidine (PCP), dizocilpine (MK-801), and ketamine. Like amphetamine, NMDA antagonists produce a psychosis-like state when administered to healthy individuals. Further

support for the glutamate hypothesis of schizophrenia also includes changes in glutamate receptor density and decreased glutamate levels in certain brain regions (Deakin et al., 1989; Toru, 1998; Tsai et al., 1998). The studies reviewed in this chapter examine the effects of the NMDA antagonist ketamine on ERPs. Like other NMDA antagonists, ketamine exacerbates positive symptoms in schizophrenia and induces schizophrenia-like symptoms in nonaffected individuals (Javitt and Zukin, 1991; Lahti et al., 1995; Newcomer et al., 1999; Lahti et al., 2001). The following studies evaluate ketamine as a pharmacological model of schizophrenia-like changes in ERPs.

Studies have demonstrated that acute ketamine administration decreases the amplitude and latency of the mouse N40 and P80, mimicking schizophrenia-like abnormalities on those components (Connolly et al., 2004; Maxwell et al., 2006b). In contrast, a study by de Bruin et al. (1999b) reported that acute ketamine had no effect on gating of the N40 and P80 components. Additionally, these same studies report that ketamine increases the amplitude and latency of the mouse P20 which is inconsistent with schizophrenia (Connolly et al., 2004; Maxwell et al., 2006b). Based on the contradictory data reported, the overall value of acute ketamine to model schizophrenia-like ERP deficits is still in question.

Although chronic ketamine (repeated acute administration for 14 days) reportedly has no effect on the amplitude or latency of any ERP components, there does appear to be some lasting effects of chronic ketamine exposure (Maxwell et al., 2006b). In a study by Maxwell and colleagues, mice were treated with ketamine daily for 2 weeks, and then tested 1 week later. Ketamine decreased N40 amplitude, but not latency 7 days after last ketamine dose. These data indicate that chronic NMDA receptor hypofunction can lead to auditory processing deficits similar to those seen in schizophrenia patients (Maxwell et al., 2006b).

4.3 Stress – Corticosterone

Some studies indicate that patients with schizophrenia have increased circulating cortisol levels (Gil-Ad et al., 1986; Whalley et al., 1989; Breier and Buchanan, 1992; Lammers et al., 1995). As a result, a number of studies have examined the ability of isolation stress in rats (Stevens et al., 1997) or chronic administration of the stress hormone corticosterone in mice (Stevens et al., 2001; Maxwell et al., 2006a) to recreate ERP amplitude and/or gating deficits of schizophrenia. These studies indicate that increased psychological stress and/or chronic corticosterone can yield reduced ERP amplitude and gating deficits similar to the patterns seen in schizophrenia.

Chronic corticosterone administered at a variety of doses produced a trend in which decreased amplitude and impaired gating of the mouse N40 was correlated with increased circulating corticosterone levels. This finding resembles a reported schizophrenia endophenotype of elevated cortisol and impaired N100 amplitude. Additionally, the trend of decreased circulating corticosterone improving normal gating provides a novel therapeutic strategy for schizophrenia (Maxwell et al., 2006b). In a study by Stevens et al. (2001), 8 days of chronic corticosterone treatment resulted in a decrease in auditory gating of the ERP. A concurrent decrease in α-BTX binding sites suggests that a decrease in α-BTX binding, and thus α7 nicotinic acetylcholinergic receptor expression, may be associated with this change in behavior (Freedman et al., 1995).

4.4 Mixed Models – Bupropion

These findings of dopaminergic hyperfunction or NMDA hypofunction producing schizophrenia-like deficits in auditory processing highlight the necessity for examination of multiple neurotransmitter systems and pathways in schizophrenia. They also illustrate the complexity of the disease and the difficulty of modeling it in animals. Despite the challenge of finding an appropriate model, mouse ERP studies have contributed significantly to our understanding of the pharmacological properties of many compounds. These studies include both pharmacological illness models, such as dopamine agonist-induced endophenotypes of schizophrenia, and various treatment models which will be explored later in this chapter. The monoamine reuptake inhibitor bupropion has been helpful in both of these respects.

