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Behavioral Neurobiology of Chronic Pain

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Behavioral Neurobiology of Chronic Pain

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Preface

The physiological pain in humans that initially results from tissue injury is an unpleasant and aversive experience with sensory, cognitive, and motivational components. These interact to precipitate behavioral and psychological responses that serve essential survival functions, including protective behaviors that allow avoidance and escape from the pain-generating stimulus, as well as recuperative behaviors that facilitate healing. This interplay between stimulus and behavioral response becomes quite complex after the transition from acute to chronic pain, and must be addressed because chronic pain represents an enormous clinical problem that is poorly served with currently available analgesic drugs. To this end, we bring together a series of authoritative chapters written by leading experts in preclinical and clinical aspects of pain neurobiology. This volume fills several gaps not previously addressed by other books or reviews dedicated to chronic pain. First, it provides comprehensive details of the physiology, pharmacology, and neurobiology of previously neglected forms of chronic pain, with a focus on chronic postoperative pain in humans ("Persistent Postsurgical Pain: Evidence from Breast Cancer Surgery, Groin Hernia Repair, and Lung Cancer Surgery") and several relatively new animal behavioral assays including those that model post-herpetic neuralgia ("Animal Models and Pharmacology of Herpetic and Postherpetic Pain"), the chronic pain of multiple sclerosis ("Mechanisms and Pharmacology of Neuropathic Pain in Multiple Sclerosis" and "Pain and Cognition in Multiple Sclerosis"), painful diabetic neuropathy ("Animal Models of Diabetes-Induced Neuropathic Pain"), visceral pain ("Visceral Pain"), latent central sensitization ("Endogenous Analgesia, Dependence, and Latent Pain Sensitization"), and striatal muscle pain ("Anatomical and Physiological Factors Contributing to Chronic Muscle Pain"). Second, this book treats chronic pain syndromes as a multidimensional disease and considers bidirectional comorbidities with factors such as cognitive deficits ("Pain and Cognition in Multiple Sclerosis"), drug dependence ("The Self-administration of Analgesic Drugs in Experimentally Induced Chronic Pain"), social interaction ("The Interaction Between Pain and Social Behavior in Humans and Rodents"), stress, anxiety and depression ("Neurobiology of Stress-Induced Hyperalgesia"), and prior injury history ("Endogenous Analgesia, Dependence, and Latent Pain Sensitization").

Third, because the evoked behavioral responses so commonly used in preclinical research fail to capture the affective and spontaneous components of pain that are most relevant to chronic pain syndromes and its effective pharmacology, this volume provides one of the first comprehensive reviews of non-evoked behavioral readouts ("Preclinical Assessment of Pain: Improving Models in Discovery Research") for the preclinical assessment of chronic pain, as well as important pitfalls and considerations for the design of animal models for pharmacological studies with adequate sensitivity and specificity ("Behavioral Pharmacology of Pain"). Further detail on operant assays is provided ("Operant Assays for Assessing Pain in Preclinical Rodent Models: Highlights from an Orofacial Assay"), along with the contention that the self-administration method of operant conditioning may be an effective approach to evaluate potential analgesics with greater sensitivity than standard reflex measures ("The Self-administration of Analgesic Drugs in Experimentally Induced Chronic Pain"). Fourth and finally, this volume is decorated with examples of new and exciting mechanisms that contribute to chronic pain, such as endogenous opioid dependence after tissue injury ("Endogenous Analgesia, Dependence, and Latent Pain Sensitization"), peripheral sensitization mechanisms underlying chronic muscle pain ("Anatomical and Physiological Factors Contributing to Chronic Muscle Pain"), and an emerging understanding of how sensitization mechanisms change from early life to adulthood ("Acute and Chronic Pain in Children"). With this increased understanding of the mechanisms of chronic pain and the body's natural ability to inhibit it with endogenous analgesia ("Endogenous Analgesia, Dependence, and Latent Pain Sensitization"), together with new animal assays and behavioral readouts, new treatment strategies now seem achievable in the near future.

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Part I
Chronic Pain in Humans

Persistent Postsurgical Pain: Evidence from Breast Cancer Surgery, Groin Hernia Repair, and Lung Cancer Surgery

Mads Utke Werner and Joakim Mutahi Bischoff

Abstract The prevalences of severe persistent postsurgical pain (PPP) following breast cancer surgery (BCS), groin hernia repair (GHR), and lung cancer surgery (LCS) are 13, 2, and 4–12 %, respectively. Estimates indicate that 80,000 patients each year in the U.S.A. are affected by severe pain and debilitating impairment in the aftermath of BCS, GHR, and LCS. Data across the three surgical procedures indicate a 35–65 % decrease in prevalence of PPP at 4–6 years follow-up. However, this is outweighed by late-onset PPP, which appears following a pain-free interval. The consequences of PPP include severe impairments of physical, psychological, and socioeconomic aspects of life. The pathophysiology underlying PPP consists of a continuing inflammatory response, a neuropathic component, and/or a late reinstatement of postsurgical inflammatory pain. While the sensory profiles of PPP-patients and pain-free controls are comparable with hypofunction on the surgical side, this seems to be accentuated in PPP-patients. In BCS-patients and GHR-patients, the sensory profiles indicate inflammatory and neuropathic components with contribution of central sensitization. A number of surgical factors including increased duration of surgery, repeat surgery, more invasive surgical techniques, and intraoperative nerve lesion have been associated with PPP. One of the most consistent predictive factors for PPP is high intensity acute postsurgical pain, but also psychological factors including anxiety, catastrophizing trait, depression, and psychological vulnerability have been identified as significant predictors of PPP. The quest to identify improved surgical and anesthesiological techniques to prevent severe pain and functional impairment in patients after surgery continues.

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Contents

1 Introduction

Persistent pain after surgical procedures remains a major surgical and medical problem (Kehlet et al. 2006; Kehlet and Rathmell 2010; Dworkin et al. 2010; Rappaport et al. 2010; Buvanendran 2012). The estimated annual surgical volumes in the U.S.A. of breast cancer surgery (BCS), groin hernia repair (GHR) and lung cancer surgery (LCS) are 480,000, 600,000, and 80,000, respectively.[1] Since 2–10 % of these patients will develop severe persistent postsurgical pain (PPP) (Kehlet et al. 2006), this is a huge and daunting problem for the individual and the community.

GHR, a seemingly minor surgical procedure with superficial, limited tissue injury, illustrates the problem. The patient has felt a lump in the groin area and has

[1] Extrapolated from http://www.cancer.gov/cancertopics/types/breast, http://www.sages.org/publication/id/PI06/, http://www.cancer.gov/cancertopics/types/lung, studies (Wildgaard et al. 2011; Walters et al. 2013) and a review (Kehlet et al. 2006).

Table 1 Prevalence of persisting postsurgical pain in BCS, GHR, and LCS

Surgical procedure	Author	Year	Number	Method	Pain intensity	Pain prevalence (%)
Breast cancer surgery	Mejdahl et al. (2013)	2013	2,411	Questionnaire	Moderate to severe	16
	Andersen et al. (2011)	2011	15,697	Review	Moderate to severe	25–60
	Gärtner et al. (2009)	2009	3,754	Questionnaire	Moderate to severe	52
	Vilholm et al. (2008)	2008	258	Questionnaire	Moderate to severe	24
Groin hernia repair	Bright et al. (2010)	2010	9,607	Questionnaire	Severe	1–3
	Loos et al. (2007)	2007	1,766	Questionnaire	Severe	2
	Haapaniemi et al. (2002)	2002	264	Questionnaire	Moderate to severe	5
	Bay-Nielsen et al. (2001)	2001	1,169	Questionnaire	Moderate to severe	3–8
Lung cancer surgery	Wildgaard et al. (2011)	2011	546	Questionnaire	Moderate to severe	11–18
	Wildgaard et al. (2009)	2009	2,586	Review	Moderate to severe	3–16
	Keller et al. (1994)	1994	238	Questionnaire	Moderate to severe	11
	Kalso et al. (1992)	1992	150	Questionnaire	Moderate to severe	44

experienced regional discomfort. The general practitioner tells the patient of the necessity of surgery, due to the risk of development of irreducibility of the hernia, a condition which may lead to strangulation of an intestinal loop and subsequent life-threatening septicemia. The patient accepts this and is admitted for open surgery. After uncomplicated anesthesia and surgery, the patient wakes up quite unprepared for a nightmare of persisting pain and disability.

About 1–3 % of postsurgical hernia patients (e.g. 6,000−18,000[2] patients each year in the U.S.A.) are affected by severe persisting and disabling inguinal post-herniotomy pain that may lead to functional and socioeconomic disability (Bay-Nielsen et al. 2001; Loos et al. 2007). In other surgical procedures, e.g., BCS, involving larger tissue volumes and including deeper structures, and when adjuvant chemotherapy or radiotherapy is added, the risk of developing persistent pain increases fivefold or more, as compared to GHR (Table 1).

In this chapter, the aftermath of three surgical procedures is studied: BCS, GHR, and LCS. These procedures are quite different in regard to patient population (age, gender, concomitant diseases), surgical techniques and composition of tissues

[2] http://www.sages.org/publication/id/PI06/

in the surgical field (nerve density), and adjuvant treatments (chemotherapy, radiotherapy). Also, each procedure presents with specific patterns of postoperative morbidities, including pain and functional impairment.

2 When is Pain Persistent?

2.1 Definitions

The prevalent definition of PPP presented in the literature, is pain in or near the surgical area, continuing beyond 3–6 months after surgery, without signs of a postsurgical complication (Kehlet et al. 2010; Macrae 2008). Attempts to use a more operative definition of chronic pain, i.e., pain that does not have a biological meaning and continues for more than 3–6 months (Cousins 2007), may seem to fail in PPP since low-grade chronic inflammation in the tissues, around foreign bodies (implants), may contribute to pain for prolonged periods of time.

Based on the current definition (Kehlet et al. 2010; Macrae 2008), updated criteria for PPP are presented in Table 2 (Werner and Kongsgaard 2014). These criteria indicate that PPP may occur after a pain-free period; that is, development of PPP cannot automatically be considered to follow a direct trajectory from acute to chronic pain. In BCS and GHR, late-onset PPP has been demonstrated (Reinpold et al. 2011; Mejdahl et al. 2013). Some tentative reasons for this bi-phasic (acute-chronic) response are: *first*, nerve damage sometimes is associated with a delayed onset of neuropathic pain symptoms. Indeed, neuropathic pain components are considered a major contributor to PPP (Kehlet et al. 2006; Borsook et al. 2013). *Second*, in implant surgery, partial dehiscence of the inguinal mesh or dislocation of orthopedic prosthetic material may lead to PPP after a pain-free postsurgical period. Some authors, however, do not consider this PPP, but rather to reflect a mechanical complication following surgery (Kehlet et al. 2012). *Third*, reinstatement of pain-like behavior has been observed in experiments following a deep tissue injury. Several weeks after complete recovery from pain-like behavior in mice, administration of naltrexone, a μ-opioid receptor antagonist, leads to reinstatement of tactile hypersensitivity, guarding behavior, and pain behavior (Campillo et al. 2011; Corder et al. 2013). During the resolution of the injury, endogenous receptor activity enhances pain inhibitory signaling. This up-regulated, tonic activation of endogenous opioid receptors, blocked by naltrexone, seems responsible for the attenuation of latent sensitization, persisting beyond the resolution of the injury. Administration of the μ-opioid receptor antagonist leads to blockade of the endogenous opioid system and uncovering of latent sensitization. Translational research in humans has not yet uncovered an analogous mechanism in man (Pereira et al. 2013), although recent data seem to indicate that use of very high doses of naloxone at least in some volunteers may lead to uncovering of latent sensitization (unpublished observations, MUW).

Table 2 Proposed criteria for PPP (Werner and Kongsgaard 2014)

1. The pain develops after a surgical procedure or increases in intensity after the surgical procedure
2. The pain should be of at least 3– 6 months' duration and significantly affect the HR-QOL
3. The pain is either a continuation of acute post-surgery pain or develops after an asymptomatic period
4. The pain is either localized to the surgical field, projected to the innervation territory of a nerve situated in the surgical field, or referred to a dermatome (following surgery in deep somatic or visceral tissues)
5. Other causes of the pain should be excluded, e.g., infection or continuing malignancy in cancer surgery

2.2 Duration of Persistent Pain

What is the trajectory of "classical" PPP, i.e., acute pain progressing directly into chronic pain? A study examining the sequential prevalence of PPP after GHR (n = 736) demonstrated that 65 % of the patients with PPP 6 months after surgery were pain free at the 5-year follow-up (Reinpold et al. 2011). The prevalence of PPP in 600 patients examined at intervals after lung surgery was 57 % at 7–12 months, 36 % at 4–5 years, and 21 % at 6–7 years (Maguire et al. 2006). However, it should be noted that only 40 % of the patients in this study had surgery performed due to malignancy. The data from BCS-, GHR-, and LCS-patients thus indicate a decrease, of considerable magnitude, in prevalence of patients with PPP at 5–6 years follow-up, compared with the prevalence at 6 months. Surprisingly, while the pain prevalence decreased with time, pain severity did not seem to lessen with time (Maguire et al. 2006). Evidence for late-onset of PPP is discussed below (Sect. 4.3).

3 Demographics

A number of scientific papers deserve particular recognition for highlighting the challenge of PPP (Kehlet et al. 2006; Macrae and Davies 1999; Perkins and Kehlet 2000). The authors of these papers unanimously called for improved research in what they perceived was an unrecognized and therefore undertreated area (Kehlet and Rathmell 2010; Kehlet et al. 2010; Macrae 2008). The papers clearly illustrated that PPP is a major problem compromising various physical, psychological, and socioeconomic aspects of life in 10–60 % of postsurgical patients, depending not only on surgical procedures and surgical techniques, but also on patient-related and pain management-related factors.

In BCS, GHR, and LCS a large number of questionnaire studies on the prevalence of PPP have been published (Table 1). Though the prevalence of severe PPP depends on pain intensity criteria, resting or dynamic assessment conditions,

and the time elapsed after the surgical procedure (Mejdahl et al. 2013), the reported prevalences of severe persistent pain in BCS, GHS, and LCS are in the order of 13 % (Gartner et al. 2009), 2 % (Loos et al. 2007), and 4–12 % (Wildgaard et al. 2011), respectively. Recently published data from a 6-year follow-up on BCS-patients indicate that "the problem is not static as it can either progress or regress with time" (Mejdahl et al. 2013).

4 Behavioral Impairment

4.1 General Issues in Chronic Pain (Non-cancer)

Physical impairment due to chronic pain may lead to a number of restrictions in everyday life. Several studies (Breivik et al. 2006; O'Brien and Breivik 2012) and reviews (Reid et al. 2011) have demonstrated a range of impaired activities of daily living (ADL) present in chronic pain patients; from cleaning, dressing, shopping, stair climbing, vacuum cleaning, walking, and maintaining social relations to engaging in sexual activity. These physical restrictions are also mirrored in psychological changes which are more prevalent in chronic pain patients than in the population; anxiety; catastrophizing traits; cognitive disturbances; depression; psychological vulnerability; and somatization traits (Fishbain 2013).

A recent study investigated the correlation between self-reported severe pain and socioeconomic issues (Morgan et al. 2011), i.e., "social deprivation," employment status and social security. Pain status was recorded for a population cohort consisting of more than 9,400 subjects; 62 % reported no pain, 33 % reported moderate pain while nearly 5 % reported severe chronic pain. The group with severe pain had significantly higher odds ratios of belonging to the lowest income group, of living in areas with overrepresentation of multiple social deprivation, compared to more affluent areas, and, of belonging to United Kingdom's National Statistics Socio-economic Classification Class 7 (routine occupations) and Class 8 (never worked/long-term unemployed). In individuals of working age with severe chronic pain, almost 45 % stated they were unable to work due to sickness or disability and 40 % claimed a state benefit, related to the disability. These socioeconomic data are corroborated in several reviews (Reid et al. 2011; Patel et al. 2012; Moore et al. 2014) and large scale studies (Langley et al. 2010a, b; Becker et al. 1997, 2000; Currow et al. 2010).

Not unexpectedly, ratings of health-related quality of life (HR-QOL) issues are associated with markedly lower scores in pain patients than in the population as a whole (Moore et al. 2014). The life expectancy for chronic pain patients also seems lower than for controls, with relative risk ratios for premature death of 1.1–2.4 (Moore et al. 2014). Decreased physical activity, depression, and disrupted sleep architecture are contributing factors to the principal causes of death: cardiovascular and respiratory failure.

Quite a number of assessments scales of psychological and physical function impairment are used in research on chronic pain. Some of the validated general assessment scales, applicable in a number of pain conditions, are Short Form Health Survey (SF-36) (Ware and Sherbourne 1992), the Brief Pain Inventory (BPI) (Cleeland 1991), the Pain Disability Index (PDI) (Tait et al. 1987) and the Multidimensional Pain Inventory (MPI) (Kerns et al. 1985). Disease-specific assessments scales are the Western Ontario and McMaster Universities Arthritis Index (WOMAC) (Bellamy et al. 1988). Functional Assessment of Cancer Therapy General Questionnaire (FACT-G[3]) (Cella et al. 1993; Webster et al. 2003) and European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC-QLQ) (Aaronson et al. 1993). The commonly used Short Form Health Survey (SF-36) questionnaire assesses several distinct domains: physical functioning, physical and emotional impediments to role functioning, pain, general health, vitality (fatigue, energy), social functioning, and mental health (Parker et al. 2007), comparable in construction to the EORTC-QLQ-C30.

4.2 Persistent Pain After Breast Cancer Surgery

In BCS, impairments in postsurgical HR-QOL and ADL issues are related to the surgical procedure, depending on several factors: magnitude of breast tissue injury [breast conserving surgery (lumpectomy, quadrantectomy), mastectomy]; invasiveness [sentinel lymph node resection (SLNR),[4] axillary lymph node resection]; adjuvant treatment (chemotherapy, hormonal therapy, immunotherapy, radiotherapy); and cancer stage (Rietman et al. 2003, 2006; Vilholm et al. 2009).

The EORTC-QLQ-C30 supplemented by the specific breast module EORTC-QLQ-BR23 has been used in more than 40 % of studies examining HR-QOL-issues in BCS-patients (Lemieux et al. 2011). These questionnaires evaluate cognitive, emotional, physical, sexual and social functioning, body image, distressing symptoms (fatigue, nausea), side-effects of treatment, and socioeconomic difficulties. The breast cancer-specific FACT-G [including the breast cancer module (FACT-B)]) has been used in 25 % of the studies. The SF-36 has been used in more than 10 % of BCS studies concerned with QoL-assessments (Lemieux et al. 2011; Chopra and Kamal 2012), but is not cancer-specific. The relationship between HR-QOL evaluations and ADL assessments are statistically significant (Rietman et al. 2003). Low HR-QOL scores are associated with impairment of daily activities.

[3] Related to FACIT-G = Functional Assessment of Chronic Illness Therapy General Questionnaire

[4] Sentinel lymph node assessment is a minimal invasive technique for detection of regional metastases. Sentinel lympnodes are identified by preoperative administration near the cancer of a radioactive isotope (Tc-99 m), followed by intraoperative tracing by a simple dye. Following excision of the sentinel lymp nodes intraoperative histological analyses are made. If signs of regional spread exist axillary lymph node excision is performed.

Reciprocally, however, the strength of the interdependence is affected by patient-related factors, domains examined, and context factors. Congruent findings have been observed in the relationship between HR-QOL and ADL, and pain (Rietman et al. 2003; Tasmuth et al. 1996).

The number of publications registered in PubMed on HR-QOL and ADL issues in BCS-patients, exceed more than 1,000, demonstrating the scientific interest and importance of this field. The hitherto largest bibliographical review on HR-QOL in breast cancer patients includes 477 studies (n = 85,000) (Montazeri 2008), published from 1974 to 2007. Obviously, the study covers a span of three decades and contains significant time-related changes in diagnosis, technology, and treatment procedures—necessitating cautious interpretation of data. However, the review concludes, *first*, that patients undergoing mastectomy compared to breast conserving surgery usually reported a more negative body image perception. *Second*, almost all studies indicated that BCS-patients receiving chemotherapy and adjuvant hormonal therapies experienced side-effects that negatively affected the HR-QOL. Persistent fatigue, pain, postmenopausal symptoms, psychological distress, i.e., anxiety and depression and upper limb morbidity, were among the most common symptoms reported. *Third*, sexual functioning was impaired, particularly in younger patients, thus negatively affecting HR-QOL.

These findings are probably best illustrated in a study from 2006 in breast cancer patients (n = 180), where upper limb morbidity [arm volume, muscle strength, range of motion (shoulder disability)], ADL disability, and HR-QOL were examined (Rietman et al. 2006). Treatment-related upper limb morbidity, associated ADL disabilities, and decreased HR-QOL were documented 2 years after surgery, and compared to the presurgery level. The patients receiving axillary lymph node dissection (ALND, 70 %) experienced significantly more upper limb morbidity, ADL disabilities and decreased HR-QOL, than patients receiving SLNR. These data have recently been corroborated in a 7-year follow-up after surgery in the same cohort (Kootstra et al. 2013).

Sensory disturbances or discomfort, potentially affecting everyday life, occurs in 60 % of BCS-patients, depending on the surgical and adjuvant treatment (Gartner et al. 2009). In patients undergoing SLNR and radiotherapy to the residual breast tissue, the prevalence of sensory disturbances or discomfort was 30 %, while in patients also receiving ALND and chemotherapy or loco-regional radiotherapy, the prevalence increased to 85 %. Most frequently reported locations were the axilla (65 %), arm (50 %), breast area (45 %), and the lateral side of the body (30 %).

4.3 Persistent Pain After Groin Hernia Repair

The sex ratio (females/males) of GHR is 1/9, and data indicate a significantly higher prevalence of PPP in females compared to males, 23 and 11 %, respectively (Kalliomaki et al. 2008). An early nationwide questionnaire study in 1,170

individuals 1 year after open (98 %) GHR, demonstrated that 17 % experienced physical limitations during work, sport, or other leisure activities, likely as a consequence of chronic groin pain (Bay-Nielsen et al. 2001). In addition, 20–32 % experienced pain from the groin when standing up more than 30 min, climbing stairs, sitting more than 30 min, and getting up from a chair. These restrictions in physical functioning are in agreement with the results of several other GHR-studies (Poobalan et al. 2001; Mikkelsen et al. 2004; Grant et al. 2004). In a follow-up study, 6 years after the GHR in the same patients, 75 % had less pain, while only 25 % had the same pain intensity level or higher (Aasvang et al. 2006a). The GHR-related impact on ADL functions decreased from 17 % at the 1-year follow-up to 6 % at the 6-year follow-up. No data were available on the management strategies, but it is reasonable to assume that there was a time effect, i.e., the longer the time from the primary injury the lower the pain intensity and impact on ADL functions. Very interestingly, the data indicated that only 15 % of the patients with pain/impaired ADL functions belonged to the same category of pain intensity (none, light, moderate, severe) both at the 1- and 6-year follow-up. In other words, of the 30 % (n = 59/174) experiencing moderate to severe intensity pain at the 1-year follow-up, only 16 % (n = 10/59) of these experienced the same intensity of pain at the 6-year follow-up. At the 6-year follow-up, 10 % (n = 18/174) experienced moderate to severe intensity pain. Obviously, the limited number of patients with pain and functional impairment at the 6-year follow-up precludes an affirmative statistical conclusion, but it would seem that a change in phenotype *may* occur with time. A recent study with a 6-month and 5-year follow-up of 645 GHR-patients, corroborates these findings with a more detailed methodology (Reinpold et al. 2011). In this study, 33 patients reported chronic pain at 6 months but no pain after 5 years. Chronic pain was recorded in 16 patients after both the 6-month and 5-year follow-up. In 36 patients, chronic pain was recorded after 5 years but *not* after 6 months. These data indicate that more than two-thirds of the PPP-patients develop chronic pain with a late onset!

Possible explanations include variability in pain assessment methods, statistical inaccuracies due to low pain ratings (NRS 0–3), changes in analgesic management, development of drug tolerance, hernia recurrence, neuropathic pain, or dehiscence or dislocation of the inguinal mesh. A particularly interesting hypothetical explanation could be related to the reinstatement of latent sensitization (the reader is referred to Sect. 2.1) (Corder et al. 2013).

Impairment in sexual activity as a complication to GHR has been well characterized (Aasvang et al. 2006b, 2007; Bischoff et al. 2012). In a nationwide questionnaire study of 805 individuals, pain during sexual activity was reported by 88 patients (10.9 %), of whom 45 (5.6 %) described the pain as mild, 34 (4.2 %) described it as moderate, and 9 (1.1 %) described it as severe (Bischoff et al. 2012). In open-surgery GHR, a prevalence of moderate to severe impairment of sexual activity has been reported in 2.8 % of patients, which is only marginally higher than in laparoscopically assisted repair: 2.4 %. Severe pain during ejaculation (dysejaculation) is seen in 4.0 % after open repair and in 3.1 % after

laparoscopic repair. In studies with different surgical procedures performed at single centers, the prevalence of dysejaculation for open and laparocopical repair have been reported to be 1.6 and 1.0 %, respectively (Aasvang et al. 2010a; Bittner et al. 2010).

These rather low prevalences should be contrasted to the large number of GHR procedures performed annually: 2,000 procedures per 1 million adults. It should also be emphasized that this routine procedure generally is performed in otherwise healthy individuals. Unfortunately, there are no detailed studies in GHR on HR-QOL including socioeconomic consequences associated with severe PPP (Kehlet et al. 2013).

4.4 Persistent Pain After Lung Cancer Surgery

A number of studies have examined impairment of ADL-functions following LCS, but very seldom comprehensively or as primary objectives (Wildgaard et al. 2009), which indicates a commonality with the studies performed in GHR. Larger studies (70–600 patients), with prevalences of severe pain in the order of 2–8 %, indicate that 40–50 % of the patients experience functional restrictions in everyday life more than 1 year after surgery (Maguire et al. 2006; Perttunen et al. 1999; Tiippana et al. 2003). Other studies demonstrate that 20 % of patients have moderate to severe impairment in their ADL-functions: carrying heavy objects, responding to changes in the weather, walking, lying on the operated side, feeling depressed, and working with the hand of the operated side (Perttunen et al. 1999). In particular, discomfort or restricted motion of range of the shoulder has been reported in 10–20 % of the patients (Dajczman et al. 1991; Khan et al. 2000; Landreneau et al. 1996). In a recent questionnaire-based study of persistent pain after LCS, carried out 2 years after the surgical procedure, the prevalence of perceived sensory changes was independent of the surgical procedure, the less traumatic video-assisted thoracic surgery (VATS) compared to open-surgery thoracotomy, with 75 % experiencing the sensory changes in the rib area (Wildgaard et al. 2011). Activities like carrying heavy bags, performing moderate and light physical activity, vacuum cleaning, lying on the operated side, and elevating arm/using arm above the horizontal level were impaired by pain in more than 60 % of the patients. The prevalence of persistent pain after LCS was similar when comparing type of adjuvant treatment, i.e., radiotherapy or chemotherapy, or, type of surgery. Clinically relevant pain (NRS $>$ 3) during rest, walking, and when physically active, was present in 39, 51, and 65 % of the lung cancer patients, respectively. Corresponding assessments for severe pain (NRS $>$ 5) were 14, 17, and 42 %, respectively.

A fairly limited number of studies are available on health-related QOL issues in LCS-patients compared to BCS-patients (Poghosyan et al. 2013). Of 19 studies, 11 did not extend the follow-up beyond 12 months, partly attributable to the low

survival rate of lung cancer patients (Walters et al. 2013). The most prevalent principal symptoms affecting HR-QOL were pain, fatigue, dyspnea, and coughing.

5 Pathophysiology

5.1 General Aspects

The putative etiologies for PPP are: a continuing inflammatory response; a neuropathic component; a late reinstatement of postsurgical inflammatory pain; or a combination of these. In particular, the neuropathic component has attracted much scientific interest (Kehlet et al. 2006; Borsook et al. 2013), as previously stated, though the prevalence of any of these pain components is currently not known for any surgical procedure.

Surgical tissue injury generates immediate activation of nociceptors; *physiological nociception*.[5] It signals presence, location, intensity, and duration of the noxious stimulus and fades rapidly once the surgical stimulus is removed (Kehlet et al. 2006). *Inflammatory-mediated nociception* is initiated minutes after the tissue trauma, by activation of complex cascade systems: classical inflammatory mediators; cytokines; TRP-channels; and neurotrophins. These responses lead to peripheral sensitization with a reduction in thresholds of nociceptors (primary hyperalgesia), to "wind-up"-like phenomena[6] and to central sensitization (Woolf 2011), with increased excitability of neurons in the central nervous system, including activation of glial cells (Scholz and Woolf 2007). These changes contribute to exaggerated responses to innocuous sensory input [secondary hyperalgesia, long-term potentiation (LTP)]. Inflammatory hypersensitivity may be present for days to weeks depending on the severity of the tissue injury.

Enhanced excitation and impaired inhibition are basic pro-nociceptive mechanisms. Severe tissue injury leads to local and systemic inflammatory responses. Behaviorally these adaptations lead to hyperalgesia and hypersensitivity, and immobilization, limiting the tissue injury and increasing the survival chance of the animal. If these changes continue after the healing process has been completed, they become maladaptive and counterproductive, leading to sustained pain with behavioral consequences (Sandkuhler and Gruber-Schoffnegger 2012). LTP is a complex amplification mechanism in spinal and thalamic neurons that constitutes a pain-retaining mechanism, i.e., pain "memory". A number of human experimental studies have demonstrated that low-frequency (2–10 Hz) and high-frequency (100 Hz) electrical stimulation (van den Broeke et al. 2010; Biurrun Manresa et al. 2011),

[5] Nociception means to pick-up signs of imminent tissue injury. Pain is the conscious perception of nociception.

[6] "Wind-up"-like activity describes temporal summation, i.e., repeated noxious stimuli will lead to progressively increasing pain.

are associated with several hours of sensitization of the nociceptive pathways that persist after termination of the primary conditioning stimulus. However, extrapolation of data from these experimental studies to PPP is limited by two factors: the time aspect, the LTP-studies only induce fairly short-lasting changes vis-à-vis a chronic pain state; and the pain model—only skin stimulation, a target organ with an inherent low propensity for development of chronic pain, has been used.

Impaired function of an endogenous pain inhibition system, the descending conditioned pain modulation system (CPM),[7] is an important contributing factor to postsurgical pain sensitivity in humans (Yarnitsky 2010; Yarnitsky et al. 2008). Deficiency in the CPM system has been associated with PPP (Yarnitsky 2010; Yarnitsky et al. 2008) as well as a number of other chronic pain conditions: chronic musculoskeletal pain (Staud et al. 2012), chronic tension-type headache (Pielsticker et al. 2005), chronic pancreatitis (Olesen et al. 2010), fibromyalgia (Price et al. 2002), irritable bowel syndrome (King et al. 2009), temporomandibular disorder (King et al. 2009) and osteoarthritis (Kosek and Ordeberg 2000). However, the causal relationship between the CPM system and chronic pain has yet not been demonstrated: is the impairment in CPM responsible for development of chronic pain or is the impairment in CPM a consequence of chronic pain, the chicken and egg problem revisited?

5.2 Quantitative Sensory Testing

Quantitative sensory testing (QST), commonly used in experimental and clinical pain research, investigates the graded psychophysical response to controlled thermal, mechanical, electrical, or chemical stimuli, allowing quantification of clinically relevant perception and pain thresholds (Werner et al. 2013). Persistent pain following surgical procedures represents a unique opportunity to identify pathophysiological contributors, i.e., neuropathic and inflammatory components, and link these to a specific postsurgical pain state (Jensen and Kehlet 2011). Most of the variables studied are associated with the testing of cutaneous sensory function, e.g., neuropathy testing. Assessments of pressure pain, vibratory thresholds, and certain laser stimuli test the function of deeper structures (dermis, fascia, nerves, vessels, musculoskeletal tissues) and may reveal inflammatory origins of pain.

5.2.1 Breast Cancer Surgery

An early, pivotal QST-study on PPP, performed in BCS-patients, demonstrated that thresholds to tactile and thermal stimuli were higher on the surgical side as compared to the contralateral side, both in pain *and* pain-free subjects (Gottrup et al. 2000). Pressure pain thresholds, assessed by pressure algometry, were lower

[7] In animals called the diffuse noxious inhibitory control system (DNIC)

on the surgical side in pain patients, while no side-to-side difference was apparent in the pain-free group. No patient in this study had received chemotherapy, which potentially could affect the sensory system, while 6 out of 15 patients had received radiotherapy. The findings indicated *first*, that sensory hypoesthesia in the skin is a common finding in the surgical field, irrespective of pain status, and *second*, that hyperesthesia from deeper structures characterize BCS-patients with PPP. *Third*, signs of an enhanced temporal summation, induced with repetitive 2 Hz pin-prick stimuli, were demonstrated on the surgical side in PPP-patients but not in pain-free subjects. These results have partially been corroborated in other studies (Vilholm et al. 2009; Edwards et al. 2013). Interestingly, a deficiency in the CPM system, i.e. a decrease in endogenous pain inhibition, has recently been reported in PPP patients (Edwards et al. 2013).

In a study with assessment of pressure pain thresholds in areas distant from the surgical field (neck, deltoid muscle, hand, and lower leg), significantly lowered thresholds were demonstrated in BCS-patients compared to healthy controls, indicating signs of widespread musculoskeletal sensitization (Fernandez-Lao et al. 2011). A high-powered study with pain (n = 102) and pain-free (n = 98) BCS-patients (Schreiber et al. 2013) confirmed these results, but could not demonstrate changes in thermal thresholds and temporal summation tests outside the surgical field.

5.2.2 Groin Hernia Repair

Quantitative sensory findings in GHR-patients (open surgical technique) and BCS-patients are quite similar. The GHR-patients demonstrated increased tactile thresholds, cool and warmth detection thresholds, and heat pain thresholds on the surgical side compared to the nonsurgical side, both in-pain patients *and* pain-free controls (Mikkelsen et al. 2004; Aasvang et al. 2008, 2010b). Sensory mapping, with a metal roller, demonstrated preferential hypoesthesia in the surgical field in both pain and pain-free groups. However, increased tactile thresholds (Aasvang et al. 2008), decreased tactile *pain* thresholds (Aasvang et al. 2010b), decreased pressure pain thresholds (Aasvang et al. 2008, 2010b), increased thermal thresholds (Aasvang et al. 2010b), and temporal summation to mechanical stimuli (Aasvang et al. 2010b), assessed on the surgical side, distinguished PPP-patients from pain-free controls. This indicates a *hyper*sensitivity to noxious mechanical stimuli and *hypo*sensitivity to thermal stimuli in the GHR-patient with PPP compared to pain-free GHR controls. Interestingly, pressure hyperalgesia and cool hypoesthesia were observed in the *non*-surgical side in GHR-patients with PPP, but not in pain-free GHR controls (mirror-image findings, please see below) (Aasvang et al. 2010b). In a study of laparoscopically operated PPP-patients, the findings of a number of pain localizations outside the inguinal region, clearly separated this group from the group with open-surgery GHR (Linderoth et al. 2011). These QST data indicate that inflammatory, mechanical, and neuropathic components are potential contributors to persistent pain in GHR-patients.

5.2.3 Lung Cancer Surgery

Thermal and mechanical detection thresholds are increased, reflecting a sensory hypofunction on the surgical side, compared to the nonsurgical side, both in-pain patients and pain-free controls after LCS. This seems to indicate that LCS is the cause of development of neuropathy in the surgical field (Kristensen et al. 2010; Wildgaard et al. 2012a, b). In LCS-patients with persistent pain, more extensive disruption of thermal sensory function (increased thresholds) is seen compared to pain-free LCS controls (Wildgaard et al. 2012a), indicating a predominantly neuropathic origin of pain. These findings are in agreement with a number of other LCS studies (Duale et al. 2011; Guastella et al. 2011; Wildgaard et al. 2013a, b).

Recent studies have demonstrated that single QST assessments in LCS-patients with PPP carry an excessively high variability in thermal thresholds, necessitating the use of repeated testing in order to acquire reliable data (Wildgaard et al. 2013b). In a companion study mapping thoracic areas by simple metal rollers, deviations of cool and warmth perception were recorded, using a test–retest technique. Mirror images of the sensory changes on the surgical side were replicated on the non-surgical side in 12 out of 14 patients (Werner et al. 2013). The exact neural mechanism implicated in this "crosstalk" between the pathologically changed side and the contralateral normal side, is not known, but animal data indicate that it depends on a glial cell inflammatory response with subsequent release of cytokines leading to the activation of central neural pathways (Obata et al. 2010).

In conclusion, the QST-profiles following BCS, GHR, and LCS exhibit common denominators for pain patients and pain-free controls: increased tactile and thermal thresholds on the surgical side compared to the nonsurgical side, probably constituting an injury-induced, stereotype imprint upon the nervous system. The QST-profiles in PPP-patients, across the three surgical procedures, also demonstrate similarities: the increases in thermal thresholds seem to be accentuated compared to pain-free individuals. In addition, BCS-patients and GHR-patients with PPP present with increased tactile thresholds, decreased pressure thresholds, and augmented temporal summation in the surgical field, indicating the presence of both inflammatory and neuropathic components with a significant contribution of central sensitization.

6 Predictive Factors

6.1 Surgical Factors

A number of surgical factors associated with PPP have been identified, including increased duration of surgery (Peters et al. 2007), low surgical volume (Tasmuth et al. 1999), repeat surgery (Aasvang et al. 2006a), use of more invasive surgical techniques (Aasvang et al. 2010a) and intraoperative nerve lesion (Wildgaard et al. 2009;

Katz and Seltzer 2009). Surgical procedures like VATS, laparoscopic cholecystectomy, or laparoscopic groin hernia surgery are associated with decreased probability of development of PPP, compared with open-surgery procedures. These surgical noninvasive techniques decrease the risk of nerve lesion in BCS, GHR, and LCS, and it is reasonable to assume that this is a major factor for the improved outcome. In addition, the sentinel node technique in BCS, with breast tissue conserving therapies, has dramatically decreased the prevalence of PPP (Gartner et al. 2009). In implant surgery like GHR, use of lightweight mesh and glue fixation has, in laparoscopic GHR, reduced the prevalence of PPP (Bittner et al. 2010). It should however, be emphasized that one of the most consistent predictive factors for PPP is high intensity acute postsurgical pain (Andersen and Kehlet 2011; Kehlet et al. 2012), implicating the necessity for joint efforts by surgeons and anesthesiologists in the prevention and management of acute pain.

6.2 Psychological Factors

Psychological factors, i.e., attentional bias to pain, anxiety, catastrophizing behavior, depression, introverted personality, and psychological vulnerability, have all been identified as significant predictors of PPP (Hinrichs-Rocker et al. 2009; Lautenbacher et al. 2011; Theunissen et al. 2012). A recent study compared dependence of specific psychological predictors (anxiety, catastrophizing behavior, and depression) on two different surgical procedures, BCS and total knee arthroplasty (Masselin-Dubois et al. 2013). The breast cancer group was younger and seldom restricted by preoperative pain, while the knee osteoarthritis group was older and suffering from impaired mobility and preoperative pain. From a psychological perspective, these groups are quite different, since breast cancer is obviously a potential life-threatening disease associated with existential thoughts and fears, while knee osteoarthritis is a slower, degenerative disease often received with relative stoicism in the elderly. Multivariate logistic regression analyses indicated that clinically significant PPP at 3 months was predicted independently by age, immediate postsurgical pain intensity, and state anxiety (as opposed to inherent [trait] anxiety). Linear regression models also showed that pain magnification, one of the three dimensions of catastrophizing, independently predicted chronic pain intensity. Interestingly, any procedure-specific dependence of these psychological factors was not demonstrated in this study.

6.3 Socioeconomic Factors

It is quite amazing, in our increasingly cost–utility conscious societies, how little attention has been paid to PPP, pain-related functional impairment, social consequences, and influence on societal cost-issues, i.e., sick leave, early retirement,

transfer incomes, and healthcare expenditures (Gilron and Johnson 2010; Kehlet et al. 2013). Swedish data, based on a population of nine million inhabitants, indicate that the annual expenditure for patients with a diagnosis related to chronic pain, in the period of 2004–2009, was estimated at 42 billion USD, corresponding to 10 % of the gross national product (GNP) of Sweden (Gustavsson et al. 2012). Unfortunately, it is not possible from these data to extract detailed expenditure data for PPP-patients, but a reasonable estimate is that 1–5 % of this cost is related to severe PPP.

6.4 Biological Factors

6.4.1 Genetics

Pain perception is genetically a very complicated trait, consisting of an aggregate of phenotypes affected by peripheral and central nervous system dynamics, inflammatory dynamics, physical stress dynamics, and distress responsiveness (Smith et al. 2012). Based on animal studies, estimates of heritability of indices of pain sensitivity generally conclude that 30–75 % of the variation in pain responding is explained by genetic factors (Young et al. 2012). Significant discoveries have been made mapping single gene characteristics, characterizing the genetic variation by single nucleotide polymorphisms (SNPs). A number of SNPs are associated with phenotypically increased sensitivity to pain: polymorphisms of the genes OPRM1, CACNAD2, ABCB1, COMT, GCH1, and SCN9A[8] (Kehlet et al. 2012; Rhodin et al. 2013). A statistically superior method, called genome-wide association (GWA) studies, focuses on associations between multiple SNPs and phenotype, e.g., pain sensitivity. The GWA studies more accurately delineate the often complex interactions between a large number of SNPs. However, GWA studies may require tissue sampling from 1,000 up to 100,000 individuals (Smith et al. 2012).

6.4.2 Quantitative Sensory Testing

Preoperatively assessed QST-variables, as potential predictors of acute and chronic postsurgical pain, have been used in a number of studies (Katz and Seltzer 2009; Ip et al. 2009; Werner et al. 2010; Werner and Kehlet 2010; Abrishami et al. 2011). However, only two predictive studies are presently available on the three surgical procedures presented in this chapter (Aasvang et al. 2010a; Yarnitsky et al. 2008).

[8] OPRM1: μ-opioid receptor; CACNA2D2: voltage-dependent calcium channel subunit $\alpha 2\delta$-2; ABCB1: ATP-binding cassette B1 transporter enzyme; COMT: catechol-O-methyl transferase; GCH1: GTP cyclohydrolase 1; SCN9A: encoding the expression of $Na_v1.7$ ion channel.

In a high-powered study, patients scheduled for GHR, by open (n = 244) or laparoscopic (n = 198) surgery, were examined preoperatively with functional assessments by a groin hernia specific pain-related activity scale (AAS-score), pain ratings, psychometrics, QST variables, and pain responses to tonic heat stimuli (Aasvang et al. 2010a). The 1- and 6-month's follow-up after GHR, demonstrated four significant risk factors for development of PPP: preoperative AAS-score, preoperative pain to tonic heat stimulation, 1-month postsurgical pain intensity, and sensory dysfunction in the groin at the 6-month follow-up. In a predictive, multiple regression model containing preoperative variables and surgical technique, three risk factors for development of PPP were identified: a preoperative AAS-score indicating a high degree of preoperative physical impairment, an increased preoperative pain response to heat stimulation, and surgery performed by an open procedure.

An interesting, predictive QST study in 62 patients scheduled for thoracotomy (number of LCS-patients not stated) examined the *pre*operative CPM-efficiency, i.e., the efficacy of the endogenous pain-inhibiting system (Yarnitsky et al. 2008). The conditioning stimulus was immersion of the hand in 46.5 °C water for one min. The test stimuli were 30-second contact heat impulses delivered at the contralateral forearm and maintained at a temperature level, initially corresponding to a pain perception of 60 on a numerical rating scale of 0–100. Patients with an efficient CPM system preoperatively experienced a reduction in pain perception of the test stimuli, during and immediately after the conditioning stimulus. The patients were followed in mean (SD) for 7 months (4 months) after the surgical procedure. The preoperatively assessed CPM-efficiency significantly predicted a lowered risk of development of PPP: odds ratio of 0.52 (0.33–0.77; 95 % CI). Thus an efficient CPM system seems to protect against PPP. Further procedure-specific studies are, however, needed to corroborate these findings (Lewis et al. 2012), but a predictive potential for PPP by assessment of efficiency of the descending inhibitory system has been demonstrated (Granovsky 2013).

6.4.3 Neuroimaging

A number of neuroimaging studies related to acute and chronic postsurgical pain have been published during recent years (Pogatzki-Zahn et al. 2010; Gwilym et al. 2010; Howard et al. 2011). Neuroimaging techniques have revealed structural and functional changes in the brain related to the chronic pain state (Borsook et al. 2013). These changes are localized to regions primarily involved in pain processing (thalamus, posterior insula, and primary somatosensory cortex) and pain modulation (anterior cingulate cortex), but also regions involved in emotional processing of pain (anterior cingulate cortex, insula, periaqueductal gray substance), premotor activity (supplemental motor areas, cerebellum), and cognitive processing (anterior cingulate cortex, prefrontal cortex) (Borsook et al. 2013). Interestingly, decreases in brain gray matter density have been demonstrated in chronic pain patients, e.g., chronic back pain, complex regional pain syndrome,

and knee osteoarthritis (Baliki et al. 2011). These morphometric changes have preferentially been demonstrated in the anterior cingulate cortex, insular cortex, prefrontal cortex, and amygdale, including the brainstem (Rodriguez-Raecke et al. 2009; Emerson et al. 2014). The exact mechanisms underlying these structural changes remain obscure and cell atrophy (affecting neurons or glia cells), cell death or synaptic loss as well as simple decreases in cell size, interstitial fluid density, or blood volume has been suggested as possible explanations (Draganski and May 2008; Rodriguez-Raecke et al. 2009). However, the decrease in brain gray matter density, in chronic pain patients seems to be a reversible process; in patients with osteoarthritis pain, successful hip replacement surgery, assessed by behavioral indices, is associated with an increase in the brain gray matter density in the involved cortical areas (Rodriguez-Raecke et al. 2009; Gwilym et al. 2010). Similar structural brain changes have also been demonstrated in patients with chronic low back pain undergoing spine surgery, facet joint injections (Seminowicz et al. 2011) or topical analgesic treatment (Baliki et al. 2008). Interestingly, in the former study, patients after treatment had an increase in cortical gray density matter in the left dorsolateral prefrontal cortex, an increase that correlated with reduction in pain and physical disability. Even more interesting was that increases in cortical gray matter density in right anterior insula and in the primary motor cortex were specifically associated with reduced pain and with reduced physical disability, respectively (Seminowicz et al. 2011). These findings illustrate very aptly the synergistic interaction between pain and physical impairment: reduction in either pain or in physical impairment may lead to improvement of the other, while increase in either pain or physical impairment may lead to deterioration of the other. A number of animal and human studies have shown cortical structural effects of exercise (Draganski and May 2008; Scheef et al. 2012), which is of critical importance for evaluation of management strategies.

7 Future Research Strategies

The literature base of this overview clearly indicates that in studies of PPP, a plethora of research methods, yielding a large number of different outcomes, has been used. Consensus on optimal study designs and psycho-physiological research methods would facilitate interpretation of data leading to improvements in diagnosis, management, and preventive measures in postsurgical pain. The recommendations from IMMPACT (Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials) (Dworkin et al. 2008, 2010) and ACTION (Analgesic Clinical Trial Innovations, Opportunities, and Network initiative) (Rappaport et al. 2010; Dworkin et al. 2011) have the potential to serve as useful platforms.

Another problem is our lack of knowledge concerning the pathophysiological mechanisms behind PPP. The principal question here is: is the pain neuropathic, inflammatory, or of mechanical origin? The accepted criteria for neuropathic pain are (Treede et al. 2008):

1 Pain with a distinct neuroanatomically plausible distribution.
2 A history suggestive of a relevant lesion or disease affecting the peripheral or central somatosensory system.[9]
3 Demonstration of the distinct neuroanatomically plausible distribution by at least one confirmatory test.[10]
4 Demonstration of the relevant lesion or disease by at least one confirmatory test.[11]

According to Sect. 5.2, it seems that the criteria for neuropathic pain in PPP-patients, across the three surgical procedures considered in this chapter, are fulfilled, with a grading certainty of the diagnosis of *definite* neuropathic pain or at least *probable* neuropathic pain (please see the footnotes). However, there is a didactic problem: pain-free, postsurgical controls, fulfill the same criteria as the PPP-patients, except for the existence of pain, meaning either, that the expression of pain in postsurgical neuropathy is highly variable (Kalliomaki et al. 2011), or that inflammatory or mechanical components, disguised as neuropathic "pain," are contributing to the pain state. The QST data seem to indicate that inflammatory or mechanical components play a substantial part in the PPP state following BCS and GHS. Future strategies in PPP should include procedure-specific research targeted at elucidating basic mechanisms underlying the pain state and, if possible, assess the relative contribution of neuropathic, inflammatory, and mechanical components.

Remember the patient with a lump in the groin introduced at the beginning of the chapter, who had an open GHR performed? The indications for performing GHR in reducible hernias are primarily preventive, as mentioned, to forestall complications and to relieve discomfort and pain (Metzger et al. 2001), and secondarily, to cosmetically improve the physical defect. Could the patient's postsurgical suffering have been foreseen? As described in Sect. 6.4.2, increased preoperative AAS-scores, increased preoperative pain to heat stimulation, and a planned open surgical procedure, increase the risk significantly for development of PPP (Aasvang et al. 2010a). If the patient's preoperative examination had revealed moderately impaired physical function prior to surgery and a pain rating of the heat stimulus as severe (NRS = 9), a preoperative risk calculation would have estimated a risk of development of PPP following open surgery of 42 % and laparoscopic surgery of 22 %. If laparoscopic surgery had been performed the risk of PPP would have been decreased by 50 % (Aasvang et al. 2010a)!

[9] The suspected lesion or disease is reported to be associated with pain, including a temporal relationship typical for the condition.

[10] As part of the neurologic examination, these tests confirm the presence of negative or positive neurologic signs concordant with the distribution of pain. Clinical sensory examination may be supplemented by laboratory and objective tests to uncover subclinical abnormalities.

[11] As part of the neurologic examination, these tests confirm the diagnosis of the suspected lesion or disease. These confirmatory tests depend on which lesion or disease is causing neuropathic pain.

Since the preoperative examinations revealed the patient to be in a high-risk group, amendments to the surgical procedure, such as use of lightweight mesh and fixation with glue, could, in addition to the laparoscopical procedure, have reduced the risk of development of PPP. Future procedure-specific research should focus on procuring simple reliable predictive indices of acute and PPP. Neuroimaging techniques should be used in experimental predictive studies. A recent morphometric MRI-study in human volunteers demonstrated a strong correlation between greater thermal and pain sensitivity and cortical thickening of the primary somatosensory cortex (Erpelding et al. 2012). It will be of interest to determine if patients with increased gray matter volume of the primary somatosensory, the posterior mid-cingulate, and the orbito-frontal cortex are at greater risk of developing PPP than controls.

8 Conclusion

With an extrapolated total global, annual volume of 235 million surgeries (Weiser et al. 2008) and estimating that 2–10 % of the patients are impaired by severe persistent postsurgical pan, major causes of human hardship and strain on healthcare resources are recognized. Surgery is pivotal in management of breast cancer, groin hernia repair, and lung cancer, and the benefits to the patients are unquestionable; but the quest of identifying improved surgical and anesthesiological techniques to prevent or mitigate severe pain and functional impairment in patients after surgery, continues.

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Part II
Pharmacology of Chronic Pain

Behavioral Pharmacology of Pain

Odd-Geir Berge

Abstract Behavioral methods are extensively used in pain research. Rodent modeling tends to rely on evoked responses but there is a growing interest in behavioral readouts that may capture elements of ongoing pain and disability, reflecting the major clinical signs and symptoms. Clinically, analgesics show greater efficacy in acute pain after standard surgery than in chronic conditions but are never completely effective on a population basis. In contrast, experimental pharmacological studies in rodents often demonstrate full efficacy, but there is variability in sensitivity between models and readouts. Full efficacy is rarely seen when more complex or multiple readouts are used to quantify behavior, especially after acute surgery or in studies of clinical pain in animals. Models with excellent sensitivity for a particular drug class exist and are suitable for screening mechanistically similar drugs. However, if used to compare drugs with different modes of action or to predict magnitude of clinical efficacy, these models will be misleading. Effective use of behavioral pharmacology in pain research is thus dependent on selection and validation of the best models for the purpose.

Keywords Pain models · Neuropathy · Osteoarthritis · Arthritis · Rodent · Rat · Mouse · Dog · Behavior · Wheel-running · Burrowing · Analgesia · Predictive validity · Opioid · Nonsteroidal · Spontaneous pain · Evoked pain · Screening · Validation

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Contents

1 Introduction

The concept of behavioral pharmacology of pain includes the use of pharmacological tools to study pain-related phenomena like nocifensive mechanisms and pain pathophysiology, as well as the use of behavioral models to predict the clinical efficacy of pharmacological agents. In spite of a heated discussion about the clinical relevance of behavioral rodent models in pain research, particularly with regard to their usefulness in predicting clinical efficacy of novel analgesics (Mao 2009; Mogil et al. 2010; Quessy 2010; Rice et al. 2008), there is evidence that their use is increasing (Mogil et al. 2009). Both the discussion and some failed predictions are due in part to a lack of appreciation for the necessity of choosing appropriate modeling strategies based on the research purpose. Investigating the functional significance of novel mechanisms, characterizing models for defined pain conditions, screening, and selection of compounds at various stages of a drug discovery project are each activities with different requirements for sensitivity (the ability to predict true efficacy) and specificity (ability to detect negative outcomes). Pain is a clinical problem both in man and animals and there is a significant overlap between veterinary and human medicine, for instance regarding procedural or post-surgery pain, which may represent a valuable translational link.

2 Publication Bias and Environmental Factors

Publication bias, i.e., the favoring of publishing positive data over negative or neutral results, has been documented in many areas of research and also in the context of pain modeling (Currie et al. 2013). Reproducibility of results is less than perfect. One study found that only 20–25 % of reported studies could be satisfactorily replicated in a target validation program (Prinz et al. 2011). Data from another group confirmed 11 % of published conclusions and success was tied to quality indicators like adequate controls, evidence of bias reduction, and complete

description of the data (Begley and Ellis 2012). Procedures may be highly standardized in a particular laboratory, especially in the pharmaceutical industry and in other settings where standard operating procedures are in place to provide consistency when models are used routinely. There are nevertheless environmental and procedural factors that create variability within and between laboratories even when all reasonable measures have been taken to enhance reproducibility. A within-laboratory analysis of thermal nociceptive data from genetically diverse mouse strains found that environmental factors, and particularly the person carrying out the experiments, were more important than genotype (Chesler et al. 2002). Season, humidity, cage density, time of day, sex, and within-cage order of testing also affected the outcome and it should be noted that only some of these factors can be controlled by adequate randomization. In a classic paper comparing behavioral outcomes related to motor and cognitive functions, anxiety, and pharmacology in eight strains of mice across three laboratories, several outcomes where highly laboratory-dependent (Crabbe et al. 1999). Factors considered important, e.g., apparatus, housing and acclimatization, age of animals, and timing of experiments, were extremely well controlled. There was an expected genetic effect on behavior, but somewhat surprisingly, less important was sex, or whether the animals were locally or externally bred with associated differences in transportation. The authors concluded that large genetic effects are likely to be reproducible across laboratories while smaller effects may be more susceptible to laboratory-specific variables.

In the context of behavioral pharmacology, it seems reasonable to assume a similar relationship between pharmacological effect and reproducibility. A high degree of standardization may increase the risk of overestimating spurious effects, and so it has been suggested that measures to systematically increase heterogeneity may improve the external validity of results (Richter et al. 2011). It may be worth considering that the use of pet animals as subjects in studies of painful veterinary conditions automatically provide heterogeneity and a degree of randomization that is difficult to obtain in a traditional laboratory setting. The impact of environmental and genetic factors will vary between paradigms. This should always be considered when different results are obtained with seemingly similar methods and before results are generalized from a particular model to clinical conditions in man or animals.

Whereas experimental variability is unavoidable, bias and inadequate reporting of methodological details add avoidable uncertainty to interpretation of the animal research literature (Kilkenny et al. 2009). Recognition of these problems has motivated recommendations and checklists, some that specifically focus on pain research (Rice et al. 2008), while others are of general scope (Kilkenny et al. 2010; Muhlhausler et al. 2013). The greatest impact has been achieved with the ARRIVE guidelines (http://www.nc3rs.org.uk/page.asp?id=1357), currently supported by more than 300 journals (June 2013; http://www.nc3rs.org.uk/page.asp?id=1796). Several pain journals have adopted these guidelines and an example of how they may be implemented can be found in Huang et al. (2013). In the future, hopefully more consistent reporting will facilitate proper meta-analysis of preclinical data in the pain field.

3 Symptoms, Signs, and Readouts

Research in behavioral pharmacology is often motivated by human disease. A thorough description of pain in man is beyond the scope of this chapter, but some points are worth mentioning. Clinically, acute pain is usually studied in the context of surgery. Postsurgical pain is characterized by ongoing pain exacerbated by movements (Brennan 2011). Pressure pain thresholds are reduced around the wound and inversely correlate with spontaneous pain and pain during movement (Møiniche et al. 1997). Minor surgery, such as removal of impacted molars, is frequently used as a human model in drug discovery, typically with readouts reflecting ongoing pain and its reduction. Clinical trials of new analgesic drug candidates are usually carried out in chronic conditions.

In osteoarthritis, the major complaint is pain at rest and during movement, although mechanical and thermal thresholds may be lower both near the affected joints and at more distant sites, possibly reflecting different pathophysiological mechanisms. Lower thresholds have been correlated with higher visual analog scale (VAS) scores and found to be normalized upon effective pain relief (Smith et al. 2012). Functional parameters are often used to quantify treatment effect, but pain reduction does not always result in improved function; in one study, walking speed declined in 20 % of patients despite reduced pain (White et al. 2011).

Neuropathic pain is associated with a range of symptoms and signs (Jensen et al. 2001). The common denominator for neuropathic pain is that it is caused by a lesion or a disease affecting the somatosensory system (Treede et al. 2008) but symptoms and pharmacology differ between patients and diagnoses. Spontaneous pain (apparently stimulus-independent), may be continuous or paroxysmal and of different qualities. Stimulus-evoked pain, most commonly involving a mechanical or thermal stimulus, can either be time locked to the stimulus or persistent, representing a sliding transition toward spontaneous pain. Sensory disturbances, either loss of sensation, elevated thresholds, or positive sensory phenomena such as allodynia, hyperalgesia, and paroxysmal pain evoked by supra-threshold stimulation in areas with elevated sensory thresholds (hyperpathia) are not specific to neuropathic pain. Other symptoms such as superficial, ongoing pain, and pain evoked by touch or cold may be more common in neuropathic patients than in non-neuropathic patients (Rasmussen et al. 2004). Spontaneous pain, whose characteristics may differ significantly between patients, is the most frequent complaint and the most commonly used primary readout in clinical studies (Novak and Katz 2010). Increased sensitivity to stimulation in any specific modality is seen in about one-third or less of patients and sensory loss is common (Maier et al. 2010).

In animal models, evoked withdrawal responses are commonly used as surrogate endpoints for pain; however, these measures are associated with a number of methodological problems. First, withdrawal may be either reflexive or voluntary. Second, stimuli that gradually increase in intensity during application will by default first activate low-threshold, non-nociceptive sensory nerves, which may be sufficient to elicit a response. Third, visual or auditory cues may cause motor

responses indiscernible from reflexive withdrawal, particularly when repeated stimulation creates an opportunity for conditioning. Fourth, both application of stimuli and assessment of responses may be difficult to standardize and reproduce between observers and laboratories. If correctly tested, interpreted, and reported, evoked responses may, nevertheless, provide relevant information about sensory functions. For an extensive review and analysis of transient nociception, including discussion of electrical stimuli and vocalization, see Le Bars et al. (2001). This chapter will briefly discuss the use of acute thermal and mechanical stimuli, and then emphasize alternative readouts.

Heat. Heat is an adequate pain stimulus that was initially used in human psychophysical studies, (discussed in (Beecher 1957)) and applied to rodents in the tail-flick assay (D'Amour and Smith 1941). In man, progressive heating of the skin causes burning pain followed by stinging pain. Stinging pain exhibits heat response-latency correlations similar to tail-flick and other withdrawal responses in animals (Le Bars et al. 2001). The effective stimulus is determined by heat source, energy supplied, initial temperature of the skin, and skin color (Berge et al. 1988; Luukko et al. 1994; Tjølsen et al. 1989; Wen et al. 2009; Winder et al. 1946). Depending on the tissue temperature achieved and the rate of heating, different classes of nociceptors are sequentially recruited (Le Bars et al. 2001; Schepers and Ringkamp 2009; Yeomans and Proudfit 1996; Yeomans et al. 1996). The current standard for heat stimulation in rodents is the radiant heat paw withdrawal assay, with response latency as the readout (Hargreaves et al. 1988). The assay is sensitive to potentially confounding factors like posture, exact focus of the beam, and the initial temperature of the skin, which may be altered by inflammation or neuropathy as well as by handling, conditions in the testing environment, and by confounding pharmacological effects (Bennett and Xie 1988; Dirig et al. 1997; Luukko et al. 1994). Preheating the floor may allow better consistency of the stimulus function. Starting from a higher temperature favors slower heating rates, a factor that may tune the assay toward C-fiber-mediated responses.

Cold. As for heat, both rate of temperature change and actual tissue temperature determine the recruitment of different cold fiber types and thus the mechanisms activated by a cold stimulus (Belmonte et al. 2009; Foulkes and Wood 2007). Commonly used in rodent neuropathic pain modeling, the application of acetone produces a cold sensation even in man and may evoke allodynia in a minority of chronic pain patients (Rasmussen et al. 2004). A testing sequence is initiated by application of a fixed volume of acetone to the plantar skin and the response, defined as brief withdrawal of the paw, may be scored after a predefined number of evenly spaced applications (Choi et al. 1994). While the tactile component may be insignificant in the conventional procedure (Taylor et al. 2007), the use of acetone or ethyl chloride spray adds a mechanical component to the cold stimulus. In these cases, the outcome variable is usually the accumulated duration of paw withdrawal during a defined poststimulus interval (Dowdall et al. 2005; Gustafsson and Sandin 2009).

Tactile, Non-Noxious. Mechanical sensitivity in rodents is most commonly determined as a response threshold or response frequency to application of von Frey filaments of different stiffness. The response criterion is usually a brisk withdrawal. The method is associated with a number of drawbacks. First, the applied pressure changes during application: contact area and geometry depend on degree of bending and compliance of the tissue; force generation varies with application speed; and unpredictable off-axis forces are created as the fiber bends (Bove 2006). Second, conventional filaments are hygroscopic and the bending properties, particularly of thinner filaments, will change rapidly in response to normally occurring fluctuations in relative humidity (Ängeby Möller et al. 1998). These factors lead to increased variability and even systematic errors if randomization is inadequate. Mechanically stable filaments with a fixed tip size can circumvent some problems (Fruhstorfer et al. 2001; Song et al. 1999) and force transducer-based devices facilitate standardization of stimulus application (Ängeby Möller et al. 1998). Protocols designed to reduce inter- and intra-observer variability have been suggested, taking into consideration duration of application, stimulus interval, degree of bending, and site of stimulation (Hogan et al. 2004; Song et al. 1999).

Tactile, Noxious. Mechanical hyperalgesia by the pinprick method is tested by application of a sharp object to the skin, causing indentation but no penetration. The response, defined as a brisk withdrawal or a "hyperalgesia-like" response with sustained elevation, licking, and grooming of the paw, can be quantified as duration of evoked behavior (Erichsen and Blackburn-Munro 2002; Hogan et al. 2004). Sensitivity to deep pressure is usually tested by application of a constant or gradually increasing stimulus to a paw with withdrawal, struggling, or vocalization as endpoints and the pressure at which the response occurs as readouts (Pradhan et al. 2010; Randall and Selitto 1957; Whiteside et al. 2008). It should be kept in mind that these endpoints may represent different sensory and emotional experiences.

4 Readouts that Do Not Involve Experimenter-Applied Stimuli and Evoked Responses

Alternatives to evoked responses have been introduced to model the subjective experience of pain and also to model pain-relevant disability. These measures can be categorized as general behaviors that are depressed by pain or disability, as behavioral manifestations that preferentially reflect pain, or as operant behaviors.

General Behaviors. Analysis of general behavior to quantify pain and analgesia is common in veterinary medicine. Therapeutic intervention in individual subjects requires readouts that allow immediate assessment, while studies of therapeutic efficacy are better served by data collection over longer periods of time. Some parameters are recorded by standardized, objective, automated procedures. This

can be done in the home cage, thus minimizing observer–subject interaction and allowing continuous data collection (Miller et al. 2011a; Roughan et al. 2009). Food and water consumption as well as motor activity may be used to characterize postsurgical pain and disability (Liles and Flecknell 1993; Jacobsen et al. 2012). Even experimental models may use automated registration of locomotion and rearing (Cho et al. 2013). Thigmotaxis (the tendency to stay near the walls, e.g., in an observation chamber) may be used as an index of increased anxiety caused by a presumably painful manipulation (Huang et al. 2013).

Wheel-running and Burrowing Activity are other innate behaviors suppressed by pain and disability. Access to a running wheel may be free, allowing continuous registration of activity, or restricted to certain time slots. Several parameters can be registered, including distance traveled, speed, and in the case of continuous monitoring, circadian activity pattern. Both in relative and absolute terms, mice are more active and regular runners than rats, and, therefore, mice may in effect get a substantial exercise effect of voluntary running (Allen et al. 2001). Mice show great individual, sex and strain differences in wheel-running time (Lightfoot et al. 2010; Siepka and Takahashi 2005), but activity is stable within subjects (Knab et al. 2009). Compared to spontaneous locomotion in the home cage or in an open field, much greater distances are covered with free access to a running wheel (Costello et al. 2010). The high level of activity in some mouse strains provides a solid basis for studying the effect of presumably painful procedures and may increase the impact of movement-related pain. Most studies find that a run-in period of a few days is sufficient to stabilize the activity level in mice, whereas a much longer time may be required for rats (Stevenson et al. 2011). Similar to other spontaneous behaviors, wheel-running in mice is generally reduced by surgery and in experimental models of inflammation (Adamson et al. 2010; Clark et al. 2004; Cobos et al. 2012; Krug et al. 2009; Miller et al. 2011b; Sluka and Rasmussen 2010). Wheel-running is also reduced in spontaneously occurring osteoarthritis in aged mice, where reduced performance is correlated with disease severity (Costello et al. 2010).

It is not clear how useful this assay is in rats. Voluntary wheel-running was reduced following carrageenan injection into the hind paw but not the tail (Loram et al. 2007). This indicates that it is possible to dissociate pain related to limb usage (after paw inflammation) from the general discomfort of inflammation (after tail inflammation). Monosodium iodoacetate-induced monoarthritis also reduced wheel-running, but the effect was small compared to decreased weight-bearing and increased sensitivity to von Frey (Stevenson et al. 2011). Stavudine is an antiretroviral drug used in the treatment of HIV where it is associated with painful neuropathy and also used to induce neuropathy in a rodent model (Huang et al. 2013). Long-term treatment with stavudine did not change voluntary wheel-running, food intake, and weight gain in rats, even though it increased sensitivity to pressure (but not heat) applied to the tail (Weber et al. 2007).

Wheel-running and other forms of motor activity may interact with nociception, but the data are not consistent; prior running has been reported both to increase and

reduce the responsiveness in the rat tail-flick assay (Kanarek et al. 1998; Spradley et al. 2012). Wheel-running exercise for 2 h, 24 h prior to testing increased bilaterally the response to plantar von Frey stimulation in mice with a unilateral muscle inflammation but not in normal mice, assumed to be an effect of muscle fatigue (Sluka and Rasmussen 2010). Effects of exercise on nociception are not limited to wheel-running paradigms. For example, repeated swimming exercise reduces the response to formalin and decreases the behavioral hypersensitivity in rat and mouse models of peripheral neuropathic pain (Kuphal et al. 2007).

Burrowing. Burrowing has been used to study neurobehavioral functions in several species, including rats and mice (Deacon 2006, 2009). The assay measures the amount of material removed from a tube in the home cage during a defined time period and does not require observer interaction. Recently, the behavior has been implemented as a readout in mice after laparotomy and in colitis (Jirkof et al. 2010, 2013). In rats, burrowing is reduced in several traumatic neuropathy models (Andrews et al. 2012; Lau et al. 2013). Although mechanical responsiveness was increased in these models, there was no correlation between burrowing deficits and mechanical response thresholds. This indicates that the measures reflect different aspects of pain. Burrowing was also reduced in stavudine-induced neuropathy (Huang et al. 2013) and in the rat model of inflammation induced by intraplantar injection of Freund's complete adjuvant (FCA; Andrews et al. 2012; Rutten et al. 2013a, b).

Ongoing Pain-Like Behaviors. Behaviors assumed to be evoked by pain, such as limping, licking, or favoring of a paw, are well established as readouts in models like the formalin test and in models of unilateral paw or joint inflammation induced by various agents (Berge 2013). Alternatives are, however, needed for quantification of behavioral changes after certain types of surgery or in multifocal pain. A comprehensive analysis of over 150 individual behaviors and behavioral sequences observed after laparotomy identified transient back arching and horizontal stretching followed by abdominal writhing, and twitching while the animal was inactive as the more robust and quantifiable activities (Roughan and Flecknell 2001). Roughly similar behaviors were affected by vasectomy in mice (Jacobsen et al. 2012). Some similar behaviors are observed in other testing paradigms, e.g., flinching in the formalin test. Other behaviors were sensitive to drug treatment, independent of surgery. These measures included behaviors associated with grooming, among others face grooming following paw licking which is a sequence typical for heat dissipating behavior in the rodent (Berge et al. 1983). The licking-grooming behavior may be a confound when forepaw licking is used as an endpoint in the hot plate test, and is frequently observed in the formalin test, where it should be differentiated from paw lick occurring independent of grooming. In mice, other visual scoring systems used to evaluate pain after surgery are based on the overall condition of the animals, the condition of the skin, fur, or eyes, and on motor coordination and posture (Adamson et al. 2010; Clark et al. 2004).

Facial Expression. Arguably, the most innovative approach to readouts reflecting pain in laboratory animals is the analysis of facial expression using grimace scales (Langford et al. 2010). These were inspired by scales used in

nonverbal humans (Williams 2002). Observers tend to focus on the face, which may be an advantage in grimace analyses but a challenge in behavioral paradigms that involve other, or multiple body parts (Leach et al. 2011).

The mouse grimace scale (Langford et al. 2010) is based on scoring of facial expression from sampled video frames. In the original publication, positive scores were synchronized with conventional readouts in inflammatory and nociceptive models of moderate duration and in surgical models. The grimace scores did not correlate to responses in models using shorter-acting stimuli nor in any of three common neuropathic models 1, 7, or 14 days after lesioning. The authors concluded that noxious stimuli of moderate duration and origin in deep structures were most likely to be associated with a "pain face." The lack of effect in the neuropathy models might indicate that these models primarily display hypersensitivity rather than ongoing pain. At the one-day readout, one would, however expect significant postoperative pain, and the data appear to be in conflict with the results from surgical models in this and another study in mice (Matsumiya et al. 2012) and from a rat study using an adaptation of the mouse scale (Sotocinal et al. 2011). In the rat study, a useful readout was obtained also in the FCA-induced paw inflammation model and in monoarthritis induced by intra-articular injection of kaolin and carrageenan. It should be noted that a baseline grimace score is obtained even in presumably pain-free subjects and the readout is not strictly specific to pain.

Accepting that the experience of pain is multidimensional, with sensory, emotional, and cognitive components, any single readout is unlikely to reflect the complete experience of the animal, although more complex readouts may integrate several dimensions. Another challenge is to understand how the intensity of the sensation is coded by the measurement variable, e.g., whether there are ceiling effects saturating the response at higher intensities of stimulation. For instance, if weight bearing is used as a readout in a monoarthritis model, the maximum observable response occurs when no weight is put on the affected limb but this will not, a priori, indicate maximal imaginable pain. At the lower end, nonpainful sensations may elicit a response or weakly painful sensations may be insufficient. In a behaving animal, the limits may be modulated by competing neuronal activity.

It is well documented that rodents may suppress pain behavior when exposed to novelty factors or stressors, including the presence of a human observer (Kavaliers 1988; Kavaliers and Innes 1988). Transportation of animals from vendor or animal quarters, handling, even when repeated for weeks, and sequential removal from common cages increase stress indicators (Olfe et al. 2010; Prager et al. 2011; Rosén et al. 1992). Other factors that may contribute to variability are single versus multiple housing, cage cleaning, environmental enrichment, and light conditions. Some stress factors are difficult to avoid in a laboratory setting, although many can be minimized by proper routines and randomization.

5 Pharmacology

For practical purposes, a model can be characterized in many ways: by stimulus type and intensity; whether a pathological state is present and if so, type and duration of pathology; and by the observable response. Ethical and practical considerations dictate that truly chronic models are uncommon in rodent behavioral pharmacology; inclusion criteria for a clinical trial in chronic pain is at a minimum 3 months of pain duration and the average in most studies is several years. "Chronic" rodent models typically last for a period of days or weeks after initial insult and only exceptionally are the animals kept for several months. This means that the models as commonly implemented show better face validity for human acute pain than for chronic conditions. Pain intensity may also be lower in animal models than in many clinical conditions and severe pain may in fact be detrimental to behavioral measurements.

Postoperative Pain. Postoperative pain is generally considered to be of less medical concern than chronic pain and has not received the same attention from the preclinical research community; however, pain after surgery is surprisingly common (Apfelbaum et al. 2003; Brennan 2011). Treatment often require individualized multimodal approaches which include a combination of drugs, but still renders a large proportion of patients with moderate to severe pain for several days. Some standardized surgical procedures such as third molar extraction resemble animal models in terms of homogeneity of subjects, absence of comorbidity, moderate intensity, and short duration of pain and are frequently used in human experimental studies of new analgesics. They have the advantage of allowing assessment of efficacy without concomitant medication (except rescue medicine). The effect sizes may be higher and the number needed to treat (NNT) values for cyclooxygenase (COX) inhibitors and other compounds may be better than in general surgery and other clinical conditions (Moore et al. 2011). Models of pain due to removal of an impacted third molar is generally considered capable of delivering highly reproducible data (Cooper and Desjardins 2010). Typically, surgery is performed under local anesthesia. Pain builds up over some hours after surgery and remains at a moderate level for about 12 h and gradually subsides over the following days. In a dose response study of valdecoxib given preoperatively, the higher doses reduced average pain scores from moderate to mild (more than 50 % compared to placebo) whereas in bunionectomy, a procedure considered to be more painful, the reduction was approximately 30 % (Desjardins et al. 2002).

In clinical studies of postsurgery analgesia, fixed doses of a nonopioid study drug is usually added to an opioid. The contribution of the nonopioid analgesic to the total analgesic effect is reflected in improved analgesia and reduced opioid consumption—this has been demonstrated for acetaminophen, nonselective COX inhibitors, and COX-2 selective inhibitors (Maund et al. 2011). Acetaminophen and a COX inhibitor may be combined for greater efficacy (Ong et al. 2010). Even gabapentanoids have an opioid-sparing effect (Tiippana et al. 2007; Zhang et al.

2011). Pain is reduced but not abolished by pharmacological treatment in these conditions, in contrast to many animal modeling studies.

Standardized surgery in animals can be used to model postoperative pain whether performed for that specific purpose (Brennan 2005), as part of an experimental manipulation (Lascelles et al. 1995) or for medical reasons, e.g., in pet animals (Hansen 2003). In rats and mice, mechanical and thermal sensitivity is increased after incision of the plantar tissue (Brennan et al. 1996; Field et al. 1997; Pogatzki and Raja 2003). Both types of responses are reduced by reference analgesics, but efficacies and potencies may differ within and between studies. For instance, morphine completely reversed thermal and mechanical hypersensitivity in mice at 10 mg/kg while a lower dose only affected the thermal response (Pogatzki and Raja 2003). A similar relationship was found in rats (Field et al. 1997) and in this study gabapentin and pregabalin yielded full analgesic efficacy on von Frey thresholds, but only partial effects on thermal readouts.

In another rat study using two different mechanical assays, COX inhibitors showed efficacy and potency slightly higher on thresholds measured by an electronic von Frey device than on paw pressure. An exception was indomethacin, which was remarkably effective in reducing responses to paw pressure (Whiteside et al. 2004). Even gabapentin, in relatively low doses, was efficacious on paw pressure but reversed the von Frey response by less than 20 % at a dose of 30 mg/kg. In this study, morphine was the only compound that completely normalized the response in both assays. A standard veterinary dose of the opioid buprenorphine showed full efficacy on mechanical sensitivity measured by paw pressure and von Frey stimulation, but the latter was sensitive to lower doses (Curtin et al. 2009).

The effects of COX inhibitors and opioids are less consistent in other types of rodent post-surgical pain. In dose response studies, the efficacy of COX inhibitors tends to reach a plateau short of full reversal of responses and readouts differ in sensitivity. For instance, in a mouse vasectomy study where several pain-related behaviors as well as corticosterone levels were quantified, meloxicam reduced surgery-induced behavior with no clear dose response relationship, indicating maximum efficacy even at the lower dose (5 mg/kg); however, the highest dose (20 mg/kg) reduced corticosterone levels, indicating an ameliorated stress response (Wright-Williams et al. 2007). Great individual and strain differences were noted both in pain behavior and corticosterone response. High doses of meloxicam and buprenorphine (20 and 5 mg/kg, respectively) were effective in another mouse study that assessed both general behavioral and grimacing (Leach et al. 2012).

In rats both pain-evoked and pain-suppressed behaviors after laparotomy were partially normalized after administration of meloxicam, carprofen, and ketoprofen (Roughan and Flecknell 2001; Roughan and Flecknell 2003). In young rats, daily administration of meloxicam (1 mg/kg) restored weight gain after thoracotomy without affecting this parameter in control animals (Brennan et al. 2009). In this study, buprenorphine had a negative effect on growth rate whether the rats had undergone surgery, general anesthesia only, or no treatment. This finding is not unique and underscores the importance of adequate controls to determine any confounding effects of test drugs. More advanced behavioral controls are possible

in dogs, e.g., after orthopedic surgery where standardized ethograms and pain scales have been developed for veterinary use (Rialland et al. 2012a).

The frequently observed nonanalgesic effects of opioids may interfere with pain assessment (Roughan and Flecknell 2002). A study in mice using an ethographic scoring system in combination with wheel-running and monitoring of food and water intake found no benefit of buprenorphine either alone or in combination with carprofen, presumably due to nonspecific interference of opioid effects with the scoring system (Adamson et al. 2010). However, in another study, repeated administration of buprenorphine or of an extended release formulation of oxymorphone after splenectomy significantly improved ethographic scores, indicating pain relief; oxymorphone also improved wheel-running performance and weight gain (Clark et al. 2004). Mild pain after surgery may in mice have less impact on spontaneous behaviors and appearance, even when changes in autonomic functions registered by telemetry as well as food intake and nest building may be evident and responsive to treatment by COX inhibitors (Arras et al. 2007).

Osteoarthritis. Osteoarthritis is a favored indication for clinical trials and there is a large number of published studies of COX inhibitors, opiates, and novel candidate drugs. In clinical practice, there appears to be little difference in analgesic efficacy between COX inhibitors in osteoarthritis pain (Conaghan 2012). A meta-analysis of responder rates found that 40–50 % of patients reported more than 50 % pain relief and 20–30 % more than 70 % after treatment with COX inhibitors; interestingly, the reduction in pain was only 8–15 mm on a 100 mm VAS (visual analog scale) compared to placebo (Moore et al. 2010). It is worth considering that a standard experimental animal study would not be powered to detect efficacies of this magnitude. Acetaminophen, often seen as first-line pharmacological therapy for OA, is considered less efficacious than nonsteroidal anti-inflammatory drugs (NSAIDS; Lee et al. 2004; Towheed et al. 2006), while opioids may be slightly more efficacious that NSAIDS, but with an unfavorable side effect profile (Nüesch et al. 2009).

Naturally occurring canine osteoarthritis deserves special attention as a possible alternative to rodent models of inflammatory pain, suggested to offer better prediction of clinical efficacy in man (Quessy 2010). Osteoarthritis is common in the dog, representing a significant clinical problem (Johnston 1997). Instruments for quantification of pain and disability are well developed, comprising both subjective rating scales for use by owners and veterinarians/researchers and objective methods for weight bearing, gait analysis, and motor activity (Rialland et al. 2012b). Although in many respects the dog would be closer to man in pathophysiology, species differences in efficacy and therapeutic window are likely. A trial cohort is likely to consist of individuals of different sex, age, strain, and body weight. All of these are factors that may interfere with disease progression, pharmacokinetics, and treatment effects. Depending on inclusion criteria, there may be variability in disease expression and comorbidity. The number and location of affected joints may differ, which could lead to interaction between the joint studied and other affected joints when using force plate and similar methods. The diversity in the population would reduce the likelihood of overestimating treatment

effects, but increases the risk of false negatives. From the practical point of view, the cost of conducting a trial, including synthesis of test compound in case of novel drugs, might be limiting, as may the availability of patients. From an ethical perspective, there is both the advantage of using subjects that may benefit from the scientific advances and the drawback that companion animals may be at risk of suffering side effects or sub-optimal treatment. Naturally occurring disease in pet animals can hardly replace rodent models but may be useful in later stage drug discovery programs and in exploratory work when acceptably safe and inexpensive test compounds are available. COX inhibitors and opioids are effective in dog osteoarthritis but efficacy depends on readout and there may be differences in the impression of efficacy between owners and investigators (Moreau et al. 2003; Vasseur et al. 1995). This is not necessarily negative since the assessment instruments, like different readouts in rodent models, would reflect different aspects of pain. Since some instruments incorporate subjective scales to be used by owners or therapists, placebo effects can be evident, however (Malek et al. 2012).

Inflammatory Pain. Rodent models of pain due to inflammation and arthritic disease are usually induced by chemical agents injected into a single joint or in the soft tissue of a paw. Depending on the nature and concentration of the induction agent, symptoms may last from several hours to several weeks, with significant differences in pathophysiology and pharmacology (Ängeby Möller et al. 2012; Ferland et al. 2011). Spontaneous and polyarthritic models may be used for pain studies but are more often applied to research on disease mechanisms (Ameye and Young 2006; Bendele et al. 1999). In the dog, surgically induced models as well as naturally occurring osteoarthritis have been used for pharmacological studies (Gregory et al. 2012).

In general, rodent models of paw inflammation and arthritis yield much greater analgesic drug efficacy than would be expected from human data, but both efficacy and potency varies. In plantar inflammation induced by FCA in rats, weight bearing and the response to pressure in a Randall-Selitto test were completely reversed by several COX inhibitors. By contrast, mechanical hypersensitivity measured by progressively increased punctate pressure to the paw was refractory (Huntjens et al. 2009). Several COX inhibitors showed full efficacy at clinically relevant plasma concentrations in a paradigm where gait analysis was used in rats with carrageenan-induced monoarthritis, while the same testing paradigm was much less sensitive when FCA was the induction agent, (Ängeby Möller et al. 2012; Ängeby Möller et al. unpublished observations).

In mice, neither analgesic drugs nor prednisolone, when given at doses that completely normalized the running wheel deficit caused by bilateral inflammation, changed mechanical hypersensitivity tested with von Frey filaments after unilateral FCA-induced paw inflammation (Cobos et al. 2012). In this study, however, the efficacy of morphine was unusually high, with an ED50 of less than 0.1 mg/kg. In another study, both rearing and locomotion deficits in carrageenan-induced bilateral paw inflammation was reduced or completely reversed by several COX inhibitors at doses that were devoid of effects on rotarod performance in controls but unfortunately, no control data were presented on spontaneous motor activity

(Cho et al. 2013). In contrast, morphine showed some efficacy in the same paradigm at 2 mg/kg, but performance deteriorated at 10 mg/kg, regardless of whether inflammation was present.

Buprenorphine reduced joint tenderness measured as the response to repetitive palpation and partially reversed the running wheel deficit in carrageenan-induced arthritis, whereas a high dose of morphine only reduced tenderness, possibly because the duration of effect was inadequate for the running wheel assay (Krug et al. 2009). In this study, treatment with botulinum toxin type A was effective on both readouts in the FCA model but not after carrageenan.

Neuropathic Pain. Overall, clinical pain of neuropathic origin responds poorly to treatment. In a comprehensive meta-analysis, the combined NNT values for polyneuropathies, the indications most frequently selected for clinical trials, varied from 2 to 3 for opioids and tricyclic antidepressants to 6.4 for gabapentin, while there was a span between 2.5 and 5 for postherpetic neuralgia (Finnerup et al. 2010). There was little evidence for efficacy of drugs other than opioids for pain due to nerve trauma. Furthermore, cancer and HIV chemotherapeutics of different types induce painful peripheral neuropathy by specific pathophysiological mechanisms, but are largely refractory to pharmacological mono-therapy with tricyclic antidepressants and anticonvulsants, including amitriptyline, gabapentin, and pregabalin (Kaley and Deangelis 2009; Phillips et al. 2010).

The more common neuropathic rodent models utilize traumatic injury of a peripheral nerve (Berge 2011, 2013). Consequently, they do not reflect the clinical trial scenario in terms of etiology, but are rather built on explicit or implicit assumption that the results can be generalized. There are, nevertheless, alternative models based on systemic neurotoxic effects of chemotoxic agents, emulating elements of diabetic neuropathy (Obrosova 2009), and neuropathy related to chemotherapy of cancer (Authier et al. 2009) and HIV (Dorsey et al. 2009; Joseph et al. 2004; Wallace et al. 2007b). It is important to keep in mind that different implementations exist and that the use of a particular agent does not guarantee identical pathology across laboratories and species. Although less common, models of postherpetic neuralgia (Fleetwood-Walker et al. 1999) and HIV (Wallace et al. 2007a) have been described.

Reference compounds show variable efficacy and potency in neuropathic pain models but as for other conditions, the data tend to be more optimistic than warranted by clinical reality (Berge 2011). This is not entirely consistent, however. In a review of data obtained in the spinal nerve ligation model, amitriptyline and duloxetine failed to show efficacy at clinically relevant exposures whereas the minimum effective doses for gabapentin and carbamazepine produced peak plasma exposures only 2–3 times higher than the maintenance doses in man (Whiteside et al. 2008). In the rat spared nerve injury model, chronic administration of pregabalin partially reduced hyperalgesia at clinically relevant plasma levels, whereas carbamazepine was ineffective even at doses several times higher than required for anticonvulsive effects (Lau et al. 2013). Whereas the Whiteside study used a conventional evoked readout, the Lau study used burrowing as well. The divergent

data on carbamazepine indicate that either models, readouts, or study designs are differentially sensitive to these drugs and their modes of action.

Drug Concentration. Ideally, pharmacological effect should be related to drug concentration in the target tissue, both to allow comparison of efficacy between studies but even more importantly, to establish that a drug is acting at a concentration compatible with its supposed mode of action. But to obtain such data is difficult and beyond the capacity of many laboratories. Plasma level is a reasonable compromise, but it should be appreciated that many compounds, including frequently used reference drugs like morphine, diclofenac, and gabapentin, have a delayed distribution from blood to target organ (Berge 2011; De Gregori et al. 2012; Torres-Lopez et al. 1997). In single administration studies, a drug with a short half-life and delayed distribution may be eliminated before the concentration in the target tissue reaches an effective level. Furthermore, the concentration in more accessible tissues may be sufficient to induce unpredicted effects, perhaps incorrectly interpreted as relevant efficacy or side effect. In repeated administration studies, the possibility for accumulation or induction of metabolism should be considered. The bottom line is that pharmacological data should be interpreted with extra caution when data on tissue or plasma concentration is lacking.

6 Modeling Strategies

The examples presented above are meant to illustrate the great heterogeneity between models and readouts in terms of pharmacological sensitivity. It is hardly possible to identify a paradigm that has general translational value on its own. This is commonly seen as a problem, but may in fact be an asset if used to select models optimal for the purpose of the research. A model may be used for screening on the assumption that the mechanism pursued will provide clinical analgesia if adequately addressed. This is a typical strategy in a drug discovery project at a stage where compounds are synthesized and optimized. This strategy is also useful in projects where clinically proven drugs are subject to reformulation and pro-drug approaches and the effect of the modifications needs to be compared to an existing drug. In these cases, it is important to establish values for potency, ideally indicating the exposure level needed for relevant target engagement in man. The primary requirement, however, is that the data allow drugs with the same mode of action to be compared. This is best served by a model that produces a full range of response, from no effect at a low dose to 100 % effect at the high end of the dose–response range. This is achievable such as for opioids in many acute models and for COX inhibitors in carrageenan-induced monoarthritis (Ängeby Möller et al. 2012; Le Bars et al. 2001). In these examples, the former would underestimate clinical potency, while the latter would provide a realistic estimate. Both models would overestimate efficacy in any clinical population (Fig. 1). The readout for screening should be pharmacodynamically linked to target engagement, but does not have to faithfully reflect a relevant pathophysiological mechanism. For

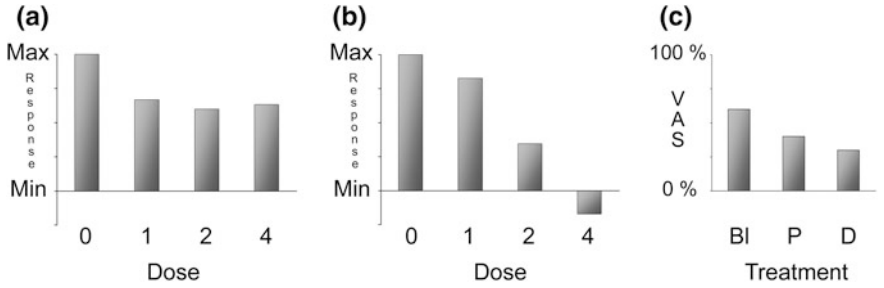

Fig. 1 A possible translational scenario. **a** Sign of efficacy in a model that incorporates some features of relevant pathophysiology. Data from several such models and different readouts would be combined with other relevant information to build understanding of and confidence in the concept. This level of modeling may not provide reliable dose–response relationships, however. **b** Optimization and selection of compounds is facilitated by a model with high sensitivity for the pursued mode of action or class of drug. **c** A successful clinical trial may demonstrate an advantage over placebo by 10–15 mm on a 100 mm VAS in terms of pain relief. *Bl* basal-level score prior to treatment, *P* Placebo, *D* Drug

example, in neurology and psychiatry research, various types of motor behavior driven by drug–target interaction but with no or little connection to the clinical symptomatology have successfully been used to develop effective therapies (Kaakkola and Teravainen 1990; Ögren et al. 1990). But the use of substitute endpoints does carry a risk of optimizing for an unwanted or irrelevant effect. A classical example would be respiratory depression, once suggested to be the most predictive modeling readout for opioid analgesia (Beecher 1957).

The use of models for screening can be validated with available ligands to the same molecular target. But when the mode of action is unprecedented, good pharmacological tools may be unavailable. Establishing the ability of the model to detect a relevant drug–target interaction would then be part of the model validation process, necessary for any novel target.

The aim of most studies in behavioral pharmacology is to predict clinical potential of novel drugs or pathophysiological mechanisms. This task is more diverse and challenging than screening, and the recent history is a mix of success and failure (Berge 2011). Correct predictions require integration of information from different sources. The simplest, but perhaps most challenging situation would be a black box project where a compound is active in some model, but with an unknown mode of action. A classic example is the inhibitory effect of gabapentin in neuropathic pain. To estimate the potential of such a compound, a possible approach would be to screen it in a battery of pharmacologically characterized models in order to establish a degree of efficacy on different sensory and behavioral parameters, including side effects, and use the information to formulate testable hypothesis concerning target indication. The test battery should include models representing different types of pathophysiology, functions, and modalities.

In a more typical situation, the molecular target is defined and to some extent validated. Ideally, there would be evidence from human studies to evaluate construct validity for both model and target. The development of analgesic therapies based on inhibition of nerve growth factor (NGF) may serve as an example. Before the publication of clinical data on analgesic efficacy in osteoarthritis (Lane et al. 2010), substantial evidence suggested a role for NGF in human nociception and joint pain. NGF and its receptor TrkA is upregulated in human joint tissue from patients with arthritis (Aloe et al. 1992) and injection of NGF causes hyperalgesia or pain in human experimental models (Dyck et al. 1997). There was also a large body of rodent data (McMahon 1996). A suitable model would incorporate some critical features of the clinical condition, e.g., upregulation of NFG in arthritic tissue and a readout reflecting movement-related pain. FCA-induced monoarthritis with gait analysis in the rat would fulfill these criteria and has been used to demonstrate convincing efficacy of an NGF antibody as well as other compounds acting on the same pathway (Ängeby Möller et al. unpublished observations). In this model, even the cyclooxygenase pathway is activated (Finn and Oerther 2010) but COX inhibitors are less efficacious.

7 Conclusions

The examples discussed in this text allow several conclusions. Pain in the clinic, when studied at a population level, is poorly treated with single drug therapies. This is clearly the case in major human indications and in veterinarian settings. Even in many experimental models, readouts vary in sensitivity to analgesics, and so the challenge is to understand how this reflects the perception and experience of the individual. Although high doses of drugs are frequently used, lack of sensitivity is not a universal problem of animal behavioral models. There are combinations of models and readout with excellent efficacy at clinically relevant drug exposure for some drug classes. However, sensitivity does not guarantee validity. In fact, good sensitivity to compounds like gabapentin or ibuprofen would indicate that the model is tuned toward a certain mechanism and may be useless for general prediction. The search for globally relevant models is probably futile—the focus should be on identifying the best models to address specific questions. Translation and prediction then have to be based on the weighted evidence derived from a variety of sources, behavioral pharmacology being just one, albeit important. Undoubtedly, many projects have been progressed into clinical development with poorly validated targets and on the assumption that the animal models were validated by their sensitivity to current analgesics. This is unfortunate, but there are excellent tools in the toolbox of behavioral pharmacology. With proper investment in the validation processes, there is reason to hope for better success rates in the future.

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Animal Models and Pharmacology of Herpetic and Postherpetic Pain

Yasushi Kuraishi and Atsushi Sasaki

Abstract Varicella-zoster virus (VZV) causes varicella upon primary infection and subsequently becomes latent in the sensory ganglia. Reactivation of latent VZV in the sensory ganglion results in herpes zoster, which usually begins with pain and dysesthesia. Pain that persists long after healing of the rash is termed postherpetic neuralgia. VZV inoculation into rats induces mechanical allodynia and thermal hyperalgesia without causing herpes zoster. As with VZV, herpes simplex virus 1 (HSV1) is an alphaherpesvirus. HSV1 also becomes latent in the sensory ganglia after primary infection, and reactivation of latent HSV1 in the sensory ganglion results in herpes simplex. HSV1 inoculation into mice causes zoster-like skin lesions together with mechanical allodynia and mechanical hyperalgesia. A marked difference between the two rodent models is whether the herpes virus proliferates in the nervous system after inoculation. VZV-inoculated rats are useful for investigating mechanical allodynia induced by latent infection with herpes virus. HSV1-inoculated mice are useful for investigating mechanical allodynia induced by the proliferation of herpes virus in sensory neurons and for assessing the effects of acute herpetic pain on the incidence of postherpetic allodynia.

Keywords Herpes zoster · Postherpetic neuralgia · Varicella-zoster virus · Herpes simplex virus 1 · Allodynia · Hyperalgesia · Hypoalgesia

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Contents

1 Introduction

Varicella-zoster virus (VZV, human herpesvirus 3) causes varicella (or chickenpox) upon primary infection and subsequently becomes latent in the sensory ganglia. Reactivation of latent VZV in the sensory ganglion results in herpes zoster (shingles), which is characterized by clustered vesicles with pain and dysesthesia (Dworkin and Portenoy 1996). The viruses replicate in the sensory ganglion and travel antidromically along primary afferents to the skin. Clustered vesicles spread over one dermatome or adjacent ones innervated by the affected nerve. The majority of patients with herpes zoster present with a prodrome of pain beginning several days before the vesicular eruption. Although herpes zoster usually resolves in 2–4 weeks, some patients experience pain and dysesthesia for a long time after healing of herpes zoster, which is termed postherpetic neuralgia (Dworkin and Portenoy 1996). VZV inoculation into rats induces mechanical allodynia and thermal hyperalgesia without causing herpes zoster.

As with VZV, herpes simplex virus 1 (HSV1, human herpesvirus 1) is an alphaherpesvirus. HSV1 also becomes latent in the sensory ganglia after primary infection, and reactivation of latent HSV1 in the sensory ganglion results in herpes simplex. Pain occurs before eruption appears (Layzer and Conant 1974), and some patients complain of pain even after vesicles disappear (Krohel et al. 1976; Gonzales 1992). These symptoms are similar to those of herpes zoster. HSV1 inoculation into mice causes zoster-like skin lesions together with mechanical allodynia and mechanical hyperalgesia. In this chapter, the characteristics of animal models of herpetic and postherpetic pain produced by VZV and HSV1 inoculation are described.

2 Behavioral and Electrophysiological Responses

2.1 VZV

VZV-infected cells are injected to cause latent VZV infection in animals. In rats, VZV inoculation into the hind paw induces static (punctate) allodynia and thermal hyperalgesia in the inoculated paw without causing herpes zoster (Fleetwood-Walker et al. 1999; Garry et al. 2005). Dynamic allodynia (pain in response to light stroking stimulation) is also induced with slower onset; the onset of static and dynamic allodynia is 6–7 and 14 days post-inoculation, respectively (Fleetwood-Walker et al. 1999; Hasnie et al. 2007). The responses of wide dynamic range neurons in the lumbar dorsal horn to brush, pressure, and pinch stimuli are increased in rats inoculated with VZV (Zhang et al. 2011).

RNAs for VZV genes can be detected in the dorsal root ganglia (DRGs) 1 week post-inoculation, and VZV DNA is observed in 5–10 % of the DRG neurons (Kennedy et al. 2001). Thus, it is possible that VZV infection of the DRGs is an underlying cause of allodynia. VZV-induced static allodynia is not inhibited by repeated administration of the antiviral agent valaciclovir (Dalziel et al. 2004; Zhang et al. 2011), suggesting that static allodynia in this model is not a result of active viral replication.

2.2 HSV1

In contrast to VZV inoculation into rats, percutaneous inoculation of HSV1 into mice induces zoster-like skin lesions in the inoculated dermatome (Fig. 1). When HSV1 is inoculated topically on the hind limb (the shin or femur), a few vesicles erupt on the animal's back on day 5 post-inoculation, zoster-like skin lesions are observed in the inoculated dermatome on day 6–10 post-inoculation, and skin lesions almost subside by day 20 (Fig. 1) (Takasaki et al. 2000a; Sasaki et al. 2008a). HSV1 DNA can be detected at low-levels in the lumbar DRGs on day 2–3 post-inoculation, markedly increases on day 4–7, and then rapidly decreases (Takasaki et al. 2000a). HSV1 replicates in 20–30 % of DRG neurons on around day 5 post-inoculation (Takasaki et al. 2000b; Sasaki et al. 2013a).

HSV1 inoculation induces mechanical allodynia and mechanical hyperalgesia in the affected hind paw in mice (Takasaki et al. 2002; Sasaki et al. 2008a). Licking of the lesioned skin, an index of superficial pain, is observed during the herpetic stage (Takasaki et al. 2000a; Sasaki et al. 2013b). Mice may also feel itchy at the herpetic stage, since they scratch the lesioned skin with their hind paw (Sasaki et al. 2013b). Licking and scratching of the affected skin are not observed after healing of the skin lesions (unpublished observation), suggesting that superficial pain and itch have subsided. These findings do not exclude the possibility that the mice experience spontaneous deep pain at the postherpetic stage.

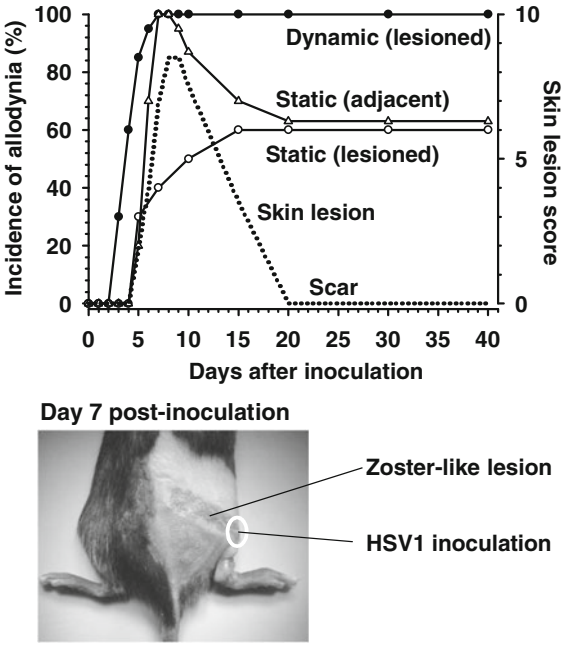

Fig. 1 Schematic representation of the time course of allodynia and skin lesion following herpes simplex virus 1 inoculation in mice. Dynamic (lesioned), dynamic allodynia in the lesioned dermatome; static (lesioned), static allodynia in the lesioned dermatome; static (adjacent), static allodynia in the dermatome adjacent to the skin lesion

Thermal hyperalgesia is not observed (Sasaki et al. 2003), but instead thermal hypoalgesia is induced at the postherpetic stage (Sasaki et al. 2013a). Dynamic allodynia becomes apparent before vesicular eruption and reaches a plateau when the rash spreads throughout the dermatome; the incidence of dynamic allodynia is higher than that of static allodynia in the lesioned dermatome (Fig. 1) (Sasaki et al. 2008a). Static allodynia becomes apparent on the day when vesicles erupt, and then it increases rapidly in the dermatome adjacent to the lesioned region and slowly in the lesioned dermatome; the incidence of static allodynia is higher in the dermatome adjacent to the lesioned region relative to the lesioned dermatome (Fig. 1) (Takasaki et al. 2000a, 2002; Sasaki et al. 2008a). In some mice, static allodynia in the dermatome adjacent to the lesioned skin resolves as skin lesions heal. In other mice, static allodynia is not relieved until at least day 40 post-inoculation (Takasaki et al. 2002).

At the herpetic stage (day 7 post-inoculation), the responses of the tibial nerve (which innervates the lesioned skin) to brush, pressure (punctate), and pinch stimuli are reduced in HSV1-inoculated mice (Nishikawa et al. 2009). The spontaneous activity of the tibial nerve is not different between naïve and herpetic mice (Nishikawa et al. 2009). However, the response of wide dynamic range neurons in the lumbar dorsal horn to brush stimulation is markedly increased in herpetic mice (Nishikawa et al. 2009). In contrast, their response to noxious pinch stimulation is markedly reduced in herpetic mice, and the responses to punctate stimuli are not different between naïve and herpetic mice (Nishikawa et al. 2009). These findings suggest that dynamic allodynia in the affected dermatome is due to

the increased excitability of wide dynamic range neurons, but not primary afferents, to brush stimulation.

When started on day 2 post-inoculation, repeated oral treatment with the antiviral agent acyclovir prevents mechanical allodynia and inhibits the development of skin lesions and HSV1 replication in the DRGs (Takasaki et al. 2000a). In contrast, when started on day 5, acyclovir treatment slightly inhibits skin lesions and viral proliferation, but not mechanical allodynia (Takasaki et al. 2000a). Thus, it is suggested that mechanical allodynia is a result of active HSV1 replication. After proliferation in the DRG neurons, HSV1 travels along the primary afferents to the skin and dorsal horn (Takasaki et al. 2000a; Sasaki et al. 2007), causing immune responses in these regions (Sato-Takeda et al. 2006; Takasaki et al. 2012). These immune responses may enhance HSV1-induced mechanical allodynia.

In contrast to mice, HSV1 inoculation does not induce zoster-like skin lesions in rats (Andoh et al. 1995), although HSV1 DNA and HSV1 antigen are detected in the DRGs (Andoh et al. 1995; Shiraki et al. 1998). HSV1 inoculation into the rat hind paw causes static allodynia on day 2–5 post-inoculation (Dalziel et al. 2004) and mechanical hypoalgesia from day 4 to at least day 10 post-inoculation (Andoh et al. 1995). Dynamic allodynia is not observed (Andoh et al. 1995). HSV1 antigen is observed in about 70 % of neurons in the DRGs on day 10 post-inoculation (Shiraki et al. 1998). When started before inoculation, repeated topical application of acyclovir on the inoculation site prevents mechanical hypoalgesia and markedly decreases the number of HSV1 antigen-positive neurons (Shiraki et al. 1998). Infection of cultured rat DRG neurons with nonsyncytial strains of HSV1 reduces evoked excitability, while infection with syncytial strains of HSV1 induces spontaneous activity (Mayer et al. 1985, 1986). Viral infection of most of the sensory neurons may be causative of mechanical hypoalgesia in rats.