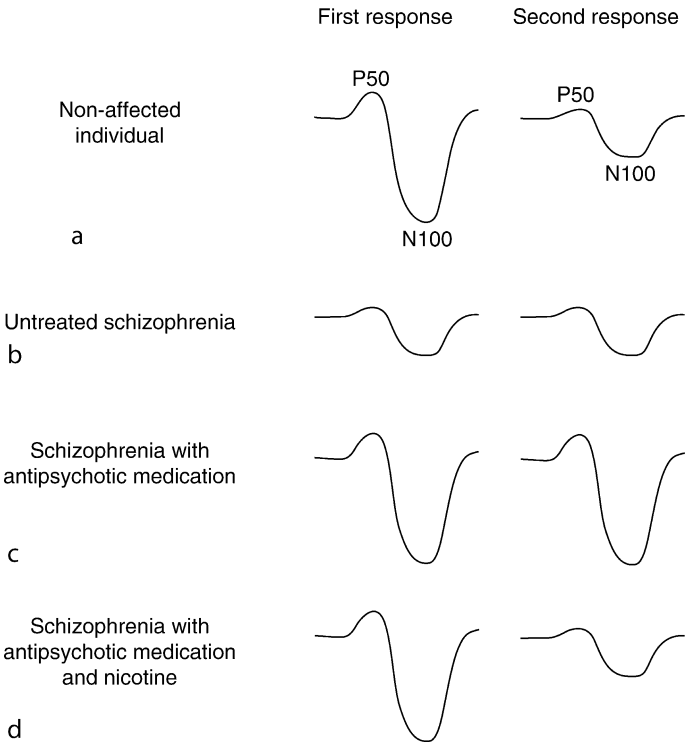
Some studies have shown that amphetamine causes a reduction in auditory gating of the P20/N40 components (Stevens et al., 1995), while others have argued that the effects are limited to amplitude of the

first response, rather than gating per se (Maxwell et al., 2004a). Additionally, there is a large body of literature that has suggested that nicotine receptor dysfunction may underlie gating mechanisms in general, and deficits in schizophrenia. Therefore, the indirect dopamine agonist and nicotine receptor antagonist bupropion has been proposed as a possible model for ERP and or gating deficits in the illness.

These studies demonstrate that bupropion alone recreates the reduction in P20 and N40 amplitude, similar to amphetamine. The addition of nicotine has no benefit to amplitude or gating under these circumstances. However, unlike amphetamine, the addition of haloperidol to bupropion yields a profile of large first response with an abnormally large second response. This combination of an illness model (bupropion) with a treatment model (haloperidol) fully recreated the pattern of large responses to both the first and second stimuli seen in some studies of schizophrenia. Furthermore, under these conditions, nicotine restored gating by selectively reducing the second response for the N40 (Siegel et al., 2005). Thus, this study provides a unified explanation for why initial studies in untreated schizophrenia concluded that small amplitude was the primary deficit in schizophrenia, but later studies performed on medicated patients suggested a gating deficit mediated by a large second response which responds to nicotine. As such, the authors concluded that "gating" may be an epiphenomenon of treatment on a background of the real primary deficit in schizophrenia, namely reduced amplitude (*Figures 5.2-5*).

Figure 5.2-5

Schematic representation of schizophrenia phenotypes for the P50 and N100. (a) The normal pattern of P50 and N100 responses in nonaffected individuals. Note that the first response is large and the second is decreased. (b) In unmedicated schizophrenia, both the first and second responses are diminished for the P50 and N100. (c) Antipsychotic treatment results in increased amplitude of both the first and second responses for the P50 and N100 among schizophrenia patients, yielding the pattern of normal amplitude with impaired gating often described among medicated patients. (d) Nicotine reduces the amplitude of the second response among medicated schizophrenia patients (Reprinted from Umbricht et al., 2004, with permission from Elsevier)



5 Genetic Models of Schizophrenia

While pharmacological models of ERP changes and gating deficits have contributed significantly to our ability to study these phenotypes in animals, there are also multiple studies using genetically engineered models. These models provide the opportunity to study behaviors without concern for drug–drug interactions and a method for mimicking potential genetic changes in the human condition.