3 Neurohistology

Activating transcription factor-3, an axonal injury marker, is induced in the affected DRGs at the herpetic stage in HSV1-inoculated mice, and then subsides before the postherpetic stage (Unezaki et al. 2012). At the postherpetic stage, calcitonin gene-related peptide immunoreactivity (a sensory C-fiber neuron marker) is markedly reduced in the scarred skin. Similarly, peripherin immunoreactivity (another sensory C-fiber neuron marker) is decreased in the DRGs, suggesting damage to C-fiber neurons and their axons (Sasaki et al. 2013a). In contrast, there are no significant decreases in immunoreactivity for neurofilament 200 (an A-fiber neuron marker) in the scarred skin and affected DRGs (Sasaki et al. 2013a), suggesting negligible damage to sensory A-fiber neurons and their axons. Postherpetic dynamic allodynia may therefore be associated with injury to sensory C-fiber neurons and little damage to A-fiber neurons (Sasaki et al. 2013a). In VZV-inoculated rats, activating transcription factor-3 is induced in the DRG

3 weeks after inoculation (Garry et al. 2005). Thus, nerve injury may occur, but there are no reports of neurohistological changes in VZV-inoculated rats.

HSV1 antigen is observed in both peripherin- and neurofilament-200-positive DRG neurons on day 5 post-inoculation, the peak time of virus replication in the DRG (Takasaki et al. 2000a, b). Therefore, selective injury to sensory C-fiber neurons cannot be explained by selective infection of A- or C-type neurons. HSV1 is capable of both inducing and inhibiting apoptosis in infected cells (Galvan and Roizman 1998; Aubert and Blaho 1999). One viral factor that has been implicated in inhibiting apoptosis is latency-associated transcript. Although latency-associated transcript is an RNA produced during latent infection, its expression occurs during the acute infection (Margolis et al. 1992). The anti-apoptotic qualities of latency-associated transcript have been documented in vitro and in vivo (Ahmed et al. 2002; Branco and Fraser 2005). In mice inoculated with HSV1 in the footpad, latency-associated transcript expression is primarily localized in DRG neurons positive for stage-specific embryonic antigen 3 (Margolis et al. 1992). Stage-specific embryonic antigen 3 is present in medium- and large-sized DRG neurons, which do not contain neuropeptides and predominantly terminate in laminae III and IV of the dorsal horn (Dodd et al. 1984), suggesting that they are A-fiber neurons. Therefore, differential regulation of apoptosis and anti-apoptosis genes between sensory C- and A-fiber neurons may be responsible for the selective injury to sensory C-fiber neurons in postherpetic mice.

After healing of herpes zoster, epidermal nerve density in the affected skin is lower in patients with postherpetic neuralgia than in patients without pain (Rowbotham et al. 1996; Oaklander 2001). Impairment of C-fiber function is correlated with dynamic allodynia in the affected skin in patients with postherpetic neuralgia (Baron and Saguer 1993). Consistent with the symptoms of postherpetic neuralgia, sensitivity to heat and capsaicin sensitivities is in the scarred skin of postherpetic mice (Sasaki et al. 2013a), which may be due to reduced cutaneous innervation of sensory C-fibers (Dai et al. 2002). The intensity of dynamic allodynia is inversely correlated with heat and capsaicin sensitivity, suggesting that injury to sensory C-fiber neurons is responsible for the development of postherpetic dynamic allodynia.

As mentioned above, static allodynia in the dermatome adjacent to the lesioned skin resolves as skin lesions heal in some, but not all, HSV1-inoculated mice. The mean number of intraepidermal nerve fibers in the scarred skin decreases in both groups (Inomata et al. 2013). In the dermis of the scarred skin, the mean numbers of nerve fibers were significantly decreased in mice with static allodynia at the postherpetic stage, but not in mice without static allodynia (Inomata et al. 2013). It is suggested that the severity of dermal denervation in the scarred skin is associated with the development of static allodynia that extends beyond the margins of the initial rash area.

Table 1 Pharmacological effects on mechanical allodynia in HSV1-inoculated mice

Agents	Doses	Route	Herpetic allodynia		Postherpetic allodynia		References
			Static	Dynamic	Static	Dynamic	
Cyclooxygenase inhibitor							Takasaki et al. 2000b, 2002, 2005
Diclofenac	10–100 mg/kg	i.p.	++	–	–	–	Sasaki et al. 2008a
NS-398	5 and 10 mg/kg	i.p.	++	nr	–	nr	
JTE-522	1–10 mg/kg	p.o.	++	nr	nr	nr	
Opioid receptor agonist							Takasaki et al. 2000b, 2002, 2006
Morphine	1–5 mg/kg	s.c.	++	nr	+	nr	
Morphine	0.3–3.0 nmol	i.t.	++	nr	+	nr	
Morphine	0.03–0.3 nmol	i.c.v.	++	nr	++	nr	
Nalfurafine	0.001–0.1 mg/kg	p.o.	++	nr	++	nr	
Anticonvulsant							Takasaki et al. 2000b, 2001, 2002
Gabapentin	10–100 mg/kg	p.o.	++	++	+++	+	
Gabapentin	10–100 μg	i.t.	++	nr	nr	nr	Sasaki et al. 2008a
Gabapentin	10–100 μg	i.c.v.	–	nr	nr	nr	Kuraishi et al. 2004
Gabapentin	10–100 μg	i.pl.	–	nr	nr	nr	
Antidepressant							Takasaki et al. 2002
Amitriptyline	3 and 10 mg/kg	i.p.	–	nr	–	nr	Kagaya et al. 2004
Amitriptyline (repeated)	1–10 mg/kg	s.c.	++	nr	nr	nr	Kuraishi et al. 2004
NMDA receptor antagonist							Sasaki et al. 2008a, b
Ketamine	25 and 50 mg/kg	i.p.	nr	–	+	–	
Sodium channel blocker							Takasaki et al. 2002
Mexiletine	30 mg/kg	p.o.	nr	–	+	–	Sasaki et al. 2008a
NOS inhibitor							Sasaki et al. 2007
7-Nitroindazole	3 and 10 mg/kg	i.p.	–	nr	+	nr	
S-Methylisothiourea	3 and 10 mg/kg	i.p.	+	nr	–	nr	
GlyT inhibitor							Nishikawa et al. 2010
ALX1393	1–5 μg	i.t.	nr	+++	+++	+++	
Sarcosine	10 and 30 μg	i.t.	nr	–	–	–	
Adrenoceptor agonist							Sasaki et al. 2003
Clonidine	0.03–0.3 mg/kg	i.p.	+++	nr	nr	nr	
Clonidine	0.03–0.3 μg	i.t.	+++	nr	nr	nr	
Clonidine	0.03–0.3 μg	i.c.v.	–	nr	nr	nr	

–, no effect; +, moderate inhibition; ++, strong inhibition; +++, strong, and long-lasting inhibition
GlyT, glycine transporter; NMDA, N-methyl-D-aspartate; NOS, nitric oxide synthase; nr, no reports; SMT, S-methylisothiourea; i.c.v., intracerebroventricular; i.p., intraperitoneal; i.pl., intraplantar; i.t., intrathecal; p.o., per os; s.c., subcutaneous

4 Pharmacology

The main pharmacological studies that describe drug effects on static and dynamic allodynia at herpetic and postherpetic stages in HSV1-inoculated mice are summarized in Table 1. The main endogenous factors that control static allodynia in the herpetic and postherpetic stages are shown in Fig. 2. The main pharmacological studies that describe drug effects on static allodynia and thermal hyperalgesia in VZV-inoculated rats are summarized in Table 2.

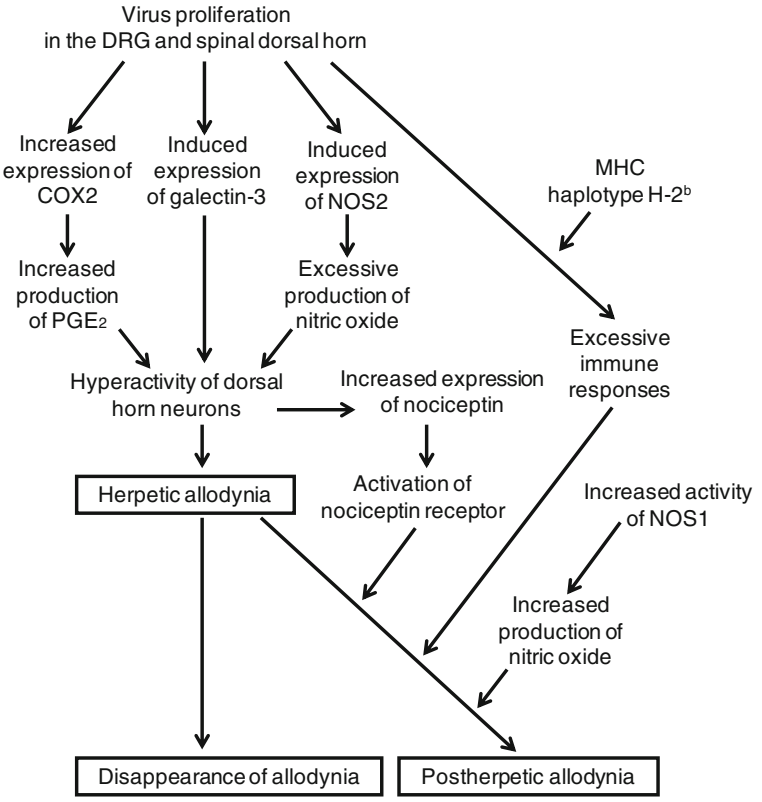

Fig. 2 Schematic presentation of endogenous factors affecting static allodynia at the herpetic and postherpetic stages in mice inoculated with herpes simplex virus 1. COX, cyclooxygenase; DRG, dorsal root ganglion; MHC, major histocompatibility complex; NOS, nitric oxide synthase; PGE_2, prostaglandin E_2

4.1 Static Allodynia

4.1.1 Cyclooxygenase Inhibitors

The nonselective cyclooxygenase (COX) inhibitor diclofenac and the selective COX-2 inhibitors NS-398 and JTE-522 reduce static allodynia at the herpetic stage in HSV1-inoculated mice, but diclofenac and NS-398 do not inhibit static allodynia at the postherpetic stage (Takasaki et al. 2000b, 2002, 2005). COX-2 is induced and the content of prostaglandin E_2 (PGE_2) is increased in the affected DRG at the herpetic stage (Takasaki et al. 2005), with COX-2-like immunoreactivities being localized around the nuclear membrane of DRG neurons (Takasaki et al. 2005). COX-2 mRNA expression and PGE_2 content in the DRG at the postherpetic stage are similar to those of naïve mice (Takasaki et al. 2005). These

Table 2 Pharmacological effects on mechanical allodynia and thermal hyperalgesia in VZV-inoculated rats

Agents	Doses	Route	Static allodynia	Thermal hyperalgesia	References
Cyclooxygenase inhibitor					Garry et al. 2005
Diclofenac	100 mg/kg	p.o.	–	–	Hasnie et al. 2007
Ibuprofen	20 mg/kg	i.p.	++	nr	
Opioid receptor agonist					Garry et al. 2005
Morphine	2.5 mg/kg	i.p.	++	nr	
DAMGO	1 μmol	i.t.	–	–	
Anticonvulsant					Garry et al. 2005
Gabapentin	100 mg/kg	p.o.	+++	++	Hasnie et al. 2007
Gabapentin	30 mg/kg	i.p.	++	nr	
Antidepressant					Hasnie et al. 2007
Amitriptyline	10 mg/kg	i.p.	++	nr	
NMDA receptor antagonist					Garry et al. 2005
AP5	40 pmol	i.t.	++	nr	Zhang et al. 2011
MK-801	100 pmol	i.t.	++	nr	
(R)-CPP	2.5 nmol	i.t.	++	++	
Sodium channel blocker					Garry et al. 2005
Mexiletine	100 mg/kg	p.o.	++	++	
Lamotrigene	100 mg/kg	p.o.	++	++	
NO scavenger					Zhang et al. 2011
PTIO	30 μg	i.t.	++	nr	
NOS inhibitor					Zhang et al. 2011
7-nitroindazole	20 μg	i.t.	–	nr	
L-NIL	1.1 μmol	i.t.	++	nr	

–, no effect; +, moderate inhibition; ++, strong inhibition; +++, strong and long-lasting inhibition AP5, (2R)-amino-5-aminophosphonovaleric acid; DAMGO, [D-Ala^2, MePhe^4, Gly-ol]-enkephalin; L-NIL, L-N6-(I-iminoethyl)-lysine hydrochloride; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; PTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; (R)-CPP, 3-((R)-2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid; i.p., intraperitoneal; i.t., intrathecal; p.o., per os

findings suggest that PGE_2 production by COX-2 induction in the DRG contributes to herpetic allodynia, but not postherpetic allodynia.

In VZV-inoculated rats, systemic administration of ibuprofen attenuates static allodynia (Hasnie et al. 2007). However, diclofenac inhibits neither static allodynia nor thermal hyperalgesia (Garry et al. 2005).

4.1.2 Opioid Receptor Agonists

In humans, morphine is inadequate for the management of pain in patients with postherpetic neuralgia after intravenous and epidural injections (Eide et al. 1994; Watt et al. 1996). Morphine inhibits static allodynia at the herpetic and

postherpetic stages in HSV1-inoculated mice (Takasaki et al. 2002). However, the antiallodynic effect of morphine is reduced at the postherpetic stage; a subcutaneous dose of 5 mg/kg almost completely inhibits herpetic allodynia, but only partially suppresses postherpetic allodynia (Takasaki et al. 2002). The anti-allodynic effect of intrathecal morphine is reduced at the postherpetic stage, although the anti-allodynic effect of intracerebroventricular morphine is similar between the herpetic and postherpetic stages (Takasaki et al. 2006). μ-Opioid receptor-like immunoreactivities in the lumbar dorsal horn are markedly decreased at the postherpetic, but not herpetic, stage (Takasaki et al. 2006). The expression level of μ-opioid receptor mRNA is significantly decreased in the affected DRGs at the postherpetic stage (Takasaki et al. 2006). Specific down-regulation of the μ-opioid receptor in the primary sensory neurons is suggested to be responsible for the reduced anti-allodynic action of morphine at the postherpetic stage. In contrast to μ-opioid receptor, the expression level of κ-opioid receptor mRNA is not decreased in the affected DRGs at the postherpetic stage compared to that at the herpetic stage (Takasaki et al. 2006). The κ-opioid receptor agonist nalfurafine suppresses herpetic and postherpetic allodynia to similar degrees (Takasaki et al. 2006). These findings suggest that the κ-opioid receptor is a potential target for the analgesic treatment of postherpetic neuralgia.

In VZV-inoculated rats, intrathecal administration of the highly selective μ-opioid receptor agonist [D-Ala^2, MePhe^4, Gly-ol]-enkephalin inhibits neither static allodynia nor thermal hyperalgesia (Garry et al. 2005), although systemic administration of morphine inhibits static allodynia (Hasnie et al. 2007).

4.1.3 Gabapentin

Although relatively high doses (900–3,600 mg) are needed, gabapentin is effective against clinical postherpetic neuralgia without producing severe adverse effects (Rowbotham et al. 1998). Oral administration of gabapentin inhibits static allodynia at the herpetic and postherpetic stages in HSV1-inoculated mice (Takasaki et al. 2001, 2002). Gabapentin at an oral dose of 30 mg/kg almost completely inhibits postherpetic allodynia, but it only partially suppresses herpetic allodynia (Takasaki et al. 2001, 2002). Intrathecal administration of gabapentin also attenuates static allodynia, whereas intraplantar, intracisternal, and intracerebroventricular administration of gabapentin has no effect (Takasaki et al. 2001).

Systemic administration of gabapentin inhibits static allodynia and thermal hyperalgesia in VZV-inoculated rats (Garry et al. 2005; Hasnie et al. 2007). The expression of the $\alpha_2\delta$-1 subunit of voltage-dependent calcium channels, the most likely molecular target of gabapentin, is upregulated in the DRG of VZV-inoculated rats (Garry et al. 2005). Alterations in the expression of the $\alpha_2\delta$-1 subunit in the primary sensory neurons may be associated with differences in the anti-allodynic potency of gabapentin between the herpetic and postherpetic stages.

4.1.4 Tricyclic Antidepressants

Tricyclic antidepressants such as amitriptyline are used in the first-line pharmacological treatment of postherpetic neuralgia (Hempenstall et al. 2005). Although a single administration of amitriptyline (3 and 10 mg/kg, intraperitoneal) does not change static allodynia at the herpetic or postherpetic stage in HSV1-inoculated mice (Takasaki et al. 2002; Kagaya et al. 2004), repeated administration of amitriptyline at a dose of 10 mg/kg for 3 days gradually attenuates herpetic allodynia (Kuraishi et al. 2004). In contrast, static allodynia in VZV-inoculated rats is inhibited by a single intraperitoneal administration of amitriptyline (10 mg/kg) (Hasnie et al. 2007). Acute anti-allodynic activity of tricyclic antidepressants may be mediated mainly by the inhibition of the reuptake of noradrenaline and/or serotonin into axon terminals of the descending monoaminergic pain inhibiting systems. Therefore, it is conceivable that the lack of acute anti-allodynic activity of amitriptyline in HSV1-inoculated mice is due to hypofunction of the descending monoaminergic systems. In this context, HSV1 replicates in the dorsal horn after percutaneous inoculation in mice (Sasaki et al. 2007), which may cause hypofunction of the terminals of the descending monoaminergic systems in the dorsal horn.

4.1.5 Glutamate Receptor Antagonists

Systemic administration of ketamine, a noncompetitive NMDA glutamate receptor antagonist, inhibits static allodynia at the postherpetic stage in HSV1-inoculated mice (Sasaki et al. 2008a). In VZV-inoculated rats, intrathecal injections of the NMDA receptor antagonists (2R)-amino-5-aminophosphonovaleric acid (Zhang et al. 2011), MK-801 (Zhang et al. 2011), and 3-((R)-2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid attenuate static allodynia (Garry et al. 2005).

4.1.6 Sodium Channel Blockers

Systemic administration of the sodium channel blocker mexiletine inhibits static allodynia at the postherpetic stage in HSV1-inoculated mice (Takasaki et al. 2002; Sasaki et al. 2008a). In VZV-inoculated rats, static allodynia and thermal hyperalgesia are inhibited by both mexiletine and another sodium channel blocker lamotrigine (Garry et al. 2005). The tetrodotoxin-sensitive sodium ion channel $Na_v1.3$ and the tetrodotoxin-resistant $Na_v1.8$ are upregulated in the DRG of VZV-inoculated rats (Garry et al. 2005). VZV infection of a rat DRG × mouse neuroblastoma hybrid cell line results in the increased gene expression of $Na_v1.8$ and tetrodotoxin-sensitive $Na_v1.6$ and $Na_v1.7$ (Kennedy et al. 2013).

4.1.7 Nitric Oxide Synthase Inhibitors

Nitric oxide (NO) is typically produced by nitric oxide synthase-1 (NOS1, neuronal NOS) in neurons (Millan 1999), while viral infection induces NOS2 (inducible NOS) expression mainly in macrophages (Fujii et al. 1999). Static allodynia at the herpetic stage in HSV1-inoculated mice is reduced by the selective NOS2 inhibitor S-methylisothiourea, but not by the selective NOS1 inhibitor 7-nitroindazole (Sasaki et al. 2007). In contrast, static allodynia at the postherpetic stage is reduced by 7-nitroindazole, but not S-methylisothiourea (Sasaki et al. 2007). NOS2 is markedly induced in the lumbar dorsal horn at the herpetic, but not postherpetic, stage and its expression is observed around HSV1 antigen-immunoreactive cells (Sasaki et al. 2007), suggesting that virus proliferation is responsible for NOS2 expression. Although the expression level of NOS1 is relatively constant at the herpetic and postherpetic stages, the activity of reduced nicotinamide adenine dinucleotide phosphate diaphorase, an index of NOS1 activity, increases in the laminae I and II of the lumbar dorsal horn of mice with postherpetic allodynia, but not in mice without postherpetic allodynia (Sasaki et al. 2007). Thus, it is suggested that herpetic and postherpetic allodynia are mediated by NO in the dorsal horn and that NOS2 and NOS1 are responsible for herpetic and postherpetic allodynia, respectively.

In VZV-inoculated rats, static allodynia is inhibited by intrathecal injections of the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide and the selective NOS2 inhibitor L-N6-(I-iminoethyl)-lysine hydrochloride, but not by an intrathecal injection of the NOS1 inhibitor 7-nitroindazole (Zhang et al. 2011). NOS2 expression is increased in the DRG and spinal cord by VZV inoculation and is localized in neurons but not in astrocytes and microglial cells (Zhang et al. 2011). VZV inoculation does not affect NOS1 expression in these regions (Zhang et al. 2011).

4.1.8 Glycine Transporter Inhibitors

Glycine is an inhibitory neurotransmitter in the spinal dorsal horn and its extracellular concentration is regulated by glial glycine transporter (GlyT) 1 and neuronal GlyT2 (Aragón and López-Corceura 2003). An intrathecal injection of the GlyT2 inhibitor ALX1393, but not the GlyT1 inhibitor sarcosine, markedly suppresses static allodynia at the herpetic and postherpetic stages in HSV1-inoculated mice (Nishikawa et al. 2010). Pharmacological blockade of GlyT2 in the spinal dorsal horn is a potential therapeutic strategy for postherpetic neuralgia.

4.1.9 Adrenergic Agents

At doses that inhibit static allodynia induced by partial sciatic nerve ligation in mice, the adrenergic neuron blocking agent guanethidine and the nonselective

α-adrenoceptor antagonist phentolamine do not affect static allodynia at the herpetic stage in HSV1-inoculated mice (Sasaki et al. 2003). Systemic administration of the α_2-adrenoceptor agonist clonidine inhibits static allodynia at the herpetic stage in HSV1-inoculated mice, which is reversed by intrathecal injections of phentolamine and the α_2-adrenoceptor antagonist yohimbine (Sasaki et al. 2003), suggesting that clonidine inhibits herpetic allodynia through the spinal action. These findings, taken together, suggest that sympathetic nerves and α-adrenoceptors are not involved in herpetic allodynia.

4.1.10 Galectin-3

Galectin-3 is a member of the animal *β*-galactoside-binding lectin family. It is secreted from monocytes/macrophages and a variety of epithelial cells (Barondes et al. 1994). HSV1 inoculation induces galectin-3 in the dorsal horn at the herpetic stage. Galectin-3 is localized in cells positive for the macrophage/microglia marker Iba-1 in the superficial dorsal horn (Takasaki et al. 2012). Deficiency in galectin-3 markedly reduces static allodynia at the herpetic stage in HSV1-inoculated mice, without effects on zoster-like skin lesions (Takasaki et al. 2012). Intrathecal injections of anti-galectin-3 antibodies also reduce static allodynia at the herpetic stage and an intrathecal injection of galectin-3 causes static allodynia in naïve mice (Takasaki et al. 2012). These findings suggest that galectin-3 in infiltrating macrophages and/or resident microglia in the spinal dorsal horn contributes to static allodynia at the herpetic stage.

4.2 Pharmacology of Dynamic Allodynia and Spontaneous Pain

In many patients with herpes zoster and postherpetic neuralgia, dynamic allodynia is a pathognomonic symptom and can dramatically decrease their quality of life. Dynamic allodynia at the herpetic and postherpetic stages is not inhibited by diclofenac in HSV1-inoculated mice (Sasaki et al. 2008a), although diclofenac inhibits static allodynia at the herpetic stage (Takasaki et al. 2000b, 2005). Mexiletine and ketamine also do not inhibit dynamic allodynia at the herpetic and postherpetic stages, although they inhibit static allodynia (Sasaki et al. 2008a). Gabapentin (30 mg/kg) inhibits dynamic allodynia at the herpetic and postherpetic stages (Sasaki et al. 2008a), but the inhibition is more marked in static allodynia than in dynamic allodynia (Sasaki et al. 2008a; Takasaki et al. 2001, 2002). Pregabalin (10 mg/kg) inhibits dynamic allodynia at the herpetic and postherpetic stages; the degree of inhibition is similar between these two stages, but the duration is longer at the postherpetic stage than at the herpetic stage (unpublished observation). Similar to the effects on static allodynia, an intrathecal injection of

ALX1393, but not sarcosine, suppresses dynamic allodynia in mice at the herpetic and postherpetic stages (Nishikawa et al. 2010). Intrathecal ALX1393 also inhibits dynamic allodynia induced by intrathecal injections of strychnine and NMDA (Nishikawa et al. 2010). These findings suggest that dynamic allodynia is more resistant to the analgesics and analgesic adjuvants than is static allodynia, and that blockade of GlyT2 in the spinal dorsal horn is a potential therapeutic strategy for dynamic allodynia in patients with postherpetic neuralgia.

HSV1 inoculation induces licking (pain-related behavior) and scratching (itch-related behavior) of the lesioned skin at the herpetic stage (Sasaki et al. 2013b). Hydrogen peroxide is increased in the lesioned skin, and herpetic licking and scratching behaviors are suppressed by the antioxidant *N*-acetyl-L-cysteine and the TRPA1 channel antagonist HC-030031 at doses that inhibit licking and scratching induced by an intradermal injection of hydrogen peroxide (Adhikari et al. 2013).

5 Factors Affecting the Incidence of Postherpetic Pain

A unique feature of HSV1-inoculated mice is that static allodynia in the dermatome adjacent to the lesioned skin decreases as skin lesions heal in only about half of mice. In the other half, allodynia lasts long after healing of the lesions (Takasaki et al. 2002; Inomata et al. 2013). Since these studies used inbred strains of mice, this result suggests that acquired factors are involved in the development of postherpetic allodynia. In this context, the intensity of mechanical hyperalgesia at the acute herpetic stage is higher in mice that have postherpetic allodynia than in mice that do not have postherpetic allodynia (Takasaki et al. 2002). Repeated administration of gabapentin or amitriptyline during the herpetic stage produces a marked reduction in the incidence of postherpetic allodynia (Kuraishi et al. 2004). Deficiency in the EP_3 prostanoid receptor that markedly diminishes the herpetic allodynia and decreases the incidence of postherpetic allodynia (Takasaki et al. 2005). These findings support the idea that severe herpetic pain is a risk factor for the development of postherpetic neuralgia.

Although nociceptin-receptor deficiency does not affect the development of skin lesions and herpetic allodynia, it prevents postherpetic allodynia (Sasaki et al. 2008b). Pronociceptin mRNA increases in the dorsal horn of the lumbar enlargement on day 6 post-inoculation, and this increase is inhibited by repeated administration of gabapentin (Sasaki et al. 2008b). Thus, the spinal nociceptin system may contribute to the mechanisms involved in the transition from herpetic allodynia to postherpetic allodynia.

Phosphorylation of the NR2B subunit of the NMDA receptor at Tyr^{1472} in the dorsal horn is suggested to be essential for the development of nerve ligation-induced pain behaviors (Abe et al. 2005). In HSV1-inoculated mice, a mutation of Tyr^{1472} of the NR2B subunit to Phe, which blocks the phosphorylation of the corresponding amino acid residue, reduces the intensity and incidence of postherpetic allodynia, without affecting the herpetic allodynia, (Unezaki et al. 2012).

Mutation of Tyr^{1472} suppresses the induction of activating transcription factor-3 in the DRGs (Unezaki et al. 2012), which may be associated with the reduction of damage to the axons of infected sensory neurons.

Human histocompatibility leukocyte antigen affects the development of postherpetic neuralgia, without affecting the onset of herpes zoster (Sato-Takeda et al. 2004). In animals, HSV1 inoculation induces static allodynia in the dermatome adjacent to the lesioned skin in all mice of C57BL/6 and BALB/c strains, whereas the incidence of postherpetic allodynia is higher in C57BL/6 mice that have a major histocompatibility complex of $H\text{-}2^b$ than in BALB/c mice that have a major histocompatibility complex of $H\text{-}2^d$ (Takasaki et al. 2005; Sato-Takeda et al. 2006). In addition, the incidence of postherpetic allodynia of BALB/b mice, a congenic BALB/c strain with $H\text{-}2^b$, is similar to that of C57BL/6 mice (Sato-Takeda et al. 2006). These findings suggest that T leukocytes play a role in the increase of the incidence of postherpetic allodynia. Endogenous factors affecting the incidence of postherpetic allodynia are shown in Fig. 2.

6 Conclusion

There are two animal models of postherpetic neuralgia, VZV-inoculated rats and HSV1-inoculated mice. A marked difference between these two rodent models is whether the herpes virus proliferates in the nervous systems after inoculation. VZV-inoculated rats are useful for investigating mechanical allodynia induced by latent infection with herpes virus. HSV1-inoculated mice are useful for investigating: (1) mechanical allodynia induced by proliferation of herpes virus in sensory neurons; and (2) the effects of acute herpetic pain on the incidence of postherpetic allodynia.

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Mechanisms and Pharmacology of Neuropathic Pain in Multiple Sclerosis

T. Iannitti, B. J. Kerr and B. K. Taylor

Abstract The neuropathic pain of multiple sclerosis is quite prevalent and severely impacts quality of life. A few randomized, placebo-controlled, blinded clinical trials suggest that cannabis- and anticonvulsant-based treatments provide partial pain relief, but at the expense of adverse events. An even smaller, but emerging, number of translational studies are using rodent models of experimental autoimmune encephalomyelitis (EAE), which exhibit pain-like behaviors resembling those of Multiple sclerosis (MS) patients. These studies not only support the possible effectiveness of anticonvulsants, but also compel further clinical trials with serotonin–norepinephrine reuptake inhibitors, the immunosuppressant drug rapamycin, or drugs which interfere with glutamatergic neurotransmission. Future behavioral studies in EAE models are essential toward a new pharmacotherapy of multiple sclerosis pain.

Keywords Multiple sclerosis · Pain · Experimental autoimmune encephalomyelitis

Contents

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1 Introduction

Multiple sclerosis (MS) is an autoimmune-inflammatory neurodegenerative disease of the central nervous system (CNS) that disrupts the myelin sheath, leading to dysfunction of the brain, spinal cord, and optic nerves. Worldwide, over 2 million people are affected by MS. It is the most common cause of acquired disability in young adults, usually presenting itself at 20–45 years of age (Ragonese et al. 2008). It is significantly more common in women, with an overall female:male prevalence ratio ranging from 2.6:1 in the United States (Schwendimann and Alekseeva 2007) to 4:1 in Canada.

The four accepted subtypes of MS are: relapsing and remitting, secondary progressive, progressive relapsing, and primary progressive, and we provide here definitions from Lublin and Reingold (Lublin and Reingold 1996). The **relapsing–remitting** subtype is present in 85 % of patients (McDonald et al. 2001) and is defined as "clearly defined relapses with full recovery or with sequelae and residual deficit upon recovery; periods between relapses characterized by a lack of disease progression." **Secondary progressive** MS develops in 65–80 % of patients previously affected by the relapsing–remitting form, and is defined as "initial relapsing-remitting disease course followed by progression with or without occasional relapses, minor remissions, and plateaus." (Lublin and Reingold 1996) **Progressive relapsing** MS is present in 5–25 % of MS patients (Lublin and Reingold 1996) and is defined as "progressive disease from onset, with clear acute relapses, with or without full recovery; periods between relapses characterized by continuing progression." **Primary progressive** MS is present in 15 % of patients (Cottrell et al. 1999) and is defined as "disease progression from onset with occasional plateaus and temporary minor improvements allowed."

Pain is a frequent and debilitating feature of MS (Beard et al. 2003; Svendsen et al. 2005; Kumpfel et al. 2007). Two recent systematic reviews indicated that pain is estimated to be present in nearly 50 % (O'Connor et al. 2008) and as high

as 63 % (Foley et al. 2012) of the MS population. The pain of MS is particularly problematic in primary progressive and progressive relapsing forms, seriously increasing disability (Nurmikko et al. 2010; Truini et al. 2011). As with other forms of neuropathic pain, the pain of MS is refractory to treatment. A typical study found that only 61 % of patients reported pain relief, and of those, the extent of pain relief was less than 40 % (Grau-Lopez et al. 2011). Here we review randomized controlled clinical drug trials in MS patients (uncontrolled studies are excluded) and experimental pharmacological studies in the experimental autoimmune encephalomyelitis (EAE) rodent model of MS.

2 Pathology of Multiple Sclerosis

MS is characterized by focal demyelinated areas (plaques) with variable shape, number, and size in the white matter of the spinal cord and brain, as well as the gray matter in cerebral cortex, basal ganglia, and thalamus (Haberland 2007). While gray matter lesions are associated with neuronal pathology, white matter plaques are associated with axonal loss (Franklin et al. 2012). Microglial activation and astrogliosis occur in the early and chronic stages of disease, respectively (Lassmann 2010). Activated microglia phagocytose myelin debris and also inhibit oligogenesis (Bauer et al. 1994; Li et al. 2005; Zhang et al. 2011). Axonal injury, cortical demyelination, perivascular inflammation, and neuronal changes have also been extensively described in MS (Hu and Lucchinetti 2009; Vercellino et al. 2009).

Patterns of demyelinating lesions have been categorized by Luchinetti et al. (Lucchinetti et al. 2000): Pattern I demyelinating lesions are characterized by the presence of macrophages and T-cells within the lesions. Pattern II demyelinating lesions are characterized by the presence of macrophages and T-cells plus activated complement antigen (C9neo) and immunoglobulin (mainly IgG) deposition. Pattern III demyelinating lesions are characterized by the presence of macrophages, T-cells and activated microglia with damage to oligodendrocytes and significant loss of myelin-associated glycoprotein (MAG) but not other myelin proteins including proteolipid protein (PLP), myelin basic protein (MBP, and 2′,3′ cyclic nucleotide 3′ phosphodiesterase (CNP). Finally, Pattern IV demyelinating lesions are characterized by the presence of T-cells and macrophages with damage to oligodendrocytes but no significant loss of MOG or other myelin components. Pattern I and II lesions have been detected in patients that present with all clinical subtypes of disease before biopsy or death. On the other hand, pattern III lesions have been observed mainly in patients with a disease of less than 2 months duration before biopsy or autopsy. Pattern IV lesions have been observed in patients affected by primary progressive MS with prominent cerebellar and brainstem involvement and are associated with cognitive deficits (Lucchinetti et al. 2000).

3 Etiology of MS

MS is a multifactorial disease of unclear origin. A leading hypothetical cause of early MS lesions includes activation of myelin-specific T-cells and macrophage/monocytes (Li et al. 1996; Huseby et al. 2012), damage to the endothelial blood brain barrier, and infiltration of T-cells into the CNS (Goverman 2009), leading to oedema formation and complement deposition (Wekerle 2008). The contribution of T-cells to the pathogenesis of MS has been extensively reviewed (Anderton and Liblau 2008; Miller 2011). Microglial activation occurs in both demyelinating and inflammatory non-demyelinated regions of the brain throughout the course of disease (Wu and Tsirka 2009; Mikita et al. 2011). Numerous other causes have also been proposed, including oedema, viral infection by Epstein-Barr virus (Pender et al. 2014), impaired cerebrospinal venous drainage (D'Haeseleer et al. 2011), and inhibition of oligogenesis by activated microglia (Bauer et al. 1994; Li et al. 2005; Zhang et al. 2011).

4 Diagnosis and Chronic Consequences of MS

Widely accepted diagnostic criteria have been summarized by McDonald et al. (McDonald et al. 2001), and revised by Polman et al. (2005, 2011). Strong evidence of MS includes 2 or more "attacks" that involve clinical events characteristic of an acute CNS inflammatory demyelinating episode. These events last at least 24 h and occur in the absence of fever or injection. Clinical events include spasticity, clumsiness of the hands, double vision (diplopia), temporary vision loss or blurred vision, numbness, pain, bladder, bowel and sexual dysfunction, impairment of coordination, and muscle weakness. A definitive diagnosis of MS includes corroboration of attacks with neurological examination and paraclinical laboratory assessments, namely magnetic resonance imaging (MRI), visual evoked potential responses (VEP), and cerebrospinal fluid (CSF) analysis. MRI quantifies the progression of white and gray matter lesions in the brain and spinal cord (Thorpe et al. 1996; Bastianello et al. 2000; Garcia-Lorenzo et al. 2012). Gray matter lesions can appear at early phases of disease, often correlating with development of cognitive disability (Calabrese et al. 2012). Evoked potentials aid the confirmation of clinically silent lesion sites (subclinical lesions). CSF is analyzed for protein and glucose, lactate, myelin basic protein, CSF/serum albumin ratio, immunoglobulin-gamma (IgG), immunoglobulin-M (IgM), kappa light chains, and oligoclonal IgG bands (Luque and Jaffe 2007). Diagnostic criterion include positive brain MRI (9 lesions or 4 lesions with a positive VEP response), positive spinal cord MRI (two focal lesions), or positive CSF (Polman et al. 2011). The above criteria are concomitantly used to formulate a diagnosis; however, misdiagnosis of other diseases as MS remains a major problem (Singhal and Berger 2012).

Consequences of MS, possibly associated with chronic pain, include spasticity, fatigue, depression, and cognitive impairment (Solaro and Uccelli 2011). The pseudobulbar affect (pathological laughing or crying) is present in 10 % of the MS population (Smith et al. 2004). The Expanded Disability Status Scale (EDSS) is widely used to monitor progression of MS-related symptoms (Iuliano et al. 2008), as it correlates strongly with laboratory diagnostic confirmations (Invernizzi et al. 2011).

Laser Evoked Potentials (LEPs) are cortical responses to a laser beam directed to the skin of the hand and foot and are often delayed or reduced in MS patients (Kakigi et al. 1992; Spiegel et al. 2003). LEPs reflect selective activation of A-delta and C nociceptors in the hairy skin (Treede et al. 2003; Cruccu and Garcia-Larrea 2004), and indicate lesions or dysfunction of the spinothalamic tract (Spiegel et al. 2003). Somatosensory Evoked Potentials (SEPs) in response to stimulation of median or posterior tibial nerves may also be delayed in MS patients, and can indicate lesions or dysfunction of the dorsal column tract (Spiegel et al. 2003).

5 Clinical Presentation of Pain in MS

The International Association for Study of Pain (IASP®) recently introduced a revised definition of neuropathic pain as ‘‘pain caused by a lesion or disease of the somatosensory system” (Jensen et al. 2011). Although MS unequivocally involves CNS lesions of motor systems, it is not established whether lesions extend to the somatosensory system. Based on pain reports in MS patients, and use of LEPs to investigate the function of nociceptive fibers in patients affected by neuropathic pain (Cruccu et al. 2008; Haanpaa et al. 2011), we argue that neuropathic pain does exist in MS. For example, Truini et al. found that, of 10 MS patients reporting ongoing extremity pain, 9 (90 %) displayed LEP abnormalities, indicative of dysfunction in nociceptive pathways (Truini et al. 2012).

Pain severely impacts the quality of life of about half of the MS population, with up to 75 % of patients reporting pain within the preceding month (O’Connor et al. 2008; Solaro and Uccelli 2011; Foley et al. 2012). Up to a third of MS patients characterize pain as one of their worst symptoms, and analgesics represent a major class of medications used by them (Stenager et al. 1991; O’Connor et al. 2008; Solaro and Uccelli 2011). Pain intensity can occur in newly or recently diagnosed cases, increases with disease severity (O’Connor et al. 2008; Truini et al. 2012), and varies widely from mild to severe across several studies. On average, most patients report moderate pain – approximately 5 on a 0–10 numerical rating scale. Pain in MS typically involves the musculoskeletal system (nociceptive pain) and/or the CNS (neuropathic pain), or both, and MS is associated with a high incidence of headache as well. The primary forms of neuropathic pain in MS are ongoing “dyesthetic” extremity pain, trigeminal neuralgia, and painful Lhermitte’s sign. These are frequently associated with painful muscle

spasms and cutaneous allodynia/hyperalgesia (O'Connor et al. 2008). It is not uncommon for patients with MS to experience multiple types of pain.

Ongoing extremity pain is usually chronic, presenting as bilateral burning sensation at the legs and feet. It is a common form of pain in MS, being reported by almost 1 in 5 patients (O'Connor et al. 2008; Truini et al. 2011). It is particularly prevalent in primary progressive and progressive relapsing subtypes (O'Connor et al. 2008; Nurmikko et al. 2010).

Trigeminal neuralgia and optic neuritis (eye pain) present in approximately 5–20 % of MS patients (Cruccu et al. 2009; Nurmikko 2009; Truini et al. 2011). Compared to classic trigeminal neuralgia, MS-associated trigeminal neuralgia is more often bilateral in presentation (O'Connor et al. 2008). MS-associated trigeminal neuralgia is often associated with lesions of the intrapontine trigeminal primary afferents. Eye pain results from inflammation of the optic nerve trunk, thereby activating intraneural nociceptors innervated by nervi nervorum.

Lhermitte's sign (symptom) is evoked by neck flexion and presents as a sudden-onset, brief, electric shock-like sensation traveling rapidly down the spine, occasionally reaching the arms or leg (Kanchandani and Howe 1982; Al-Araji and Oger 2005; Nurmikko et al. 2010). It is frequently reported in MS patients with a prevalence of 9–41 % (Solaro et al. 2004). Truini et al. reported that of 18 MS patients with painful Lhermitte's, 13 (72 %) exhibited abnormal SEPs (Truini et al. 2012), indicative of neuropathic dysfunction within the dorsal column/medial lemniscus system (Treede et al. 2003; Cruccu et al. 2008).

In addition to spontaneous pain, MS is often associated with cutaneous hypersensitivity, including allodynia and hyperalgesia. Many patients report heat hypersensitivity (58 %) or paradoxical sensation of warmth elicited by a cold stimulus; such abnormalities likely contribute to fatigue, cognitive problems (*see Benson and Kerr in this volume*), and pain (Hansen et al. 1996; Morin et al. 2002; Flensner et al. 2011). Other patients present with cold allodynia (Osterberg et al. 2005; Svendsen et al. 2005), and some report tactile allodynia (Svendsen et al. 2004; Osterberg et al. 2005, Cruccu et al. 2009), namely brush-evoked allodynia (Sakai et al. 2004; Osterberg and Boivie 2010).

Approximately 75 % of MS patients present with a velocity-dependent increase in muscle reflexes (Rizzo et al. 2004), and muscle spasms are often associated with musculoskeletal pain. For example, approximately 10 % of MS patients present with cramping and/or pulling descriptions of arm pain that are associated with tonic spasms (Solaro and Messmer Uccelli 2010; Truini et al. 2011). Uncontrolled spasms can contribute to damage of descending motor pathways and motor neurons, leading to a vicious cycle of increased tone, gait abnormalities, fatigue, and painful tonic spasms (Beard et al. 2003; Boissy and Cohen 2007; Ben-Zacharia 2011). On the other hand, in human subjects with CNS lesions, intrathecal baclofen suppressed dysesthetic and spasm-related pain with a different time course, suggesting that different mechanisms contribute to each pain type (Herman et al. 1992).

6 Clinical Pharmacology of Pain in MS

Pharmacological studies and treatments for MS typically target motor dysfunction, and often neglect neuropathic pain. For example, out of 134 patients reporting pain associated with MS, only 38 % received treatment for their pain (42 % NSAIDs, 27 % anticonvulsants, 7 % homeopathic, 24 % others (Grau-Lopez et al. 2011). This is due in large part to the fact that clinicians often fail to recognize the clinical features of pain, in part because they are difficult for patients to describe (Solaro and Uccelli 2011). Furthermore, clinical trials in MS do not typically evaluate pain as an outcome measure. Of the clinical trials that have measured pain, the small cohorts of patients used, the open-label design of most studies, and the lack of placebo controls does not allow for definitive conclusions to be drawn regarding analgesic efficacy of currently prescribed analgesic drugs. Indeed, MS patients with neuropathic pain report low satisfaction with pain management (Solaro and Uccelli 2011). Thus, we have a suboptimal situation where pharmacological management of neuropathic pain in MS is driven by anecdotal reports and by findings of clinical trials conducted in patients with very different forms of neuropathic pain, such as spinal cord injury or peripheral nerve injury (Nurmikko et al. 2010). In Table 1, we summarize the results of blinded, randomized controlled trials (RCT) that have assessed pharmacological approaches to manage pain in MS. In general, discussions of open-label studies are omitted from this review.

6.1 Cannabinoid Receptor Agonists

Sativex. Initial RCTs of this drug class assessed the analgesic efficacy of Sativex, an oromucosal spray of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). This preparation was thought to antagonize some THC-induced adverse events such as intoxication, sedation, and tachycardia, while contributing its own analgesic and anti-emetic properties (Russo and Guy 2006). Conflicting results have been reported in double-blind RCTs using Sativex. While two studies showed no reduction in MS pain using Sativex (Wade et al. 2004, Conte et al. 2009), several others have yielded promising results. First, a small RCT reported that Sativex decreased VAS by greater than 50 % in 16 out of 34 MS patients, but also produced several side effects (Notcutt et al. 2004). Similarly, Rog and colleagues reported that Sativex reduced the intensity of neuropathic pain (Rog et al. 2005), although again, dizziness and nausea were reported as common side effects in a follow-up study (Rog et al. 2007). During the randomized-withdrawal phase, Sativex was still significantly effective (Langford et al. 2013). Open-label follow-up studies report that Sativex-responsive patients continue to receive pain relief for over a year (Wade et al. 2006; Rog et al. 2007). A more recent placebo-controlled study found that a 10-wk regimen of Sativex significantly decreased pain numerical rating scale

Table 1 Clinical pharmacology of chronic pain in MS: randomized, placebo-controlled, blinded clinical trials

Reference	Treatment	#subjects	Pain scale	Outcome	Pain as the primary endpoint?
Cannabinoid-based drugs					
Zajicek et al. (2003)	Cannabis extract or THC	611	CRS	Reduced patient-reported pain	No
Notcutt et al. (2004)	Sativex®	34	VAS	Decrease VAS, $p < 0.05$ Decrease > 50 % in 16/34 patients	No
Svendsen et al. (2004)	Marinol, 3 weeks	24	NRS	Decreased NRS, increased pain relief, increased SF-36	No
Wade et al. 2004	Sativex®	160	VAS	No change, $p > 0.05$	Yes[a]
Rog et al. (2005)	Sativex®	64	NRS	Sativex® reduced mean intensity of pain ($p = 0.005$).	No
Conte et al. (2009)	Sativex®	17	RIII reflex; VAS	Decrease in RIII reflex. No change in VAS	Yes
Corey-Bloom et al. (2012)	Cannabis cigarettes (4 % THC)	30	VAS	VAS pain reduced by an average of 5.28 points versus placebo	No
Zajicek et al. (2012)	Cannabis extract, 2.5–25 mg THC	279	NRS	Self-reported relief from body pain at 4, 8 and 12 weeks	No
Langford et al. (2013)	Sativex®	339	NRS	Significantly reduced pain by week 10; otherwise, no effect	Yes
Anticonvulsants					
Breuer et al. (2007)	lamotrigine	12	BPI, NPS, MSQOL-54	No effect of drug	Yes
Rossi et al. (2009)	**Levetiracetam**	20	VAS	Significant VAS pain reduction ($p < 0.05$)	Yes
Falah et al. (2011)	**Levetiracetam**	**27**	6-point verbal scale pain relief	No effect except in patients with lancinating or without touch-evoked pain	Yes

This table only lists clinical trials that were randomized and controlled, containing 12 or more subjects, and for which multiple reports within a particular drug class are available.

VAS Visual Analog Scale, *NRS* Numerical Rating Scale, *CRS* Category rating scale for self-reported pain, *BPI* Brief Pain Inventory, *NPS* Neuropathic Pain Scale, *MSQOL* Multiple Sclerosis Quality of Life Inventory, *SF-36* SF-36 quality of life scale, *THC* Δ9-tetrahydrocannabinol

[a] pain as the primary endpoint only in a subset of 36 patients

on a 10-point numerical rating scale (improvement equal or superior to 30 %) (Langford et al. 2013).

Other cannabinoid preparations. Oral cannabis extract was assessed in 211 MS patients and THC was assessed in a similar sized cohort of 206 MS patients. Both treatments significantly reduced pain scores as assessed by category rating scale (Zajicek et al. 2003). Furthermore, a synthetic THC, dronabinol, significantly reduced median spontaneous pain intensity and radiating pain when compared to placebo (Svendsen et al. 2004). Nabilone, a synthetic cannabinoid, has also been found to reduce the pain associated with spasticity, without serious adverse events (Wissel et al. 2006). Cannabis preparations that contained THC and cannabidiol improved self-reported pain assessed by an 11-point category rating scale in a randomized placebo-controlled clinical trial. At 4 and 8 weeks, body pain was significantly decreased and, at 12 weeks, the proportion of relief in patients receiving cannabis extract was nearly double that reported by subjects receiving placebo (Zajicek et al. 2012). Furthermore, a randomized double-blind clinical trial of smoked cannabis showed a significant improvement in VAS pain (Corey-Bloom et al. 2012). Thus, in contrast to the mixed effects of Sativex, administration of other cannabinoid receptor agonist preparations typically reduces the pain of MS. In summary, cannabinoid receptor agonists hold considerable promise for the treatment of MS pain. However, these compounds are often associated with a significant amount of side effects including dizziness, headache, fatigue, and impaired judgement, and so have been restricted to 2nd or 3rd-line treatments for neuropathic pain, or to adjunctive treatments as in the case of Sativex (Attal et al. 2010; Dworkin et al. 2010).

Despite an enormous amount of interest in cannabinoids for the neuropathic pain of MS, all clinical trials to date have been limited by a low number of patients. Studies are also limited by the selection of patients with just the secondary progressive subtype of MS and the absence of discrimination between MS subtypes.

6.2 Opioids

Despite the widespread use of opioids for chronic pain, remarkably few studies have investigated their efficacy for the neuropathic pain of MS. A single dose of intravenous morphine decreased MS pain (>50 % pain reduction) in 29 % of the 14 patients involved in a single-blind nonrandomized placebo-controlled trial (Kalman et al. 2002). Opioid receptors appear to mediate the analgesic effect of morphine, as it was reversed by naloxone. However, analgesia was apparent only at high doses of morphine (mean = 41 mg), indicating weak efficacy. Thus, strong opioid analgesics have been relegated to 2nd line treatment for the pain of MS (Dworkin et al. 2010).

6.3 Anticonvulsants

Anticonvulsants are a first-line treatment for several forms of neuropathic pain, including trigeminal neuralgia, postherpetic neuralgia, and painful diabetic neuropathy (Jensen 2002). As a result, they are also prescribed for the pain of MS, particularly carbamazepine for MS-related trigeminal neuralgia. However, the small number of RCTs conducted so far does not demonstrate substantial pain relief or tolerability in MS patients. Levetiracetam (500 mg) was shown to significantly reduce VAS scores in MS patients with pain in a randomized double-blind placebo-controlled clinical trial (Rossi et al. 2009). In this study, degree of pain reduction was correlated to the severity of pain at baseline (r squared = 0.55, $P < 0.05$). Higher doses of Levacetiram (3000 mg) also reduced pain on a 10-point numeric scale in a randomized double-blind placebo-controlled clinical trial (Falah et al. 2011). However, this effect was limited to a subgroup of patients without touch-evoked or lancinating pain. By contrast, a well-done pilot study found that the anticonvulsant lamotrigine had no efficacy for pain in MS, and the authors concluded that larger scale studies were not needed (Breuer et al. 2007). Oxacarbazepine, a keto-analog of carbamazepine, was found to reduce painful paroxysmal symptoms of MS in an open-label pilot study (Solaro et al. 2007). Gabapentin, a commonly prescribed drug for neuropathic pain also reduced MS-related pain in an open-label study, but several of the patients experienced dose-dependent side effects including mental cloudiness, confusion, dizziness, and irritability (Houtchens et al. 1997).

6.4 Tricyclic Antidepressants (TCA)

Despite sparse clinical trials, TCAs are recommended as first-line medicines for the management of the pain of MS (Solaro and Uccelli 2011, Truini et al. 2011). One randomized trial compared nortriptyline with self-applied transcutaneous electrical nerve stimulation (TENS) rather than placebo (Chitsaz et al. 2009). Nortriptyline was as effective as TENS in reducing VAS scores, but produced substantially more side effects. A promising Phase 3 RCT found that duloxetine reduced 24-hour pain scores on an 11-point Likert scale as compared to placebo (EliLilly&Co 2011).

6.5 Muscle Relaxants

Intrathecal administration of the γ-aminobutyric acid class B ($GABA_B$) receptor agonist baclofen is used for the treatment of spasticity, and so was tested in patients with MS. In a double-blind RCT, baclofen decreased dysesthetic pain (continual,

spontaneous burning, lancinating, shooting, radiating knife-like feelings), as well as spasm-related pain and pinch-induced pain (Herman et al. 1992). Baclofen, or newly available $GABA_B$ receptor agonists such as arbaclofen, could be interesting candidates for future RCTs for MS pain.

7 Animal Models of Multiple Sclerosis

Experimental autoimmune encephalomyelitis (EAE). EAE models in mice and rats remain the most widely used translational tools in multiple sclerosis research (Baxter 2007). Although important differences between EAE and MS pathologies have been pointed out (Croxford et al. 2011), there are many similarities including clinical course, pathological CNS lesions, glial activation, and axonal demyelination (Mix et al. 2010; Handel et al. 2011). For example, EAE rodents exhibit widespread demyelination, anti-MOG antibodies, and MOG-reactive T-cells, similar to the CNS of patients with MS (Storch et al. 1998; Iglesias et al. 2001). Thus, EAE has provided fundamental advances in our understanding of MS pathology (Linker and Lee 2009) and in the development of new pharmaceutical therapies (Kieseier and Hartung 2003; Steinman and Zamvil 2006). Most EAE models involve the systemic administration of encephalitogenic myelin antigens to a range of species including mice, rats, guinea pigs, and monkeys. Bacterial components such as heat inactivated mycobacteria are added to an adjuvant (Freund's adjuvant) to activate the innate immune system and to increase sensitization to myelin antigens (Gold et al. 2006; Constantinescu et al. 2011). Pertussis toxin (PTx) is co-injected as it elicits a type 1 differentiation of T-cells and opens the blood brain barrier (BBB), facilitating the migration of pathogenic T-cells into the CNS (Hofstetter et al. 2002; Darabi et al. 2004). Histopathology and clinical course of the disease varies considerably with the type and dose of antigens and adjuvants, and species (Lassmann 1983; Khan and Smith 2013).

EAE using MOG. Myelin oligodendrocyte glycoprotein (MOG) is a component of the outer myelin layer. MOG is the most commonly used antigen to model MS because its expression is restricted to the CNS, and because it produces clinical symptoms similar to MS that generally are stable or gradually worsen—they do not resolve over time. Subcutaneous injection of a fragment of MOG peptide (MOG_{35-55}), induces encephalitogenic T-cells, demyelination, axonal loss, and clinical signs of neuromuscular dysfunction in C57BL/6 and Biozzi ABH mice (Linker and Lee 2009). Patterns of EAE-induced pathology vary considerably with the dose and frequency of antigen administration. For example, a single injection of low dose MOG_{33-55} (50 μg) and PTx (200 ng) produces a mild monophasic form of relapsing remitting EAE in which remission is not followed by subsequent symptoms. By contrast, higher doses of MOG_{33-55} (300 μg) and PTx (300 ng) produce a more chronic form of EAE characterized by larger inflammatory lesions, myelin loss, axonal damage, and clinical signs that worsen with time (Berard et al. 2010). Each of these models has been used to study different forms of MS. Recent

work, however, suggests that changes in pain sensitivity are a common feature of MOG_{35-55} induced EAE regardless of the source of the MOG or the concentration of adjuvant used to induce the disease (Olechowski et al. 2009, 2013).

Other EAE models. Other commonly used antigens include proteolipid protein (PLP) and myelin basic protein (MBP). These are not as commonly used as MOG because they are expressed not only in the CNS, but also in the peripheral nervous system. PLP causes CNS inflammation, neuritis, and radiculitis in the absence of frank demyelination (Pender 1988). MP4 is an MBP-PLP fusion protein that causes demyelination at the onset of clinical symptoms (Kuerten et al. 2011). A severe form of EAE results from the daily administration of cyclosporine for 21 days after MOG-induced EAE (Thibault et al. 2011). A model of chronic-progressive MS can be generated by intracerebroventricular inoculation with Theiler's murine encephalomyelitis virus (TMEV). TMEV inoculation leads to spinal cord and brain inflammation, demyelination, and axonal damage (Fuller et al. 2004; Oleszak et al. 2004; Tsunoda and Fujinami 2010). The TMEV model supports the hypothesis that the etiology of MS includes viral infection (Olson et al. 2005).

8 Behavioral Signs of Neuropathic Pain in EAE

An emerging body of work demonstrates that pain-like cutaneous hypersensitivity at the tail and hindpaws is a reliable symptom associated with EAE models, and mimics the lower extremity pain reported by MS patients (Svendsen et al. 2005; O'Connor et al. 2008; Nurmikko et al. 2010; Truini et al. 2011). The following sections summarize the characteristics of these behaviors, describe part of the neuropathology underlying them, and then illustrate the limited preclinical studies designed to generate new targets for pain relief in MS patients.

Tactile hypersensitivity. Mechanical allodynia, as assessed with calibrated von Frey hair monofilaments applied to the dorsal or plantar surface of the hind paws, has been described in several EAE models. Compared to controls, MOG_{35-55} consistently increases tactile sensitivity. For example, compared to complete Freund's adjuvant (CFA), MOG decreased response threshold (Olechowski et al. 2009; Rodrigues et al. 2009). Mechanical allodynia has also been described in a rat model of chronic MOG_{1-125}-induced EAE (Ramos et al. 2010). On the other hand, using higher doses of MOG_{35-55} to induce more severe clinical symptoms, mechanical allodynia may not manifest (Lu et al. 2012). The reasons for this are unclear, but it is quite possible that the motor deficits associated with higher doses of MOG are severe enough to interfere with the observation of pain behaviors. Indeed, as further discussed below, the interpretation of pain behaviors and dorsal horn neurophysiology in EAE models requires a careful consideration of motor and reflex function at the ventral horn and brain.

Cold hypersensitivity. As observed in MS patients, rodents with EAE exhibit increased sensitivity to cold stimuli (Morin et al. 2002 check refs, Osterberg et al. 2005; Svendsen et al. 2005). Compared to vehicle (CFA) controls, MOG_{35-55} consistently increased the response duration to innocuous cold stimuli delivered to the ventral surface of the hindpaw (Olechowski et al. 2009). Cold hyperalgesia at the tail or paw have also been observed in MBP-induced EAE models as assessed by the cold plate test (Thibault et al. 2011).

Heat hypersensitivity. As observed in MS patients (Flensner et al. 2011), rodent EAE models exhibit heat hypersensitivity. For example, $PLP_{139-151}$-induced EAE in SJL/J mice led to a heat hypersensitivity in the tail of male or female mice (Aicher et al. 2004). Both $PLP_{139-151}$-induced relapsing–remitting EAE in SJL mice and MOG_{33-55}-induced chronic EAE in C57BL/6 mice exhibited heat hypersensitivity during the chronic phase (day 35 to 45 after EAE induction) (Lu et al. 2012). TMEV infection also induced heat hypersensitivity in male and female SJL/J mice, as compared to control noninfected mice in the thermal tail-immersion test (Lynch et al. 2008). In a rat MBP model of EAE, heat hypersensitivity developed at the tail (Thibault et al. 2011). An exception was reported by Olechowki et al., who found no difference in hindpaw heat withdrawal thresholds of mice treated with MOG_{35-55} (Olechowski et al. 2009).

Relative time course of EAE pain and motor dysfunction. MOG_{35-55} produces mechanical hypersensitivity in mice within 7 days of immunization, well before the onset of frank neuromuscular deficits (Olechowski et al. 2009; Rodrigues et al. 2009; Yuan et al. 2012). Similarly, mechanical allodynia in male and female SJL/J mice occurs before or during the onset of motor deficits in the TMEV model (Lynch et al. 2008). Also, heat and cold hypersensitivity in rat MBP EAE models begins before the onset of motor dysfunction, and heat hyperalgesia continued even after clinical signs had disappeared (Thibault et al. 2011). However, a smaller number of studies indicate that thermal and mechanical hypersensitivity can develop after the onset of motor dysfunction in severe mouse MOG and PLP and rat MBP models of EAE (Thibault et al. 2011; Lu et al. 2012). Furthermore, a clinical study reported that MS patients present with sensory and motor symptoms of MS a year before complaints of pain (Osterberg et al. 2005). Still, the majority of studies in animal models indicate that pain hypersensitivity typically precedes motor dysfunction in EAE models.

Sex differences in EAE pain. Despite the large difference in the prevalence of MS in women as compared to men, only a small number of rodent studies have compared EAE pain in both sexes. In two studies, thermal hyperalgesia after $PLP_{131-151}$ or TMV was similar between male and female mice (Aicher et al. 2004, Lynch et al. 2008). By contrast, TMEV mice develop mechanical allodynia faster in females than in males, despite the more rapid disease progression in males (Lynch et al. 2008). These findings support the idea, discussed below, that neuromuscular deficits do not prevent the study of pain hypersensitivity in EAE.

9 Neuropathological Mechanisms of Neuropathic Pain in EAE-MOG Models

EAE models are associated with damage to spinal and supraspinal pain transmission pathways. MOG_{33-55} rapidly (within 7 d of administration) and dramatically (>50 %) damages the fasciculus gracilis of the medial dorsal column, with no significant remyelination at 1 year (Jones et al. 2008). This can lead to sensory deficits, since the dorsal columns normally carry non-noxious tactile information from large myelinated primary afferent neurons to the dorsal column nuclei. However, under certain pathological conditions, this information may be perceived as painful (Ossipov et al. 2002). Pain inhibitory $GABA_B$ receptors are expressed at high concentrations in the superficial dorsal horn (Price et al. 1987; Margeta-Mitrovic et al. 1999; Castro et al. 2004), and clinical studies in patients with CNS lesions indicate that spinal $GABA_B$ dysfunction may contribute to central pain, including MS pain (Herman et al. 1992).

EAE models are also associated with early changes in the reactivity of glial cells residing within the superficial dorsal horn. Activation of microglia and astrocytes correlates with the emergence of aberrant pain behaviors. For example, the mechanical and cold allodynia following MOG_{35-55}-induced EAE was associated with an increase in glial fibrillary acidic protein (GFAP) immunoreactivity and the number of $CD3^+$ T-cells in the superficial dorsal horn of the spinal cord. GFAP and $CD3^+$ T-cells were significantly increased not only during the onset and peak phases of EAE, but also during the chronic phase of stable motor dysfunction, 28 days post-MOG. MOG also increased F4/80 expression (labeling of microglia/macrophage) in the dorsal horn, particularly during the chronic phase (Olechowski et al. 2009). Similarly, in a model of relapsing–remitting EAE in SJL mice, the number of GFAP- and Iba1-immunoreactive cells in spinal cord dorsal horn was increased at the onset, peak, and chronic phases of disease (Lu et al. 2012). Rat models of chronic and relapsing–remitting EAE also are associated with increased GFAP and CD11b immunoreactivity in the dorsal horn (Ramos et al. 2010). Thus, activated astrocytes and microglia may contribute to neuropathic pain in MS, just as they do in neuropathic pain models involving traumatic nerve injury.

For an excellent, more extensive review of additional pathobiology of MS-neuropathic pain in EAE models, see Khan and Smith (Khan and Smith 2013).

10 Pharmacological Studies in Animal Models of MS Pain

Despite the prevalence of neuropathic pain in MS and the need for new treatments, pharmacological studies in experimental models are remarkably sparse. As noted above, several small clinical trials have investigated the effects of cannabinoid receptor agonists and anticonvulsants in MS-related pain, and single controlled studies have evaluated other targets. Only a few EAE studies, however, have been

conducted to validate these approaches with currently available drugs, or to investigate new drugs for the future pharmacotherapy of central neuropathic pain. Below we outline the results of these pharmacology studies, most of which are limited to a single dose of a single drug class. Surprisingly, none have reported on the effects of cannabinoid receptor agonists on EAE pain. For a more comprehensive list of potentially "druggable" targets in MS-associated pain, see Kahn and Smith (Khan and Smith 2013).

Anticonvulsants. Gabapentin is a first-line therapy for the neuropathic pain associated with painful diabetic neuropathy and postherpetic neuralgia. In a rat EAE model, two weeks of daily gabapentin administration reduced mechanical hyperalgesia but not neuromuscular dysfunction (Thibault et al. 2011). Although only a single dosing regimen was used, the results of this study support the early clinical studies assessing the efficacy of anticonvulsants for pain in human EAE.

Serotonin-norepinephrine reuptake inhibitors (SNRIs). Both tramadol and duloxetine inhibit the uptake of serotonin and norepinephrine in neuronal terminals, and this action is thought to contribute to their efficacy in reducing clinical neuropathic pain. Both of these drugs prevented the development of cold allodynia at 21 and 35 days after induction of EAE in rats (Thibault et al. 2011). Like gabapentin, neither tramadol nor duloxetine reduced motor dysfunction. Although only a single dosing regimen was used, this study points to the need for further clinical studies assessing the efficacy of SNRIs in human EAE.

mTOR inhibitors. Cytokine-driven T-cell proliferation likely contributes to the induction of MS, as well as to the induction of motor dysfunction in EAE models (Huseby et al. 2012). Repeated administration of the immunosuppressant and mTOR inhibitor, rapamycin (Mondino and Mueller 2007), begun 2 days after induction of EAE, significantly reduced mechanical allodynia at a single time point 2 weeks later, and produced a more complete prevention of motor impairment (Lisi et al. 2012). Additional studies are needed to determine whether rapamycin inhibits neuropathic pain in EAE by reductions in T-cell activity and proliferation coincident with reductions motor impairments, or by a distinct mTOR activation mechanism within spinal pain transmission pathways (Geranton et al. 2009; Asante et al. 2010; Norsted Gregory et al. 2010).

Inhibitors of glutamatergic neurotransmission. Glutamatergic neurotransmission within spinal pain transmission pathways or at sclerotic lesion sites may contribute to the pain of EAE and MS. Neuropathological data indicate that EAE animals exhibited decreased excitatory amino acid transporter 2 and increased glutamate type 1 transporter (Olechowski et al. 2010; Ramos et al. 2010) and MS patients exhibit greater concentrations of excitatory amino acids in cerebrospinal fluid (Sarchielli et al. 2003), glutamate receptor expression on oligodendrocytes at the borders of active lesion sites (Newcombe et al. 2008), and other signs of altered glutamate homeostasis (Werner et al. 2001). An emerging pharmacology is supporting the idea that inhibition of glutamatergic signaling decreases neuropathic pain in EAE. For example, ceftriaxone is not only a third-generation cephalosporin antibiotic, but also upregulates the spinal expression of excitatory amino acid transporters (EAAT), thus reducing synaptic glutamatergic neurotransmission.

In both mouse and rat MOG models of EAE, daily intrathecal injection of ceftriaxone prevented behavioral signs of neuropathic pain (Ramos et al. 2010; Olechowski et al. 2013). In the rat, ceftriaxone also slowed the progression of paralysis and reduced molecular signs of astrocyte and microglial activation, which may contribute to sensitization of spinal pain transmission (Ramos et al. 2010).

11 Conclusions

Although ignored for many years, pain in MS is now recognized as an important feature of the disease that significantly impacts quality of life in roughly half of MS patients. Up to a third of MS patients characterize pain as one of their worst symptoms (Stenager et al. 1991, 1995). Sclerotic lesions in MS likely impinge on the somatosensory system, and therefore the MS pain associated with ongoing extremities pain, trigeminal neuralgia, and L'hermitte's sign can be classified as neuropathic. Other forms of MS-associated pain may arise from peripheral somatosensory dysfunction or a mixed nociceptive, neuropathic pain (Khan and Smith 2013).

Of the handful of clinical studies that have addressed pain in MS, most are severely limited by an open-label design. Of the very small subset that were designed with proper randomization, placebo control, and blinding, insufficient numbers of subjects prevent any firm conclusion. Clinical studies with anticonvulsants are promising, and studies with cannabinoid-based drugs suggest the CB1 and CB2 cannabinoid receptors to be quite a compelling target for future studies.

Conventional EAE mouse models, particularly those involving administration of MOG as the antigen, yield robust tactile and thermal hypersensitivities that mimic evoked pain in MS patients. MOG-based models are particularly attractive because MOG expression is restricted to the CNS, clinical symptoms are stable or increase over a long time period, and neuropathic pain-like behaviors are robust. Importantly, the majority of studies in EAE models indicate that behavioral manifestations of pain hypersensitivity typically precede motor dysfunction. We suggest that neuroplasticity of nociceptive transmission in the dorsal horn develops quickly in EAE as in other animal models of neuropathic pain, while relatively more time is required for the accrual of adequate demyelination and/or axonal injury before the behavioral manifestation of motor impairments. The practical ramifications of this temporal dissociation are that EAE-induced motor impairment does not prevent the observation and study of pain hypersensitivity, particularly in the earlier course of the disease. Future studies will likely reveal specific neuroplastic mechanisms within nociceptive pathways of the dorsal horn that contribute exclusively to the behavioral manifestations of chronic neuropathic pain.

Such neuropathological mechanisms of EAE pain are just beginning to be understood, and could involve dysfunction of glia or glutamatergic systems within the spinal cord dorsal horn. MOG-based EAE models have yielded some promising candidates from clinically available drugs including anticonvulsants, SNRIs,

and mTOR inhibitors, and these studies provide proof-of-principle that studies in EAE hold great promise for the development of a new pharmacotherapy for the neuropathic pain associated with MS.

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Part III
Preclinical Models of Chronic Pain

Preclinical Assessment of Pain: Improving Models in Discovery Research

Tamara King and Frank Porreca

Abstract To date, animal models have not sufficiently "filtered" targets for new analgesics, increasing the failure rate and cost of drug development. Preclinical assessment of "pain" has historically relied on measures of evoked behavioral responses to sensory stimuli in animals. Such measures can often be observed in decerebrated animals and therefore may not sufficiently capture affective and motivational aspects of pain, potentially diminishing translation from preclinical studies to the clinical setting. Further, evidence indicates that there are important mechanistic differences between evoked behavioral responses of hypersensitivity and ongoing pain, limiting evaluation of mechanisms that could mediate aspects of clinically relevant pain. The mechanisms underlying ongoing pain in preclinical models are currently being explored and may serve to inform decisions towards the transition from drug discovery to drug development for a given target.

Keywords Ongoing pain · Affective · Motivational behaviors · Translation

Contents

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Pain in humans is a multidimensional experience with cognitive, motivational, and sensory components (Melzack and Casey 1968). Nociceptive pain, typically resulting from traumatic injury (e.g., bone fracture), serves protective functions including escape/avoidance of the pain-generating stimulus and promotes recuperative and protective behaviors to facilitate healing (Costigan et al. 2009). Injuries can sometimes lead to chronic pain that reflects maladaptive plasticity of the nervous system. Unlike nociceptive pain, chronic pain does not offer survival advantages and is often associated with pain that occurs in the absence of external stimuli (ongoing or spontaneous pain), from normally innocuous stimuli (allodynia) and with enhanced and longer lasting pain due to normally painful stimuli (hyperalgesia). Examples of chronic pain states include chronic non-malignant inflammatory (e.g., low back pain) and neuropathic (e.g., post-herpetic neuralgia) pain conditions as well as multimodal conditions such as cancer pain. Additionally, dysfunctional pain can occur in the absence of apparent tissue injury (e.g., fibromyalgia) (Clauw 2009; Goldenberg 2009).

Current treatments for pain continue to rely largely on non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, therapies that have been in existence across millennia. These drugs remain the gold standard for pain management, but are associated with a large array of adverse side effects that can compromise the patient's quality of life. This limits the therapeutic goal of providing complete pain relief by limiting dosing to effect, thus impairing the beneficial outcome of pain management. Advances in pain therapeutics are needed and depend on understanding of the neurobiology associated with specific pain conditions. Animal models with apparent relevance to clinical pain conditions have been developed and have informed the basics of our understanding of mechanisms associated with pain syndromes. While our knowledge of biological mechanisms of pain has been immensely aided by the use of animal models, there is increasing concern that these models are not sufficiently "predictive" to gain insight into mechanisms relevant to the human experience of pain (Vierck et al. 2008; Mogil 2009; Mao 2012; Percie du Sert and Rice 2014). Thus, the disproportionate lack of availability of new treatment strategies relative to our gains in understanding the neurobiology underlying pain have been fairly or unfairly linked to a failure of animal models to capture essential features of clinical pain. It should be noted that the issue of translation of mechanism to novel therapy is complex and the impediments associated with preclinical models represent only one of many hurdles. While it is essential to recognize the limitations of animal models in providing insights into the multidimensional human experience of pain, it is also important to recall their contribution to the tremendous advances in the understanding of sensory

neurobiology including the molecular underpinnings of transduction, transmission, and modulation in response to stimuli that typically elicit sensations of pain.

Most preclinical studies of pain have emphasized output measures that rely on responses to evoked stimuli (Vierck et al. 2008; Mogil 2009). While such stimuli engage the nociceptive pathway and likely accurately reflect the mechanisms associated with acute nociceptive pain, these reflexive responses are unlikely to capture components of pain that are most relevant to clinical chronic pain syndromes. Reflexive behaviors can often be observed in decerebrated animals (Woolf 1984) and do not require learning (Vierck et al. 2008), an essential feature of physiological pain. For this reason, investigators involved in preclinical pain research have developed a number of novel strategies aimed at capturing features of pain that might have increased translational relevance. Such approaches are generally intended to measure features of pain without the need for an evoked reflexive withdrawal response. In this review, we highlight some of the recent advances in how "pain" is measured in the preclinical setting.

1 Preclinical Studies: Animal Models

Animal models are not intended to mimic the human pain experience. Many models have been developed that allow measurement of neurochemical and neurophysiological mechanisms of nociception and of behavioral responses that likely have relevance to aspects of pain in humans. Models allow for evaluation of output measures before (baseline readings) or at multiple time points following an injury for testing of mechanistic hypotheses that form the basis of novel chemistry and drug discovery. Mogil has suggested that a preclinical pain model is comprised of three basic components: the subjects, the assay, and the outcome measure (Mogil 2009). Each component requires careful consideration in order to optimize potential translational value to the proposed human pain syndrome being modeled.

2 Preclinical Studies: Subjects

The predominant subjects used in preclinical studies within basic science focusing on mechanisms underlying pain, as well as for drug development and testing of proof of concept for improved analgesics are rodents, especially rats and mice. Subjects are chosen to elicit reliable and reproducible responses to represent the pain state of interest and for this reason, most experiments are performed in only a limited number of rodent strains in laboratories around the world. However, it is known that many strains do not respond well to injuries presumed to be eliciting pain syndromes (e.g., nerve injury models of neuropathic pain) (Mogil et al. 1997, 1998, 1999a, b; Yoon et al. 1999). This is especially notable given observations that injuries in humans can lead to chronic pain, but in most cases they do not.

Thus, in patients, the incidence of chronic pain resulting from amputation or coronary artery bypass surgery is estimated to be approximately 30–50 % while Caesarean section or inguinal hernia repair produces an incidence of chronic pain of 10 % (Kehlet et al. 2006). Thus animal subjects that do not develop chronic pain in the setting of injury are likely to more accurately reflect the human condition and important mechanistic insights may be gained by comparisons across strains within a species. Ethical and practical considerations may limit widespread evaluation of animal strains that are "resistant" to the development of chronic pain. Nevertheless, strains demonstrating variable outcomes in response to injury may provide important information regarding mechanisms that are necessary, but not sufficient, for driving pain, chronification of pain as well as for pain relief (Mogil et al. 1997, 1998, 1999a, b; Yoon et al. 1999; De Felice et al. 2011).

3 Preclinical Studies: Pain Assays

Historically, the first pain assays used were observations of behavioral responses to acute administration of a noxious stimulus to the hindpaw, tail, or abdomen. The measured behaviors are typically reflexive withdrawal from the noxious stimulus, or other simple behaviors (e.g., writhing, flinching, licking) that can be easily observed and scored. These assays relate to acute noxious experience in humans, for example touching a hot stove, and have served to elucidate many of the basic neurobiological mechanisms that underlie transduction, transmission, and modulation of acute pain states (Basbaum et al. 2009; Woolf 2011; von Hehn et al. 2012). However, their relevance to persistent or chronic pain states typically found in the clinical setting is limited.

Animal assays of persistent or chronic pain often involve the induction of inflammation and/or nerve injury. These assays are artificial by design, and meant to reveal mechanisms driving pain in the experimental setting. These assays have been successful in dissociating biological mechanisms likely to be associated with inflammation or nerve injury (Schaible et al. 2011; Xu and Yaksh 2011; von Hehn et al. 2012) and provide insight into time-dependent mechanisms of some clinical syndromes. Persistent pain can be elicited by localized administration of noxious inflammatory agents (e.g., capsaicin, formalin, mustard oil), resulting in immediate behavioral responses such as flinching and licking and associated with neurochemical and neurophysiological changes within the spinal cord and the brain. Other agents (e.g., carrageenan, CFA) have diminished immediate effects, but produce longer lasting responses in peripheral tissues and in the central nervous system (CNS) that are characterized behaviorally as hypersensitivity to evoked stimuli (e.g., thermal and tactile stimuli). Such hypersensitivity likely reflects mechanisms of peripheral and central sensitization that can be explored to reveal plasticity and adaptive responses to noxious stimuli (Woolf 2007, 2011). Multiple assays of nerve injury have also been developed with differential patterns of hypersensitivity to evoked stimuli possibly reflecting different driving mechanisms

(Dowdall et al. 2005; Campbell and Meyer 2006; Mogil 2009). The differences between these assays may provide insight into specific injury-induced changes that may reflect clinical observations of differences in pain phenotype. This possibility is supported by differences in pain phenotype characterized clinically by the German Neuropathic Pain Network suggesting the need for individualized treatment strategies in individual neuropathic pain patients (Baron 2006; Backonja and Woolf 2010; von Hehn et al. 2012).

The assays described above are mostly designed to capture mechanisms driving persistent inflammatory pain. In addition, assays have been developed that are designed to mimic, at least in part, disease processes that can produce pain including diabetes, bone cancer or chemotherapy-induced neuropathic pain. Additionally, assays have now been characterized to gain insight into disease-specific mechanisms associated with migraine, bone fracture, osteoarthritis, low back pain, spinal cord injury, pancreatitis, oral cancer, bowel pain, and others (Kesslak and Keirstead 2003; Vera-Portocarrero et al. 2003; Rosenzweig and McDonald 2004; Freeman et al. 2008; Bove et al. 2009; Meng et al. 2011; Okun et al. 2012; Farrell et al. 2014; Mantyh 2014a, b). These assays allow the examination of time-dependent mechanisms associated with the progression of the disease as well as the discovery of disease modifying treatments that may influence pain. For example, Denosumab, a human IgG2 monoclonal antibody with affinity and specificity for human RANKL, has been used to treat cancer-induced remodeling of the bone and was shown in clinical trials to have a strong consequent effect on pain (Honore et al. 2000; Luger et al. 2001; Lipton and Balakumaran 2012; Cleeland et al. 2013). An example of a potentially disease modifying mechanism is anti-NGF antibodies that have shown clinical efficacy for back pain and osteoarthritis (Seidel et al. 2013). Preclinical studies demonstrated that anti-NGF antiserum blocks thermal and tactile hypersensitivity following nerve injury (Ugolini et al. 2007; Wild et al. 2007), fracture pain, and cancer-induced bone pain (Halvorson et al. 2005; Sevcik et al. 2005; Koewler et al. 2007). Another important example is the discovery and development of anti-CGRP antibodies that have been clinically validated for migraine prophylaxis (Peroutka 2014). This work was built on preclinical observations of the cardinal role of CGRP in migraine (Meng et al. 2011; De Felice et al. 2013) and clinical observations that CGRP receptor antagonists were effective in migraine (Olesen et al. 2004; Doods et al. 2007).

4 Preclinical Studies: Pain Outcome Measures

4.1 Reflexive Withdrawal

The most commonly used behavioral measures are easily scored and rely on recording reflexive withdrawal of a limb, usually a hindpaw or the tail in rats and mice (e.g., tail-flick test), in response to exposure to a noxious (e.g., heat, high intensity mechanical) or non-noxious (e.g. tactile) stimulation in an inflammatory

or injured condition. These behaviors correspond to responses to acute nociceptive stimuli in humans. These assays allow for relatively rapid analysis of time-course of drug effect as well as determination of dose response curves for test compounds (i.e., PK/PD relationships). Other measures rely on spinal-bulbospinal reflexes in response to a noxious stimulus (e.g., hot plate test, formalin, acetic acid), such as licking, flinching, biting or scratching, abdominal stretching, or ultrasonic vocalization as measures of pain. However, many of these responses persist in decerebrated animals (Woolf 1984; Xu et al. 1992), indicating that they do not require cortical processing of the nociceptive stimuli, a critical aspect of the pain experience. Therefore, the relevance to understanding mechanisms promoting ongoing pain in humans is questionable (Costigan et al. 2009; Mogil 2009). Of note, it should be emphasized that pharmacological mechanisms that modulate nociception at the spinal level have shown a very strong correlation to efficacy in humans (e.g., opioids, α2 adrenergic agonists, N-type calcium channel blockers, local anesthetics) and that spinal delivery of drugs is an important, and in some cases necessary strategy for management of pain in many patients (Mercadante et al. 2012; Pope and Deer 2013).

Reflexive measures remain important for drug discovery and for initial proof of concept. Their predictive value for translation across species for spinal modulation of acute pain is unquestionable. However, these measures may not sufficiently capture dimensions of pain that are important in humans including affective or cognitive components (Melzack and Casey 1968; Fields 1999). This deficiency has led to increased efforts to develop novel measures based on existing assays in an effort to improve the drug discovery process and to enhance translation of mechanism identified in preclinical studies to clinical settings.

5 Measures of Use and Function

Decreases in use or function of an injured body part likely reflect the presence of either ongoing pain or of tenderness to evoked stimuli. For example, outcome measures in preclinical assays of osteoarthritis (OA) usually rely on evaluation of relative weight bearing of the rat hind limbs following injection of chemicals (e.g. monoiodoacetate, MIA) or surgical damage to the knee (Schott et al. 1994). Whether weight bearing differences reflect evoked hypersensitivity or the presence of ongoing pain is not completely clear. Okun and colleagues (Okun et al. 2012) demonstrated that systemic administration of NSAIDs could reverse weight bearing in a preclinical assay of advanced OA pain but failed to block ongoing pain (see below). Guarding has been used as an outcome measure in assays of post-operative, inflammatory and cancer pain (Schwei et al. 1999; Djouhri et al. 2006; Xu and Brennan 2009, 2010). Notably, guarding behavior has been linked to increased spontaneous activity of nociceptive fibers and dorsal horn neurons in rats with hindpaw incision of skin and deep tissue, linking the behavior with spontaneous neural activity associated with nociception (Xu and Brennan 2009, 2010).

Gait patterns have also been employed in assays of cancer-induced bone pain and in nerve injury-induced neuropathic pain (Schwei et al. 1999; Vrinten and Hamers 2003). These measures provide information on time-course of pain related mechanisms and potential recovery to pre-injury states. However, whether these measures accurately reflect the presence of ongoing pain or hypersensitivity resulting from ambulation is not clear. Finally, these behaviors may also reflect learned avoidance of activities that evoke pain in the hypersensitive injured area.

5.1 Depression of Voluntary Behaviors

Measures of pain-induced suppression of voluntary behaviors as indicators of a more global impact of pain on the animal have been recently introduced and are in the process of being characterized. One approach has been to assess behavioral outcome measures that are ethologically relevant to a social and prey species. These include species-specific behaviors, such as burrowing behaviors in the laboratory rat, proposed to be a measure of pain-induced suppression of an ethologically relevant behavior (Andrews et al. 2012; Huang et al. 2013; Lau et al. 2013; Rutten et al. 2013a, b). Several studies have demonstrated injury-induced decreases in these behaviors that can be reversed by treatments used in the clinical setting. For example, gabapentin reverses nerve-injury (tibial nerve transection) induced reductions in burrowing, and ibuprofen, naproxen, gabapentin, and morphine, reversed inflammation (CFA)-induced reductions in burrowing (Andrews et al. 2012; Rutten et al. 2013a, b). A suggested advantage of burrowing over evoked reflex testing is that drug-induced sedation or motor impairment would further dampen the behavior, rather than produce an increase as would be expected with analgesics, reducing the possibility of a false positive in these instances. Initial characterization of burrowing behavior revealed sensitivity to a wide array of conditions and the behavior was reported to be altered by "anything that affected the well-being of the animal" (Deacon et al. 2001; Guenther et al. 2001; Deacon 2006, 2009). Reductions in burrowing have been reported due to prion disease and Scrapie disease as well as in response to lipopolysaccharide, which induces nausea (Deacon et al. 2001; Guenther et al. 2001; Deacon 2006, 2009). Therefore, further characterization of this behavior is required to demonstrate that alterations in burrowing behavior are specific to pain and not the consequence of other factors such as anxiety, stress, or illness.

Similar confounds are associated with suppression of exploration, particularly of open areas such as the center of an open field. Recent studies have demonstrated increased thigmotaxis, or increased time spent in the peripheral zone close to the walls, in preclinical assays of herpes zoster and nerve injury-induced pain (Huang et al. 2013). Notably, however, this behavior was altered by anxiolytic drugs, such as diazepam, (Huang et al. 2013), raising concerns of specificity of mechanism of action to pain (Ablin and Buskila 2013; Borsook et al. 2013; Finan and Smith 2013; Goesling et al. 2013).

Other studies have reported pain-induced depression of voluntary wheel running behavior (Stevenson et al. 2011; Cobos et al. 2012). Cobos and colleagues recently demonstrated a notable decrease in voluntary wheel running in rats following injection of CFA into the hindpaw (Cobos et al. 2012). An important factor is that the diminished wheel running was observed only following bilateral injection of CFA, likely reflecting the ability of animals that are quadrupeds to compensate by running on uninjured legs. Cobos and colleagues characterized the effects of therapeutically available agents commonly used to treat inflammation-associated pain in patients in the wheel running measure. Opioids were the most effective in blocking the CFA-induced diminished wheel running, followed by corticosteroids and then NSAIDs, wherein diclofenac was more effective than ibuprofen or celecoxib (Cobos et al. 2012). These observations are consistent with the relative efficacy of these drugs in patients (Cobos et al. 2012). An important observation in this study is that the effective dose of ibuprofen that produced recovery of wheel running behavior was approximately 20-fold lower than the dose required to reverse evoked tactile hypersensitivity (Cobos et al. 2012). This is consistent with the 20-fold higher plasma concentrations observed at doses required to reverse tactile hypersensitivity in preclinical assays compared to therapeutic plasma concentrations in humans (Cobos et al. 2012). The higher sensitivity of voluntary wheel running to drug effects compared to reflexive withdrawal measures, such as tactile hypersensitivity, has been suggested to more accurately predict efficacious drug doses in humans. It was acknowledged by the authors that the reduction in wheel running could be due to ongoing pain, injury-induced touch sensitivity, or to avoidance of activity that produced pain (e.g., evoked pain) (Cobos et al. 2012). However, this situation was suggested to be similar to humans where a painful condition may induce a loss of motivation and avoidance of activities that may evoke pain in the injured area or aggravate pain already there (Cobos et al. 2012) reflecting the "everyday pain experience". As this measure required inflammation of both hindpaws to produce a measurable decrease in wheel running that allowed analysis of drug effects or creation of dose response curves, its use may be limited in other types of pain (e.g., nerve injury, trigeminal pain).

6 Affective Pain Measures

Pain in humans is assessed on the basis of its "intensity" by self-report using a variety of rating scales (e.g., VAS, numerical rating scale, etc.). Very few studies assess sensory thresholds in pain patients as a primary endpoint, though such changes are well documented (Rolke et al. 2006; Maier et al. 2010; Pfau et al. 2014). Pain is fundamentally aversive and it is this feature that is the main complaint of patients (Fields 1999; Vierck et al. 2008). Our relative inability to study mechanisms mediating affective, or unpleasant, dimensions of pain in the preclinical setting is likely to have been an important barrier to the discovery of

new medications. Until recently, assays that focused predominately on the unpleasant/affective component of chronic pain were lacking. Collective preclinical success in measuring affective components of pain will likely provide critical complementary information to studies emphasizing sensory neurobiology.

6.1 Measuring the Affective/Motivational Aspect of Pain

A proposed measure of the affective component of pain is the facial grimace scale developed by Mogil and colleagues (Langford et al. 2010; Sotocinal et al. 2011; Matsumiya et al. 2012). Facial expressions were characterized by comparing videos of mice before or after administration of acetic acid known to induce abdominal constriction (Langford et al. 2010). The changes to facial features including orbital tightening, nose bulge, cheek bulge, ear position, and whisker change were coded on a 3-point scale as a measure of pain (Langford et al. 2010). This scale was applied across a variety of preclinical assays ranging from transient pain lasting seconds to pain models lasting minutes to hours to chronic pain models associated with pain lasting days, weeks, months, or longer, with testing occurring 1, 7, or 14 days following injury. While nociceptive stimuli of moderate duration, lasing 10 min to 12 h produced changes within the facial grimace scale (Langford et al. 2010), assays of longer duration such as nerve injury-induced pain did not produce changes in facial grimace (Langford et al. 2010). Lesions of the insular cortex were found to block pain-induced facial grimace but lesions of the amygdala and the anterior cingulate cortex, areas implicated in affective and motivational components of pain by imaging studies (Rainville et al. 1997; Rainville 2002) as well as preclinical studies (Johansen et al. 2001; Nandigama and Borszcz 2003; Johansen and Fields 2004; LaGraize et al. 2004, 2006; Harte et al. 2011; Qu et al. 2011) failed to do so (Langford et al. 2010).

Vocalization after-discharge in response to noxious tail shock has been characterized as a measure of pain affect by Borszcz and colleagues (Borszcz 1993, 1995; Nandigama and Borszcz 2003; Harte et al. 2011; Spuz and Borszcz 2012). Electrical shock-induced avoidance was found to correlate with induction of vocalization after-discharge, but not with shock-induced spinal reflexes (Borszcz 1993) suggesting correspondence with the motivational aspects of the painful shock. Administration of opioids suppressed vocalization after-discharge at doses significantly lower than those required to block vocalization during shock or the spinal motor reflex; however, this was also true with the anxiolytic diazepam (Borszcz et al. 1994). Lesions of the rostral anterior cingulate cortex (rACC) and medial thalamus were found to block shock-induced vocalization after-discharge linking this measure to affective components of pain (Harte et al. 2011). Other key brain sites that play a role in this measure include the amygdala (Nandigama and Borszcz 2003; Spuz and Borszcz 2012), periaqueductal gray (PAG) and ventral medulla (Borszcz 1995), hypothalamus (Borszcz 2006), ventral tegmental area (VTA) (Kender et al. 2008), and parafascicular nucleus (Harte et al. 2005).

Four recently developed measures attempt to capture pain affect in assays of chronic pain. These measures exploit the motivational behaviors elicited by ongoing pain following tissue injury. The place escape avoidance paradigm (PEAP) measures the motivation to escape and avoid unpleasant painful stimulation applied by the experimenter by withdrawing, or moving away from the stimulus, and is based on the assumption that if an organism escapes and/or avoids a noxious stimulus, then the stimulus is aversive to the organism (LaBuda and Fuchs 2001; LaGraize et al. 2004; Fuchs and McNabb 2012; Uhelski et al. 2012). Conditioned place aversion (CPA) to a chamber paired with normally non-noxious tactile stimulation following nerve injury or inflammation injury demonstrates the negative affective component of repeated tactile hypersensitivity (allodynia) (Hummel et al. 2008). Notably, this tactile stimulation-induced CPA was reversed by doses of morphine that did not produce analgesia (Hummel et al. 2008). The self-administration paradigm measures the reinforcing effects of drugs that induce pain relief, and demonstrates that animals in pain will work to acquire pain relief (Martin et al. 2006, 2007). Similarly, conditioned place preference (CPP) measures negative reinforcement associated with removal of the aversive component of pain and corresponding reward from pain relief (Navratilova et al. 2012). These measures have been characterized within several pain assays using various drugs and manipulations known to be clinically effective in modulating the aversiveness of pain in humans (LaBuda and Fuchs 2001; Martin et al. 2006; Hummel et al. 2008; King et al. 2009; De Felice et al. 2013). Moreover, these measures allow dissociation of the affective/motivational and sensory components of pain.

King et al. demonstrated that pairing a context with an effective and rapidly acting pain relieving treatment can produce single-trial CPP (King et al. 2009, 2011; Qu et al. 2011). Pain has a strong emotional component exemplified by its unpleasantness. The unpleasantness of pain serves as the "teaching signal" that leads to avoidance of stimuli that can potentially produce damage to tissues (Johansen et al. 2001; King et al. 2009). Chronic pain can be envisioned as an aversive state that provides strong motivation to seek relief. Moreover, pain relief is rewarding, as indicated by human imaging demonstrating that offset of an acute pain stimulus produces a positive BOLD signal in the nucleus accumbens, an area associated with reward-aversion processing in humans (Becerra and Borsook 2008). Reward achieved from removal of an aversive stimulus produces "negative reinforcement" and is applicable to alleviation of an aversive state induced by chronic pain. Pairing pain relief with a distinct context increases time spent in that context (King et al. 2009). Importantly, conditioned place preference (CPP) to a context that is paired with pain relief is only observed in rats with injury, demonstrating the "unmasking" of ongoing or spontaneous experimental inflammatory or neuropathic pains (King et al. 2009, 2011; Qu et al. 2011).

The concept that pain relief may induce conditioned place preference (CPP) was proposed by Sufka (1994). In this initial study, CPP was observed in rats with hindpaw injection of complete Freund's adjuvant (CFA) following multiple learning (e.g., conditioning) trials with systemic MK-801 but not with indomethacin. Additionally, systemic morphine produced CPP in both CFA and saline-treated rats

suggesting that the effect might not be specific for pain and could result from the inherently rewarding properties of the drug rather than the reward associated with pain relief (Sufka 1994). Reasons for these variable results are not clear but could be related to uncertain kinetics associated with systemic delivery where possible changes in pain state occur at times at which associations with the context are not easily made. Validation of single trial CPP as a measure for detecting ongoing, or non-evoked, pain, has been performed with rats across preclinical assays of experimental nerve injury (i.e., spinal nerve ligation or spared nerve injury) (King et al. 2009), inflammation-induced pain (Okun et al. 2011); incision pain (Navratilova et al. 2012), and in a preclinical assay of osteoarthritis pain (Liu et al. 2011; Okun et al. 2012). In all cases, the route of administration of the treatments were carefully chosen to avoid confounding influences of pharmacokinetics or direct stimulation of the reward pathways.

One important aspect of developing measures with potential clinical translation abilities is validation through comparison with clinical findings and reports. Consistent with observations in the self-administration measure, clonidine, but not adenosine, delivered spinally produced CPP selectively in rats with nerve injury and not in sham operated controls (Martin et al. 2006; King et al. 2009). Such observations support specificity for pain-induced motivational behaviors revealing the presence of ongoing pain and allowing for the study of underlying mechanisms. Notably, spinal administration of ω-conotoxin was also effective in producing CPP selectively in animals with nerve injury. These preclinical observations are consistent with human observations. In a small clinical trial, spinal clonidine was effective against ongoing neuropathic pain (Eisenach et al. 2000; Wermeling and Berger 2006), whereas adenosine blocked secondary hyperalgesia, but did not block ongoing pain (Eisenach et al. 2003). Additionally, ω-conotoxin is marketed as ziconotide (Prialt), as an effective pain reliever in humans. The findings that spinal clonidine and ω-conotoxin produce CPP preclinically suggest that this measure could facilitate translation of new therapeutics.

Axotomy of the sciatic nerve has been useful as an assay for electrophysiological evaluation of injured nerves but has been difficult to study behaviorally as it produces denervation of the hindpaw (Devor 1991, 2009). As a consequence of denervation, it has been difficult to conclusively demonstrate whether ongoing pain is actually present since: (a) ectopically discharging axons in a neuroma are not identified nociceptors, (b) evoked behavioral hypersensitivity following axotomy is difficult or impossible to measure due to denervation, and (c) axotomy-induced autotomy or self-mutilation might be due to loss of sensation in the limb rather than pain (Rodin and Kruger 1984; Devor 1991). However, CPP can be demonstrated selectively in animals with either partial or complete hind paw denervation following either spinal clonidine or RVM lidocaine confirming an aversive state likely reflecting spontaneous neuropathic pain (Qu et al. 2011). These data also suggest that spontaneous pain arises from injured nerve fibers, consistent with findings in humans (Qu et al. 2011). Additionally, these observations in animals with complete denervation of the hind paw also provide an important control, eliminating concerns for pain resulting from tactile stimulation potentially arising

from ambulation within the testing apparatus (Qu et al. 2011). These data cannot address, however, whether additional contributions to spontaneous pain, or evoked hypersensitivity, may result from uninjured, but abnormal adjacent fibers in partial nerve injury assays.

Using the CPP measure, a new preclinical assessment of advanced OA pain was recently reported. Advanced OA pain in patients is associated with a constant dull/aching pain punctuated by short episodes of often unpredictable intense pain (Hawker et al. 2008). Notably, patients with advanced OA pain are resistant to NSAIDs and must undergo joint replacement therapy (Hawker et al. 2008). Within an established and well-characterized preclinical measure of osteoarthritis in which monosodium iodoacetate (MIA) is injected into the intra-articular space of the knee joint, Okun and colleagues demonstrated that commonly used doses of MIA that produce weight asymmetry and hindpaw tactile hypersensitivity failed to elicit ongoing pain (Okun et al. 2012). A higher dose of MIA was required to elicit persistent ongoing pain that is characteristic of advanced OA pain (Liu et al. 2011; Okun et al. 2012). Notably, NSAIDs at a dose sufficient to block MIA-induced weight asymmetry failed to block ongoing pain, observations consistent with patients with advanced OA pain (Okun et al. 2012). Further, the MIA-induced ongoing pain was not blocked by either a TRPV1 or TRPA1 receptor antagonist (Okun et al. 2012), two molecular targets with compounds in clinical development for pain management, including for potential use for OA pain (Honore et al. 2009; Puttfarcken et al. 2010).

An important feature of the PEAP, CPA, self-administration, and CPP approaches is that they are based on the aversive aspect of the injured state. The unpleasantness of pain is perhaps the most important facet of the pain experience. An important aspect of validating these measures as reflecting the affective/motivational aspect of pain is assessing the role of the rACC. Early studies demonstrated that the affective and sensory components of pain are distinguishable, and rely on differential processing within cortical areas. Fields and colleagues initially demonstrated that lesions of the rostral, but not caudal, anterior cingulate cortex blocked CPA to a distinctive context paired with hindpaw formalin, but failed to block the formalin-induced licking and flinching of the formalin treated hindpaw (Johansen et al. 2001). Similar observations have been made in the PEAP and CPP measures of spontaneous/ongoing pain (LaGraize et al. 2004; Qu et al. 2011).

Using the PEAP assay, Fuchs and colleagues demonstrated that bilateral lesions of the ACC did not alter mechanical hypersensitivity induced by tight ligation of the L5 spinal nerve, but significantly attenuated the shift from the dark side of the chamber to the light side of the chamber in response to mechanical stimulation of the injured hindpaw (LaGraize et al. 2004). Further, morphine microinjection into the ACC caused an attenuation of place escape/avoidance behavior with no alteration in mechanical hypersensitivity (LaGraize et al. 2006). A similar dissociation in the affective and somatosensory aspects of nerve injury-induced pain was observed in the CPP measure of SNL-induced spontaneous pain. Lesions of the rACC failed to alter SNL-induced hypersensitivity, but blocked the

SNL-induced spontaneous pain (Qu et al. 2011). Notably, the rACC lesions did not alter CPP to a positive reinforcing agent such as cocaine (Qu et al. 2011).

The importance of limbic system structures in pain processing is supported by human brain imaging studies that have found a positive relationship between the self-reported unpleasantness of experimental pain with ACC activation (Rainville et al. 1997, 1999, 2002; Hofbauer et al. 2001; Rainville 2002). Indeed, the altered perception of unpleasantness in the absence of a change in stimulus intensity correlated with activity in the ACC, but not the somatosensory cortex (Rainville et al. 1997; Rainville 2002). Moreover, brain imaging studies studying basal (non-evoked) activity in patients with chronic neuropathic pain indicate increased activity in the insula and ACC without significant changes in the somatosensory cortices (S1 and S2) (Moisset and Bouhassira 2007), whereas brush evoked allodynia is predominately associated with changes in the lateral thalamus and S1, S2 somatosensory cortices rather than the ACC and insula (Peyron et al. 1998, 2002, 2013; Ducreux et al. 2006; Witting et al. 2006). In addition, patients with cingulotomies report diminished pain related unpleasantness, but discrimination of stimulus intensity or localization of the noxious stimulus was unaltered (Foltz and White 1962; Ballantine et al. 1967; Hurt and Ballantine 1974). The consistency between the preclinical observations of selective blockade of motivational aspects of pain without alteration of behavioral signs of evoked hypersensitivity with these clinical observations strengthen the argument that these assays are capturing affective components of injury-induced pain.

7 Enhancing Discovery Through Improved Animal Models

An animal model reflects variables of subjects, assays, and outcome measures, each of which contributes to the ultimate validity of conclusions about the clinical pain syndrome of interest. It is important that models undergo rigorous validation (e.g., lesion of pain pathways, selective response to clinically effective drugs) for specificity to pain rather than other related conditions (e.g., anxiety, depression). Lack of clarity that the measure is reflective of pain makes interpretation of mechanisms, circuits, and drug effects difficult. Reverse translation may be achieved through characterization of drug effects reflecting clinical experience; one example is the observation that corticosteroids were more effective in restoring CFA-suppressed wheel running compared to NSAIDs demonstrating corresponding efficacy across drug families (Cobos et al. 2012). There should also be evidence for corresponding efficacy across pain syndromes. For example, current treatment guidelines for inflammatory pain states recommend NSAIDs, acetaminophen, or local steroids while those for treatment of neuropathic pain recommend different classes of agents such as serotonin-norepinephrine reuptake inhibitors or pregabalin (Attal et al. 2006; Dworkin et al. 2007; Sarzi-Puttini et al. 2012; Whittle et al. 2012; Ablin and Buskila 2013). Another way to increase confidence in the predictive value of the animal model is through correspondence of clinical

observation of differential drug effects on evoked hypersensitivity and ongoing or spontaneous pain, as was observed in response to adenosine (Eisenach et al. 2003; Martin et al. 2007; King et al. 2009). Finally, the dose range of drug effects should correspond to clinically effective doses, as observed in ibuprofen-induced restoration of wheel running (Cobos et al. 2012), blockade of the PEAP response to mechanical stimulation following incision injury (LaBuda and Fuchs 2001), and blockade of CPA to tactile stimulation following nerve injury or carrageenan by non-analgesic doses of morphine (Hummel et al. 2008). In conclusion, it is clear that mechanistic differences exist between evoked behavioral responses of hypersensitivity and ongoing pain. The mechanisms underlying ongoing pain in preclinical models are currently being explored and may help in the process of filtering targets informing decisions to engage in the transition from drug discovery to drug development for a given target.

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Operant Assays for Assessing Pain in Preclinical Rodent Models: Highlights from an Orofacial Assay

Niall P. Murphy, Richard H. Mills, Robert M. Caudle and John K. Neubert

Abstract Despite an immense investment of resources, pain remains at epidemic proportions. Given this, there has been an increased effort toward appraising the process by which new painkillers are developed, focusing specifically on why so few analgesics make it from the benchside to the bedside. The use of behavioral assays and animal modeling for the preclinical stages of analgesic development is being reexamined to determine whether they are truly relevant, meaningful, and predictive. Consequently, there is a strengthening consensus that the traditional reflex-based assays upon which several decades of preclinical pain research has been based are inadequate. Thus, investigators have recently turned to the development of new preclinical assays with improved face, content, and predictive validity. In this regard, operant pain assays show considerable promise, as they are more sensitive, present better validity, and, importantly, better encompass the psychological and affective dimensions of pain that trouble human pain sufferers. Here, we briefly compare and contrast reflex assays with operant assays, and we introduce a particular operant orofacial pain assay used in a variety of experiments to emphasize how operant pain assays can be applied to preclinical studies of pain.

Keywords Operant · Pain · Orofacial · Testing · Assay · Preclinical

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1 Introduction

Pain is a deeply personal experience that virtually everyone has experienced at some point in their lives, and most people have benefited from its adaptive value. However, there are numerous situations, pathological and otherwise, where uncontrolled pain is counterproductive and debilitating. As such, chronic pain is an epidemic public health problem, costing individuals their well-being and costing society billions of dollars annually. Thus, the quest to find effective and safe analgesics stretches back millenia, and despite the time and resources dedicated to this quest, it is arguable that we still have a limited number of appropriate methods for safely, effectively, and permanently ridding ourselves of pain. Acute pain control is relatively straightforward with the use of nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and local anesthetics; however, the management and relief of chronic pain is much more troublesome, as opiates, the gold standard for care in many chronic pain patients, are liable to inducing dependence, tolerance, and, to some extent, addiction.