5.1 Dysregulation of c-AMP Binding

Deficits in auditory processing in schizophrenia have been localized to cortical forebrain structures as indicated by the decreased amplitude and gating of the cortically generated N100. In addition, auditory ERPs are reportedly modulated by compounds that influence the cyclic AMP (cAMP) signaling cascade and the G-protein-coupled cAMP signaling cascade has been identified as a target to help understand sensory processing deficits similar to those in schizophrenia (Maxwell et al., 2006c). A transgenic mouse line was generated with forebrain-specific expression of a constitutively active G-protein, Gαs, which paradoxically produced decreased cAMP levels. Transgenic mice displayed decreased N40 amplitude relative to wild type that was ameliorated by haloperidol. This mouse line also exhibits deficits in prepulse inhibition of the acoustic startle reflex that are consistent with deficits observed in individuals with schizophrenia (Gould et al., 2004). These data provide one of the first genetic models of schizophrenia that can elucidate biochemical contributions to sensory processing impairments associated with schizophrenia (Maxwell et al., 2006c).

5.2 DBA/2 Strain

DBA/2 mice have been identified as one strain that exhibits impairments in both prepulse inhibitions of the acoustic startle response and gating of ERP components, suggesting that it shares some relevant neurobiology with abnormal neuronal function in schizophrenia (Stevens et al., 1996a; Paylor and Crawley, 1997; Ellenbroek et al., 1999; de Bruin et al., 1999b). DBA/2 mice share several endophenotypes that are seen in people with schizophrenia, including reduced PPI, reduced gating of the P20/N40 evoked potential, and decreased P3a following a deviant tone (Siegel et al., 2003). For example, a study by Stevens et al. (1996a) reported that DBA mice displayed significantly disrupted gating of the P20/N40 ERP components relative to several other strains of mice. The authors equated low expression of the α7 nicotinic acetylcholinergic receptor (nAChR) subunit in DBA/2 mice with similar reduced expression in schizophrenia. A second study found that two substrains of DBA/2 mice differ in the gating of evoked potentials, with the DBA/2J substrain (Jackson Lab, Bar Harbor ME) demonstrating enhanced gating relative to DBA/2Hsd 2Hsd (Harlan Sprague-Dawley, Indianapolis, IN) mice (Connolly et al., 2003). These data suggest that differences within substrains of the DBA/2 mice may allow for a more selective dissection of the genetic, neurobiological, and perhaps environmental factors that contribute to the expression and transmission of schizophrenia.

6 Pharmacological Models of Treatment

There have been a number of studies examining the ability of antipsychotic medication to affect ERPs and to reverse amplitude and gating deficits in mouse ERP models of schizophrenia. It is common to use reversal of pharmacologically induced disruptions with treatment compounds and medications as a method for validating animal models of human conditions, including schizophrenia.

6.1 Typical Antipsychotics – Haloperidol

Haloperidol is a typical antipsychotic with strong antidopaminergic properties. The effects of chronic haloperidol on auditory gating of the mouse P20, N40, and P80 were measured in a paradigm in which trains of clicks were presented ranging from 0.25 to 8 s ISI. Chronic haloperidol had no effect on gating for any of the ERP components in this study (Maxwell et al., 2004b). Although acute haloperidol dramatically reversed amphetamine-induced decreases in amplitude of the mouse P20 and N40 (Maxwell et al., 2004a), it was unable to attenuate strain-related gating reductions in the N40/P20 in DBA/2 mice (Simosky et al., 2003). Also, as noted earlier, haloperidol reversed amplitude reductions following bupropion, but the combination produced the classic gating deficit described among medicated schizophrenia patients. These findings suggest haloperidol has a varied and unclear effect on ERP disruptions in schizophrenia.