So, why are we still without the perfect analgesic? Modern development of analgesics depends on a discovery process that ultimately relies on in vivo testing, which almost invariably assesses the effectiveness of painkillers in experimental animals. Some of these assay methods date back many decades, and while they have certainly been useful in the discovery process, there has recently been considerable interest in re-evaluating the utility of these assays, particularly in terms of their ability to model pain states relevant to the way pain is experienced by people (Vierck et al. 2008). Moreover, as time has passed, the realization that pain is not simply a reflex, but a complex psychological and emotional experience that can derive from a variety

of causes (i.e., nociceptive, inflammatory, neuropathic, or idiopathic) has called for more relevant assays and models that present more than face validity.

2 Pain Assays

The generation of animal models of neuropathic and inflammatory pain mimicking clinical pain symptoms has been steadily increasing. However, in vivo pain evaluation has been susceptible to problems that have hindered the development of models with strong content, face, and predictive validity. Until recently, most preclinical pain assays have consisted of exposing experimental animals to some type of painful stimulus, and then measuring the intensity, frequency, rate, or duration of a behavior believed to reflect the experience of pain. Noxious heat stimulation, such as exposure to a hot plate, has been used extensively, though a gamut of others have been applied over the years, including formalin or acetic acid injection and physical stimulations such as tail pinch and von Frey filaments. Generally speaking, the responses measured in these assays fall into one of four categories (1) simple withdrawal reflexes, such as paw withdrawal; (2) unlearned or innate behaviors, such as licking or guarding; (3) spontaneous behaviors, such as grooming; or (4) learned or operant behaviors, such as lever pressing to receive an analgesic (Chapman et al. 1985). While a brief discussion of the appropriateness of established animal models in pain is warranted here, the reader is also referred to recent reviews for a more in-depth discussion on this specific topic (Mogil 2009; Rice et al. 2008; Vierck 2006). In short, simple withdrawal reflexes and innate responses generally offer the advantage of being relatively reliable and open to objective scoring, but lacking in clinical face validity. This is largely due to an over-reliance on simple spinal reflexes; furthermore, these assays do not consider the complex central neural circuitry responsible for the affective experience, and executive control that animals must have to adapt to pain states. While reflex responses can be evoked in even decerebrate animals (Woolf 1984), their utility is exemplified by the efficacy of a few classes of established analgesics, particularly opioids such as morphine. Aside from the lack of clinical face validity, the one-dimensional nature of the outcome measures for reflex responses (e.g., latency to paw withdrawal) makes them susceptible to misinterpretation or overinterpretation. For instance, many drugs have sedative and/or psychomotor properties that can readily confound any outcome measure that relies solely on a motor response.

3 Operant Pain Assays

Here, we define “operant” as a voluntary behavior modified by the consequences of emitting that behavior, regardless of whether those consequences are positive or negative. For instance, a patient with chronic back pain may report their pain levels differently, if they know their spouse will support them differently (e.g., provide

massages). In this instance, alleviation of the painful state is the consequence of the voluntary report of higher pain. Another example is a person with a history of migraine headaches who is motivated to seek medication, as they have learned such behavior helps alleviate their pain. Importantly, characteristics of operant behaviors are that they are intentional, motivated, learned, and typically involve complex "higher" processing (Vierck 2006).

Conflict paradigms are an example of an instance where animals emit operant responses. For instance, Mauderli and colleagues (Mauderli et al. 2000) subjected rats to an avoidance/escape paradigm by providing them a choice between two compartments—one with a floor set to an aversive temperature (hot or cold), or an alternative escape compartment with a neutral floor temperature, but brightly lit. By determining the time spent in the heated versus the brightly lit compartment, Mauderli and colleagues inferred the amount of pain being experienced by the rats. Reward-conflict assays provide another type of operant testing paradigm and will be further highlighted in this chapter using the Orofacial Pain Assessment Device (OPAD). In a reward-conflict assay, an animal is given a choice between receiving a reward in the presence of an aversive stimulus or choosing not to pursue the reward and avoiding the stimulus.

In terms of pain testing, operant assays are characterized by their integration of the entire neuraxis. A stimulus sufficient to activate primary sensory afferents generates signals that propagate through spinothalamocortical projections leading to a subsequent cortically mediated influence over the behavioral response (Vierck 2006). The integration of these different levels of the nervous system better reflects human pain behavior because the outcomes depend on both nociceptive and motivated, learned processes (Vierck 2006). In this regard, operant assays differ markedly from reflex-mediated (e.g., tail flick) or unlearned behaviors (e.g., paw licking, guarding), in that spinalized and decerebrate animals cannot display these highly integrated pain outcomes (Woolf 1984). As pain is a complex sensation, one needs to utilize comprehensive assays to gain a thorough understanding of underlying mechanisms. Given this, interest in operant measures of pain is increasing, and recent years have seen the development of various approaches, including avoidance, conditioned place preference, escape, and drug self-administration. Some of the most common approaches are summarized in Table 1. As outlined in the table, operant measures typically monopolize on either the desire of animals to avoid or terminate pain (e.g., avoidance/escape, drug self-administration) or their willingness to endure pain for a reward (e.g., the OPAD system). Generally speaking, avoidance/escape paradigms are easier to execute experimentally, are arguably easier to interpret, and are considered by some to be the gold standard in preclinical operant pain testing (Vierck et al. 2008). However, reward-conflict paradigms can offer graded responses because the magnitude of the response can be carefully controlled by titrating the magnitude of the reward against the noxious stimulus conflicting with the reward.

Operant assays offer several advantages in addition to their ability to address the higher central pain circuits that underlie the complex learned and motivated behaviors used by humans to avoid pain. These assays have the ability to reveal

Table 1 Summary of major preclinical operant pain tests

Assay	Description	Outcome measures	Advantages	Disadvantages	Example references
Intracranial self-stimulation (ICSS)	Electrodes implanted into lateral hypothalamus; assess pain-depressed impairment of a motivated behavior	Intensity of applied current or frequency of stimulation	Pain-depressed behavior is representative of human clinical pain	Technically challenging implantation surgery; relatively new pain measure	Pereira Do Carmo et al. (2009) see Ewan and Martin (2013)
Escape test	Animals are given a choice between a non-noxious aversive stimulus (e.g., light) against an alternative noxious aversive stimulus (e.g., temperature)	Time spent in aversive light or on aversive thermal plate	Assessment of thermal pain versus innate behaviors	Observed effects could be due to effects on either of the two conflicting stimuli	Mauderli et al. (2000), Ding et al. (2005)
Dolognawmeter	Animals gnaw through polyethylene foam and ethylene vinyl acetate resin dowels	Time to gnaw through dowels	Use of innate behavior; minimal/no training; measure of deep pain (e.g., muscle, joint)	Vulnerable to drugs that induce gnawing; does not distinguish between pain and dysfunction	Dolan et al. (2010)
Orofacial Pain Assessment Device (OPAD)	Animals are given an opportunity to consume a palatable reward in the presence of a noxious stimulus (e.g., temperature) applied to their face	Reward licking number, face stimulus contact number, reward/stimulus pain ratio	Innate behavior, thermal and mechanical pain assessment	Specific to orofacial region; hairy animals must be shaved for thermal testing; observed effects could be due to effects on either of the two conflicting stimuli	Nolan et al. (2011), Anderson et al. (2013), Neubert et al. (2005, 2008), Rossi and Neubert (2009)

(continued)

Table 1 (continued)

Assay	Description	Outcome measures	Advantages	Disadvantages	Example references
Place conditioning	Animals physically avoid locations in which they experience pain, or gravitate to locations in which they have received relief from pain	Time spent in a physical locality where the stimulus was presented	Can assess both the rewarding and pain relieving properties of stimuli. Animals can be drug free during final testing	Can be confounded by simple reward or aversion effects independent of pain relief	Cahill et al. (2013) King et al. 2009), Sufka (1994) see Navratilova et al. (2013)
Lever pressing	Animals press levers to obtain analgesics	Number of presses made/ willingness to increase effort	Well-established paradigms. Strong content validity	Extensive training required. Easily confounded by motor impairments or psychomotor activation	See Ewan and Martin (2013), Martin and Ewan (2008)

ongoing and spontaneous pain states, such as inflammatory or neuropathic pain. For instance, a given drug may have no inherent motivating properties in an animal that is not in pain, and therefore animals will not seek out places where they have experienced this drug. In psychological parlance, they do not show a "conditioned place preference". In contrast, animals in pain will seek out such a place, thus revealing that they are in a pain state. Secondly, given the complex nature of operant measures, animals are afforded the opportunity to develop flexible response strategies for avoiding pain. For instance, animals may choose to expose themselves to pain in short frequent bursts, rather than in long occasional bursts to obtain the reward. This response flexibility more closely mimics the pain states of humans who also must develop novel strategies (e.g., frequently sitting down to avoid arthritic pain) to allow them to adapt and continue their lives. Thirdly, some pain-related responses consist of complex cognitive aspects that can potentially only be uncovered by operant assays in experimental animals. Examples of this could include social aspects, placebo effects or the ability of complex environmental sensory stimuli, such as noise or odor, to interfere with pain responding. Fourthly, as operant pain assays are often fully automated, they fully remove observer bias. In addition, in the context of studying orofacial pain and trigeminal neurobiology, these assays do not require restraining animals, which is necessary for some of the more traditional reflex tests, likely significantly reducing the contribution of stress and stress-induced analgesia. Finally, the dynamic nature of the assay may allow rapid and automated determination of otherwise laborious measures such as the temperature at which 50 % responding (i.e., an ET_{50}) occurs, as pain stimulus intensities can be controlled rapidly and titrated to instantaneous responding of animals.

However, operant assays are not without limitations. Firstly, their complex cognitive nature often requires special consideration of relevant and sometimes confounding psychological processes, such as learning, memory recall, anxiety, attention, salience, motivation, and reward efficacy. Particularly in the case of reward-conflict paradigms, two opposing processes are integrated, making it difficult to ascribe any change in behavioral responding to a specific process. To some degree, a well-designed experiment with adequate controls can tease these effects apart, but this may require a larger commitment of resources. Indeed, much of the central neural circuitry mediating affective states associated with pain, or the relief from pain, is shared with other affectively-laden stimuli, such as food and drugs. Secondly, the effect of drugs on motor systems requires consideration. Though, locomotor effects are generally more readily recognized in operant assays than traditional reflex assays, and can be more easily avoided by virtue of the higher sensitivity of the assay. Thirdly, relative to reflex-based assays that can be performed with equipment as simple as a water bath or a syringe and hypodermic needle, operant assays often require specialized equipment, such as Skinner boxes and computers, and more space. Fourthly, in comparison to reflex assays that measure innate unconditioned responses, operant assays rely on learned and conditioned behaviors that can require extensive training before reaching a stable baseline.

4 The Orofacial Pain Assessment Device (OPAD)—a Preclinical Operant Pain Assay

We have introduced preclinical operant pain assays and compared them with traditional reflex assays. The remainder of this review will highlight a new preclinical operant pain assay recently developed in our laboratory. This assay uses an Orofacial Pain Assessment Device (OPAD) and was conceived to address many of the concerns outlined above and, consequently, offers several advantages over reflex pain assays. We have completed a series of studies in both rats and mice using the OPAD (and previous prototypes) to demonstrate its utility.

At the heart of the OPAD is a reward-conflict assay whereby rodents express their willingness to withstand thermal pain applied to their face in order to gain access to a palatable liquid reward, such as sucrose solution or sweetened condensed milk. Thus, the primary outcome measure is the number of successful lick events. The device applies mild noninjurious temperatures (typically in the range 8–48 °C) using two "thermodes" against which the animal must place its muzzle to access the reward. The Peltier-based thermode can be adjusted in several ways, including width—to accommodate the size of the animal (e.g., from mouse to rat) and temperature—using a computer-controlled delivery of either static or dynamic stimulus temperatures. Additionally, we have added nickel titanium wires in front of the thermodes that provide sharp, punctate mechanical stimulation to assess mechanical allodynia and hyperalgesia (Nolan et al. 2011). This utilizes the same pain outcomes across different stimuli modalities (i.e., thermal vs. mechanical) and enables direct comparison of the relative impact of each stimulus on operant pain behavior. We recently published the detailed methodology and practical aspects of setting up, programing, and using the OPAD that includes a video reference (Anderson et al. 2013).

Aside from the above, the OPAD has several additional desirable features. In particular, the OPAD can detect failed access attempts to the reward by recording when thermode contacts are made without the animal successfully obtaining the reward. These failed attempts provide an indirect measure of motivation and pain sensitivity. Secondly, as outlined below, the OPAD produces a robust and smooth stimulus response curve, unlike reflex-based assays that depend largely on threshold behaviors. Thirdly, the assessment of pain-related outcomes can be exported in computer data files in a ready-for-analysis format.

5 Summary of Experiments Using OPAD

The breadth of studies completed using the OPAD demonstrates the range and utility of this system for assessing pain and analgesics. We have applied the OPAD to study the most commonly used pain models, including inflammatory pain (Neubert et al. 2005, 2006), neuropathic pain (Rossi et al. 2012), central pain

(Caudle et al. 2010), and chemotherapy-induced peripheral neuropathic pain (Mustafa et al. 2013). With these models, we have investigated several hypotheses related to physiology (Neubert et al. 2008), expectations (Nolan et al. 2012), environment (Rossi and Neubert 2008), and pharmacological treatments (Chapman et al. 1985; Neubert et al. 2005; Caudle et al. 2010; Mustafa et al. 2013; Kumada et al. 2012; Rossi et al. 2009). A summary of studies to date that have utilized the OPAD is provided in Table 2. Independent investigators have recently adopted OPAD-type methodology to complete studies relating to cold sensitivity via transient receptor potential (TRP) channel modulation and nerve injury, (Zuo et al. 2013; Cha et al. 2012) and we anticipate further increases in research employing the OPAD. The following five examples illustrate the many studies completed using this operant orofacial reward-conflict paradigm and highlight the breadth of research that can benefit from this type of behavioral assessment.

6 Study 1: Relating Behavior to Physiology—Characterization of Mouse Orofacial Pain and the Effects of Lesioning TRPV1-Expressing Neurons on Operant Behavior

A particularly important but difficult aspect of behavioral studies is relating the behavior of an animal back to the function of specific cells and proteins. As highlighted above, reflex assays generally depend on thresholds for initiating a response. Consequently, once the threshold is crossed, the difference between stimulus intensities is lost as they all evoke the expected motor response. Even if the precise force or temperature of the stimulus on the skin is known, there is little difference in behavioral response characteristics that can distinguish the stimuli. Thus, correlation of reflex behavior with cell or protein function, which can be measured with great precision, is handicapped by the floor effect of the behavioral assay. In essence, once the reflex is initiated the sensory stimulus is irrelevant. In reward/conflict operant assays, behavioral responses to the stimulus are modified in a more graded fashion by animals in response to the stimulus intensity, the stimulus unpleasantness, and the desire of the animal to acquire the reward. Furthermore, strategies to obtain the reward may change as the variables change and these strategies are easily and distinctly measured from the strategies used to evaluate the response to nonaversive stimuli. For example, as the OPAD thermode temperature moves into the aversive range, animals reduce the amount of time that they press their muzzle onto the thermodes, yet they increase the total number of times that they contact the thermodes. The new strategy results in consumption of a similar amount of the reward solution in the aversive conditions, but a clear difference in the duration and number of stimulus contacts.

Table 2 Examples of operant orofacial pain studies. Bolded references indicate additional information is provided in the text

Study design	Results	Reference
Substance P-Botox A conjugate; ICM; CIPN	Control animals developed thermal sensitivity post-paclitaxil treatment; SP-Botox A animals demonstrated analgesia	Mustafa et al. (2013)
N-INF; thermal ramping protocol	OPAD methodology, online video available	Anderson (2013)
High-fat or regular diet; C57Bl/6 J, SkH1-E mice	No effect of diet-induced obesity on acute thermal nociception in the absence of inflammation or injury	Rossi et al. (2013)
Trigeminal CCI; thermal, mechanical	Development of cold sensitivity and aversive mechanical behaviors after infraorbital nerve injury	Rossi et al. (2012)
Conditioning with morphine or PBS	Placebo effect produced in morphine-conditioned animals; naloxone reversed placebo response	**Nolan et al.** (2012)
Chronic morphine administration to induce tolerance	Chronic opioid use induced changes in NMDA receptor composition; differential pain sensitivity based on NMDA subunit change	Anderson et al. (2012)
Botox A; intramuscular; CCI	Botox A blocks development of CCI-induced heat hyperalgesia	Kumada et al. (2012)
N-INF; morphine; mechanical, thermal	Thermal versus mechanical stimuli with same outcome measures; demonstration of mechanical sensitivity using varying diameters of nickel titanium wire	Nolan et al. (2011)
Naïve; sucrose and sweetened condensed milk reward	Differing concentrations of noncaloric (sucrose) versus caloric (sweetened condensed milk) rewards can modify operant pain outcomes	Nolan et al. (2011)
SP-CTA; ICM; Naloxone; rats and mice	Central pain model (SP-CTA); naloxone sensitive; mu-opioid receptor knockout mice display sensitivity; endogenous opioid system implicated	Caudle et al. (2010)
Naïve; face place preference	Motivated behavior can be modulated based on hot/cold face preference	Rossi and Neubert (2009)
Icilin; ICM	Comparison of TRPA1/TRPM8 agonism on cold and heat sensitivity; low dose icilin (0.025 mg) induces cold sensitivity, but decreases heat sensitivity	Rossi et al. (2009)
RTX; TRPV1 KO and wild-type mice (C57BL/6 J, SKH1-)	Comparison of effects of pharmacologic and genetic TRPV1 manipulation on heat sensitivity; first mouse paper using orofacial operant assay	**Neubert et al.** (2008)
Sex differences; heat sensitivity	Males were hypersensitive to nociceptive heat	Vierck et al. (2008)

(continued)

Table 2 (continued)

Study design	Results	Reference
Environmental enrichment; general activity (rearing)	Environmental enrichment reduces exploratory behavior and increases pain thresholds (reduces sensitivity)	**Rossi and Neubert** (2008)
Mu and Kappa opioid agonists; N-INF; rearing	Comparison of reflex versus operant measures; demonstrated sensitivity of operant assay; GR89,696 ineffective for operant pain reduction	**Neubert et al.** (2007)
Naïve; menthol	Less robust stimulus response in the cold temperature range compared to heat; menthol can produce cold sensitivity	Rossi et al. (2006)
N-INF; morphine	Capsaicin-induced allodynia versus hyperalgesia demonstrated with varying temperature	Neubert et al. (2006)
INF; morphine	First paper describing the orofacial operant assay; inflammation produced thermal hyperalgesia reversed by morphine	Neubert et al. (2005)

Botox A Botulinum neurotoxin A
CCI Chronic constriction injury
CIPN Chemotherapy-induced peripheral neuropathy
ICM Intracisternal injection
INF Inflammation (carrageenan)
N-INF Neurogenic inflammation (capsaicin)
PBS Phosphate buffered saline
RTX Resiniferatoxin
SP-CTA Substance P-Cholera Toxin subunit A

An example of the above is shown in the previously unpublished data in Fig. 1, which illustrates the longest thermode contact as a function of thermode temperature (Neubert et al. 2008). The altered strategy produces a smooth sigmoidal stimulus response relationship. Figure 1 also shows the stimulus response relationship for the current evoked by heat through transient receptor potential vanilloid 1 (TRPV1) channels. The inward currents evoked in response to heating the buffer solution were measured by whole cell voltage clamp (−60 mV) in HEK293 cells that were transiently transfected with TRPV1. Previous work demonstrated that TRPV1 knockout mice exhibit a rightward shift in their stimulus response profiles indicating that the noxious range of 42–48 °C is likely mediated by TRPV1 receptors (Neubert et al. 2008; Mitchell et al. 2014). The effect of temperature on behavior and neurophysiological responses to heat are correlated (r^2 = 0.82, p = 0.036) suggesting that TRPV1 may transduce the stimulus that initiates the change in behavior. It is clear from Fig. 1 that the protein is activated at approximately the same temperature that animals start to reduce their contact time with the stimulus probes. As the temperature increases, the current flowing through the ion channel increases and the animals demonstrate a proportional decrease in their stimulus contact times. In thermal-evoked reflex assays, the temperature initiating the behavioral response is typically unknown and the behavioral response is

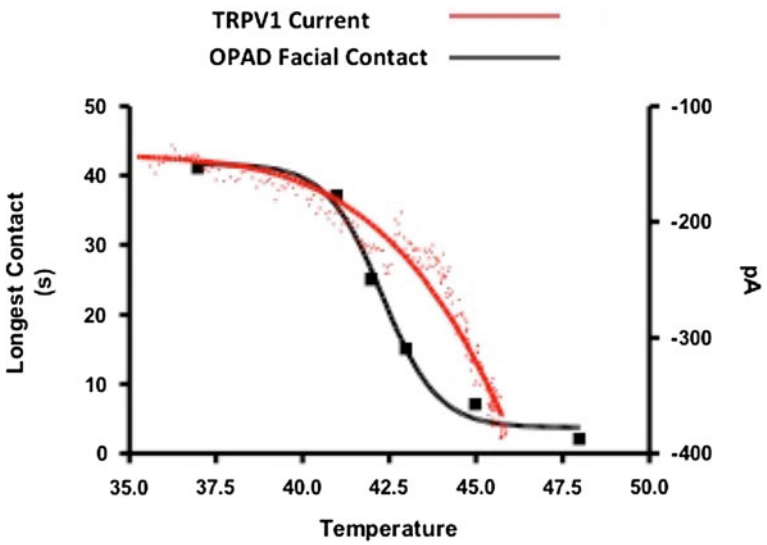


Fig. 1 Relationship between operant behavior and physiology. A representative temperature/current relationship for TRPV1 (*red*) was plotted with the temperature/response relationship for rats' longest contact with the thermal stimulus while performing in the OPAD (*black*). TRPV1 currents were obtained from whole cell voltage-clamped HEK293 cells that were transiently transfected with TRPV1. The bath solution was slowly raised as the current was monitored at −60 mV. The OPAD data were collected in separate experiments at multiple temperatures to evaluate pain tolerance (longest contact duration during a 10 min session)

measured only by response latency. In one of the few studies to examine skin temperature as it relates to reflex initiation, Hargreaves and colleagues found that hind paw stimulated reflexes in normal animals were evoked when skin temperatures reached approximately 45 °C (Hargreaves et al. 1988). As Fig. 1 illustrates, 45 °C is well above the temperature at which the TRPV1 ion channels are engaged and the animals begin to make behavioral adaptations in the operant assay. Thus, the graded responses and adaptive behaviors of rodents in operant assays provide more information with which to correlate behavior with protein or cell function than reflex-based assays.

To further evaluate the role of TRPV1 in operant pain, we used the OPAD system to evaluate thermal sensitivity after pharmacological intervention or gene deletion mutant mice (Neubert et al. 2008). Figure 2a shows that wild-type C57BL/6 J displays a typical thermal stimulus response, in that reward licking events decreased significantly as the stimulus temperature reached noxious temperatures (≥45 °C). TRPV1 KO mice (Fig. 2c) were insensitive to the thermal stimulus through the temperature range corresponding to TRPV1 activity, as displayed by the relatively flat response up to 52 °C (Neubert et al. 2008). Both genotypes showed a significant decrease in reward licking events at 55 °C (Fig. 2b, c), a temperature mediated by TRPV2 (Caterina et al. 1999). When wild-type C57BL/6 J animals were treated

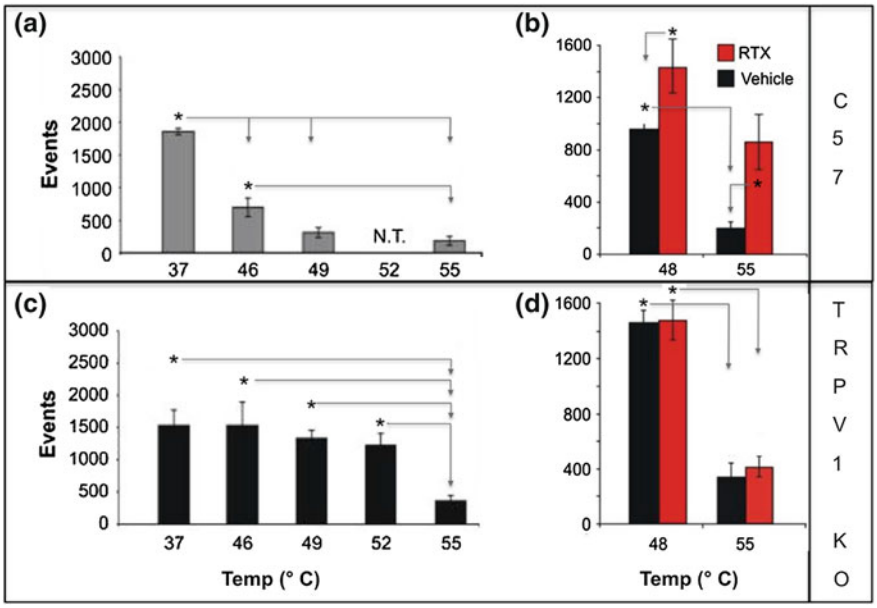


Fig. 2 Use of genetic and pharmacological manipulation of TRPV1 to assess the relationship between physiology and pain operant behavior in mice. **a** Operant reward licking events in naïve wild-type C57BL6 J mice decreased as stimulus temperature increased. *N.T.* not tested. **b** Wild-type C57BL6 J mice injected intracisternally with the TRPV1 agonist, RTX, had significantly higher licking events compared to vehicle-treated animals at both 48 and 55 °C. **c** TRPV1 KO mice were relatively insensitive to temperatures ≤52 °C, as their responses in the noxious heat range of 46–52 °C produced responses similar to baseline 37 °C testing conditions. **d** TRPV1 KO mice treated with RTX responded similarly to vehicle-treated KO mice at both 48 and 55 °C. Data are shown as mean ± S.E.M. * = $P < 0.05$. This figure was reproduced and modified from a previously published study (Neubert et al. 2008)

intracisternally with resiniferatoxin (RTX), a potent TRPV1 agonist used to molecularly lesion TRPV1-expressing neurons, the response increased significantly such that the C57BL/6 J animals resembled KO animals at 48 °C (Fig. 2b). As expected, RTX had no effect on TRPV1 KO animals (Fig. 2d). Interestingly, the C57BL/6 J RTX-treated animals also showed insensitivity at 55 °C, which we hypothesize is due to lesioning of TRPV2 neurons that coexpress TRPV1 (unpublished data).

These studies provide strong, direct evidence that the behavioral measures assessed using the OPAD are linked to pain processing. For example, setting the thermal stimulus at temperatures that are noxious (>42 °C) elicits the expected avoidance behavioral strategy. Notably, the ability to precisely control stimulus temperature with a ± 0.1 °C tolerance allows us to finely discern pain responses at 42 °C, the lower limit of TRPV1 activation.

7 Study 2: Action of an Established Analgesic (Morphine) on OPAD Operant Responses

Despite the immense cost involved with developing novel analgesics, only a few established classes of drugs—including antiepileptics, acetaminophen, opioids, and NSAIDs—remain staples for pain control. To validate the OPAD, we sought to demonstrate that morphine is an effective agent for increasing operant behavioral outcomes and is reflective of analgesic responses (Neubert et al. 2005, 2006, 2007; Nolan et al. 2012; Anderson 2012). Importantly, morphine at doses as low as 0.25 mg/kg can produce significant antihyperalgesic effects, and doses as low as 2 mg/kg can produce analgesia. We reasoned that the relatively high sensitivity of the OPAD allows for detection of responses in dose ranges that are clinically relevant (humans require approximately 0.15 mg/kg for pain relief) (Plone et al. 1996). The vast majority of reflex-based measures typically require 5- to 50-fold higher doses of morphine (5–50 mg/kg) to detect an "analgesic" response. At these high doses, confounding behavioral responses, such as sedation or hyperlocomotion, are likely to occur. This is important when considering the predictive value and translatability of these models and methods to human clinical care.

To illustrate the efficacy of low doses of morphine to reduce hyperalgesia, Fig. 3 shows results from two individual naïve animals given either phosphate buffered saline vehicle (PBS, Fig. 3a) or morphine (2 mg/kg, Fig. 3b). These are typical examples of OPAD output response tracings for nonanalgesic and analgesic treatments, respectively. The figure shows the use of a ramping protocol whereby animals are initially tested at 32 °C for 2 min, before the thermode is ramped to 43 °C, held there for 10 min, and then returned to 32 °C for 2 min. These data show that both animals completed the operant task when the thermodes were set to the neutral temperature (32 °C), but only the morphine-treated animal could maintain this behavior when the temperature reached nociceptive (43 °C) levels. Note that individual events (e.g., licks, and stimulus contacts) can be patterned over time to generate a complex behavior based on the external stimulus and internal processing of the animal as they form their response during the session. Nonetheless, the overall pattern quickly and simply distinguishes a painful from a nonpainful response.

8 Study 3: The Effect of Environmental Enrichment on Thermal Sensitivity in the OPAD Assay

The experience of pain is influenced by numerous factors, including molecular makeup (e.g., C- vs. A-delta nociceptors), genetics, sex, and epigenetics. Pain and stress are tightly related, and studies show that acute and chronic stress can modulate nociceptive responses (King et al. 2003, 2007; Gameiro and Gameiro 2006; Gameiro 2005; Khasar et al. 2005; Butler and Finn 2009; Olango and Finn 2014).

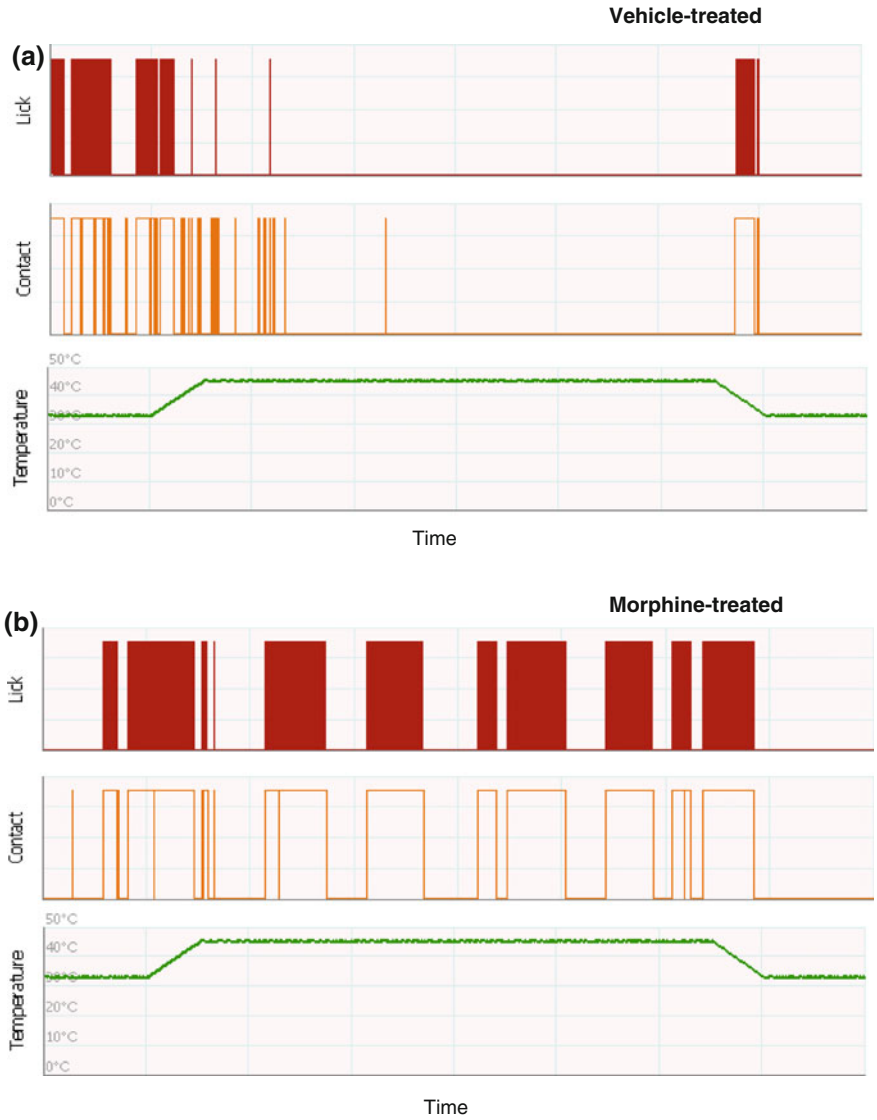


Fig. 3 An example of an analgesic versus painful response measured in the OPAD. The three traces in each panel display reward licking events (Lick), thermode contact events (Contact), and thermode temperature (Temperature). **a** Rat treated with vehicle (PBS) 30 min prior to testing. **b** Rat administered morphine (2 mg/kg, i.p.) 30 min prior to testing. These are typical traces for an animal that displays a normal "nonanalgesic" (**a**) and an "analgesic" (**b**) pain response. The stimulus was held at 32 °C for 2 min, ramped to 45 °C and held for 10 min, and then ramped back down to 32 °C. Note that both animals have similar responding during the lower neutral temperature period, characterized by long bouts of licking and few, but long, stimulus contacts. As the temperature becomes painful (45 °C), only the morphine-treated animal maintained responding

A straightforward approach to modify stress in laboratory animals is to change their housing to include environmental enrichment. Environmental enrichment allows for supplemental cognitive and physical tasks with additional sensorimotor activity (Duan et al. 2001) that can improve cognition and reduce anxiety (Benaroya-Milshtein et al. 2004; Nilsson et al. 1999). Conversely, isolation and environmental deprivation may increase or decrease pain, in the form of stress-induced hyperalgesia (Becker et al. 2006) or stress-induced analgesia (Coudereau et al. 1997; Puglisi-Allegra and Oliverio 1983), respectively. Given that operant assays depend on affective processes, we assessed the role of environmental enrichment on pain behavior. This was accomplished by evaluating two cohorts of Sprague Dawley rats, the first housed under standard conditions with two animals per cage and no enrichment, and the second housed in groups in an environment enriched with crawl spaces, chew toys, and an exercise wheel. The enriched group was also provided opportunities for increased social interactions that may be important for reducing stress and pain (Will et al. 2004; Pham et al. 2002). When evaluated for general exploratory (rearing) behavior (Fig. 4a, b), we found that rats in the enriched environment reared significantly less than the environmentally deprived animals (Fig. 4c). Over a range of temperatures (2–48 °C), environmentally enriched animals exhibited a significantly lower reward licking/stimulus contact ratio compared with the deprived rats (Fig. 4d).

A growing trend in pain management is the search for alternatives to pharmacotherapy for chronic pain control. Biofeedback, behavior modification, and stress relieving techniques are among these alternatives. Given the cognitive dependence of many of these techniques, it becomes important to utilize assays that depend on these cognitive processes. These data support the idea that environmentally enriched animals were both less stressed and displayed less pain than their deprived counterparts. Therefore, a change in living conditions may have an effect similar to a drug. Certainly, this is only one of many possible explanations regarding the role of environmental enrichment, but these results indicate that there may be alternatives to pharmacotherapy, such as cognitive-based techniques that rely on stress control that may be effective for pain control. Use of operant assays can better incorporate cognitive processes governing pain and allow for the evaluation of these pain management strategies.

9 Study 4: Effects of Mu- and Kappa-2 Opioid Receptor Agonists on Pain and Exploratory Behaviors

We have highlighted the differences between reflex and operant assays, but it is vital to address how these disparities may impact drug evaluation. We addressed this question by directly comparing the response in a thermal hindpaw withdrawal assay with the OPAD thermal operant assay. We used rearing as an index of exploratory activity to compare drug effects on general behavior. This also provided a secondary metric, in addition to analgesic potency, to assess dose selection in the pain assays.

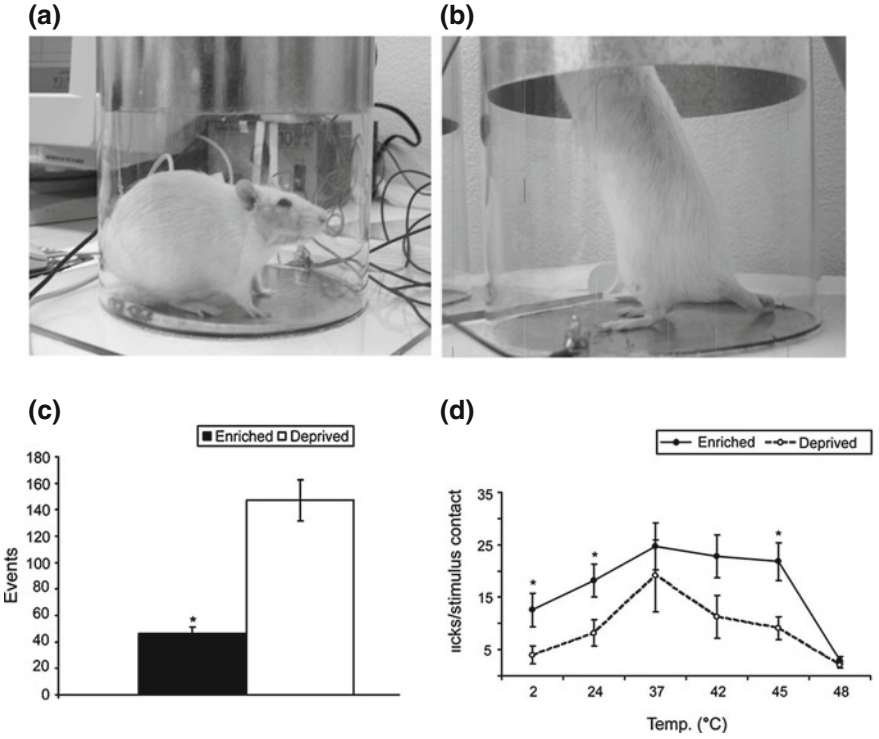


Fig. 4 The effect of environmental enrichment on general exploratory and operant pain behavior. Rats were housed either in standard Plexiglass cages (2/cage) or communal housing (3/cage) in an enriched open environment consisting of a metal cage containing cardboard boxes, two shelves, a hammock, PVC tubing, chew toys, and an exercise wheel. Vertical locomotion (rearing) behavior was automatically recorded and expressed as the number of reaching events in a 10 min session. **a** and **b** Representative images show a rat at rest and rearing in the cylinder. **c** Rats in enriched housing had significantly fewer rearing events compared with the deprived rats in standard housing. Data are shown as mean ± S.E.M., $*P < 0.05$. **d** When tested for orofacial pain sensitivity across a wide range of temperatures, there was a significant main effect of housing condition on the reward licking/stimulus contact ratio. Overall, the enriched animals had a higher reward licking/stimulus contact ratio, indicative of lower pain relative to the deprived group. Data are shown as mean ± S.E.M. $*P < 0.05$. This figure was reproduced from a previously published study (Rossi and Neubert 2008)

We compared the efficacy of the mu-opioid receptor agonist, morphine (0.5–5 mg/kg, s.c.), and the kappa-2 opioid receptor agonist, GR89,696 (0.000125–1 mg/kg, s.c.). We found that all doses of GR89,696 tested, except 0.000125 mg/kg, significantly reduced rearing (Fig. 5). At higher doses, catatonia was observed. Morphine at higher doses (≥2.5 mg/kg) also significantly reduced rearing, seemingly due to sedation.

For the reflex assay, the highest dose of each drug (1 mg/kg GR89,696 or 5 mg/kg morphine, s.c.) was required to observe an analgesic response in the hindpaw withdrawal test (Fig. 6a, b). In comparison to the rearing data shown in Fig. 5, the delayed response in the reflex pain assay may be due to factors other than pain relief,

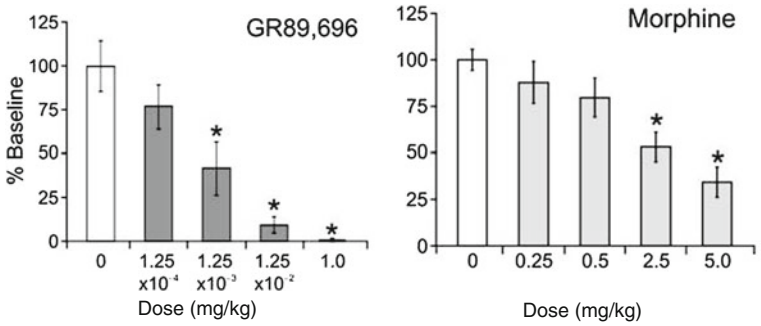


Fig. 5 The effects of the kappa opioid receptor agonist GR89,696 and mu-opioid receptor agonist morphine on rearing activity as a measure of general exploratory behavior. There was a significant dose-related decrease in the number of rears following GR89,696 or morphine administration compared with baseline values. Data are shown as mean ± S.E.M (*P < 0.05 versus vehicle [0] treated animals). We identified the decrease in locomotor activity as a potential confounding issue as animals are required to be mobile and motivated to complete operant testing. This figure was adapted from previously published data (Neubert et al. 2007)

such as general motor sedation, that leave the animals incapacitated. Conversely, a relatively low dose of morphine (0.5 mg/kg) can be antihyperalgesic against capsaicin-induced thermal hyperalgesia and sensitivity (Neubert et al. 2005, 2006). Based on the rearing behavior of animals dosed with GR89,696, and that animals would remain in whatever position they were placed in the OPAD after higher dose GR89,696 administration, we used only the lowest dose (0.000125 mg/kg) of GR89,696 to complete the operant task (Fig. 6c). As with the previous studies using morphine, the TRPV1 agonist capsaicin was used to produce a pain-depressed behavior by decreasing operant outcomes (e.g., licking reward/stimulus contact ratio). Pain-depressed behavior is characterized as a decreased response to a noxious stimulus (Negus and Bilsky 2010; Pereira Do Carmo et al. 2009). To be considered analgesic, GR89,696 should block this pain-depressed behavior as reflected by an increased lick/face ratio; however, we observed no such change, indicating a lack of analgesic effect at a dose that does not affect general behavior (Fig. 6c). These data emphasize the necessity of multifaceted approaches for drug evaluation and reveal how drugs and treatments can affect nonpain-related processes to alter the assay outcome itself and yield false positives.

10 Study 5: Placebo-Induced Analgesia in an Operant Pain Model in Rats

There has been much recent interest in psychosomatic effects and the role that expectancy states play in perceived experience and compliance (see for example, Wilson 2010; Horwitz and Horwitz 1993). In particular, the placebo effect has

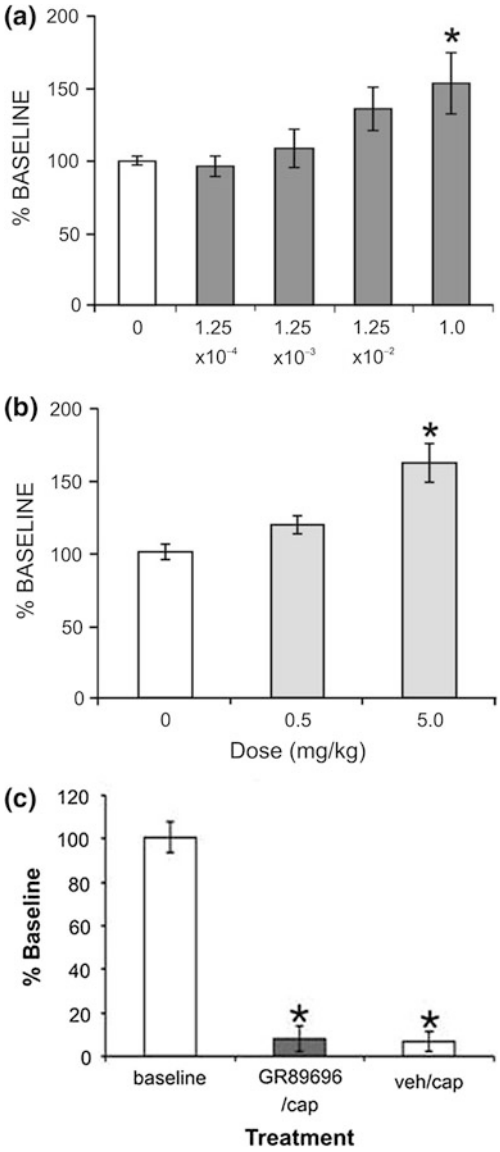
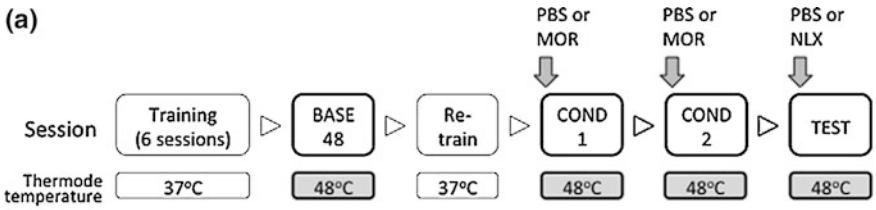


Fig. 6 Discordant responses between reflex- and operant-based pain behaviors following opioid receptor activation. To test reflex responses to thermal pain stimuli, we administered (**a**) GR89,696 or (**b**) morphine and then measured the hindpaw withdrawal latency of the animals. There was a significant increase in withdrawal latency only at the highest doses tested for GR89,696 and morphine. These doses severely impair general exploratory behavior, as shown in Fig. 5. **c** Animals treated with either GR89,696 (0.000125 mg/kg, subcutaneous) or PBS (veh, subcutaneous) prior to facial capsaicin (cap) application did not significantly differ from each other when evaluated using the reward licking/facial stimulus operant pain outcome measure. Note that a direct comparison of %baseline values between panels A/B and C is not possible, as different pain assays are used. Both groups were significantly lower (*$P < 0.05$) than baseline values. Data are shown as mean ± S.E.M. Figure adapted from previously published data (Neubert et al. 2007)

garnered much interest because pain is particularly susceptible to placebo-induced analgesia and likely makes significant contributions to genuine therapeutic effects in humans. Indeed, if such effects could be harnessed, they would be highly beneficial (Price et al. 2008). Expectancy states, such as the placebo effect, partially rely on higher levels of brain circuitry that underlie learning, prediction, and affective experience (see Price et al. 2008). The emotional components of pain are of particular interest here because they are one of the most debilitating aspects for chronic pain sufferers (Merskey 2007; Treede et al. 1999). Thus, we hypothesized that operant assays would be particularly effective at detecting placebo-induced analgesia. To test this hypothesis with the OPAD, we trained rats to expect morphine-induced analgesia, and then administered a placebo. Indeed, our results show that that rats exhibit placebo-induced analgesia (Fig. 7), and the characteristics of this analgesia bore many similarities with that seen in humans (Nolan et al. 2012). Namely, we found a strong inter-animal variability in the response and a significant positive predictive relationship between the genuine analgesic effect of morphine and the placebo effect; furthermore, this effect was suppressed by the opiate antagonist naloxone. These data suggest that operant assays are particularly well suited to probe higher brain circuits that underlie cognitive and affective functioning.

11 Do Results Using the OPAD Correspond with Reflex-Based Assays?

The search for better analgesics comes down to our belief and trust that preclinical assays truly reflect the human condition. One must be wary not to overly anthropomorphize findings in animal models and infer that an animal is feeling or experiencing those same emotions that encompass the affective aspect of pain experienced by humans. Even in humans it can be challenging to assess pain using standard assays, such as VAS given the large between-subject variability. Therefore, attempts have been made to rationalize designs that encompass as many pain pathways (i.e., primary sensory afferents, spinothalamic tract, cortex) as possible in preclinical models in the hope that this corresponds to the neurobiology of pain in humans. We and others (Vierck et al. 2008; Mogil 2009; Mogil et al. 2010; Negus et al. 2006) have argued that reflex-based assays generally fall short of this requirement and are inadequate for preclinical pain assessment compared with operant assays. Indeed, there are several examples of discordance when evaluating a drug using a reflex versus an operant assay. Previous studies in the field of social isolation demonstrated an increased pain threshold (i.e., lower pain) response in reflex tests that was hypothesized to be due to increased endogenous opioid levels (Coudereau et al. 1997; Tuboly et al. 2009). But, in our operant study, animals deprived of environmental enrichment showed relative increases in pain sensitivity (i.e., more pain) when tested across a range of temperatures. Additionally, previous preclinical studies of kappa opioid receptor agonists assessed by reflex-based measures were promising (Pasternak 1980); however when



(b)

Treatment group	Treatment prior to COND1 and COND 2	Treatment prior to TEST
PBS → PBS (n = 8)	PBS	PBS
MOR → PBS (n = 19)	MOR	PBS
MOR → NLX (n = 10)	MOR	NLX

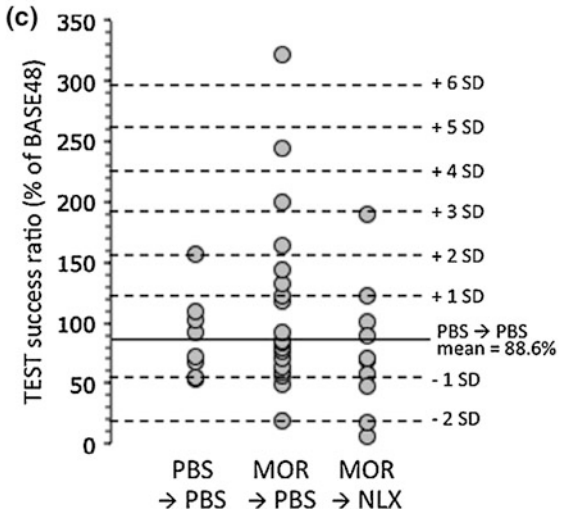


Fig. 7 Placebo-induced analgesia in an operant pain assay. **a** Schematic representation of the behavioral paradigm used for studying placebo effects. Boxes represent exposure to training in the OPAD. Variations of phosphate buffered saline (PBS) vehicle, morphine (MOR, 1 mg/kg), or naloxone (NLX, 5 mg/kg) were administered 30 min prior to each of the 3 conditioning [COND] sessions. **b** Summary of treatment groups studied. **c** Operant licking responses for palatable reward in the OPAD in individual rats during the TEST session. The solid horizontal line indicates the mean response in the PBS → PBS group. There were an increased number of animals with high responding in the MOR → PBS group, which was reversed by administration of naloxone. This figure was reproduced from a previously published study (Nolan et al. 2012). The figure has been reproduced with permission of the International Association for the Study of Pain® (IASP). The figure may NOT be reproduced for any other purpose without permission

tested in the OPAD operant assay, it was clear that these compounds had confounding locomotor properties, and ultimately clinical testing of these same compounds failed due to undesirable psychotomimetic and dysphoric side effects (Pfeiffer et al. 1986). Compared to a reflex assay, if a drug produces an adverse effect, the animal will simply be unable to perform the operant task and this is readily apparent. This highlights the importance of choosing an appropriate assay predictive of human pain.

12 Summary

Here, we have highlighted the utility and sensitivity of the OPAD, and its suitability for measuring pain-related behaviors in rodents. We do not suggest that the OPAD is the only method suitable for preclinical pain research. Rather, we wish to stress that operant assays might yield richer and more interpretable data sets that address the involvement of higher psychological processing in pain responding, such as the contribution of expectancy states. At its extreme, application of inappropriate pain assays may lead to incorrect interpretation of the effects of drugs, as exemplified by the case of GR89,696. However, we are mindful that operant assays do have their limitations, such as the possibility that neural circuits mediating orosensory reward may overlap with pain-related circuitry. Nonetheless, we suggest that operant pain assays should be at the vanguard of preclinical pain research, and we look forward to their contribution to the discovery of more effective and safer analgesics.

Conflicts of Interest Statement The authors are all employees of Velocity Laboratories, a company that provides fee-for-service behavioral testing using operant pain assays.

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Animal Models of Diabetes-Induced Neuropathic Pain

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Abstract Neuropathy will afflict over half of the approximately 350 million people worldwide who currently suffer from diabetes and around one-third of diabetic patients with neuropathy will suffer from painful symptoms that may be spontaneous or stimulus evoked. Diabetes can be induced in rats or mice by genetic, dietary, or chemical means, and there are a variety of well-characterized models of diabetic neuropathy that replicate either type 1 or type 2 diabetes. Diabetic rodents display aspects of sensorimotor dysfunction such as stimulus-evoked allodynia and hyperalgesia that are widely used to model painful neuropathy. This allows investigation of pathogenic mechanisms and development of potential therapeutic interventions that may alleviate established pain or prevent onset of pain.

Keywords Diabetic neuropathy · Painful neuropathy · Diabetic rodents

Contents

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1 Clinical Context

In order to appreciate the relative strengths and weaknesses of the current animal models of painful diabetic neuropathy, it is important to first consider the clinical presentation of diabetes and diabetic neuropathy.

1.1 The Diabetes Epidemic

Diabetes mellitus comprises a group of metabolic diseases that are characterized by hyperglycemia caused by either impaired insulin production or a reduced ability to respond to insulin. It has been estimated that there were 347 million people worldwide who suffered from diabetes in 2008 and the incidence of diabetes is predicted to increase dramatically over the next few decades (Danaei et al. 2011). The majority of cases (90–95 %) represent type 2 diabetes, also called insulin-independent or adult-onset diabetes, with a smaller proportion being type 1, also known as insulin-dependent or juvenile-onset diabetes.

While both forms of diabetes share the common presentation of hyperglycemia, they differ in their underlying pathophysiology. Type 1 diabetes mellitus occurs as a result of autoimmune damage to the β-cells of the pancreas, leading to insulin deficiency (Nokoff et al. 2012). Because type 1 diabetes is the result of compromised insulin secretion, insulin replacement is required. In contrast, hyperglycemia in type 2 diabetes is primarily due to insulin resistance arising from an impaired ability of insulin receptors to signal correctly in response to insulin. Early stages of type 2 diabetes are characterized by hyperinsulinemia such that patients usually do not require insulin supplementation. However, long-term insulin resistance drives the β-cells of the pancreas to produce excess amounts of insulin, resulting in cellular stress and ultimately apoptosis (Prentki and Nolan 2006). When the β-cells become compromised, a hypoinsulinemic state develops and insulin supplementation becomes necessary (Ramlo-Halsted and Edelman 1999). Type 1 diabetes is caused by a combination of genetic, immunological and environmental insults, whereas susceptibility to type 2 diabetes is conferred by genetic and lifestyle factors such as poor diet, obesity, and reduced physical activity (Cheng 2005; Ramarao and Kaul 1999).

1.2 Diabetic Neuropathy

Diabetes disrupts function and structure of all parts of the nervous system, leading to diabetic encephalopathy and myelopathy in the central nervous system (CNS: Biessels et al. 2008) and peripheral neuropathy in all divisions of the peripheral nervous system (PNS). Diabetic peripheral neuropathy is among the most debilitating consequences of chronic diabetes and ultimately affects 50 % or more of all people with diabetes (Thomas 1999). Autonomic neuropathy is most commonly associated with dysregulation of cardiovascular and gastrointestinal functions while peripheral sensory neuropathy can manifest as pain, dysesthesias, and/or loss of sensation (Boulton et al. 2005). Numbness in the extremities is the most common symptom described by diabetic patients and loss of sensation to pain, particularly when accompanied by vascular disease and poor wound healing capacity, is a primary cause of foot ulceration and amputation in diabetic patients (Boulton 2012). Motor nerves are also impaired by diabetes (Andersen 2012), but their dysfunction is less frequently noted by patients or clinical examination until later stages of disease. Diabetic sensory neuropathies may be subclassified as acute sensory or chronic sensorimotor neuropathy (Boulton et al. 2004). Acute sensory neuropathy is also known as insulin neuritis and is characterized by rapid onset and intense painful sensations. It is most prevalent in poorly controlled diabetic patients immediately after sudden improvement of glycemic control, and may be reversible once stable glycemic control is achieved (Boulton et al. 1982; Gibbons and Freeman 2010). In contrast, chronic sensorimotor neuropathy is broadly characterized by a slow onset of numbness with a symmetrical and length-dependent distribution. A subset of patients also experience early "positive" symptoms such as paresthesiae and shooting or burning pain that may be spontaneous or evoked. Consequently, patients with chronic sensorimotor neuropathy can experience a spectrum of symptoms that varies between individual patients and exhibits an unpredictable time course.

Symptoms such as pain or sensory loss may alert diabetic patients to the onset of neuropathy. Pain can be scored using visual analog scales while simple clinical tests such as perception of touch, provided by a 15 g monofilament, and vibration, provided by a tuning fork, are commonly used to confirm overt sensory loss. More sensitive assays such as large fiber motor and sensory nerve conduction velocities (NCV) and the quantitative sensory test (QST) panel are frequently used to identify subclinical neuropathy and follow disease progression on the basis that dysfunction may predict, and then reflect, the ultimately irreversible structural damage of diabetic neuropathy. Quantitative imaging of distal regions of small fibers by skin biopsy or corneal confocal microscopy may also provide an alternative means for diagnosing and stratifying diabetic neuropathy that is at least as sensitive as traditional measures of nerve dysfunction (Tavakoli et al. 2010).

Diabetes causes structural damage to many cell types within the peripheral nervous system (PNS) including both axons and Schwann cells of nerve fibers, perineurial cells and vascular endothelial cells. Large diameter myelinated fibers

exhibit segmental demyelination and remyelination (Behse et al. 1977), as well as atrophy and degeneration of their distal regions (Brown et al. 1976; Archer et al. 1983). Schwann cells undergo independent reactive and degenerative changes (Kalichman et al. 1998) that may also indirectly contribute to axonal pathology. Myelinated axons are not unique in their susceptibility to diabetes, as small unmyelinated fibers also show signs of degeneration and attempted regeneration within distal nerve trunks (Brown et al. 1976) and there is a reduction of the unmyelinated sensory intra-epidermal nerve fibers (IENF) in the skin (Kennedy et al. 1996). The distal regions of the longest nerves in the body have been widely considered to be the most susceptible to injury, in part due to the initial "stocking and glove" presentation of neuropathy symptoms. However, recent findings demonstrating that injury to distal regions of the relatively short trigeminal nerve in the cornea is as sensitive an indicator of neuropathy as loss of epidermal sensory nerves in the feet and legs (Quattrini et al. 2007), may question the concept of length-dependent neuropathy.

1.3 Painful Diabetic Neuropathy

The prevalence of painful symptoms in the diabetic population is estimated to be as high as 34 % (Harris et al. 1993; Partanen et al. 1995; Boulton et al. 1985; Sorensen et al. 2002; Abbott et al. 2011), with a slightly higher incidence among type 2 diabetic patients (Abbott et al. 2011). However, patients may underreport their symptoms (Daousi et al. 2004), such that estimates of prevalence may be low. Painful symptoms are described as pricking, tingling, stabbing, and/or constricting, and may be spontaneous or stimulus evoked. These symptoms impair quality of life and frequently coexist with sleep disturbances and depression (Lustman et al. 2000; Grandner et al. 2012). Pain can be present in conjunction with negative symptoms and it is likely that part of the difficulty in estimating the prevalence of painful symptoms may be due to presence of both pain and loss of sensation in the same patient (Boulton et al. 2004; Sorensen et al. 2002).

1.4 Pathogenic Mechanisms

Poor glycemic control and duration of diabetes are the major risk factors for development of diabetic neuropathy (Tesfaye et al. 1996; Group, D.C.a.C.T.R 1993, 1995). Severity of neuropathy is also correlated with increased height, cigarette smoking, high cholesterol, obesity, elevated triglycerides, hypertension, and cardiovascular disease (Tesfaye et al. 1996, 2005; Reese et al. 2006). Elevated triglycerides correlate with myelinated fiber loss, independent of other risk factors (Wiggin et al. 2009). Many pathogenic mechanisms linking these risk factors to diabetic neuropathy have been proposed and investigated. The majority of

preclinical studies have focused on the potentially damaging consequences of long-term hyperglycemia, such as protein glycation and glucose metabolism by aldose reductase (Tomlinson and Gardiner 2008) and their cellular consequences (Calcutt et al. 2009). Interventions against such presumed pathogenic mechanisms have been widely investigated in both preclinical and clinical studies. Unfortunately, there remains no FDA-approved interventional therapy for diabetic neuropathy and only limited approvals for the aldose reductase inhibitor Epalrestat in Japan and alpha lipoic acid, an antioxidant with diverse cellular actions, in Germany (Hotta et al. 2012; Ziegler 2011). The pathogenic mechanism(s) underlying diabetic neuropathy identified by preclinical studies therefore remain largely unproven in the clinical setting.

There is a similar lack of understanding of the mechanisms that produce neuropathic pain. It remains uncertain whether pain is a direct consequence of degenerative neuropathy, shares some pathogenic aspects with degenerative neuropathy, or is an independent entity. Early suggestions that nerve fiber degeneration could promote pain, presumably either by intrinsic ectopic activity, ephaptic activation of adjacent undamaged fibers or during attempted regeneration (Dyck et al. 1976; Asbury and Fields 1984; Britland et al. 1990) were not supported by detailed biopsy studies of nerve trunks (Llewelyn et al. 1991). The recent focus on peripheral terminals of small unmyelinated fibers in the epidermis as an early indicator of nerve damage has reopened this debate, as skin biopsies from patients with pain show increased depletion of IENF (Quattrini et al. 2007). However, putative mechanisms linking small fiber damage with pain have yet to be established.

The general working assumption that perception of pain in the extremities implies that pain originates from damage to the PNS has recently been challenged by recognition that diabetes has effects on the central nervous system (CNS) and is increasingly linked to cognitive dysfunction (McCrimmon et al. 2012). Painless diabetic neuropathy is associated with microvascular impairment of the thalamus, while painful diabetic neuropathy is associated with increased thalamic vascularity (Selvarajah et al. 2011). Diabetic patients with neuropathic pain show evidence of altered metabolic profile of the thalamus relative to patients without pain (Selvarajah et al. 2008; Sorensen et al. 2008). Additionally, application of a heat stimulus to the foot causes greater activation of the pain matrix in patients with painful diabetic neuropathy, particularly in the prefrontal cortex and anterior cingulate cortex (Selvarajah et al. 2011). Nevertheless, it remains unclear whether changes in the brain associated with diabetic neuropathy are a cause of pain or the consequence of altered function elsewhere in sensory pathways such as the spinal cord (Selvarajah et al. 2006) and PNS.

The variable presentation of painful diabetic neuropathy (Baron et al. 2009) may implicate a complex pathogenesis. Indeed, the diverse manifestations of pain described above may have entirely distinct mechanisms that happen to coincide in the same patient. Such difficulties have directed investigators toward investigating specific mechanisms and potential therapeutics in animal models of diabetes.

2 Animal Models of Diabetic Neuropathy

Animal models of diabetes are widely used to investigate pathogenic mechanisms of diabetic neuropathy and evaluate potential therapeutic interventions. None are perfect replicates of the human condition in terms of either the general physiology associated with diabetes or the impact of diabetes on the nervous system. Data obtained in these models may be useful, but should always be handled with appropriate caution before extrapolating to human diabetic neuropathy. Because mechanisms underlying painful diabetic neuropathy in human patients remain unclear, it is important to consider how closely each animal model replicates features of general diabetic neuropathy in order to evaluate usefulness to the study of painful diabetic neuropathy.

2.1 Commonly Used Models

Rats and mice are the most widely used models of diabetic neuropathy, with less frequent use of rabbits, pigs, and primates. Type 1 diabetes is primarily induced by chemical or genetic means, while type 2 diabetes is usually modeled by genetic or nutritional manipulation. To date, the majority of studies have used rodents with chemically induced, insulin-deficient diabetes. However, use of models of type 2 diabetes is increasing to reflect the dominant prevalence of this form in the human population and suspicions that there may be differences in the pathogenesis or phenotype of neuropathy in type 1 and type 2 diabetic patients.

Chemical models of diabetes rely upon ablation of the pancreatic β-cells with diabetogenic drugs: initially alloxan, but now more commonly streptozotocin (STZ). Both drugs cause β-cell apoptosis, leading to a hypoinsulinemic and hyperglycemic state that models type 1 diabetes (Szkudelski 2001). Rats are usually made diabetic with a single dose of STZ (40–60 mg/kg, depending on body weight (Calcutt 2004)) after an overnight fast to reduce competition between glucose and STZ for uptake into β-cells. In mice, a total dose of 180–200 mg/kg STZ, given across 1 or 2 injections, can be used, with the higher dose reflecting correction for surface area (Freireich et al. 1966) and a degree of trial and error. High doses of STZ can initially produce a severe hyperinsulinemia as insulin is released from degenerating β-cells, followed by marked insulinopenia. Both of these phenomena can cause early death in a substantial proportion of a cohort of mice unless mitigated by first sugar, then insulin, replacement. To counter high cohort mortalities in STZ-injected mice, lower doses of STZ given daily for anywhere from 2 to 5 days may be used to induce diabetes. Such multiple low-dose regimens produce a less aggressive hyperinsulinemia and subsequent insulinopenia that allows animals to survive for months. However, these regimens may also produce a mild and inconveniently slowly developing neuropathy, with some animals reverting to normoglycemia over time (Kennedy and Zochodne 2005).

Advantages of using STZ-diabetic rodents include low cost, rapid induction of diabetes, certainty of duration of diabetes, and an extensive background literature. Disadvantages include an uncontrollable degree of β-cell death and subsequent insulinopenia that can lead to between-study variability in the neuropathy phenotype. The potential for direct STZ toxicity to organs is also occasionally raised as a caveat and is a particular concern for nephropathy studies. However, it is not a factor in the onset of neuropathy (Davidson et al. 2009) while the neuropathy reported occasionally in STZ-injected but normoglycemic animals reflects moderate insulinopenia (Romanovsky et al. 2006). Progressive muscle wasting and ultimate cachexia of STZ-diabetic animals can interfere with behavioral studies and cause premature death in rodents with long-term (3+ months) diabetes. This can be offset by insulin replacement regimes that maintain body weight without promoting normoglycemia (Willars et al. 1989), with the caveat that trace insulin supplementation may also modify the neuropathy phenotype (Calcutt 2004; Singhal et al. 1997; Hoybergs and Meert 2007).

Genetic models of spontaneous type 1 diabetes include non-obese diabetic (NOD) mice, Akita mice and the Bio-Breeding (BB) rat. NOD mice develop frank autoimmune-induced diabetes between 13–30 weeks of age but do not require insulin supplementation for survival (Makino et al. 1980). Concerns have been raised about the contribution of autoimmune damage to neuropathy in this model (Bour-Jordan et al. 2013). Akita mice express a mutant, nonfunctional isoform of insulin (Izumi et al. 2003) and have a reduced number of β-cells (Ron 2002). They develop diabetes around 3–4 weeks of age and show indices of peripheral neuropathy (Choeiri et al. 2005). The BB rat progresses to severe diabetes around 12 weeks of age, requires insulin supplementation for survival (Marliss et al. 1982) and also develops peripheral neuropathy that has a somewhat different phenotype to that of matched models of type 2 diabetes (Sima et al. 2000; Kamiya et al. 2005). In general, genetic models of type 1 diabetes show a neuropathy phenotype similar to that of STZ-diabetic rodents but have to date been primarily used to study the pathogenesis of diabetes itself, rather than its complications (Rees and Alcolado 2005).

The common genetic models of type 2 diabetes typically have deficiencies in either the gene encoding for leptin, or that for its receptor. These include ob/ob mice, db/db mice, Zucker fatty (ZF) rats and Zucker diabetic fatty (ZDF) rats. Ob/ob mice and ZF rats are characterized primarily by hyperinsulinemia and impaired glucose tolerance, indicative of insulin resistance, with only mild to moderate hyperglycemia (Liu et al. 2002; Srinivasan and Ramarao 2007). Db/db mice and ZDF rats display early hyperinsulinemia and impaired glucose tolerance that rapidly progresses to hypoinsulinemia and frank hyperglycemia as β-cell mass diminishes (Kjorholt et al. 2005; Pick et al. 1998). Rats and mice with a conditional knockdown of the insulin receptor have also been bred to model type 2 diabetes (Seibler et al. 2007; Kotnik et al. 2009), but have yet to be used in neuropathy studies.

Diet-induced models of diabetes can be used to mimic the progression from metabolic syndrome to type 2 diabetes in humans. C57/Bl6 J mice or

Sprague–Dawley rats fed a high fat (45–60 %) diet develop marked obesity, hyperinsulinemia, and insulin resistance but rarely attain glucose levels above those accepted as illustrating overt diabetes (usually >15 mmol/l in rodents). Nevertheless, they exhibit a number of indices of peripheral neuropathy and are used as models for metabolic syndrome (Davidson et al. 2009, 2010) and to study potential pathogenic mechanisms related to dyslipidemia (Vincent et al. 2009). A model that combines a high fat diet and low-dose STZ has also been developed to generate frank hyperglycemia accompanied by obesity and hyperinsulinemia (Zhang et al. 2008; Srinivasan et al. 2005).

2.2 Neuropathy Phenotype and Relevance to Humans

Slowing of large fiber motor and sensory nerve conduction velocity (NCV) is usually the primary endpoint used in clinical trials of drugs designed to prevent or reverse diabetic peripheral neuropathy (Airey et al. 2000; Pfeifer and Schumer 1995). NCV slowing is associated with increased duration of diabetes, severity of neuropathy and loss of sensation (Carrington et al. 2002; Redmond et al. 1992; Morimoto et al. 2012) and provides a reproducible, reliable, and objective measure of nerve dysfunction (Bril 1994; Bril et al. 1998). NCV slowing may have a metabolic, reversible component in early stages of disease, as indicated by the modest efficacy of aldose reductase inhibitors (Bril et al. 2009), but is also likely to increasingly involve axonal and Schwann cell pathology as the disease progresses.

In rodents, NCV slowing generally develops within weeks of the onset of diabetes in models of both type 1 (Greene et al. 1975; Ng et al. 1998) and type 2 (Sima and Robertson 1978; Shimoshige et al. 2000) diabetes. Conduction slowing precedes detectable physical damage to large myelinated fibers and this early metabolic dysfunction is readily reversible by many interventions. Indeed, by increasing local temperature, nerves from short-term STZ-diabetic rats can immediately display normal conduction velocities, suggesting that there is no fundamental obstruction to faster conduction at this stage of the disease (Fig. 1). Reductions in myelinated fiber axonal caliber, representing impaired axonal radial growth or atrophy can be detected after 8–12 weeks of diabetes in rats (Britland et al. 1985) and likely contribute to progressive NCV slowing. While some models of diabetes in rats (Powell et al. 1977) and mice (Kennedy and Zochodne 2005) do eventually develop overt axonal degeneration in nerve trunks, this requires months to years of diabetes. There are few credible reports of segmental demyelination in peripheral nerves of STZ-diabetic rodents, although myelin splitting occurs in the spinal roots (Tamura and Parry 1994) and an increase in axons with thin myelin are seen when STZ-induced diabetes is superimposed on hypertension (Gregory et al. 2012), a separate risk factor for neuropathy in diabetic patients. While diabetic rodents and humans share a common disorder in NCV slowing, they may not always share common pathogenic mechanisms, with rodents illustrating primarily the consequences of acute metabolic dysfunction and impaired axonal

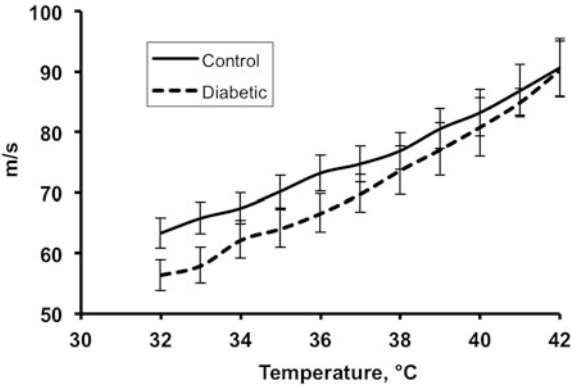

Fig. 1 Sciatic motor NCV in isoflurane anesthetized control and 4-week STZ-diabetic female Sprague–Dawley rats during manipulation of local nerve temperature. Data are mean ± SEM of N = 5–6/group. Andrade and Calcutt, unpublished data

radial growth while human NCV slowing includes a major contribution from segmental demyelination and axonal degeneration. This may explain the poor translation of therapeutics that target NCV slowing from preclinical studies to the clinical arena.

There is a growing interest in developing alternative assays of small fiber neuropathy to accompany large fiber electrophysiology in diagnosing and staging the progression of diabetic neuropathy. Measuring IENF density in skin biopsies is emerging as a valuable technique with which to diagnose and assess the progression of small fiber neuropathy in a variety of diseases, including diabetes (Kennedy et al. 1996). Standard measurement techniques have been adopted (Lauria and Lombardi 2012) and normative values established (Lauria et al. 2010). IENF depletion occurs in both type 1 and type 2 diabetic subjects, appears early in the disease and indeed has been reported in subjects with impaired glucose tolerance and metabolic syndrome (Sumner et al. 2003; Pittenger et al. 2005; Smith et al. 2006). IENF depletion may not be easily reversible, as IENF density does not significantly increase 12–40 months after a simultaneous kidney/pancreas transplant that normalized hyperglycemia and hypoinsulinemia (Boucek et al. 2008; Tavakoli et al. 2013), although there are reports of increased IENF density following drug (Boyd et al. 2010) or lifestyle interventions (Smith et al. 2006; Kluding et al. 2012). It appears reasonable to assume that loss of small sensory fibers in the epidermis leads to loss of sensation in diabetic patients and IENF density inversely correlates with both loss of thermal sensation (Pittenger et al. 2004; Sorensen et al. 2006) and severity of clinical neuropathy (Quattrini et al. 2007). However, these correlations are rather weak and it is worth noting that subjects exposed to different concentrations of capsaicin to deplete IENF were only able to discriminate loss of heat sensation when 50 % or more of the IENF were ablated (Malmberg et al. 2004). As many diabetic subjects report numbness with less dramatic IENF depletion, factors other than IENF depletion may contribute to sensory loss in early disease.

IENF depletion also occurs in rat and mouse models of both type 1 (Beiswenger et al. 2008; Bianchi et al. 2004) and type 2 (Brussee et al. 2008; Underwood et al. 2001)

diabetes. In our experience, IENF depletion in STZ-induced type 1 diabetes can occur within weeks of onset of insulinopenia and hyperglycemia (Beiswenger et al. 2008) but may also take many months to develop during conditions of equivalent hyperglycemia but milder insulinopenia (Calcutt, unpublished observations), suggesting that insulin deficiency per se contributes to IENF depletion. As with humans, it is tempting to associate IENF depletion with loss of thermal sensation. In STZ-diabetic rats, initial paw thermal hyperalgesia may be followed by a progression to thermal hypoalgesia (Calcutt 2004) over a time course that coincides with the onset of IENF depletion (Bianchi et al. 2004) while some treatments that reverse thermal hypoalgesia also attenuate IENF depletion (Roy Chowdhury et al. 2012). However, thermal hypoalgesia and IENF depletion do not always coincide so conveniently (Gregory et al. 2012), and STZ-diabetic mice (Beiswenger et al. 2008) and ZDF rats (Fig. 2) exhibit thermal hypoalgesia before onset of IENF depletion. Changes to sensory neuron phenotype, such as reduced expression (Diemel et al. 1994), axonal transport, (Tomlinson et al. 1988) and spinal release (Calcutt et al. 2000) of neuropeptides may contribute to sensory loss before onset of detectable IENF depletion.

Evaluation of corneal nerve density and morphology by corneal confocal microscopy (CCM) is also attracting interest as a noninvasive surrogate for the evaluation of small fiber neuropathy in diabetes and other diseases (Tavakoli et al. 2011). Loss of corneal nerve length and density agree closely with both IENF depletion and clinical indices of diabetic neuropathy (Quattrini et al. 2007) and is improved by pancreas and kidney transplantation before any impact is seen on IENF depletion (Tavakoli et al. 2013). Diabetic rodents share a similar phenotype, as there are recent reports of reduced innervation in the cornea of rats with type 1 (Davidson et al. 2012) and type 2 (Davidson et al. 2012) diabetes, and reduced corneal nerve occupancy measured by CCM in STZ-diabetic rats and mice, that preceded IENF depletion in the paw skin from the same animals (Chen et al. 2013).

3 Measuring Pain in Diabetic Rodents

Spontaneous pain and dysesthesias are common complaints in patients with diabetic neuropathy (Vinik 2005). In diabetic rodents, there have been reports of spontaneous primary afferent activity that could promote peripherally driven spontaneous pain (Burchiel et al. 1985; Khan et al. 2002). However, spontaneous primary afferent activity is not universally reported (Ahlgren et al. 1992; Pertovaara and Wei 2001; Russell and Burchiel 1993) and spontaneous neuronal activity in the spinal cord and brain also offer the possibility of central generator sites (Fischer et al. 2009; Pertovaara et al. 2001) or modification of descending inhibitory systems (Silva et al. 2013). Unfortunately, there is no clear behavioral evidence that diabetic rodents experience spontaneous pain. They do not change ultrasonic vocalization patterns (Jourdan et al. 2002), do not autotomize limb extremities and do not

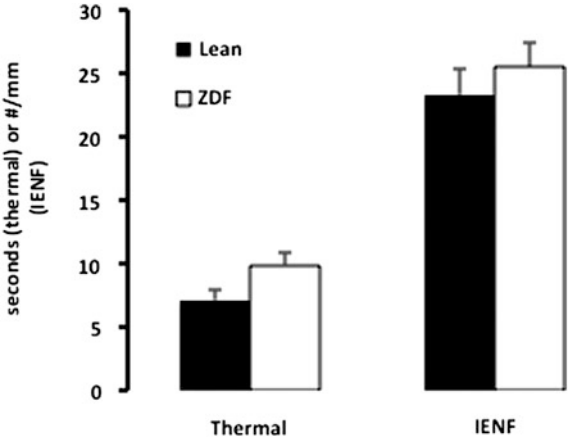

Fig. 2 Paw thermal response latency and IENF density in 6-month old type 2 diabetic ZDF rats and nondiabetic littermates. Data are mean ± SEM of N = 5–7/group. For the thermal test, ZDF rats were significantly hypoalgesic compared to lean, non-diabetic, littermates ($p < 0.01$ by unpaired t test). There was no significant difference in IENF values between the two groups. Arballo and Calcutt, unpublished data

vocalize or adopt nocifensive postures upon handling. Reduced spontaneous motor activity (Courteix et al. 1993) and grooming could reflect either pain or cachexia. As prey animals such as rodents are considered to be adept at disguising illness, injury, and pain, the presence of persistent pain cannot be discounted. Nevertheless, there is currently no quantifiable change in behavior to indicate that diabetic rodents experience the spontaneous neuropathic pain described by diabetic patients.

Because of the apparent absence of spontaneous pain-associated behaviors in diabetic rodents, studies wishing to model painful diabetic neuropathy have relied upon measuring evoked behavioral responses to sensory stimuli. Underlying this approach is the assumption that evoked behaviors in animals are equivalent to pain as humans perceive it (Wiech and Tracey 2009). However, a number of commonly used behavioral tests are likely to measure spinal reflexes rather than pain perception (Le Bars et al. 2001). Diabetes adds a further complication to the interpretation of behavioral assays because it is a systemic disease that affects tissues in which sensory terminals are embedded and that transduce external stimuli, as well as the motor neurons and muscle components of the effector system. While all behavioral studies performed in rodent models of diabetes should strive to use otherwise healthy animals, data from behavioral tests indicating a reduced or slowed response to a given stimulus should be viewed with particular caution due to the motor dysfunction and cachexia associated with chronic diabetes. The latter concern can be mitigated by using animals with less extreme insulin deficiency or using trace insulin therapy to maintain muscle mass (Calcutt et al. 1996, 2004). Findings may be most reliable when validated by concurrent tests of motor function such as the rotarod test.

Painful neuropathy in diabetic rodents is commonly quantified using nocifensive reactions to sensory stimuli applied by mechanical, thermal, or chemical means to the paws or tail of unrestrained animals. Behavioral tests that require handling or restraint are also used, but may be complicated by the role of stress in

nociception (Le Bars et al. 2001; Akil et al. 1986; Vidal and Jacob 1982). Of all the tests employed, those that mirror aspects of clinical QST may be most appropriate for comparisons between diabetic rodents and humans.

3.1 Evoked Responses to Mechanical Stimuli

The von Frey filaments used to quantify allodynia in humans (Haanpaa et al. 2009; Walk et al. 2009) may also be used in rodents. Increased sensitivity to light touch occurs in a number of nerve injury models (Chaplan et al. 1994) and as a result of certain aspects of animal husbandry such as maintenance on wire grates (Mizisin et al. 1998) or specific types of bedding (Fig. 3). Tactile allodynia has been reported in 18 % of patients with painful diabetic neuropathy (Baron et al. 2009) and can be sufficiently disruptive that patients will build tents over their feet to avoid the gentle touch of their bed sheets (Tavee and Zhou 2009). Rat models of type 1 (Calcutt et al. 1996) and type 2 (Brussee et al. 2008) diabetes develop allodynia within weeks of onset of hyperglycemia that is not a consequence of muscle wasting and is prevented by insulin therapy (Calcutt et al. 1996). Increased sensitivity to von Frey filaments relies upon activation of myelinated sensory fibers (Khan et al. 2002) and has been attributed to consequences of insulin depletion rather than hyperglycemia (Hoybergs and Meert 2007; Romanovsky et al. 2004). The assay has also been adapted for use in mice but reports to date are inconsistent, with diabetes being variably described as producing allodynia (Drel et al. 2007) or hypoalgesia (Christianson et al. 2003; Cheng et al. 2009; Wright et al. 2007). Factors contributing to this discrepancy may include differences in mouse strain, duration of diabetes, rate of progression of concurrent distal degenerative neuropathy and the testing paradigm used. One methodological variation between studies that may have functional implications is the use of manual or automated filaments. In our experience, allodynia is detected earlier in the course of diabetes when using manual von Frey filaments, while time of peak efficacy of gabapentin in alleviating allodynia varies with the filament type used (Fig. 4). It is plausible that different afferent subclasses are stimulated by each filament type.

Repeated testing of paw withdrawal with von Frey filaments does not cause behavioral adaptation in diabetic rodents, making the test useful for assessing time course of efficacy of acute pharmacological interventions against established allodynia (Calcutt et al. 2000). Drugs commonly used for treating pain such as gabapentinoids (Backonja et al. 1998), lidocaine (Baron et al. 2009) tricyclic antidepressants (Morello et al. 1999), alpha lipoic acid, (Ziegler et al. 2006) and the serotonin-norepinephrine reuptake inhibitor duloxetine (Raskin et al. 2005) effectively alleviate tactile allodynia in diabetic rodents (Calcutt et al. 1996; Field et al. 1999; Cameron et al. 2001; Yamamoto et al. 2009; Mixcoatl-Zecuatl and Jolivalt 2011), emphasizing the value of this preclinical test for predicting clinical efficacy. Many other studies have used allodynia in diabetic rodents as a tool for investigating the pathogenesis of painful diabetic neuropathy (reviewed in

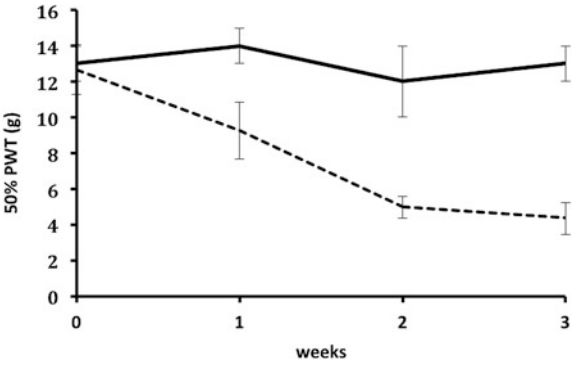

Fig. 3 Progressive increase in rat paw sensitivity to von Frey filaments after transfer from paper to corn chip bedding (*dashed line*) compared to those maintained on paper (*solid line*). Data are group mean ± SEM of N = 6/group. Jolivalt and Calcutt, unpublished data

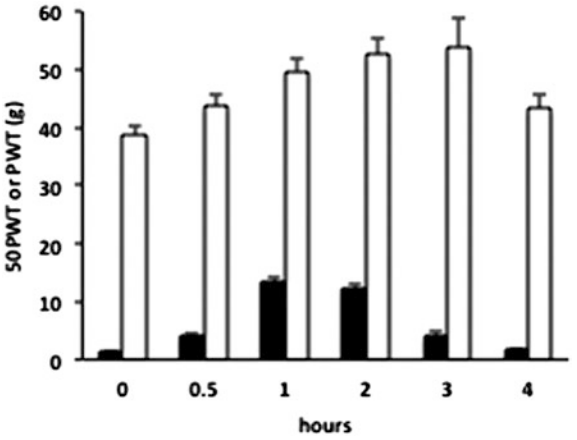

Fig. 4 Efficacy of gabapentin (100 mg/kg sc) in acutely and transiently alleviating allodynia in STZ-diabetic rats measured using manual (*black bars*) and electronic (*white bars*) von Frey filaments. Data are mean ± SEM of N = 6/group. Mixcoatl-Zecuatl and Calcutt, unpublished data

Obrosova 2009). However, as allodynia in diabetic rodents can also be alleviated by diverse agents that interfere with normal nociceptor function in the periphery or spinal cord (Calcutt and Chaplan 1997), demonstration of the acute reversal of established allodynia, while potentially of symptomatic benefit, need not identify pathogenic mechanisms underlying onset of allodynia. Unfortunately, chronic treatment studies frequently fail to evaluate the time course of efficacy in response to a single dose of therapy, so it can be unclear as to whether once-daily treatments given over many days prevent onset of allodynia or acutely suppress allodynia following the last treatment.

The Randall-Selitto test, which involves measuring limb withdrawal during delivery of increasing force to the paw of a manually restrained animal, is a commonly used alternative to use of von Frey filaments. There is no equivalent in the QST battery, although it has been argued that this test may model pain described by diabetic patients upon standing and walking (Tesfaye et al. 2011). Diabetic rats display hyperalgesia in this paw pressure test (Ahlgren et al. 1992; Courteix et al. 1994; Ahlgren and Levine 1993). Like tactile allodynia, mechanical hyperalgesia has been attributed to insulin deficiency rather than hyperglycemia

(Romanovsky et al. 2006, 2010) and in insulin-replete ZDF rats the disorder is slow to develop (Piercy et al. 1999; Sugimoto et al. 2008). Use of this method to evaluate neuropathic pain may be less than ideal due to potential interference from activation of the stress-axis in response to restraint (Le Bars et al. 2001; Akil et al. 1986; Vidal and Jacob 1982). Concerns that muscle wasting in the foot causes the illusion of hyperalgesia due to reduced cushioning of applied pressure have also yet to be fully addressed.

3.2 Evoked Responses to Heating

While heat hyperalgesia is infrequent in diabetic subjects (Baron et al. 2009), loss of heat sensation is a common feature of diabetic neuropathy, coexisting with both painful and painless neuropathies (Boulton et al. 2004; Sorensen et al. 2002). The mechanisms underlying impaired heat nociception are not clear, although heat hypoalgesia correlates with reduced IENF density in patients with both painful and painless neuropathies (Shun et al. 2004; Dyck et al. 2000).

Small fiber neuropathy is commonly assessed in diabetic rodents by measuring the latency of withdrawal from a thermal stimulus. A hot plate may be used (Forman et al. 1986), but the complexity of signals induced by stimulating all limbs and the tail makes interpretation difficult (Le Bars et al. 2001). The response latency of a single paw can be measured using a focused heat stimulus that selectively activates C-fibers at a heating rate of 1 °C/s or Aδ fibers when faster than 3 °C/s (Yeomans and Proudfit 1994, 1996; Yeomans et al. 1996). Unfortunately, heating rate is rarely reported, impeding data interpretation. Heat hyperalgesia develops within weeks of onset of diabetes in most models of type 1 and type 2 diabetes (Calcutt 2004; Kamiya et al. 2005; Calcutt et al. 2004; Piercy et al. 1999; Sugimoto et al. 2008; Gabra et al. 2005; Stevens et al. 2004) and persists in animals that retain residual endogenous insulin production or are supplemented by exogenous insulin (Calcutt 2004), whereas there is a progression to heat hypoalgesia in more insulinopenic animals (Calcutt et al. 2004; Sugimoto et al. 2008). This progression to hypoalgesia may reflect diminished trophic support by insulin that initially compromises sensory function and ultimately leads to depletion of sensory nerve terminals in the epidermis (Beiswenger et al. 2008; Guo et al. 2011). Increased polyol pathway flux is involved in the pathogenesis of both heat hyperalgesia and the subsequent progression to hypoalgesia (Calcutt et al. 2004), although the mechanistic details remain to be resolved.

3.3 Evoked Responses to Chemical Stimuli

The formalin test measures defined behavioral responses, such as flinching, licking or biting, to a chemical irritant. Unlike the tests discussed above, there is no

equivalent QST assay. Nevertheless, formalin-evoked behavior is useful because it can provide insight into processing of sensory information by both peripheral nerves and the spinal cord. Injection of formalin into the hind paw generates a dose-dependent, biphasic flinching response separated by an intervening quiescent period, with very little flinching observed in response to 0.5 % formalin, and maximal flinching frequency generated with 5–10 % formalin (Calcutt et al. 1995). The first phase of behavior represents activation of all classes of primary afferents at the injection site (Puig and Sorkin 1996). Behavioral activity during phase 2 also requires ongoing peripheral input (Taylor et al. 1997) and is accompanied by release of inflammatory mediators in the spinal cord that contribute to the behavioral response (Malmberg and Yaksh 1992; Svensson and Yaksh 2002).

Diabetes increases nocifensive behavior during the quiescent phase and phase 2 of the formalin test in rats without markedly altering phase 1 activity (Calcutt 2004; Calcutt et al. 1996; Malmberg et al. 1993). Interestingly, release of the excitatory neurotransmitter glutamate and the neuropeptide substance P in the spinal cord is paradoxically reduced during the formalin test in diabetic rats (Calcutt et al. 2000; Malmberg et al. 2006) suggesting that this form of hyperalgesia is central, rather than peripheral, in origin. In diabetic rats, increased flinching frequency is accompanied by increased spinal expression of the prostaglandin-producing enzyme cyclooxygenase (COX)-2 and prolonged release of prostaglandin E, while phase 2 flinching is suppressed by spinally-delivered inhibitors of either COX-2 or prostaglandin receptors. The pathogenic mechanism underlying this hyperalgesia involves hyperglycemia-driven glucose metabolism by aldose reductase located in oligodendrocytes that induces spinal COX-2 expression (Freshwater et al. 2002; Ramos et al. 2007). Failure of spinal GABAergic inhibitory systems induced by local BNDF release also contributes to the hyperalgesic state (Jolivalt et al. 2008; Lee-Kubli and Calcutt 2013). In a variant of this assay, low concentrations of formalin (0.2 %) that lack any behavioral effect when injected into the paw of normal rats, produce an extended monophasic flinching response in diabetic rats that corresponds to phase 2 of the usual response to formalin without activity during the phase 1 period (Calcutt 2004). Interestingly, low concentrations of formalin induce flinching behavior in normal rats via activation of TRPA1 receptors (Stucky et al. 2009). These receptors are also activated by methylglyoxal, a glycolysis derivative that is increased by diabetes (Eberhardt et al. 2012). Whether diabetes-induced hyperalgesia in this low-dose formalin test selectively reflects activation of TRPA1 by an additive combination of injected formalin and diabetes-induced local methylglyoxal remains to be determined.

In contrast to rats, diabetic mice do not show increased behavior during phase 2 of the formalin test, and indeed responses are reduced (Christianson et al. 2003; Wright et al. 2007; Johnson et al. 2007; Kamei et al. 2000). This is not a feature of species-specific spinal COX-2 expression, as the increased COX-2 seen in the spinal cord of diabetic rats (Freshwater et al. 2002) is replicated in diabetic mice

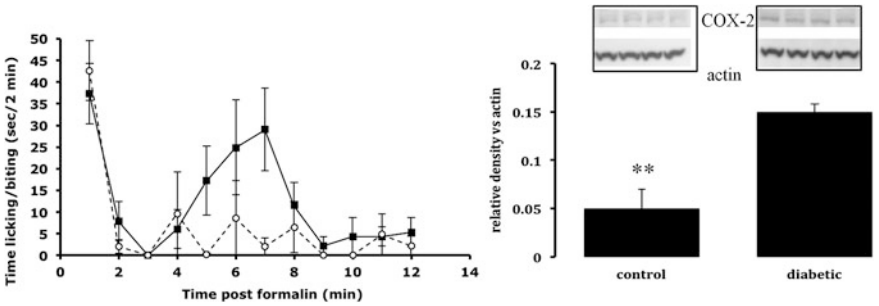

Fig. 5 Formalin (20 µl, 5 %) evoked nocifensive behavior (*left panel*) and diabetes-induced spinal COX-2 protein expression (*right panel*) in control (*black line*) and STZ-injected (*dotted line*) male C57Bl/6 J mice after 4 weeks of diabetes. Data are mean ± SEM of 5-6/group. Statistical analysis by unpaired t test. **$p < 0.01$ vs. diabetic. Note that images of blots for both groups and both proteins were taken from the same original gel. Ramos and Calcutt, unpublished data

(Fig. 5). What causes this loss of nocifensive behavior in diabetic mice is not known although increased spinal protein kinase C activity that may suppress flinching behavior has been proposed (Ohsawa et al. 1998).

4 Conclusions

Current animal models of diabetes are imperfect as they do not replicate all aspects of the human condition that may contribute to the pathogenesis of diabetic neuropathy and consequently do not display all features of nerve pathology. There is also a danger that some aspects of nerve dysfunction common to diabetic rodents and humans may arise from species-specific pathogenic mechanisms. Nevertheless, rodent models of diabetes do develop an acute onset sensorimotor dysfunction that can be equated to components of sensory neuropathy in diabetic patients, along with similarities in small fiber pathology as detected by skin biopsy and corneal confocal microscopy. Many pathogenic mechanisms for painful neuropathy have already been proposed using these models and their demonstration of indices of evoked pain such as allodynia and hyperalgesia. The diverse presentation of pain in diabetic patients and unpredictable efficacy of drugs such as gabapentinoids and duloxetine suggest that the pathogenesis of pain may not be uniform. The future challenge lies not only in translating interventions developed in rodents to clinical use, but also in identifying which mechanisms are dominant in any given patient with painful diabetic neuropathy, so that a personalized therapeutic strategy may be implemented to replace the current iterative and speculative approach.

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Visceral Pain

Erica S. Schwartz and G. F. Gebhart

Abstract Modeling visceral pain requires an appreciation of the underlying neurobiology of visceral sensation, including characteristics of visceral pain that distinguish it from pain arising from other tissues, the unique sensory innervation of visceral organs, the functional basis of visceral pain, and the concept of viscero-somatic and viscero-visceral convergence. Further, stimuli that are noxious when applied to the viscera are different than stimuli noxious to skin, muscle, and joints, thus informing model development and assessment. Visceral pain remains an important and understudied area of pain research and basic science knowledge and mechanisms acquired using animal models can translate into approaches that can be applied to the study and development of future therapeutics.

Keywords Visceral organ distension · Inflammation · Functional disorders · Afferent innervation · Sensitization · Hollow-organ · Solid-organ

Contents

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1 Introduction

It has long been appreciated that the principal conscious sensations arising from the internal organs are discomfort and pain. In chronic visceral pain states, which are common, discomfort and pain are the major reasons for physician visits in the United States. Persons with chronic abdominal and pelvic pain disorders comprise the largest proportion of patients seeking relief from gastroenterologists, urologists, and gynecologists. Interestingly, many chronic visceral pain states (e.g., irritable bowel syndrome, non-ulcer dyspepsia, bladder pain syndrome) exist in the absence of apparent structural, biochemical, or inflammatory causes to explain symptoms, and are thus characterized as "functional," whereas other chronic visceral pain states (e.g., pancreatitis, colitis) are associated with clear evidence of tissue insult.

Chronic visceral pain is generally poorly localized, typically associated with strong autonomic reactions and changes in visceral function. Patients typically exhibit significantly lower response thresholds to provocative stimuli (e.g., cystometry, rectal distension), complain of increased sensitivity during normal organ function, and exhibit increased tenderness in areas of somatic referral which, in addition, are expanded in size. Despite the prevalence of chronic visceral pain states, management of the most troubling symptoms is notoriously poor, principally because mechanisms contributing to the pain and hypersensitivity are still incompletely understood. Over the past few years, there has been an increase in interest in addressing such mechanisms, which has involved development of improved models of visceral pain.

2 Neurobiology of Visceral Sensation

2.1 Characteristics of Visceral Pain

Unlike pain from skin, joints, and muscle that is generally easy to localize, chronic visceral pain characteristically differs from non-visceral, somatic pain in several important ways (see Ness and Gebhart 1990 for comprehensive review). Chronic visceral pain is:

- diffused and poorly localized. The diffuse character and poor localization of chronic visceral pain is a result of extensive intraspinal spread of visceral afferent terminals, including to the contralateral spinal cord, as well as convergence of

inputs from other viscera and from non-visceral tissues onto second-order spinal neurons (viscero-visceral and somato-visceral convergence is discussed below):

- referred ("transferred") to somatic structures and not felt at the source,
- associated with autonomic and emotional responses typically greater than associated with non-visceral pain, and
- produced by stimuli different from those that produce pain in other tissues. In contrast with skin, muscle, or joint, tissue-damaging stimuli are not reliable, adequate noxious stimuli when applied to the viscera. For example, crushing, cutting, or burning stimuli applied to viscera normally produce little conscious sensation and rarely produce pain (see Ness and Gebhart 1990). Adequate noxious visceral stimuli include stretch (or spasm) of the smooth muscle layers of hollow organs (e.g., by distension), traction on the mesentery, ischemia, and inflammation.

2.2 Sensory Innervation of the Viscera

The sensory innervation of the viscera is unique among tissues of the body. Each internal organ is innervated by two anatomically distinct pairs of nerves, which share some functions, but importantly are also associated with different functions, knowledge of which is important in understanding mechanisms of visceral pain and also to interpretation of results in visceral pain models. Anatomically, the visceral sensory innervation is physically associated with nerves comprising the efferent sympathetic and parasympathetic divisions of the autonomic nervous system (Langley 1921). Visceral afferent fibers are contained in nerves that terminate in the spinal cord (spinal afferents) or in the brainstem (vagal afferents) (Fig. 1).

The cell bodies of spinal visceral afferents are distributed bilaterally in dorsal root ganglia along the length of the spinal cord. Spinal afferents thus innervate all thoracic, abdominal, and pelvic organs. Their axons typically travel with sympathetic efferent fibers (except for the "parasympathetic" pelvic nerves) and traverse both pre and paravertebral ganglia on the way to the spinal cord. While passing through prevertebral ganglia, some visceral afferent axons extend collateral branches which synapse on intraganglionic neurons to influence organ function (e.g., motility, secretion, etc.). In paravertebral ganglia, visceral afferents often ascend and/or descend a spinal segment(s) before entering the spinal cord (see Ness and Gebhart 1990).

The bilateral vagus nerves innervate most internal organs, including all of the thoracic viscera, most of the abdominal viscera, and (in rodents) some of the pelvic viscera. Like other visceral nerves, the vagus nerves contain both efferent and afferent axons, most of which ($\sim$80 %) are sensory (afferent) fibers. The larger nodose and smaller jugular ganglia contain the cell bodies of vagal afferent fibers; their central projections terminate bilaterally in the medullary brainstem nuclei of

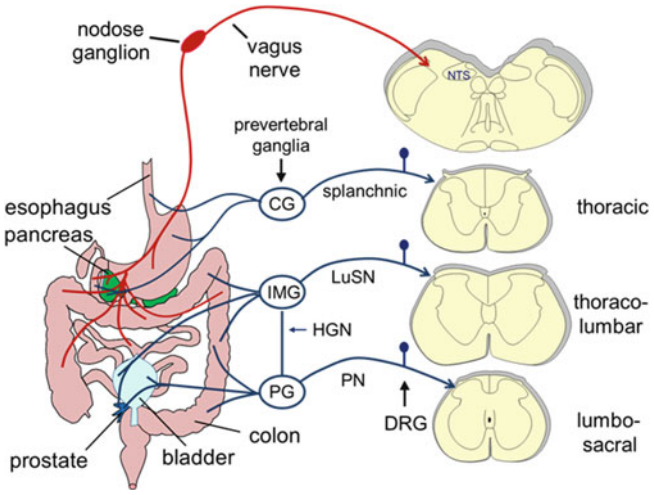

Fig. 1 Illustration of visceral afferent innervation. The vagus nerve, composed largely of afferent fibers (~80 %), is the most far-reaching nerve in the body. The cell bodies of the vagus nerve are contained bilaterally in nodose ganglia (and smaller jugular ganglia; not shown) with central terminals in the brain stem nucleus tractus solitarii (NTS). The vagus nerve innervates all of the organs in the thoracic cavity (e.g., heart, lungs, great vessels, etc.) and most of the organs in the abdominal cavity, including the stomach, gall bladder, small bowel, liver, spleen, pancreas, kidney, and proximal part of the colon. The spinal nerve innervation of the viscera is associated with cell bodies in dorsal root ganglia distributed from cervical to sacral segments of the spinal cord. Spinal visceral afferents anatomically associated with the sympathetic division of the autonomic nervous system pass through prevertebral ganglia, where they commonly give off collaterals that synapse on secretory or motor neurons contained therein and thus influence organ function. These spinal visceral afferents access the spinal cord through paravertebral ganglia (not shown), where afferents can travel rostrally or caudally in the paravertebral sympathetic trunk to enter distant spinal segments. In addition to innervation by vagal afferents, the esophagus, stomach, gall bladder, and pancreas are innervated by thoracic splanchnic (spinal) nerves. The distal colon, urinary bladder and prostate (as well as female reproductive organs, not shown) are innervated by lumbar splanchnic (LuSN) spinal afferents and pelvic nerve (PN) afferents. *CG* celiac ganglion, *HGN* hypogastric nerve, *IMG* inferior mesenteric ganglion, *PG* pelvic ganglion

the solitary tract. In nonhuman primates and rats, some vagal afferents project to the upper cervical spinal cord (C1–C2), where they apparently modulate nociceptive processing within the spinal cord (Chandler et al. 1999) and likely contribute to the referred sensations.

The dual innervation of internal organs results in widespread patterns of termination in the central nervous system. For example, vagal afferents that innervate thoracic organs terminate in the brainstem whereas spinal afferents that innervate the same thoracic organs terminate in cervical and thoracic spinal cord. For the urinary bladder, uterus, distal colon, and prostate, visceral afferents in the pelvic nerve terminate in lumbosacral spinal cord but splanchnic afferents innervating the same organs terminate in thoracolumbar spinal cord (see Ness and Gebhart 1990).

Afferents that innervate the viscera are comparatively few in number relative to afferents that innervate non-visceral tissues. It is estimated that visceral afferent input into the spinal cord is less than 10 % of the total afferent input from all tissues (Cervero and Connell 1984; Janig and Morrison 1986; Grundy 2006), although ~50 % or more second-order neurons in the spinal cord respond to visceral afferent input. This incongruity is the result of significant arborization and spread of visceral afferent terminals within the spinal cord. Spinal visceral afferent terminals typically spread several segments rostral and/or caudal from the spinal segment of entry and also can extend to the contralateral side of the spinal cord (Sugiura and Tonosaki 1995). In contrast, non-visceral somatic inputs are generally restricted to one or a few spinal segments.

2.3 Functional Basis of Visceral Pain

The study and modeling of visceral pain has lagged behind the study of more easily accessible tissues like skin or even joints. Although balloon distension of hollow organs in humans provided considerable insight into visceral pain mechanisms, early models of visceral pain in nonhuman animals typically employed a chemical stimulus injected into the peritoneal cavity. It has been well documented that balloon distension of hollow organs in humans reproduces the distribution of referred sensations from the organ as well as the quality and intensity of sensation (Ness and Gebhart 1990). Accordingly, balloon distension of hollow organs is an adequate, noxious stimulus that is easy to control in duration and intensity. Chemical stimulation is generally more difficult to deliver and to control experimentally than balloon distension of hollow organs, although chemical stimulation can be noxious, particularly in the presence of organ insult or inflammation.

2.4 Viscero-Somatic and Viscero-Visceral Convergence

Visceral afferent input into the spinal cord is characterized by convergence of inputs. Virtually all second-order spinal neurons that receive a visceral input also receive a convergent non-visceral input from skin and/or muscle as well as a convergent input(s) from other organs (Fig. 2). Convergence of inputs offers an explanation for referral of visceral sensations to non-visceral structures (i.e., viscero-somatic convergence, an example being angina, which is associated with deep retrosternal pain that radiates to the neck, left shoulder, or jaw). Viscero-visceral convergence is also common (e.g., colon and urinary bladder convergence and urinary bladder and prostate/uterus convergence onto spinal neurons are common) and likely contributes to cross-organ sensitization (see, Brumovsky and Gebhart 2010 for review).