6.2 Atypical Antipsychotics

6.2.1 Olanzapine

The effect of chronic olanzapine, an atypical antipsychotic with high affinity for dopamine and serotonin receptors, on auditory gating of the mouse P20, N40, and P80 were measured in a paradigm employing trains of clicks ranging from 0.25 to 8 s ISI. Although chronic olanzapine failed to change gating of the P20, it improved gating of the mouse N40 and P80 suggesting that olanzapine may improve gating of the human N100 and P200 (Maxwell et al., 2004b). Again, this effect was mediated entirely by effects on S1 (first stimulus) strongly suggesting the main effect of medication is to reverse the deficit in component amplitude rather than enhance response to the second stimulus.

6.2.2 Risperidone

There is also preliminary data that risperidone, an atypical antipsychotic that antagonizes multiple receptor types including dopamine receptors and serotonin receptors, may affect certain ERP components. A study of chronic risperidone delivery via subcutaneous implants reported increased P20 amplitude but no effects on the N40 component. Risperidone in this study was unable to block the amphetamine-induced decreases in the P20 component, but attenuated amphetamine-induced reduction of the N40 (Siegel et al., 2004).

6.2.3 Clozapine

Clozapine, an atypical antipsychotic with antagonistic properties at a variety of receptors including dopamine, may improve P300 to a greater extent than other antipsychotic medication in patients (Umbricht et al., 1998). Very few studies have examined clozapine and ERPs in rodents, however. A study by Simosky et al. (2003) reported that clozapine attenuated strain-related deficits in P20/N40 gating in the DBA/2 strain of mice, but there are no reports of clozapine's effects in normal model of ERPs. These findings suggest that clozapine may be working to attenuate schizophrenia-related ERP deficits in humans, but more data is needed to draw further conclusions.

6.3 Novel Therapeutics

Animal models also provide a means for testing novel compounds and can be beneficial screening devices for new therapeutics and medications. Several studies have explored the efficacy of various compounds in enhancing ERP components and gating and in reversing schizophrenia-like deficits in a variety of models.

6.3.1 Rolipram

Patients with schizophrenia are thought to have decreased c-AMP levels that may contribute to certain cognitive deficits, including sensory gating issues. Consequently, compounds that modulate c-AMP have been targeted as potential therapeutics for these symptoms of the disorder. A study by Maxwell et al. (2004a) investigated the ability of rolipram, a phosphodiesterase inhibitor that produces increases in intracellular c-AMP by preventing its hydrolysis, to attenuate amphetamine-induced changes in ERP components and gating. Rolipram alone increased the amplitude and gating of the mouse P20 and N40. Additionally, both rolipram and haloperidol, reverses the effects of amphetamine on both the mouse P20 and N40. This finding further supports the hypothesis that rolipram, in addition to other compounds that increase intracellular c-AMP, is a potential novel therapeutic that, unlike all other antipsychotic drugs, is nonreceptor-based. These data also support the theory that the c-AMP second messenger system may play a role in the etiology of schizophrenia (Maxwell et al., 2004a).

6.3.2 Nicotine

The high prevalence of smoking in individuals with schizophrenia has led to speculation regarding the potential role of nicotine in attenuating cognitive disruption associated with the disorder. As a result, the relationship between nicotine and sensory gating has been well-researched. Several studies, reviewed later, have investigated the relationship between nicotine and ERPs as well as the role of the nAChR system in gating and other deficits (Freedman et al., 1995).

A study by Metzger et al. (2006) assessed the effects of acute nicotine on the P20 and N40 components of the mouse ERP. Investigators found that acute nicotine administration had opposite effects on the P20 and N40 components; increasing the P20 amplitude and gating and reducing N40 amplitude and gating. These results reflect diverse activity of nicotine across the different brain regions and circuits that contribute to each ERP component (Metzger et al., 2006) and are consistent with previous reports of the effects of acute nicotine on ERP components (Siegel et al., 2005). Additional studies of the effects of acute nicotine (Phillips et al., 2006, 2007) also reported increases in P20 amplitude and decreases in N40 amplitude following nicotine administration. Additionally, the nicotine-induced increases in P20 were blocked by the nonspecific nAChR antagonist mecamylamine, suggesting a role for this receptor system in nicotine's effects of ERPs (Phillips et al., 2006, 2007).