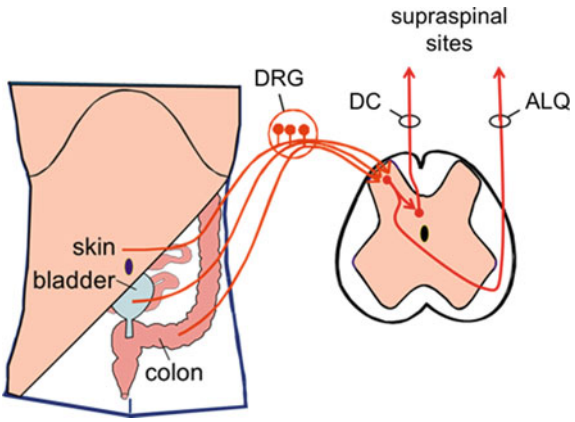

Fig. 2 Viscero-somatic and viscero-visceral convergence. Illustrated is a second-order neuron in the spinal dorsal horn receiving convergent input from the distal colon, from the urinary bladder and also from skin. Visceral sensory information is transmitted rostrally in the contralateral anterolateral quadrant (ALQ) of the spinal cord or ipsilateral dorsal column (DC) to supraspinal sites

In addition, an earlier concept of dichotomizing afferents has been refreshed by evidence that some visceral afferent endings in adjacent organs arise from a common dorsal root ganglion cell body. Studies in rodents using retrogradely transported dyes reveal that dichotomizing afferents innervate the pelvic organs (e.g., colon–bladder, bladder–uterus, bladder–prostate, e.g., Christianson et al. 2007). Because the proportion of dichotomizing afferents among the total organ afferent innervation is small, their role in cross-organ sensitization is uncertain at present.

3 Models of Visceral Nociception

What attributes of the visceral stimulus are important in developing a useful visceral pain model for use in nonhuman animals? The stimulus:

- must be an adequate (in the Sherringtonian sense) noxious stimulus that would produce pain when applied to humans and be related to human pathological pain,
- must be reliable, reproducible, and ethical in that it is under experimenter control with respect to duration and intensity and can be readily terminated either by the experimental subject or experimenter,
- should affect behavior consistent with it being aversive to the experimental subject,
- should evoke pseudaffective reflexes consistent with those evoked in humans (e.g., increase in heart rate, blood pressure, and/or respiration),
- should produce responses that are modulated by known antinociceptive/analgesic drugs.

The foregoing applies to a controllable, provocative stimulus that produces responses that can be reliably measured and quantified. The same provocative stimulus is applicable in models of visceral hypersensitivity where a leftward shift in

the stimulus–response function is expected. Assessing "spontaneous" or "ongoing" visceral pain is considerably more difficult (see Solid Organs), although avoidance and choice models may be informative.

Presented below are a selection of models; in each, we focus on the behavioral neurobiology associated with the stimulus and responses and reliability of the model. We and others have previously reviewed visceral pain models, to which the reader is referred for additional information (Ness 1999; Ness and Gebhart 2001; Robinson and Gebhart 2008).

3.1 Chemically Induced Models

3.1.1 Writhing Test

This model has long been employed as the "standard" pharmaceutical screening tool since its initial description in the 1950s (Siegmund et al. 1957; Carroll and Lim 1958). The intraperitoneal (i.p.) administration of a chemical irritates serous membranes within the abdominopelvic cavity and provokes in mice and rats characteristic alternating abdominal flexion–extension, movements of the body (particularly the hindpaws), twisting of the dorso-abdominal muscles, a reduction in motor activity, and motor incoordination. This model also is referred to as the "abdominal contortion test," the "abdominal constriction response," or the "stretching test."

The original test described in 1957 by Siegmund et al., utilized the chemical phenylbenzoquinone. In current practice, an irritating chemical is chosen based on the desired duration of action and includes acetylcholine, hypertonic saline, dilute hydrochloric or acetic acid (Eckhardt et al. 1958; Niemegeers et al. 1975), bradykinin (Emele and Shanaman 1963), adrenaline (Matsumoto and Nickander 1967), adenosine triphosphate, potassium chloride, tryptamine (Collier et al. 1968), and oxytocin (Murray and Miller 1960). The writhing test is normally carried out in unanesthetized rodents using an i.p. injection of either a fixed dose (e.g., 0.2 ml/mouse or 0.5–2.5 ml/rat) or weight-adjusted dose (e.g., 10 ml/kg) of dilute acetic acid (0.6–9 % V/V) or phenylquinone (0.1–0.3 %) solutions. Responses are quantified as all-or-none, though the number of writhes is also counted in 5-min intervals for 30–60 min. Schmauss and Yaksh (1984) reported improved reliability and reproducibility in the measurement of writhing responses to i.p. acetic acid when they employed a "0–3" scale: 0 = normal body position; 1 = a leaning position favoring one body side; 2 = stretching of hind limbs and dorsiflexion of hind paws, frequently with the pelvis rotated sideward; and 3 = contraction of abdominal muscles followed by stretching of the body and extension of the hind limbs (the classic writhing response).

The writhing test meets the criterion of analgesic drug attenuation of the writhing response, but lacks specificity in that non-analgesics (e.g., atropine, naloxone; Hendershot and Forsaith 1959; Chernov et al. 1967; Taber et al. 1969) are also

"effective." Reliability is also problematic as ≥8 % of animals may not demonstrate writhing responses (Hendershot and Forsaith 1959). Similarly, i.p. administration of bradykinin to human subjects (Lim et al. 1967) failed to evoke pain in all subjects. Further, because animals are typically sacrificed at the end of the experiment, within-animal reproducibility cannot be tested. There are two serious limitations of the writhing test. First, the writhing test is questionably "visceral;" it is highly unlikely that only viscera are stimulated in this model and it remains uncertain exactly what is stimulated. Second, the chemical stimulus is an aversive, inescapable noxious stimulus that likely is associated with significant stress. The writhing test continues to be used, but on ethical grounds and in the absence of evidence of selectively stimulating viscera, it should be abandoned.

3.1.2 Ureteral Calculosis (Artificial Kidney Stones)

Models producing artificial kidney stones have the virtue of specificity, limiting effects to the visceral ureter, but also the significant liability of inescapability. The best characterized and currently most widely used model was developed by Giamberadino and colleagues (Giamberardino et al. 1990, 1995). In a surgical procedure, 20 μl of liquid dental cement is injected into the upper-third of one ureter. The cement hardens and blocks the ureter, resulting (after recovery from surgery) in referred lumbar muscular hypersensitivity and episodic pain behaviors, thus correlating well with the human experience of kidney stones. Visceral pain episodes in the rat consist of three or more of the following behaviors, which must occur in succession and last for 2 min or longer: (1) a humped back position; (2) licking of the lower abdomen and/or the ipsilateral (to the ureter) flank; (3) repeated waves of contraction of the flank muscles and inward movement of the hind limbs; (4) stretching of the body; (5) pressing of the lower abdomen against the floor; and (6) supine position with the ipsilateral hind limb adducted and compressed against the abdomen (Giamberardino et al. 1995). Each episode is further scored using a four point scale: score 1, three types of behaviors; score 2, four types of behaviors; score 3, etc. Episodes last from 2 to 45 min and increase in frequency with time after surgery. Thus, this model employs an adequate noxious visceral stimulus, reasonably reflecting the human experience of kidney stones, and the opioid morphine dose dependently reduces the number of visceral episodes. However, it suffers from variability (visceral episodes range between none and ≥60/rat) and inescapability from a day's long duration of an episodic noxious stimulus.

3.2 Hollow Organ Distension

Models of esophageal, gastric, small intestine, large intestine/rectum, vaginal/uteral, and urinary bladder distension have been described. Early experiments by clinicians on human subjects provided the foundation for the subsequent development and

rationale for use of hollow organ distension to model visceral pain in nonhuman animals (reviewed in Ness and Gebhart 1990). Studies in humans confirmed that constant-pressure distension (not constant volume) reproduced the distribution of referred sensations from the organ as well as the quality and intensity of sensation. It also became clear that spatial summation was important in generating pain from the large bowel; longer length balloons more reliably produced pain at lower, physiologically relevant distending pressures than did shorter balloons.

Both human and nonhuman animal models are similar in that distension of hollow organs produces a visceromotor response that can be recorded (from an appropriate striated muscle) in unanesthetized subjects and be readily quantified. The visceromotor response in humans, described by MacKenzie (1909), is a contraction of abdominal (or other) striated muscle in response to organ distension. The visceromotor response is a spinal-bulbo-spinal pseudaffective reflex (Woodworth and Sherrington 1904), not a spinal nociceptive reflex (like the tail flick reflex). That is, the visceromotor response is unaffected by mid-collicular transection (i.e., responses are robust in decerebrated animals) but absent after either C1 or T6 spinal cord transection. Accordingly, organ distension produces (in unanesthetized or lightly anesthetized subjects) an increase in blood pressure, heart rate, and respiratory rate. All of these pseudaffective responses can be recorded and quantified, but the visceromotor reflex in nonhuman animals is most amenable to long-term (weeks) recording (Fig. 3). Quantification of the response is accomplished by surgically implanting electromyographic (EMG) recording electrodes into the external oblique musculature (for colorectal or urinary bladder distension) or acromiotrapezius musculature (for esophageal or gastric distension). Detailed methods describing EMG electrode and colonic balloon materials and fabrication, aseptic surgical procedures, method of distension, data acquisition, and data analysis have been fully described (Christianson and Gebhart 2007).

In addition to the justifications derived from human experimentation, organ distension as a valid model of visceral pain offers several advantages.

1. Experiments can be carried out in unanesthetized animals. After recovery from surgical implantation of EMG recording electrodes (or indwelling arterial catheters to record heart rate and blood pressure), insertion of intracolonic balloons or intravesical catheters requires only brief isoflurane sedation, after which repeated organ distension can be performed over the course of the next 60–120 min in an experimental session.
2. Stimulus intensity and duration are easily controllable. Typically, the duration of phasic, constant-pressure organ distension is 10–20 s, and more commonly 10 s. Importantly, stimulus intensity can be varied from innocuous to noxious pressures, thus allowing assessment of encoding of the stimulus by construction of stimulus–response functions. Typically, each distension pressure is presented as a discrete stimulus (inter-stimulus interval, 3–4 min) and a stimulus–response function is constructed from visceromotor responses to 15, 30, 45, and 60 mmHg distension in mice or 20, 40, 60, and 80 mmHg distension in rats.

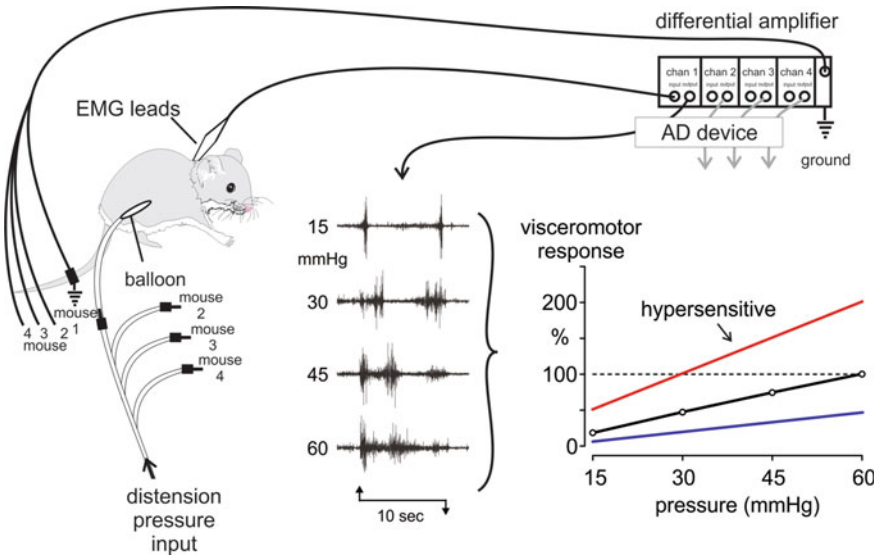

Fig. 3 The visceromotor response to balloon distension of the colon or urinary bladder can be recorded simultaneously in two or more rodents. The electromyographic (EMG) recording electrodes are surgically placed in advance in the external oblique musculature and externalized for subsequent access at the back of the head. Typical EMG records are illustrated in response to graded intensities of colon distension in the mouse. These records are then rectified and quantified as area under the curve to generate (at *right*) a stimulus–response function. The VMR can be attenuated by analgesic drugs and is enhanced in the presence of organ insult (hypersensitivity)

Alternatively, intra-organ pressure can be presented as an increasing, ramped stimulus over 60–90 s (e.g., from 0 to 60 or 80 mmHg). Rodents typically respond to organ distension by ceasing movement during the short duration of distension which, moreover, can be terminated by the experimenter at any time.

3. Within-animal experiments are the rule, permitting assessment of responses before and then in the same subject periodically for weeks after a treatment. For example, after introduction of a treatment that leads to organ hypersensitivity or selective ablation of some target of interest. Characteristic and unique features of distension of specific organs are discussed below.

3.2.1 Esophageal and Gastric Distension

Visceromotor responses to gastric distension and esophageal distension in rats are recorded from EMG electrodes implanted into the acromiotrapezius muscle. Esophageal distension is achieved by inflation of a Swan-Ganz catheter balloon (size 7F) orally inserted to the distal-third of the esophagus. Unlike distension of the stomach, large intestine/rectum, or urinary bladder, rats are anesthetized during esophageal distension. Further, the esophagus is fairly rigid and not easily

distensible and so its use has been largely restricted to electrophysiological experiments (Euchner-Wamser et al. 1993) and information about behavioral neurobiology in nonhuman animals is scant. Experimental evidence in humans is much more extensive (e.g., Hobson and Aziz 2004).

Gastric distension (GD) can be performed in unanesthetized rats. Gastric balloons have to be surgically implanted in the stomach in advance of an experiment and placed with care so that the transit of ingested food into the small intestine is not impeded. Visceromotor responses to GD are intensity-dependent and stimulus–response functions are shifted leftward after gastric ulceration or irritation, revealing the development of gastric hypersensitivity (Ozaki et al. 2002). Importantly, acquisition of a passive avoidance behavior to GD was blocked after splanchnicectomy, but unaffected by vagotomy, revealing that noxious gastric mechano-nociceptive information is conveyed to the central nervous system by spinal afferent nerves. In contrast, visceromotor responses produced by intragastric instillation of HCl were blocked by vagotomy, but not splanchnic nerve resection, revealing that gastric *chemo*-nociceptive information is conveyed to the central nervous system by the vagus nerves (Lamb et al. 2003).

3.2.2 Colorectal Distension

Colorectal distension (CRD) was initially characterized in rats by Ness and Gebhart (1988) and subsequently in mice (Kamp et al. 2003) and has been used extensively in visceral pain research. Visceromotor responses to CRD in rodents are reliably intensity-dependent and appropriately attenuated by analgesic drugs (e.g., Ness and Gebhart 1988; Bjorkman et al. 1990). Further, the peripheral and central neural pathways mediating the visceromotor response have been documented (Ness and Gebhart 1988; Kyloh et al. 2011). Transection of either the lumbar splanchnic or hypogastric nerves were without effect on visceromotor responses or passive avoidance acquisition to CRD whereas transection of the pelvic rectal nerves abolished visceromotor responses and passive avoidance acquisition to CRD. This result was obtained regardless of whether the lumbar splanchnic or hypogastric nerves were also transected, revealing that colorectal mechano-nociceptive transmission to the spinal cord is conveyed via the pelvic and not hypogastric/lumbar splanchnic nerves.

Within the spinal cord, colorectal nociceptive transmission ascends in both dorsal midline and lateral spinal pathways (e.g., Ness 2000). Lateral spinal pathways (e.g., spinothalamic tract) have long been held to be the principal, if not exclusive ascending conveyors of nociceptive (and noxious thermal) information to supraspinal loci, and while visceral nociceptive information also ascends in lateral spinal pathways, an ipsilateral post-synaptic dorsal column pathway may be more important for transmission of visceral pain (e.g., Willis et al. 1999; Palecek et al. 2002). In humans, a limited midline myelotomy at T10 that interrupts the ascending fibers in the dorsal columns leads to substantial relief of visceral pain originating from pelvic cancer (Kim and Kwon 2000; Nauta et al. 2000). A midline

myelotomy initially was performed with the intent to eliminate the crossing fibers of the spinothalamic tract in the anterior white matter commissure, but it has subsequently been documented anatomically that post-synaptic dorsal column fibers originating in the area of spinal cord lamina X are the conveyors of visceral nociception from abdominopelvic organs. In nonhuman animals, lesions of the dorsal midline reduce thalamic neuronal responses to noxious colon distension or inflammation (Al Chaer et al. 1996, 1998; Ness 2000). Interestingly, pseudaffective visceromotor and cardiovascular responses in rats to CRD were vigorous after dorsal midline lesions, but significantly attenuated or absent in rats with bilateral lateral spinal lesions (Ness 2000), suggesting that the qualities of visceral nociceptive information ascending in the spinal cord may differ between ipsilateral midline and contralateral pathways.

What is a Noxious Intensity of CRD? Electrophysiological recordings from stretch-sensitive colorectal pelvic nerve afferents revealed the presence of afferents with low thresholds for response to CRD as well as a smaller proportion of afferents with high thresholds for response in the rat (Sengupta and Gebhart 1994), findings that were subsequently replicated in the mouse (Feng and Gebhart 2011). Results from such experiments suggested that colorectal distending pressures $\geq$30 mmHg were at the threshold of the noxious range. Using a passive avoidance step-down task, the colorectum of the rat was distended to different pressures (in different groups) when stepping down from a 10 cm high platform in an open field and placing both forepaws on the floor. Rats rapidly learned to avoid distending pressures $\geq$40 mmHg by not stepping down from the platform (Ness et al. 1991). Furthermore, in the presence of colorectal inflammation, the acquisition of avoidance behavior was facilitated; an intensity of CRD that was previously without effect (30 mmHg) led to rapid acquisition of the avoidance behavior (Ness and Gebhart 1988; Ness et al. 1991, Fig. 4). These results complemented inferences made from electrophysiological studies and support to the assertion that CRD is a valid, noxious stimulus for the study of visceral pain.

Modulation of responses to CRD. CRD as a selective visceral stimulus is subject to modulation and thus to probing mechanisms of organ hypersensitivity typically present in gastrointestinal disease states (e.g., irritable bowel syndrome, inflammatory bowel disease). Characteristically, stress is a factor in human visceral pain states and responses to CRD are enhanced by a variety of stressors—maternal separation (e.g., Coutinho et al. 2002), water avoidance stress (e.g., Bradesi et al. 2005), CRF (e.g., Tache et al. 2004), and early life stress (e.g., Al Chaer et al. 2000; Christianson et al. 2010).

Other approaches selectively directed at the colorectum include intracolonic treatment with inflamogens or irritants such as acetic acid, acidified hypertonic saline, trinitrobenzene sulfonic acid (TNBS), zymosan, deoxycholic acid (DCA), etc., or oral ingestion of dextran sodium sulfate (5 %) (e.g., Jones 2007; Traub et al. 2008; La et al. 2012; Feng et al. 2012; Low et al. 2013). Models of mild to severe colitis, hypersensitivity in the absence of inflammation, or post-inflammatory colitis have been described. The severity and duration of inflammation can be manipulated by varying the concentration of inflamogen/irritant and the frequency

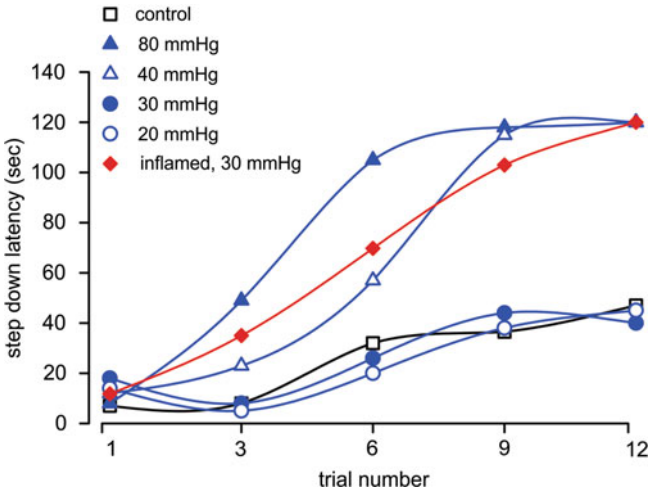

Fig. 4 Conditioned avoidance behavior in rats produced by colorectal distension. Rats were placed on a 15 × 15 × 10 cm platform in an open field and the latency to step down (both forepaws on the floor) from the platform measured. Distending pressures of 20 and 30 mmHg did not produce behavior different than in control rats, which received no colorectal distension. Distending pressures of 40 and 80 mmHg, however, led to acquisition of avoidance behavior. In the presence of colonic inflammation, 30 mmHg distension led to acquisition of avoidance behavior, revealing the presence of colorectal hypersensitivity. Adopted from (Ness et al. 1991)

of treatment. Behaviorally, key to these approaches is documentation of onset, duration, and termination of colorectal hypersensitivity, evidenced by a leftward shift in the stimulus–response function to CRD, including decreased visceromotor response thresholds, increased vigor/magnitude of visceromotor and cardiovascular pseudaffective responses to CRD, and an increase in the rate of acquisition of an avoidance behavior. Histological, biochemical, and/or molecular biological evaluation of colorectal tissue or electrophysiological assessment of the colorectal innervation typically complement the study of behavioral hypersensitivity. In the aggregate, CRD meets well all criteria described above (see Sect. 3) and is a valid model of visceral pain.

Figure 5 illustrates differences between two models of colorectal hypersensitivity in the mouse. Both intracolonic TNBS and zymosan, in the concentrations employed (see figure legend), produced robust colorectal hypersensitivity that persisted for 2 and 7 weeks, respectively. Intracolonic TNBS produces significant colorectal inflammation that resolves in 10–14 days whereas intracolonic zymosan is not associated with persistent colorectal inflammation (as determined by myeloperoxidase assay or macrophage infiltration). Importantly, sensitization of stretch-sensitive and reduced proportions of mechanically insensitive colorectal afferents in the pelvic nerve outlasts the duration of colorectal hypersensitivity, assessed by visceromotor responses to CRD. Two points merit emphasis. First, organ inflammation is not required for either the development or persistence of hypersensitivity.

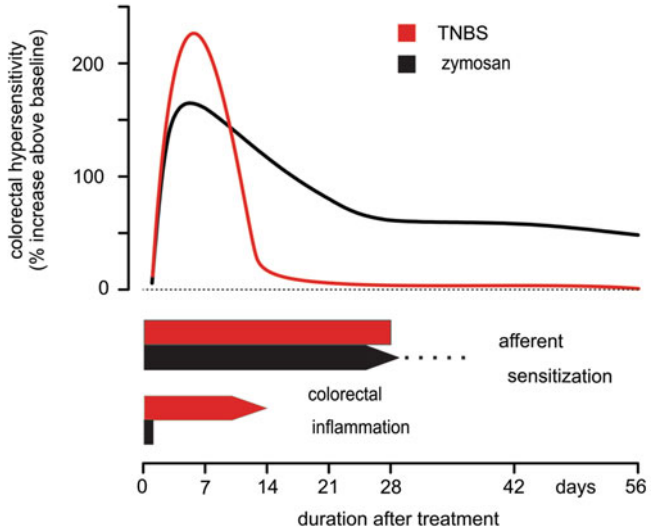

Fig. 5 Colorectal hypersensitivity, pelvic nerve afferent sensitization, and colorectal inflammation produced in two models of intracolonic insult in mice: TNBS (0.2 ml, 10 mg/ml given once) and intracolonic zymosan (0.1 ml, 30 mg/ml given daily for 3 consecutive days). Intracolonic TNBS produces a robust hypersensitivity to balloon distension of the colon that resolves by 14 days after treatment. Intracolonic TNBS is also associated with inflammation in the colorectum that also resolves by day 14 after treatment. Changes in pelvic nerve afferent excitability/sensitization persist longer than either the duration of colorectal hypersensitivity or colon inflammation. Intracolonic zymosan produces a longer lasting colorectal hypersensitivity that persists for up to 7 or 8 weeks after intracolonic treatment in the absence of colorectal inflammation; hypersensitivity of pelvic nerve afferents persists for 3–4 weeks after intracolonic treatment (longer times have not been tested). Data are summarized from experiments in C57BL/6NTac mice (Feng et al. 2012a, b, 2013; Jones et al. 2007)

And second, changes (e.g., sensitization) in the afferent innervation of the organ outlast both the duration of organ inflammation and colorectal hypersensitivity.

3.2.3 Urinary Bladder Distension

In the clinical setting, bladder infection leading to cystitis is the most common cause of painful bladder symptoms, followed closely by overdistension of the bladder due to acute urinary retention. Mechanisms underlying these human conditions have been addressed in rodent models of bladder pain (distension of the bladder) and bladder function (cystometry).

Urinary bladder distension (UBD), like CRD described above, evokes pseud-affective reflexes, including a visceromotor response (contractions of the hindlimb and abdominal musculature). The validity of the visceromotor response as a measure of bladder nociception in animals is supported by similarities between the visceromotor response to UBD in animals and verbal reports of painful sensations

produced by UBD in human subjects (Ness et al. 1990). Like CRD, UBD reliably produces pain/discomfort in humans, and also produces cardiovascular, visceromotor, and respiratory responses (Ness 2005; Powell-Boone et al. 2005). Further, the threshold to evoke a visceromotor response to UBD in rats occurs at similar intravesical pressures (20–40 mmHg) that produce discomfort during UBD in healthy female volunteers (24.4 ± 2.4 cm H_2O; Ness et al. 1998, 2005). Thus, UBD produces robust, reliable, and reproducible, stimulus-linked visceromotor responses in rat and mouse models that are readily quantifiable (Ness and Castroman 2001; Ness and Elhefni 2004; DeBerry et al. 2007; Hu et al. 2009; Ball et al. 2010), reproducible after an initial sensitization period (Ness and Castroman 2001; Ness et al. 2001), and attenuated by commonly used analgesics including morphine, fentanyl, and lidocaine (Ness and Castroman 2001; Ness et al. 2001). Accordingly, UBD satisfies criteria for a valid model of visceral nociception in nonhuman animals.

Like the visceromotor response to CRD, analysis of the visceromotor response to UBD is typically reported as area under the curve of rectified EMG activity recorded from the external oblique musculature during UBD (Ness and Elhefni 2004). The visceromotor response to UBD increases in a graded manner to increasing distending pressures, thus permitting construction of stimulus–response functions. Thus, one can, within the same animal, determine how a treatment (e.g., analgesic drug, bladder inflammation, etc.) alters responses to innocuous (10–30 mmHg), noxious (30–60 mmHg), and supra-physiological (60–80 mmHg) distending pressures. Bladder inflammation (Randich et al. 2006), including early-in-life bladder inflammation (DeBerry et al. 2010), stress (Robbins and Ness 2008; Black et al. 2009) and endogenous modulation (DeBerry et al. 2007; Randich et al. 2008) of responses to UBD have been reported, which further attest to the utility of this animal model.

Initial studies of UBD in rats and later in mice were carried out as acute, terminal experiments. Like CRD, one can perform longer term, within-animal UBD experiments (unpublished). EMG electrodes are permanently implanted into the external oblique musculature as for CRD. After recovery from surgery (~5–6 days), the mouse is briefly sedated (isoflurane) and a 24 gauge angiocatheter is inserted into the bladder via the urethra. After recovery from isoflurane (~30 min), EMG activity is recorded before (resting) and during (10 s) phasic bladder distension (via air pressure). EMG activity is differentially amplified, rectified and an area under the curve analysis of responses to graded UBD (e.g., 15, 30, 45, and 60 mmHg, minus any resting EMG activity) is used to generate a stimulus–response function.

Cystometry. Whereas UBD represents a model of bladder nociception, bladder function is evaluated by cystometry, which is a useful complement to the study of bladder pain mechanisms and, independently, to assess bladder function related to other bladder disorders (e.g., overactive bladder). Measurements during cystometry include detrusor contraction frequency and amplitude, voiding frequency and intervals, intravesical pressure, micturition onset (time to first micturition-producing contraction), pressure, frequency, and contraction/pressure amplitudes. Rats have been widely used for cystometric investigations and the rat bladder has

been well characterized in vitro, although the rat bladder demonstrates important morphological and functional differences when compared to the human bladder. For example, the rat bladder is devoid of intramural ganglia (Uvelius and Gabella 1995). While both male and female rats are used, females are preferred for transurethral cystometry due to the ease of inserting a catheter via the urethra. There are, however, differences between male and female rat micturition. Male rat voiding consists of a fast spike-like urine flow, whereas female rat voiding is ongoing but interrupted for short periods when bladder pressure is increased (Streng et al. 2002). While these sex differences are believed to be of minor relevance in the normal rodent, they may have significance in genetically modified animals (Cornelissen et al. 2008).

Mice are technically more difficult to use for cystometry because the diameter of the tubing inserted into the relatively small mouse urethra/bladder may not accurately transmit pressure changes to a transducer, but otherwise is generally approached in a manner similar to the rat (Pandita et al. 2000; Smith and Kuchel 2010). Morphologically, the mouse bladder appears more similar to the human bladder, primarily because intramural ganglia are present. For cystometry in anesthetized (urethane 1.2 mg/kg, i.p.) rodents, an intravesical catheter either can be inserted via the urethra or the dome of the bladder, where it is externalized suprapubically by a midline incision (Uvin et al. 2012). In the latter case, topical local anesthetic ointment should be applied to the incised tissue to eliminate potentially confounding nociceptive input. Studies suggest the bladder should be drained and saline infused at a constant rate of 20 μl/min for 20 min (0.4 ml total volume) in mice, and 0.1–0.3 ml/min for 20 min (2–6 ml total volume) in rats.

For cystometry in conscious mice, a polytetrafluoroethylene catheter with a blunted end is placed through the dome of the bladder under isoflurane anesthesia two days prior to recordings (Uvin et al. 2012). The catheter is exteriorized at the nape of the neck, inserted into the end of a 22-gauge angiocatheter, affixed to the angiocatheter with super glue, capped, and anchored to the fascia and skin of the neck. For cystometry, the exteriorized catheter is connected to a pressure transducer and infusion pump to deliver room temperature saline into the bladder at a rate of 10 μl/min. Urodynamic values are recorded continuously for hours and data about bladder capacity, voided volume, pressure at the start of micturition, intravesical pressure, micturition rate, intermicturition interval and number of non-micturition contractions are collected.

3.3 Solid Organs

3.3.1 Pancreas

The symptom that most often brings patients with pancreatitis to the clinic is pain. The clinical course of acute pancreatitis (AP) is highly variable, but reversible, although in 20 % of patients pain can be severe with associated morbidity and a

mortality of ~5 % (Papachristou et al. 2007). In cases of recurrent AP, which increases the likelihood of developing chronic pancreatitis (Demir et al. 2011; Puylaert et al. 2011), the disease progresses to an irreversible state in which pain is very difficult to control (Papachristou et al. 2007; Muddana et al. 2009). Chronic pancreatitis (CP) is characterized by continuous or recurrent inflammation of the pancreas, progressive and irreversible fibrosis and atrophy, and in severe cases partial or total loss of pancreatic function (Ceyhan et al. 2010; Demir et al. 2011). Severe and uncontrolled abdominal pain is the cardinal feature of CP. Pain may initially be episodic, but increases in occurrence and intensity as the disease develops. The development of models to study mechanisms of pancreatic pain is challenging because it is difficult to probe directly the cause of the pain (i.e., the pancreas). Consequently, assessment of pancreatic pain in rodents is generally indirect.

Models. Most models rely on chemically induced inflammation or destruction of the pancreas. In rodents, trinitrobenzene sulfonic acid (TNBS), bile acids, and sodium taurocholate have been given by retrograde infusion into the pancreatic duct (e.g., Perides et al. 2010). Studies have also employed repetitive overstimulation of the pancreas with intraperitoneal injections of caerulein or chronic alcohol feeding (Schwartz et al. 2013; Perides et al. 2005). Other models involve obstruction or ligation of the pancreatic duct (Vigna et al. 2011) while newer models employ genetic manipulations in rodents (Aghdassi et al. 2011). In most models of CP, areas of focal necrosis and subsequent perilobular and intralobular fibrosis develop as severity of the insult increases (see Fig. 6).

Mechanical Hypersensitivity. In humans, pain from the pancreas is referred to the upper (epigastric) abdominal area and radiates to the back (Z'graggen et al. 1998; Graham and Bonica 2001). These areas of referred sensation are typically sensitive to palpation (mechanical hyperalgesia) if not tender to touch (mechanical allodynia) (Z'graggen et al. 1998). Accordingly, referred mechanical hypersensitivity has been commonly used as the principal means of assessing of pain in animal models of pancreatitis (Vera-Portocarrero and Westlund 2004). It is important to appreciate that the pancreas (like the esophagus and stomach) is innervated by vagal and splanchnic afferents that terminate, respectively, in the brainstem and thoracic spinal cord. Thus, hypersensitivity testing in animal models of pancreatitis is associated with the splanchnic innervation of the pancreas. Mechanical probing in areas of referral is characterized as a lowered threshold of response or increased frequency of response to mechanical stimulation (e.g., von Frey-like filaments). In this assay, von Frey-like filaments requiring different forces to bend are used to probe the thoraco-abdominal area or referral. Mechanical hypersensitivity is quantified either by establishing that the filament producing a withdrawal response is less in rodents with experimentally induced pancreatitis than controls, or by measuring the number of withdrawal events (or consequent licking of the abdominal area, or whole body withdrawal) in response to normally innocuous or subthreshold probing (Choy et al. 1994). Von Frey-like filament probing is fraught with concern about the validity, reliability, and reproducibility of the stimulus

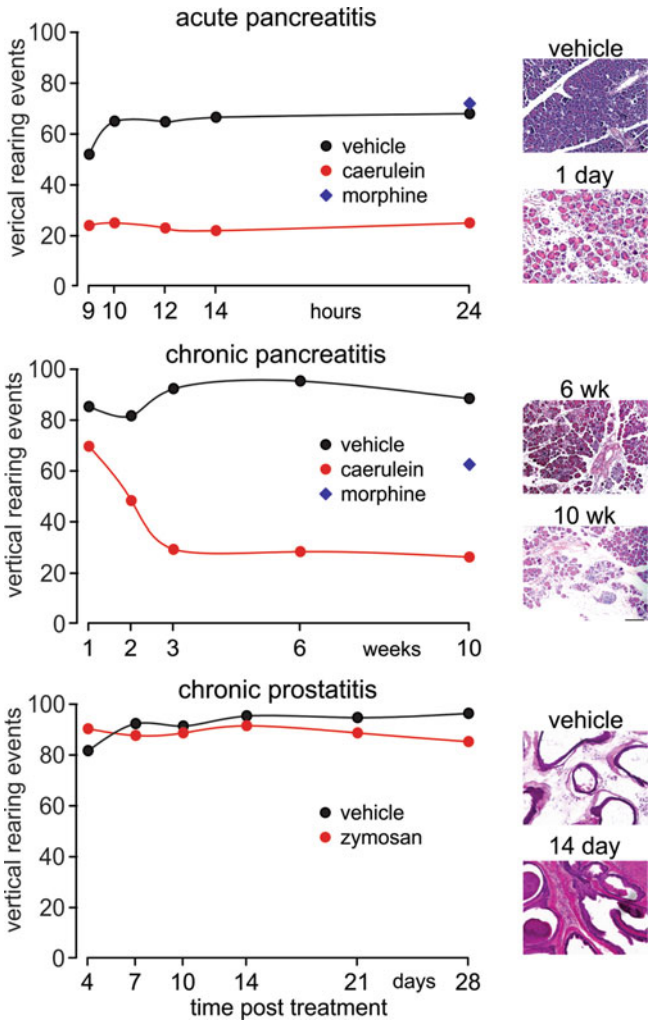

Fig. 6 Exploratory activity assessed in models of acute pancreatitis, chronic pancreatitis, and chronic prostatitis in mice. Illustrated are data associated with vertical rearing in a 25 cm square, 40 cm high activity box. Acute pancreatitis was produced by eight hourly injections of caerulein (50 μg/kg, 0.1 ml, i.p.), after which vertical rearing was assessed starting 1 h after the last caerulein injection. Activity was significantly attenuated in caerulein-treated relative to vehicle-treated mice. An intraperitoneal injection of morphine (2.5 mg/kg) 30 min before testing at 24 h reversed the attenuated behavior. Histological sections to the right illustrate the effect of treatments. The model of chronic pancreatitis involved recurrent episodes of acute pancreatitis (as above) given twice weekly over the course of 10 weeks. Vertical rearing events were significantly attenuated in mice with chronic pancreatitis relative to vehicle-treated mice, and morphine (2.5 mg/kg) attenuated the suppressed rearing activity. Histological sections of pancreas after 6 weeks and 10 weeks of treatment are illustrated to the right. The chronic prostatitis model was produced by injection of zymosan (0.1 mg/kg in 10 μl) into the dorsal lobe of the mouse pancreas. Histological sections to the right establish the presence of inflammation, which did not have an effect on vertical rearing events. Data summarized from Schwartz et al. (2011, 2013) and Xie et al. (2013)

(e.g., see Bove 2006), concerns that are amplified when applied to the hairy skin of the thorax or abdomen of a rodent.

Thermal Hypersensitivity of the abdomen also has been assessed in models of pancreatitis (Vera-Portocarrero and Westlund 2004). A noxious intensity radiant heat stimulus from a lamp is concentrated as a beam of light through a hole (1×1 cm for rats) in a light box onto the abdomen and the latency (seconds) to respond is determined. The response is defined as abdominal withdrawal (either abdominal musculature contraction or lifting of the abdomen through postural adjustment) accompanied by head turning toward the stimulus and licking of the abdominal area. The application of radiant heat to the hairy skin of the abdomen is associated with concerns about reliability as the distance of the abdominal skin from the radiant heat source as well as the rate of increase in temperature are variables affecting experimental outcomes. Further, the relevance of thermal hypersensitivity in the area of referral to the human experience is uncertain as this is not a common complaint.

Electrical Stimulation. Winston et al. (2005) adapted a previously reported method (Bliss et al. 1950; Griesbacher 1994) and applied electrical stimulation directly to the pancreas. In the rat, a pair of surgically implanted electrodes were placed on the pancreas and externalized behind the head. Subsequently, successive applications of 2, 5, and 10 mA current were applied for 5 min (with a 20 min inter-stimulus interval) and the number of nocifensive behaviors observed during stimulation was counted. Behaviors consisted of stretching, licking the abdomen, contraction of abdominal wall muscles, and extension of the hind limbs. At a later time, in the same rats, TNBS was infused via the pancreatic duct and the electrical stimulation paradigm repeated, confirming the development of pancreatic hypersensitivity as nocifensive behaviors were increased at each of the three intensities of stimulation.

Exploratory Activity. The preceding assessment methods all use a provocative stimulus. More recently, rodent exploratory activity has been employed to assess nociception in models of pancreatitis (Houghton et al. 1997; Vera-Portocarrero and Westlund 2004; Zhang et al. 2004; Schwartz et al. 2011, 2013). We assessed pain-related behaviors in mice with caerulein-induced acute (Schwartz et al. 2011) and chronic (Schwartz et al. 2013) pancreatitis. Mice were placed in Plexiglas activity boxes (25 cm square by 40 cm high) and exploratory behaviors were monitored photoelectrically for 15-minute periods at times before, during and after treatments. Photoelectric beams are spaced 1.5 cm apart, providing 0.75 cm spatial resolution. Software simultaneously analyzes time spent in different parts of the activity box, path information, distance traveled, and total movements in the X-Y plane. Furthermore, one can analyze the amount of time spent in the vertical plane or "standing" position that requires stretch of the abdominal muscles, a position that is assumed to be uncomfortable in the presence of abdominal hypersensitivity. In similar fashion, home cage activity can also be measured. Home cage activity is normally assessed for three 10-minute trails at hourly intervals. Grooming and rearing behavior is also measured by noting the number of times each of these behaviors occurred and how long they lasted. Locomotion is measured by counting

the number of times the rodent crosses markers, which divide the cage into three equal sections. Limitations to this model are that changes in exploratory behavior can often be confounded by effects due to sickness, disorientation, anxiety/depression, and/or motor defects (Mogil and Crager 2004).

We found in cases of both acute and chronic experimental pancreatitis that activity was significantly reduced relative to controls that received saline, particularly movement in the vertical plane. Further, administration of morphine restored exploratory activity, suggesting relief of pain (Fig. 6).

Provocative stimuli provide information about changes in stimulus response thresholds and response magnitude and have been used to establish the development and persistence of hypersensitivity. In contrast, exploratory activity cannot provide such information, and rather addresses what is perhaps more relevant to human visceral pain conditions, namely ongoing or spontaneous pain. Reductions in activity are assumed to represent the presence of ongoing or intermittent discomfort/pain associated with organ insult, although the frequency and intensity of nociceptor activity and nociceptive transmission cannot be determined. Presumably, because exploratory activity is still present, nociceptive intensity is "acceptable," or limited. In addition to exploratory activity," choice" paradigms (e.g., place preference) are gaining in use to assess ongoing or spontaneous pain. For example, King et al. (2009) reported that spinally administered analgesic drugs (spinal clonidine or ω-conotoxin MVIIA) to rats with a spinal nerve ligation (SNL) increased the time spent in chambers previously paired with drugs only in rats with SNL (and not sham control rats), unmasking a tonic, aversive state (i.e., the presence of ongoing or spontaneous pain). Presently, there are no published reports using such paradigms in rodents with visceral pain.

3.3.2 Prostate

Chronic prostatitis is a prevalent condition (2–10 %, with an overall lifetime prevalence estimated to be 9–16 %; (Nickel et al. 2009) with detrimental effects on quality of life. The National Institutes of Health identifies four categories of prostatitis, the most common of which is category III, or chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), characterized by genitourinary pain, with or without voiding symptoms, in the absence of evidence of a urinary tract infection (e.g., Pontari and Ruggieri 2004). Pain can be significant, even debilitating, and typically radiates to the back, abdomen and/or colorectum. CPPS is a significant clinical problem, accounting for 90–95 % of all cases of chronic prostatitis (Habermacher et al. 2006). Like other abdominal and pelvic pain syndromes (e.g., bladder pain syndrome, irritable bowel syndrome), CP/CPPS has no defined or recognized cause. CP/CPPS is difficult to manage (Cohen et al. 2012), as are other abdominal and pelvic pain syndromes, principally because mechanisms underlying the pain are poorly understood. Among the models of visceral pain discussed here, prostatitis is the least studied with respect to underlying mechanisms of discomfort and pain.

There are several animal models of non-bacterial CP/CPPS. These models are well characterized for basic histopathology and inflammatory mechanisms, but not for nociception (Vykhovanets et al. 2007). As a solid organ, studying discomfort/pain associated with prostatitis is challenging because it is difficult to probe directly the cause of the pain. Thus, like the pancreas, assessment of pain in models of prostatitis in rodents is indirect.

Mechanical Hypersensitivity. As with pancreatitis, testing for organ hypersensitivity in prostatitis has generally employed von Frey-like probing in the area of referred hyperalgesia. The prostate is innervated by the lumbar splanchnic and pelvic nerves and thus the lower abdomen/pelvic area is the area to which sensation is referred. In this model, three behaviors were described as representative of referred hypersensitivity to probing: (1) sharp retraction of the abdomen; (2) immediate licking or scratching of the area of stimulation; and/or (3) jumping (Quick et al. 2013). Response frequency of withdrawal responses was calculated as the percentage of positive responses to probing. Quick et al. (2013) also tested "secondary" hyperalgesia at a distant site, the plantar hind paw, using von Frey-like filaments and an up-down method where testing is started with a 0.04 g filament and subsequently increased in ascending order until a positive response is observed (e.g., either a sharp withdrawal of the paw or licking of the paw), after which the previous weaker filament is applied and, if no response is observed, the next stronger filament is applied (Chaplan et al. 1994).

Exploratory Activity. As described above for assessing ongoing discomfort/pain in pancreatitis, exploratory activity has also been employed in a model of prostatitis (Xie et al. 2013). For up to 4 weeks after injection of an inflamogen into the dorsal lobe of the mouse prostate, during which mechanical hypersensitivity was evident in the area of referred sensation on the lower abdomen, exploratory behavior (total activity in the X-Y plane, distance traveled, and vertical rearing) was found not to be different than in naïve mice or mice which had received an injection of saline into the dorsal lobe of the prostate (Fig. 6). Histologic evaluation of the prostate confirmed the presence of inflammation, consistent with the mechanical hypersensitivity assessed in the same mice. One interpretation of these outcomes is that any ongoing discomfort/pain was insufficient to affect exploratory behaviors, despite the presence of significant mechanical hypersensitivity in the appropriate area of referral.

4 Summary

In humans, visceral pain exhibits a number of characteristics that distinguishes it from pain that originates within other tissues. These differences are likely responsible for the unique symptoms experienced by patients with visceral disease, as well as for the lack of efficacy of pain management strategies developed for other pain conditions—e.g., neuropathic, musculoskeletal, arthritic conditions. Because internal organs receive dual afferent innervation, a more precise understanding of

nociceptive behavior and visceral nociceptor properties is needed to understand the fundamental mechanisms of visceral pain. Experimental efforts thus far have focused on mechano-nociception (e.g., balloon distension), revealing that spinal nerves convey mechano-nociceptive information from the esophagus and stomach (splanchnic innervation) and colorectum (pelvic nerve). The vagus nerves have long been considered to have no role in nociception, but chemo-nociception from the esophagus and stomach is conveyed in the vagus nerves. It is clear then that different nerves have overlapping but also distinct roles in the transmission and perception of visceral pain.

In this chapter, we described several models of visceral "pain" intended to reasonably represent human visceral pain states—kidney stones, acute and chronic pancreatitis, colitis, bladder pain syndrome, etc. Behavioral assessment of "pain" in nonhuman animals is always a challenge to investigators, who are able only to infer from withdrawal and pseudaffective responses the presence of pain. Because noxious intensity stimuli are typically reported as "painful" when applied to human subjects, we reasonably assume they are also "painful" in nonhuman animals. However, non-nociceptive components of the response to noxious stimuli are more difficult to assess. Recently, avoidance and choice paradigms have been employed to assess the "emotional valence" of noxious stimuli and, most recently, spontaneous or ongoing pain. However, with the exception of passive avoidance strategies, conditioned choice/place preference paradigms have yet to be widely employed in models of visceral pain, most of which have focused on studying underlying mechanisms of nociceptor and behavioral hypersensitivity. Such ongoing efforts, we hope, will lead to a better understanding of the fundamental mechanisms through which humans experience visceral pain, which remains a significant burden in the clinical setting.

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Part IV
Cognitive and Affective Aspects

Pain and Cognition in Multiple Sclerosis

Curtis Benson and Bradley J. Kerr

Abstract There is a high prevalence of pain, depression, and cognitive dysfunction in patients with Multiple Sclerosis (MS). These symptoms affect daily tasks resulting in a significant reduction in quality of life. Pain and cognitive changes have been studied across various animal models of MS. In these models the onset of pain and cognitive dysfunction occur early, and do not coincide with the pattern of motor deficits. This is likely underpinned by a number of different mechanisms including changes in glutamate transmission, glial cell activation, and increased levels of pro-inflammatory cytokines. Changes in pain and cognition have been described as belonging to a cluster of symptoms and have been linked through centrally driven processes. In particular, the overactive immune response can induce a state of "sickness-like behaviours" that can influence both pain and cognition. Investigating the mechanism of inflammatory sickness behaviors in MS could lead to a better understanding of the links between pain and cognition. There are currently few effective treatments for pain and cognition dysfunction in MS. Studying the relationship between these symptoms will allow better management of both symptoms.

Keywords Multiple sclerosis · Pain · Cytokine · Glial cell · Inflammation · Sickness behavior

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Contents

Multiple sclerosis (MS) is a degenerative disease where central nervous system (CNS) myelin is attacked by autoimmune processes. Progressive demyelination leads to the loss of proper neuronal signaling causing weakness and ultimately, paralysis. Demyelinating lesions can occur throughout the white matter in the brain and spinal cord, leading to a varied number of symptoms that are hallmarks of the disease. The initial symptoms of MS include muscle weakness, optic neuritis (due to demyelination of the optic nerve), reduced dexterity, and ataxia (Hauser 2006). In addition to these classic motor disabilities, cognitive impairments, increased pain, and depression are also common as the disease progresses (Grasso et al. 2008; Langdon 2011).

There are four established presentations of MS: relapsing remitting (RRMS), secondary progressive (SRMS), primary progressive (PPMS), and progressive relapsing. The majority of patients present with RRMS. RRMS is characterized by acute periods of disability followed by periods of remission of symptoms. If left untreated, a significant number of patients with RRMS will develop PPMS, which is characterized by persistent disability. A subset of people will present with PPMS, where the disease progresses quickly and there are no periods of remission (Compston and Coles 2002). Progressive relapsing multiple sclerosis is the rarest form of MS and is characterized by a diagnosis of primary progressive disease with distinct relapses in symptoms (http://mssociety.ca/en/information/types.htm).

Many immune cell types have been implicated in the progression of MS. T-cells play a significant role in the pathology of the disease. In particular, a specific subset of $CD4^+$ T-helper cells that secrete the cytokine Th17 has been identified to play a central role in the development of MS (Fletcher et al. 2010). Other

peripheral immune cell types such as $CD8^+$ T-cells, B-cells, and a variety of innate immune cells are also involved over the course of the disease (Friese and Fugger 2009; Krumbholz et al. 2012; Mayo et al. 2012). Microglia, the resident immune cells in the CNS, also have a significant influence on the pathophysiology of MS. Microglia have been shown to be involved in demyelination and are also implicated as mediators of neuronal injury (Huizinga et al. 2012). Interestingly, microglia have the capacity to secrete both pro- and anti-inflammatory cytokines (Ransohoff and Perry 2009) and there is an intensive, ongoing research effort to identify the conditions or specific factors, which determine these distinct microglial phenotypes (David and Kroner 2011; Ransohoff and Perry 2009).

The wide variety of cell types involved in the pathophysiology of MS is highlighted by the failure of many cell type-specific therapies to effectively control the disease course in MS. In addition, the role of many of these cells types has been implicated in generating chronic pain states and cognitive deficits in other disorders of the CNS (Beggs et al. 2012; Blank and Prinz 2013; Ji et al. 2013). How these cell types influence chronic pain and cognitive dysfunction in MS currently remains poorly understood.

In this chapter, we discuss the various types of chronic pain and cognitive dysfunction commonly observed in MS patients. We also examine specific animal models used to study the disease and how these models are being extended to study the pathological mechanisms leading to these changes in pain processing and cognitive disturbances.

1 Prevalence of Pain in MS

There are multiple types of painful syndromes associated with MS. Recently, the overall prevalence of pain in MS was estimated to be 62.8 % based on a systematic review of 17 studies (Foley et al. 2013). However, within this analysis the estimates of pain in the disease ranged from 40 to 86 %. The wide variability in these different studies is generally attributed to differences in methods and classification of painful syndromes. This variability aside, pain in MS has been associated with increased disability, disease duration, and age. Although MS occurs more frequently in females, the few studies that have examined it suggest that sex does not appear to be a factor in whether pain develops in MS patients (Archibald et al. 1994; Beiske et al. 2009; Foley et al. 2013; Hirsh et al. 2009).

2 Types of Pain in MS

Pain in MS can be classified as either acute or chronic. The most common types of acute pain associated with MS are tonic spasms, Lhermitte's sign, and trigeminal neuralgia (TN). Although technically a chronic disorder, TN is characterized by

episodes of acute, lancinating pain affecting the innervation territory of one or more branches of the trigeminal nerve (Love and Coakham 2001).

2.1 Trigeminal Neuralgia

TN involves frequent intense pain following the trigeminal innervations in the face, jaw, and cheek. The clinical presentation of TN in MS patients is similar to the TN in non-MS patients. However, TN occurs at a much higher rate in people living with MS compared to the general population. The prevalence of TN in MS patients is somewhere between 2 and 6 % (Foley et al. 2013). This prevalence is more than 20 times higher than that found in the general population. In addition, TN in MS patients differs in that it has an earlier age of onset and a greater chance of presenting bilaterally (Foley et al. 2013).