The role of the nAChR system in schizophrenia has been extensively studied as a potential target for new therapeutics. Particular interest has been paid to the hippocampal α7 receptor based on an observed correlation between hippocampal α7 receptor density and the level of sensory inhibition. Strains with reduced numbers of these receptors, particularly DBA/2 mice, showed deficient sensory gating while strains with larger numbers had normal gating profiles (Stevens et al., 1996a). Subsequent studies with the DBA/2 strain have reported improvement in sensory gating following treatment with nicotine or selective α7 nicotinic agonists (Stevens and Wear, 1997; Stevens et al., 1998; Simosky et al., 2001). These findings have led to speculation that α7 nicotinic receptor agonists are potential treatment candidates for attenuating deficiencies in P20 processing in schizophrenic patients (Simosky et al., 2002; Leonard and Freedman, 2003; Martin et al., 2004).

A recent study, of the α7 nicotinic receptor antagonist methyllycaconitine (MLA) and the α4β2 nicotinic receptor antagonist dihydro-β-erythroidine (DHBE), however, suggests that there may be multiple nAChR subtypes involved in the changes in ERP components observed following nicotine administration (Phillips et al., 2006). Both MLA and DHBE blocked nicotine-induced decreases in the amplitude of the N40 component. Nicotine-induced increases in the amplitude of the P20 component were attenuated only by DHBE. These data suggest that nicotine's effect to reduce the amplitude the N40 component require stimulation of multiple nicotinic receptor systems, including α7 and non-α7 subtypes. Moreover, nicotine's effects to increase the amplitude of the P20 component are specifically mediated by non-α7 nicotinic receptors. Thus, an agonist at non-α7 nicotinic receptors would potentially meet the goal of increased P20 without decreased N40 and potentially would aid in smoking cessation and in alleviating schizophrenia-related neurocognitive dysfunction (Phillips, 2006).

7 Other Models for Schizophrenia

7.1 Abnormalities in Gamma Oscillations

An animal model of impaired gamma band oscillation (GBO) which resembles the deficits seen in schizophrenia patients would be very useful as another measure to test current or novel pharmacological treatments. Evaluation of transgenic or knockout mouse lines for the presence of GBO abnormalities would also add another level of depth to those models. An initial study by Siegel et al. (2006) examined the effects of pharmacological treatments that are thought to either ameliorate or mimic schizophrenia endophenotypes in mice to evaluate their effect on GBO. Acute treatment with the antipsychotics haloperidol and risperidone increased GBO immediately following the auditory stimulus. Although amphetamine had no significant effect on GBO, the NMDA receptor antagonist ketamine caused a significant increase in gamma evoked by an auditory stimulus as well as background gamma between stimulus presentations. These findings suggest that gamma abnormalities in schizophrenia may not be a result of a hyperdopaminergic state but rather are influenced by NMDA and cAMP hypofunction (Siegel et al., 2006). This further illustrates the complexity of the illness and may indicate the need to develop treatments which act on nontraditional pathways to accompany the common antipsychotic regiment.

8 Conclusions

There is an extensive body of literature that establishes impairments in ERPs as an endophenotypic marker of schizophrenia. A significant amount of research has been dedicated to identifying and refining animal models to study the potential mechanisms of these impairments. A variety of models, including pharmacological and genetic versions, have contributed to our understanding of the mechanisms and causes of the ERP changes associated with schizophrenia. They also have provided the means to screen novel medications and treatments such as rolipram and nicotine and to refine our understanding of the mechanisms of typical and atypical antipsychotics. Rodent models of ERPs have contributed significantly to the field of schizophrenia and new animal models of related impairments, such as changes in gamma oscillations, are promising tools for further study of schizophrenia-related cognitive changes and impairments.

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