TN in MS patients occurs due to demyelinating lesions around the trigeminal nerve after it enters the pons in the brainstem (Cruccu et al. 2009; Solaro et al. 2013) Recently, an MRI study compared MS patients with painful TN and MS patients with trigeminal sensory disruption, but who reported no abnormal pain sensations. Using voxel-based 3D analysis, this study found that most MS patients with painful TN had a lesion in the ventrolateral mid pons. In contrast, MS patients without painful TN had lesions affecting other second order brainstem neurons (Cruccu et al. 2009). Electron microscopic analysis of six rhizotomy samples taken from MS patients showed demyelination in the proximal root that extended up to the junction with the distal part of the root. This demyelination was associated with gliosis and immune activation (Love et al. 2001). Biopsies of the nerve in TN patients without MS also show focal demyelination, however, unlike MS patients, little inflammatory processes are observed (Abhinav et al. 2012; Love et al. 2001). Vascular contacts have been observed in MS patients with TN, indicating that "classical TN" can occur in MS patients and that TN in MS may not be due solely to demyelination.

2.2 Lhermitte's Sign

Lhermitte's sign presents as a painful "pins and needles" sensation down the neck and back into the legs. It is usually triggered by movement in the neck. Treatment for Lhermitte's sign is rare as it usually subsides within several weeks (O'Connor et al. 2008). Lhermitte's sign can occur in the absence of MS, however, the prevalence of Lhermitte's sign in MS patients is estimated at approximately 16 % (Foley et al. 2013). One study reported that 41 % of MS patients reported having Lhermitte's sign at one point in their disease course (Al-Araji and Oger 2005). The mechanism underlying Lhermitte's sign is similar to TN and MRI: studies have associated Lhermitte's signs with lesions in the posterior column of the cervical spinal cord (Al-Araji and Oger 2005).

2.3 Pain Within the Extremities

Neuropathic pain or "dysesthesia" in the extremities of people living with MS is generally described as a burning pain within the legs and feet. It is considered the most common form of neuropathic pain in MS (Foley et al. 2013; O'Connor et al. 2008; and Osterberg et al. 2005). This type of pain occurs as the initial symptom in MS in 1–2 % of patients. It has been estimated that 23 % of MS patients report having dysesthesia at some point in the course of the disease (O'Connor et al. 2008). It can occur acutely with a relapse, but is usually a chronic form of pain for MS patients (Osterberg et al. 2005). When dysesthesia occurs acutely during a relapse, it is very likely associated with a focal demyelinating lesion within the dorsal column of the spinal cord. While chronic dysesthesia is typically bilateral, acute onset pain is unilateral depending on the location of the lesion. The acute onset of neuropathic pain can be accompanied by numbness and sensory loss. Similar to other symptoms associated with an MS relapse, dysesthesia can resolve over time. However, it is unclear if acute onset dysesthesia is a predictor or even a risk factor for developing chronic extremity pain as the disease progresses.

Extremity pain in MS includes painful tonic spasms that are a specific type of episodic spasm associated with MS. They usually occur in the limbs and are accompanied by radiating pain. There are multiple triggers for such painful spasms including touch, movement, and heightened emotions. They often can occur without any defined cause (Maloni 2000). It has been reported that pain occurs prior to the spasm and it has been suggested that the pain is not caused by the spasm itself (O'Connor et al. 2008). Painful tonic spasms have been associated with MRI lesions in the brain and spinal regions mediating motor control that result in increased excitability of motor neurons (Truini et al. 2013).

3 Animal Models for MS

There are a variety of animal models used to replicate different aspects of MS pathophysiology and the behavioral deficits associated with the disease. The animal model that is most widely used is experimental autoimmune encephalomyelitis (EAE). This model has contributed significantly to our understanding of MS pathophysiology (Ransohoff 2012). EAE can be generated in multiple ways and is therefore actually a group of MS disease models. EAE was first discovered in 1925 during experiments exploring the neurological side effects of the rabies vaccine. At the time, the rabies vaccine was generated using neural tissue that in some cases led to pathologies not associated with rabies. Experiments with non-human primates determined that the components of the vaccine that included neural tissue induced acute CNS inflammation (Ransohoff 2012). This CNS inflammation generated a disorder that was characterized by impaired gait and paralysis, which was associated with an inflammatory reaction and spinal demyelination, hallmark symptoms of EAE (Rivers et al. 1933).

Significant improvements in the EAE model(s) have occurred over the past 90 years, with multiple antigenic peptides and strains of animals being used to induce the disease. The most common peptides used are components of myelin: myelin basic protein (MBP), proteolipid protein (PLP), or myelin oligodendrocyte glycoprotein (MOG). Currently, the majority of studies induce EAE in the C57BL/6 strain of mice using the antigenic $MOG_{35–55}$ peptide fragment that is emulsified in complete Freund's adjuvant. In addition, mice are treated with pertussis toxin given on the day of induction and 2 days later. Classically, this protocol generates a monophasic disease progression with little or no relapses. However, by varying the concentration of the MOG peptide and the CFA in the emulsion, a relapsing and remitting disease phenotype can be produced (Berard et al. 2010).

4 Pain in Animal Models of MS

In all forms of EAE, the inflammatory response is thought to be driven primarily by $CD4^+$ T-cells. $CD4^+$ T-cells from a myelin peptide immunized animal can be adoptively transferred and are able to induce EAE. Mice with EAE develop an ascending paralysis that begins with weakness in the tail and progresses to include weakness and paralysis in the hindlimbs and occasionally the forelimbs depending on the severity of the disease. This presents problems when conducting assays for pain sensitivity in the model, as the majority of sensory tests in rodents are dependent on the measurement of reflex latency or duration (Olechowski et al. 2009).

4.1 PLP-Induced EAE

Aicher and colleagues conducted the first significant examination of neuropathic pain behaviors in an EAE model in 2004 (Aicher et al. 2004). In this study, EAE was induced both actively and through passive transfer using the $PLP_{139–151}$ peptide fragment. PLP-induced EAE generates a relapsing and remitting clinical course, which has increased disability followed by periods of recovery. Prior to the initial peak in motor dysfunction these mice showed hyposensitivity to noxious heat in the tail. However, it was observed that PLP-EAE mice switch and become hypersensitive to the noxious thermal stimuli as the disease progressed into the chronic stages (Aicher et al. 2004). This experiment was done in both male and female mice and both sexes showed a similar degree of thermal hyperalgesia. The authors also noted that the forepaw did not show an increase in painful responses and that hyperalgesia occurred in the clinically symptomatic areas (Aicher et al. 2004).

4.2 MOG-Induced EAE

Changes in pain sensitivity have also been characterized in the $MOG_{35–55}$ EAE model. In this model, mice develop a significant hypersensitivity to both mechanical and innocuous cold stimuli (Olechowski et al. 2009). Interestingly, these abnormal pain behaviors developed prior to the onset of clinical motor symptoms. These data indicate that processes that occur before frank demyelination or neuronal injury, such as spinal inflammation and the activation of resident glial cells, are contributing to the early sensory dysfunction in the model (Olechowski et al. 2009). The changes in pain responses in $MOG_{35–55}$ EAE correlate with the increases in astrocyte and microglial/macrophage activation, as well as $CD3^+$ T-cells infiltration into the superficial dorsal horn of the spinal cord prior to the onset of motor impairments (Olechowski et al. 2009).

The role of astrocyte activation in the maintenance of neuropathic pain in EAE has recently been shown to be affected by the bradykinin system (Dutra et al. 2013a). Specifically, the B1 bradykinin receptor subtype was shown to colocalize with astrocytes in the spinal cord of EAE mice and pharmacological antagonism of B1 receptor function inhibited mechanical and noxious heat hypersensitivity (Dutra et al. 2013a). In astrocyte cultures, antagonism of the B1 receptor blocked pro-inflammatory cytokine release and nitric oxide synthase-2 expression suggesting that the B1 receptor has a direct role on astrocytes and can modulate pain sensitivity in EAE.

In addition to the immune-driven changes in pain sensitivity in the $MOG_{35–55}$ EAE model, pain behaviors have also been associated with dysfunction in the glutamate transporter system (Olechowski et al. 2010). Using a noxious, persistent pain model, the formalin test, Olechowski and colleagues demonstrated that EAE mice actually exhibit less pain behaviors in response to formalin. The decrease in pain behaviors with formalin stimulation was correlated with a decrease in the levels of a spinal glutamate transporter subtype, EAAT-2. Reductions in EAAT-2 levels are postulated to lead to excessive amounts of extracellular glutamate upon intense noxious stimulation (i.e., formalin) that can then activate pre-synaptic, inhibitory, metabotropic glutamate receptors. Olechowski and colleagues demonstrated that acutely increasing glutamate transporter function could normalize the formalin-induced pain behaviors in mice with EAE (Olechowski et al. 2010). In a separate set of experiments, treating $MOG_{35–55}$ EAE mice chronically with the antibiotic ceftriaxone to prevent the reduction in glutamate transporter levels prevented tactile hypersensitivity associated with EAE from developing (Olechowski et al. 2013). These findings highlight the important role of the glutamate transporter and receptor systems in regulating pain sensitivity in EAE.

4.3 Theiler's Murine Encephalomyelitis Virus

MS is restricted to humans and does not normally occur in other animals. However, a naturally occurring viral infection of the CNS in mice can produce demyelination and MS-like pathologies (Pachner 2011). The most studied are Theiler's murine encephalomyelitis virus (TMEV) and mouse hepatitis virus (MHV). These models have been primarily used to examine the immune response to a persistent neurotropic virus. However, changes in pain sensitivity and cognition have more recently been examined in this model.

Similar to the increased pain sensitivity observed in EAE, mice infected with TMEV display hyperalgesia to heat and have a hypersensitivity to mechanical stimulation (Lynch et al. 2008). Female mice infected with TMEV have a more rapid onset and profound mechanical allodynia compared to male TMEV mice. In either sex, TMEV infected mice displayed a higher amount of C-fiber innervation within the hind paw. In addition, TMEV decreased mRNA expression of opioid receptors in the spinal cord, which was linked to a reduced effectiveness of opioid treatment in the model (Lynch et al. 2008).

4.4 MBP-Induced EAE

Changes in sensory function have also been examined using the MBP Lewis rat model of EAE. Thibault et al. (2011) compared rats induced with MBP and rats induced with MBP that was supplemented with cyclosporin A. Supplementing the MBP with cyclosporin A produces a more severe and relapsing disease. Even with the differences in disease progression, both these EAE models lead to increased mechanical and thermal sensitivity (Thibault et al. 2011). These two models were also characterized using standard treatments for pain. Treatment with acetaminophen had no inhibitory effect on the pain behaviors in the models but first line treatments for neuropathic pain, gabapentin, and tramadol, reduced mechanical hyperalgesia. Tramadol also lessened the hypersensitivity to cold stimuli. The serotonin–norepinephrine reuptake inhibitor duloxetine, that has a similar mechanism as tramadol, only prevented cold allodynia (Thibault et al. 2011).

5 Cognitive Dysfunction in MS

Recently, cognitive dysfunction has been recognized as a major contributing factor to the disability associated with MS (Butler et al. 2009). At the start of a 3-year follow-up study, 30 % of the enrolled MS patients displayed cognitive impairment. Over a 3-year study period, one-third of the MS patients that originally had cognitive deficits deteriorated significantly (Amato et al. 2010). The pattern of

cognitive impairment in MS involves deficits in information processing speed, verbal and visual memory, and executive function. Depression is also a significant concern.

5.1 Information Processing Speed

Information processing speed refers to the ability to maintain, process, and manipulate information over short periods of time (Guimaraes and Sa 2012; Julian 2011). Decreases in information processing speed are common, occurring in 20–30 % of MS patients with reported cognitive deficits (Bergendal et al. 2007). Deficits in information processing speed may be the fastest progressing MS-related cognitive impairment (Denney et al. 2008). Processing information is also influenced by deficits in working memory; however when studied separately, information processing speed is more severely affected in MS patients compared to working memory (Genova et al. 2012).

5.2 Memory

Memory impairment is a significant problem associated with MS. The prevalence of memory deficits is reported to be between 40 and 65 % (Maurelli et al. 1992). Problems include deficits within both working memory and long-term memory (Rogers and Panegyres 2007). It is unclear how memory is exactly affected by MS—it may not reflect a deficit in long-term memory recall. Current research indicates that deficits in memory are due to difficulties in acquiring new memories and not memory recall (Demaree et al. 2000). The quality of the memory acquisition is critical in determining the ability of an MS patient to recall the information (Thornton et al. 2002). More rehearsals of a memory task are often required for an MS patient to have error-free trials compared to healthy controls. However, once the acquisition of the memory associated with the task is complete, MS patients showed no deficits of recall and recognition (DeLuca et al. 1998). Similarly, retrieval practice serves as a significant aid for MS patients with delayed memory recall (Sumowski et al. 2013). Again, these observations indicate that strong initial memory acquisition facilitates strong memory recall.

5.3 Depression

Estimates of the prevalence of depression in MS vary widely, ranging from around 15–50 % (Chwastiak et al. 2002; Feinstein 2011; Motl et al. 2010a; Wood et al. 2013). The variability in estimates could be due to methods of study, such as the

use of self-reporting scales or clinical determination of depression. It is unclear whether depression in MS is linked with physical disability and MS disease progression (Chwastiak et al. 2002; Lester et al. 2007; Patten et al. 2005). However, depression in MS is associated with lesion load. Depression is more prevalent in patients with brain lesions compared to patients with lesions limited to the spinal cord (Rabins et al. 1986). More recently, studies have looked at the location of MS lesions in relation to MS-associated depression. Feinstein et al. showed that depressed MS patients had a greater lesion volume in the left medial interior temporal lobe compared to non-depressed and noncognitively impaired people with MS (Feinstein et al. 2004).

Besides the strong correlation between white matter lesion size and MS depression, cortical lesion load has been reported to be involved in MS-associated cognitive impairment (Calabrese et al. 2009). However, Papadopoulou et al. recently indicated that the amount of cortical lesions does not correlate with symptoms of depression in MS (Papadopoulou et al. 2013). The authors did find that cortical lesions were linked with other neuropsychological outcomes. They suggested that this might indicate a link between cortical lesions and impairment in information processing speed.

An interesting study by Gold et al. demonstrated that the severity of depression in MS is linked to decreased hippocampal volume that is restricted to the right side of the hippocampus. In addition to volume, the authors also used surface rendering analysis that indicated that the shape of the right hippocampus was changed in depressed MS patients (Gold et al. 2014).

5.4 EAE and Cognitive Dysfunction

Similar to the study of pain in EAE models, changes in cognitive function have only recently been examined in animal models of MS. One difficulty in examining cognition in the EAE model is the confound of significant motor impairments in EAE animals. One of the first studies of changes in cognitive function in EAE examined rats in the later stages of the disease that had recovered motor function. Despite the recovery, EAE rats had significant deficits in the Morris Water Maze memory task. This correlated with declines in choline acetyltransferase activity and nerve growth factor expression in the cerebral cortex, hippocampus, and basal forebrain. The cholinesterase inhibitors donepezil and rivastigmine reversed these deficits (D'Intino et al. 2005). This study suggests that, similar to changes in pain sensitivity, memory deficits are also independent of clinical score and motor function. In support of this, memory and cognitive deficits occur early in EAE disease, prior to the onset of motor symptoms. Dutra and colleagues, using the Morris Water Maze and object location test, found mice with EAE had spatial memory deficits 7–11 days post disease induction, a time point prior to any motor dysfunction. These early memory changes were also associated with down-regulation of choline acetyltransferase (Dutra et al. 2013b).

In mice with EAE, the pro-inflammatory cytokine interleukin-1 beta (IL-1β) can disrupt the balance between long-term potentiation (LTP) and long-term depression (LTD) in the hippocampus (Nistico et al. 2013). IL-1β was expressed in activated microglia within the hippocampus of EAE mice correlated with an enhancement of LTP. Hippocampal slices treated with IL-1β also showed an enhancement of LTP. In both EAE and IL-1β-treated hippocampal slices the change in LTP was associated with an inhibition in GABAergic signaling (Nistico et al. 2013). This imbalance between excitatory (LTP) and inhibitory (LTD) processes in the hippocampus may have profound effects on memory formation (Nistico et al. 2013). These observations highlight the role of an aberrant immune response in the processes underlying cognitive dysfunction.

In addition to deficits in cognition before the presence of motor dysfunction, increases in pain and memory dysfunction can occur on the day of onset of clinical signs in the $MOG_{35–55}$ EAE model (Olechowski et al. 2013). Onset of clinical signs was defined as the first day of observation of tail paralysis. Importantly, EAE mice with tail paralysis had no significant deficits in gross motor function in the hindlimbs. Changes in pain sensitivity and memory were associated with decreases in the glutamate transporter EAAT-2. As described above, EAAT-2 is predominantly expressed on astrocytes and removes extracellular glutamate. Dysfunction of EAAT-2 causes excess glutamate and has been implicated in other neurodegenerative diseases (Kim et al. 2011). Increasing glutamate transporter levels in EAE mice using the antibiotic ceftriaxone normalized pain behaviors and cognitive performance (Olechowski et al. 2013).

6 Cognitive Dysfunction and Chronic Pain

There is a strong link between chronic pain and disturbed cognition. This is underpinned by the fact that pain has a variety of underlying cognitive components. Processing pain involves learning, memory, and decision making (Moriarty et al. 2011). In both animal studies and clinical populations, pain has been described to affect cognition in multiple domains, including attention, decision making, and memory (Low 2013, Moriarty et al. 2011). In MS and EAE, the role of inflammation must be considered when examining the relationship between pain and cognition. The significant role of the immune system in modulating cognition and affect has been described as an "EAE-associated behavioral syndrome" (Pollak et al. 2002). As described above, pain and cognitive function can also be significantly affected during active inflammation. It has been theorized that the behavioral changes that occur with inflammation represent a novel motivational state, which drives an organism's actions to increase its chances of survival during an infection (Dantzer 2001). This novel state is often described as sickness-like behavior (Dantzer 2001).

Despite the association between cognition and excess glutamate described by Olechowski and colleagues, deficits in memory could be due to increased sickness-like behaviors. Sickness-like behaviors have been well documented in EAE

(Musgrave et al. 2011; Pollak et al. 2003; Pollak and Yirmiya 2002). In both people and animals, sickness induces behavioral changes and depression through a CNS response to inflammatory cytokines (Dantzer 2001). Olechowski et al. observed that EAE had reduced total exploration during assays of memory (novel object recognition). Sickness could influence the motivation to explore during the novel object recognition test and thus effect the formation of memories during the assay (Olechowski et al. 2013).

In addition to memory changes induced by inflammation, sickness behaviors have been linked to increased anxiety in EAE. Using an open field test and the elevated plus maze to measure anxiety levels, tumor necrosis factor alpha (TNF-α) appears critical in the development of anxiety behaviors in EAE (Haji et al. 2012). Elevations in TNF-α were associated with increased microglia activation in the striatum. Inhibiting the expression of TNF-α in EAE mice had an anxiolytic effect. Interestingly, administration of TNF-α to non-diseased mice mimicked the anxiety behaviors displayed by mice with EAE (Haji et al. 2012). Similarly, the central or peripheral administration of pro-inflammatory cytokines IL-1β or TNF-α can induce sickness behaviors (Kelley et al. 2003). Cytokine-induced sickness may contribute to behavioral changes in EAE and other auto-immune diseases.

7 Summary

While significant amounts of research have examined the effects of fibromyalgia, diabetic neuropathy and chronic lower back pain on cognitive functioning, the influence of MS-related pain on cognition has received little attention (Moriarty et al. 2011). In the majority of these disorders, chronic pain has an impact on a variety of mental processes including: attention, information processing, memory, learning, and executive function (Hart et al. 2000; Moriarty et al. 2011). As discussed above, these cognitive domains are also disrupted in MS. A recent study has suggested that in MS, pain, fatigue, depression, and cognitive impairment are parts of a cluster of symptoms (Motl et al. 2010b; Newland et al. 2012). Sickness behavior may be one element involved in the clustering of these symptoms. In MS patients and animal models, inflammatory sickness behaviors have a role in these affective symptoms (Heesen et al. 2006; Musgrave et al. 2011; Olechowski et al. 2013). Therefore, further study of the mechanisms underlying sickness behavior may provide substantial insight into the relationship between pain and cognition.

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The Self-administration of Analgesic Drugs in Experimentally Induced Chronic Pain

Carrie L. Wade and Carolyn A. Fairbanks

Abstract Systemically and centrally delivered opioids have been comprehensively studied for their effects both in analgesic and addiction models for many decades, primarily in subjects with presumptive normal sensory thresholds. The introduction of disease-based models of persistent hypersensitivity enabled chronic evaluation of opioid analgesic pharmacology under the specific state of chronic pain. These studies have largely (but not uniformly) reported reduced opioid analgesic potency and efficacy under conditions of chronic pain. A comparatively limited set of studies has evaluated the impact of experimentally induced chronic pain on self-administration patterns of opioid and non-opioid analgesics. Similarly, these studies have primarily (but not exclusively) found that responding for opioids is reduced under conditions of chronic pain. Additionally, such experiments have also demonstrated that the condition of chronic pain evokes self-administration or conditioned place preference for non-opioid analgesics. The consensus is that the chronic pain alters responding for opioid and non-opioid analgesics in a manner seemingly related to their respective antiallodynic/antihyperalgesic properties under the specific state of chronic pain.

Keywords Chronic pain · Reinforcement · Opioid · Analgesic · Self-administration · Conditioned place preference

Contents

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1 Introduction

The experience of chronic pain is increasingly recognized as a public health concern to the USA alongside a global public health matter. The intense and comprehensive search to improve our understanding of the complex mechanistic changes associated with various chronic pain syndromes continues alongside global efforts to optimize delivery and use of currently available chronic pain medications. These investigations have been enabled for decades by the use of well-established animal models of sensory function and analgesic pharmacology. Our knowledge of sensory plasticity, neuroanatomical, and physiological changes has been greatly advanced in the last several decades by the introduction of a wide variety of animal models specific for pain conditions including inflammation (Colpaert et al. 1980), nerve injury (Bennett and Xie 1988; Seltzer et al. 1990; Kim and Chung 1992; Decosterd et al. 2002), tumor invasion (Wacnik et al. 2001; Sasamura et al. 2002), chemotherapeutic exposure (Polomano et al. 2001; Authier et al. 2003), and muscle pain (Kehl et al. 2000; Sluka et al. 2001), among many others. Many of these have been reviewed extensively previously (Gregory et al. 2013; Wang and Wang 2003) and in this collection of essays. With the establishment of each of these models, acute opioid pharmacology has been widely assessed (Sasamura et al. 2002; Kehl et al. 2000; Hylden et al. 1991; Mao et al. 1995; Bian et al. 1995; Yaksh et al. 1995; Ossipov et al. 1997; Nichols et al. 1995; Fairbanks et al. 2000; Yamamoto and Sakashita 1999; Wacnik et al. 2000; Yaksh 2002; Petraschka et al. 2007). We have significant knowledge of how opioids either reduce or fail to reduce hypersensitivity under these particular acute conditions, but minimal information on how subjects respond to these agents chronically.

We also have a substantial literature of opioid pharmacology in models of addiction that spans many decades (Koob and Le Moal 2008). Quite understandably, it is this extensive knowledge base of the neurobiology of addiction that is consulted when considering the impact of opioid pain medications on central nervous system (CNS) centers of reward and addiction (Ballantyne and LaForge 2007; Bailey et al. 2010). The vast majority of studies of opioid reward and addiction, however, have taken place in naïve subjects with presumptive normal sensory thresholds. This notation is important because what has been learned through the last 20 years of specific pain condition

modeling is that the central (Urban and Gebhart 1999; Kuner 2010; Zieglgansberger et al. 2005) and peripheral nervous systems (PNS) (Koltzenburg et al. 1999) are altered under conditions of persistent hypersensitivity. CNS alterations in subjects with chronic pain have been suggested to account for corresponding changes in chronic pain subjects' overall response to opioids ranging from analgesic effect to the propensity to transition to an addictive state. It is essential, therefore, to consider the total opioid pharmacology in the context of the chronic pain condition of interest. A comparatively limited number of studies of opioid responding under conditions of chronic pain have, in fact, demonstrated altered responding for opioids in either an enhanced or reduced responding direction. This chapter reviews and compares the observations from these studies to provide a comprehensive description of what has been learned to date. Taken collectively, the data tend to converge upon a general pattern that the state of analgesia, regardless of the reinforcing effects of the drug under normal conditions, is, in and of itself, reinforcing under conditions of established chronic pain.

2 Adjuvant-Induced Arthritis

Some of the earliest work evaluating analgesic self-administration in a model of chronic pain came out of efforts to characterize the establishment of a model of arthritic chronic pain. In order to assess whether complete Freund's adjuvant (CFA)-treated subjects would demonstrate a preference for analgesics, Colpaert et al. (1980) instituted a two bottle choice paradigm where rats had available to them either a bottle containing suprofen (a clinically used NSAID, since discontinued) or a bottle containing an alternative sweet solution. In this first study, arthritic rats demonstrated increased intake of the suprofen-containing solution relative to control rats. It is important to note that NSAIDS are not thought to be reinforcing in normal subjects (Hoffmeister and Wuttke 1975). Colpaert and colleagues followed this report with a similarly designed study (Colpaert et al. 1982) demonstrating that arthritic rats self-administered oral fentanyl significantly more than control rats comparable to the outcomes observed with suprofen. Taken together, these results suggested that the analgesic state itself, rather than the opioid, serves as the reward. Consistent with that proposal, Colpaert et al. (2001) later showed increased fentanyl consumption (two bottle choice model) in rats with mycobacterial-induced inflammation. However, in this case, they observed attenuation of the elevation in fentanyl consumption when the rats were provided with concurrent, non-contingent delivery of dexamethasone. This outcome suggested that the rats chose fentanyl for analgesic purposes rather than for its other rewarding properties. One aspect not represented in the design of this initial series was an assessment of chronic pain. However, it is noteworthy that the time course of the elevation in suprofen self-administration in arthritic rats relative to control rats closely followed the time of maximal elevation in paw and joint diameter (Colpaert et al. 1980). Once the paw and joint diameter elevation decreased, so declined the elevation in suprofen intake. This time-course correspondence between elevation in

fentanyl intake and paw and joint diameter was even tighter in the fentanyl study (Colpaert et al. 1982), as was the time course of body weight decrease and vocalizations, measures interpreted to be indicative of pain.

Shortly thereafter, a third study conducted by Lyness et al. (1989) compared the self-administration of intravenous morphine (5.0 mg/kg/injection, 24-h access) in rats with CFA versus vehicle-injected controls. In this experiment, the tail pressure test was applied to document the development of mechanical hyperalgesia and it was systematically shown that the self-administered morphine resulted in an anti-hyperalgesic response. However, in contrast to the previous studies, the rats with established chronic inflammatory pain self-administered significantly *less* morphine than control rats. Further, non-contingent delivery of indomethacin (an NSAID) by the experimenter significantly reduced morphine self-administration specifically in the arthritic, but not the control rat. Finally, as the pathology of the inflammation resolved, the arthritic rats began to escalate morphine intake to a level more comparable to that of control. These outcomes were interpreted by the authors as suggestive of the state of chronic pain resulting in an apparent *reduction* in the reinforcing properties of morphine, which, in this case, were able to be dissociated from the motivation for analgesic relief.

3 Neuropathic Pain

The nerve injury models of neuropathic pain (Bennett and Xie 1988; Seltzer et al. 1990; Kim and Chung 1992; Decosterd et al. 2002) were developed in the late eighties and early nineties and shortly thereafter a number of studies demonstrated that the opioid analgesics in these models were reduced in potency (Mao et al. 1995; Bian et al. 1995; Yaksh et al. 1995; Ossipov et al. 1997; Nichols et al. 1995; Fairbanks et al. 2000; Yamamoto and Sakashita 1999; Yaksh 2002; Petraschka et al. 2007) with some dependence on route of administration (Bian et al. 1995; Nichols et al. 1995; Lee et al. 1995). The introduction of these models enabled the evaluation of opioid self-administration under conditions of neuropathic pain, as had been previously assessed under conditions of inflammatory pain. Kupers and Gybels (1995) used the two bottle choice fentanyl self-administration protocol established by Colpaert to compare fentanyl self-administration between subjects with partial sciatic nerve ligation (Seltzer et al. 1990) and adjuvant arthritis (*Mycobacterium butyricum*) (Colpaert et al. 1980) as well as their respective controls. In the adjuvant arthritis model, an elevation in fentanyl consumption in the 3rd week corresponded precisely with the time of elevation in spontaneous pain indicators (paw elevations and shaking) measured in a separate group of arthritic rats. Von Frey thresholds decreased and signs of spontaneous pain elevated by 1 week post-surgery in neuropathic rats as expected. However, in contrast to the arthritic adjuvant experiments, fentanyl consumption did not elevate in neuropathic rats (not tested behaviorally) and, in fact, remained comparable to that of controls throughout the four-week testing period. The authors attributed these outcomes to

potential reduction in analgesic effectiveness of opioids under conditions of chronic pain, although opioid analgesic pharmacology was not assessed in this particular study.

In a later report, Martin and colleagues described their comprehensive and systematic evaluation of opioid self-administration of four clinically relevant prescription opioids (morphine, fentanyl, hydromorphone, methadone) and the gold standard opioid reinforcer heroin in rats with L5/L6 spinal nerve ligation (Kim and Chung 1992). In this study, the development of mechanical hypersensitivity was confirmed by von Frey threshold analysis by days 5–7 post-surgery for each subject included in the study and monitored twice weekly for the duration to ensure persistent hypersensitivity. Nerve-injured and sham-operated control rats were then trained to maintain lever pressing behavior for varying doses of i.v.-infused opioid reward (1 dose per hour within a 4-h session). Dose–response curves for each opioid were constructed and compared between the neuropathic and control conditions. Sham-operated rats developed standard inverted U-shaped dose–response curves that are characteristic of fixed-ratio drug self-administration experiments. In the case of the neuropathic rats, the inverted U-shaped dose–response curve was shifted rightward for all the opioids, meaning that the lower doses that were effective in eliciting operant responding behavior in control rats were ineffective in neuropathic rats. In other words, at the lower opioid dose range, rats with chronic pain did not respond with behavior indicative of addiction. The higher doses that did elicit responding in neuropathic rats were notably comparable to those that reverse neuropathic mechanical allodynia. Further, and importantly, non-contingent delivery of a dose of intrathecal clonidine (an alpha-2 adrenergic agonist) that alleviates nerve injury-evoked mechanical hypersensitivity significantly reduced heroin-maintained responding in nerve-injured, but not control, subjects. Taken together, these data support the proposal that the antihyperalgesic effects of opioids contributed to the motivation to maintain responding in subjects with chronic pain. That interpretation is consistent with the prior views advanced by Colpaert et al. (1980, 2001), Lyness et al. (1989), and Kupers and Gybels (1995).

Martin and colleagues further demonstrated in a separate study that rats with chronic neuropathic pain (Kim and Chung 1992) (but not normal rats) will develop maintained responding for intrathecally delivered clonidine, although not for adenosine (Martin et al. 2006). Spinal clonidine-maintained responding in nerve-injured rats (Martin et al. 2006) was extinguished either by inclusion of the alpha-2 adrenergic receptor antagonist idazoxan with intrathecal clonidine or substituting saline for the clonidine intrathecal infusion. Since clonidine is typically considered to have minimal abuse liability (Martin et al. 2006) and humans do not abuse clonidine for euphoric effect, it is thought that the rats with chronic pain likely self-administered spinal clonidine for its analgesic properties. It is noteworthy that a similar pattern was later observed where nerve-injured (but not control) rats demonstrated conditioned place preference [a model often used as a measure of reward (Tzschentke and Schmidt 1995)] in a chamber associated with intrathecally delivered clonidine, but not adenosine (King et al. 2009). This difference was proposed to be explained by the observation that adenosine reduces evoked hyperalgesia in

human subjects with neuropathic pain but not spontaneous ongoing pain (Eisenach et al. 2003). Taken together, these observations are supportive of the proposal that operant measures may be able to distinguish between analgesics effective for spontaneous and ongoing pain versus hypersensitivity evoked by sensory stimuli.

4 Neuropathic Pain, CFA, Vincristine

Consistent with the work of both Kupers and Gybels (1995) and Martin et al. (2007), we (Wade et al. 2013) recently demonstrated that mice with chronic pain induced by either nerve injury (Fairbanks et al. 2000), adjuvant arthritis, or chronic exposure to the chemotherapeutic vincristine do not establish oral fentanyl-maintained responding in contrast to their respective controls with normal sensory thresholds. In these experiments, all mice were given the opportunity to lever press for oral fentanyl reward (active lever) or no reward (control lever) in daily 2-h sessions for a 3–4-week period following the establishment of chronic hyperalgesia. Importantly, and consistent with similar observations in rat (Martin et al. 2007), mice with nerve injury, paw inflammation, or chemotherapy exposure demonstrated food-maintained responding indicating that the failure to develop opioid-maintained responding was specific for the drug and not indicative of a generalized inability to acquire the behavior. These studies are distinguished from the previous reports in that the development of opioid-maintained responding in control versus chronic pain conditions was monitored daily during the initiation and maintenance phases of all chronic pain conditions.

5 Long-Access Self-administration

It is noteworthy that the prior studies (Martin et al. 2007; Wade et al. 2013) evaluating opioid self-administration under conditions of chronic pain might best be characterized as short-access sessions, meaning that the subjects had access to the opioid in increments of 1–4 h. Wade et al. (2012) have since expanded analysis to evaluate the self-administration of intravenous oxycodone in CFA-treated rats in long-access sessions (12 h in duration). Consistent with the prior reports, responding for oxycodone is significantly diminished in CFA-treated rats versus saline-injected controls under conditions of long-access sessions over a period of at least 13 days. Further, evaluation of the motivation for reward by examining breakpoints under a progressive ratio of reinforcement was conducted at the conclusion of the study. It was observed that the breakpoint (the limit in the lever presses necessary to receive the next reward) is significantly lower in animals with CFA-induced inflammation relative to controls.

6 Cannabinoid-Maintained Responding in Neuropathic Pain

Consistent with the proposal that the self-administration method of operant conditioning may be an effective approach to evaluate potential analgesics with greater sensitivity than standard reflex measures (Colpaert et al. 2001; Martin et al. 2007), Gutierrez et al. (2011) conducted a comparison of CB2 receptor-selective agonist (*R, S*)-AM1241-maintained responding between neuropathic and control rats (both sham-operated and naïve). Using the spared nerve injury (SNI) model in this case, mechanical hypersensitivity was established and the rats were allowed to enter 4 consecutive daily sessions with the opportunity to press two levers, one of which resulted in delivery of i.v.-infused (*R*, *S*)-AM1241. A key observation is that naive rats did not develop lever preference for (*R*, *S*)-AM1241, whereas rats with established neuropathic pain significantly increased preference for the lever associated with (*R, S*)-AM1241. Neuropathic rats were further evaluated for sensory thresholds 15–20 min following the operant session when it was observed that mechanical hypersensitivity was significantly alleviated, indicating that a sufficient amount of drug was self-administered to achieve an antiallodynic/antihyperalgesic effect. Such a paired set of observations (measurement of operant responding and sensory thresholds within the same subjects) provides strong evidence in support of the proposal that the state of analgesia is a reinforcing condition. Additionally, two important observations were introduced by this study: First, when the reinforcer was switched to vehicle, neuropathic rats ceased responding for reward and showed an allodynic response to presentation of von Frey fibers. Interestingly, the removal of the cannabinoid did not result in an initial elevation in lever responding prior to extinction. This pattern contrasts with that typically observed with abused drugs in rats with presumptive normal sensory thresholds. Second, subjects that received sham operation as a control for the nerve injury demonstrated comparable active lever responding for CB2 receptor-selective cannabinoid as did neuropathic animal during the FR1 schedule, although their mechanical withdrawal thresholds (at normal sensory levels) were not affected by the drug session. Consideration must be made that sham-operated controls (while viewed as a control for neuropathic pain) perhaps should not be considered "pain-free" control subjects given the fact that, by definition, they undergo surgical procedures involving muscle damage and skin incision and can be presumed to have experienced post-operative pain. The motivation for sham-operated, but not naïve subjects to lever press for cannabinoid reward, may be associated with analgesic effects not detectable by the reflex methods. It is also noteworthy that the responses of the sham-operated subjects were less than those of the neuropathic rats with increased schedules of reinforcement (e.g., FR6), suggesting that the neuropathic rats worked harder for (*R*, *S*)-AM1241 than sham-operated controls. These data indicate that, although the sham subjects may be representative of a post-operative pain state, the response to pain

was distinguishable between the two populations, as might be expected. These observations highlight the limitations of the sensory reflexes in fully detecting chronic pain responses and feature the operant self-administration model as an approach to screen potentially clinically relevant analgesic medications.

7 Analgesic-Induced Conditioned Place Preference

In contrast to operant studies of self-administration of analgesics under conditions of neuropathic pain, which have been comparatively limited, recent years have seen an increase in studies involving the conditioned place preference (CPP) model of reward to examine a variety of analgesic substances [e.g., clonidine and lidocaine in chronic pain models of inflammation and nerve injury (He et al. 2012)]. Some of the recent studies using CPP to assess non-opioid analgesic drugs have been recently reviewed (Navratilova et al. 2013) and a pattern similar to the previously described self-administration studies has emerged, consistent with the long-standing proposal that relief from pain is a rewarding state. Interestingly, however, studies of morphine-induced CPP yield notably contrasting results. Morphine-induced CPP has been shown to be significantly reduced in neuropathic rats (Ozaki et al. 2004) and mice (Niikura et al. 2008) relative to their sham-operated counterparts as well as reduced in mice with established CFA-(Betourne et al. 2008) and carrageenan-induced (Suzuki et al. 1996) inflammation and hindpaw thermal hyperalgesia. Morphine-induced CPP has been demonstrated to be reduced in mice with either formalin-induced inflammation (Suzuki et al. 1996; Narita et al. 2005) or neuropathic pain (sciatic nerve ligation) (Petraschka et al. 2007; Ozaki et al. 2002, 2003). In contrast, a recent study (Cahill et al. 2013) reported that low doses (1, 2 mg/kg, sc) of morphine significantly induced CPP in nerve-injured (spared nerve injury model), but notably not in control rats. These doses were demonstrated to be antiallodynic using von Frey mechanical stimulation and it is suggested that the analgesic rather than the hedonic properties may account for the rewarding effects. It is noteworthy that higher doses (4, 8 mg/kg) were less effective, consistent with the inverted U-shaped dose–response curves typical of opioid agonists in operant self-administration studies. The difference between this most recent report (Cahill et al. 2013) of morphine-induced CPP in chronic pain versus the prior morphine-induced CPP literature in various states of chronic pain (Ozaki et al. 2002, 2003, 2004; Niikura et al. 2008; Betourne et al. 2008; Suzuki et al. 1996; Narita et al. 2005) is not clear. However, taken collectively, these results are in agreement that under the condition of chronic pain, the reward properties of morphine are altered relative to normal control subjects.

8 Studies on CNS Alterations Under Conditions of Chronic Pain

At this point, two general themes should be evident from accumulated evidence from 30 years of operant studies of opioid responding in chronic pain states: (1) opioid responsiveness is frequently diminished (although sometimes increased) under conditions of chronic pain and (2) the state of pain relief is, itself, a reinforcing event. It should follow that chronic pain-induced alterations in the CNS could contribute to such alterations in pharmacological response. This question is an area of increasing investigation and, while a comprehensive review is beyond the scope of this chapter, some featured observations will be noted for consideration. It is increasingly recognized that persistent chronic pain causes functional (Low et al. 2012) and structural alterations throughout the CNS, some of which can result in cognitive deficits that are reversible with effective pain treatment (Seminowicz et al. 2011). Given the operant behavior reviewed here demonstrating altered responding for analgesics under conditions of chronic pain, it might be expected that some of these changes take place in the CNS locations where reward and addiction intersect with modulation of pain and/or are directly mediated. In fact, a literature is emerging that examines molecular and functional alterations at some of these centers under conditions of persistent chronic pain. For example, peripheral nerve injury results in altered DNA methylation in pre-frontal cortex (Alvarado et al. 2013; Tajerian et al. 2013), a brain region that contributes to the affective component of pain (Tracey and Bushnell 2009; Schweinhardt and Bushnell 2010) and is also known to contribute to the development of addiction (Goldstein and Volkow 2011). It has been well established that the mesolimbic dopaminergic system projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) drives the rewarding effect of morphine (Koob 1992; Nestler 1996; Narita et al. 2001). Specifically, opioids are thought to inhibit GABAergic interneurons in the VTA which in turn disinhibit dopamine cells in the VTA resulting in elevated dopamine levels in NAcc. Not surprisingly, a number of mechanistic studies of alterations in VTA and in NAcc under conditions of chronic pain have emerged.

8.1 NAcc

In normal mice, morphine-induced CPP results in a elevation of dopamine in NAcc; this elevation is reduced under conditions of chronic pain (Narita et al. 2005). However, in formalin-treated mice, inhibition of morphine-induced CPP is also accompanied by a decrease in NAcc dopamine levels, a decrement that can be reversed with intrathecal delivery of an immunoneutralizing antibody to dynorphin, suggesting involvement of the kappa opioid system (Narita et al. 2005). Similar observations were recently reported by Taylor et al. (2013) who observed that chronic constriction injury also led to an overall reduction in basal and morphine-stimulated

dopamine levels in the NAcc. Through fMRI, Baliki et al. (2010) have shown that patients with chronic low back pain present a distinct pattern of connectivity within the NAcc that corresponds to the magnitude of chronic spontaneous pain. They have further shown that anticipation of relief of pain (analgesia) results in activation of NAcc core (Baliki et al. 2013). These data are congruent with a recent study (Navratilova et al. 2012) of peripheral nerve block (PNB)-induced CPP in rats with incisional pain (Brennan et al. 1996). In this experiment, incised rats were injected 24 h post-surgery with lidocaine to induce peripheral nerve block in a paired chamber manner to evoke CPP. Lidocaine-mediated analgesia results in place preference presumably from relief of the ongoing spontaneous pain. This observation suggests that relief of post-surgical spontaneous pain is also rewarding, consistent with the previous interpretation of the observations of Colpaert et al. (1980, 1982, 2001), Lyness et al. (1989), Kupers and Gybels (1995), Martin et al. (2006), and Gutierrez et al. (2011).

8.2 VTA

Extracellular signal-related kinase (ERK) derives from the serine/threonine protein kinases and contributes to the cellular processes involving protein phosphorylation and gene expression. ERK activity in the reward centers of the brain has been examined under conditions of non-contingent chronic morphine administration with and without chronic pain. Berhow et al. (1996) showed that ERK activity increased in the VTA following implantation of a morphine pellet (75 mg, 5-day time course) and this ERK activity subsequently increased tyrosine hydroxylase activity, a biomarker for increased dopamine production in the reward centers. Ozaki et al. (2004) have similarly evaluated this pathway as a mechanism underlying their observation that neuropathic pain reduces morphine CPP (Ozaki et al. 2002). They observed that rats with neuropathic pain exhibit decreased ERK activity (lower levels of p-ERK) in the VTA compared to their sham-operated controls (Ozaki et al. 2004). Complementarily, morphine-induced CPP was also inhibited as a result of i.c.v. injection of a specific MEK inhibitor, PD98059, which blocks ERK activity. These data suggest a mechanism for the reduction of opioid-induced CPP in neuropathic pain.

8.3 Intracranial Self-stimulation of the VTA

To determine whether the alterations in opioid responding under conditions of neuropathic pain were due to a concomitant change in the dopaminergic input from the VTA to the NAcc and/or the ability of opioid agonists to modulate these inputs, Ewan and Martin (Ewan and Martin 2011) applied a model of intracranial self-stimulation (ICSS) specifically to the VTA. It has been demonstrated that rats will

develop and maintain lever pressing for electrical stimulation to the VTA, which results in elevation of dopamine in the NAcc (Hernandez and Shizgal 2009). Nerve-injured subjects revealed equivalent stimulation–response curves as controls, suggesting that the effects of nerve injury on opioid reinforcement may be specific to opioids and not general reinforcers. In support of that proposal, the ICSS-potentiating effects of morphine and heroin (but not cocaine) were both reduced in neuropathic rats compared to controls. These data in essence identify a mechanism of diminished opioid responding to the VTA and illustrate the specificity of the effect to the opioid system.

9 Summary and Conclusions

Taken collectively, the 30 years of operant studies of analgesic drugs (both opioid and non-opioid) suggest two organizing principles. First, by and large, states of chronic pain induced by diverse manipulations (inflammation, nerve injury, tumor invasion, or chemotherapeutic exposure) tend to reduce opioid self-administration. It seems that alterations in opioid sensitivity of dopaminergic neurons projecting from the VTA to the NAcc are a likely explanation for this phenomenon. Second, subjects with established chronic pain tend to seek the state of analgesia. The analgesic state itself is a rewarding stimulus. Evidence in support of this principle is found in elevated ingestion of oral analgesics (both opioid and non-opioid), reduced responding for one analgesic when another is provided non-contingently, and the self-administration of non-opioid analgesics and CPP of non-opioid analgesics, and some analgesic doses of opioids under conditions of chronic pain. This phenomenon is consistent with the human clinical experience where it is common that patients appropriately seek pharmacological treatment for relief of their malignant or chronic pain (Walsh 1984). Sometimes, the manner of that pursuit resembles inappropriate drug-seeking behavior (Marks and Sachar 1973; Weissman and Haddox 1989; Kirsh et al. 2002; Weissman 2005; Lusher et al. 2006). This phenomenon, termed "pseudoaddiction" (Weissman and Haddox 1989), is often resolved by adequate pain management (Marks and Sachar 1973; Weissman and Haddox 1989; Kirsh et al. 2002), not unlike reduced opioid self-administration with non-contingent delivery of the non-opioid analgesics reported in the aforementioned chronic pain rodent models (Colpaert et al. 2001; Lyness et al. 1989; Martin et al. 2007).

The question is often asked, what is the percentage of chronic pain patients that develop an opioid addiction and how does that compare to the general population? There is considerable variability in the clinical data responding to this question (Ballantyne and LaForge 2007). However, some patterns have emerged that merit attention. An evidence-based structured review (Fishbain et al. 2008) of sixty-seven studies is well recognized (Garland et al. 2013; IOM 2011; Minozzi et al. 2012) as having contributed useful progress toward this question. Within this review, 24 studies comprising 2,507 chronic pain patients exposed to opioids found that the abuse/addiction rate was 3.27 % (range 0–45 %). A number of variables [e.g., history

of prior non-opioid (Pletcher et al. 2006) and opioid substance abuse, mental illness, duration on opioids (Edlund et al. 2007)] are identified as likely to influence the establishment of opioid misuse/addiction following implementation of chronic opioid analgesic therapy for chronic pain. Taking at least one of these into consideration, the data were analyzed separately for those chronic pain patients without a prior history of substance abuse/addiction. The abuse/addiction rate to opioid medication was 0.19 % when considering only subjects without a prior history of abuse and addiction. These values are sometimes compared to the prevalence of addiction in the general population (~10 %) (Fishbain et al. 2008); perhaps a more useful comparison would be to more recent data collected from a survey (Huang et al. 2006) of 43,000 adults which revealed that the prevalence specifically of prescription opioid non-medical use to be 4.7 % and conversion to opioid addiction as 1.4 %.

This same review (Fishbain et al. 2008) also considered seventeen studies representing 2,655 chronic pain patients that evaluated aberrant drug-related behaviors (ADRBs, e.g., unauthorized dose escalation, aggressively requesting medication, hoarding medication, among others); these can be indicators of the development of addiction. The percentage of chronic pain patients exposed to opioids that displayed ADRB (11.5 %, range 0–44.6 %)) was reduced to 0.59 % when assessing only subjects without a prior history of substance abuse and addiction. As mentioned above, behaviors associated with pseudo-addiction can resemble ADRBs; this possibility was not considered by the reviewed studies and so the prevalence noted in the structured review may be an overestimate, as the authors noted. Further, it is noteworthy that the distinctions between definition and/or diagnosis of opioid abuse versus true opioid addiction are often obscured in the broader social and clinical discussion (Fields 2011). Therefore, the question posed above is highly complex and whether reliable conclusions can be drawn from or compared between the existing clinical literature remains controversial (Ballantyne and LaForge 2007; Minozzi et al. 2012; McAuliffe 2012).

The ongoing search to further our understanding of the neurobiological mechanisms underlying potential chronic pain-induced alteration of analgesic self-administration is essential. Such information may help guide and optimize chronic medication management for specific forms of chronic pain.

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The Interaction Between Pain and Social Behavior in Humans and Rodents

Loren J. Martin, Alexander H. Tuttle and Jeffrey S. Mogil

Abstract Pain elicits behaviors in humans and nonhuman animals that serve as social cues. Pain behaviors serve a communicative function in humans, and this may be true as well in other animals. This review considers the current evidence for modulation of acute pain in different social contexts in humans and rodents, with a focus on dyadic social interactions. Increasing data supports the ability of social buffering, emotional contagion (a form of empathy), vicarious learning, and social stress to modulate pain sensitivity and pain behavior in mice and rats. As in humans, many of these social factors operate, and affect pain, in a sex-dependent manner. The development of a true social neuroscience of pain, with detailed explication of the underlying neurochemistry and genetics, now seems achievable.

Keywords Pain · Social interaction · Empathy · Stress

Contents

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1 A Social Neuroscience of Pain in Animals?

It is well known that social context can robustly affect pain levels and outcomes in chronic pain patients. Direct effects of varying social context on laboratory pain sensitivity can also be demonstrated, but prove to be complex. It is of considerable surprise to many that social contexts and social interactions can also affect pain sensitivity in laboratory animals—but such observations have been made, and interest in the topic is growing. The converse is also true: the presence of pain can affect the social interactions of laboratory animals, serving as a useful stimulus in social neuroscience studies. The present review will briefly summarize the relevant human data, and then consider existing evidence relating to the interaction of pain and social behaviors in rodents.

2 Social Effects on Pain in Humans

In addition to representing a physical sensation, pain precipitates psychological and behavioral responses. Organisms share a conserved repertoire of distinct pain behaviors. Humans, for example, respond to pain using verbal and nonverbal expression. These pain behaviors are useful not only because they allow an individual to escape from further injury and/or protect an injured body part, but also in that they may elicit help from other members of their social network (Hadjistavropoulos et al. 2011). That is, pain can serve a social communication function, serving as a distinct social cue. The ability of others to perceive such social cues is commonly called empathy. Empathy, or the "mirroring of complex emotional states in another" has been proposed by some as a uniquely human ability (Decety and Jackson 2004), requiring the use of memory, mentalization, and cognitive reasoning to divine the thoughts and feelings of others in the social environment. Recent years have seen increased interest in examining the effects of social interactions, including empathy, on pain behavior in the laboratory and the clinic.

2.1 Social Buffering of Pain?

Even the most basic pain-relevant social interaction, simple observation of pain by another, yields a complex pattern of results. The amelioration of aversive stimuli through social interaction (i.e., social buffering) can reduce acute pain ratings. In one study, participants rated cold pressor pain as less painful when they were accompanied by a friend or stranger who provided specific support, compared to participants who were tested alone or with a merely talkative, but unsupportive partner (Brown et al. 2003). A supportive partner may also buffer other pain measures including pain tolerance (López-Martínez et al. 2008) and emotional expressions of fear (see Epley 1974). In the clinic, social support can provide analgesic effects on labor pain (Cogan and Spinnato 1988) and postoperative pain (Kulik and Mahler 1989).

However, the effect of social support on pain appears to be based on the context of the pain, level of stress associated with the painful event, and the communication and relationship status between individuals (see Krahe et al. 2013). For example, pain tolerance in dyads depends on what the person in pain and their partner believe about the context or potential threat of pain. Specifically, a participant aided by a confederate who told the participant that sensations he or she was feeling were "safe" yielded lower pain ratings than a participant threatened during the same pain test (Jackson et al. 2009). Additionally, perceived level of social support correlates with pain rating (Kerns et al. 2002) and pain behavior (Gil et al. 1987). Solicitousness from strangers or friends may lead to analgesia in certain contexts. However, the context of the interaction—familiarity, transparency of intentions, perception of the aid-giver's ability to aid (Krahe et al. 2013)—appears to be a primary factor in dictating whether a solicitous partner ameliorates or actually worsens a participant's reported pain. Studies that document detrimental effects of pain solicitation include female chronic pain patients who report worse pain when they are with a "supportive friend" (McClelland and McCubbin 2008), spouses who feel worse when they are with a solicitous partner (Flor et al. 1987; Fillingim et al. 2003), or children in acute or chronic pain when in the presence of a solicitous parent (Chambers et al. 2002).

2.2 Empathy for Pain

Pain is a reliable stimulus eliciting empathic responses in others, and vicarious influences on pain (e.g., social modeling) have been long demonstrated (Craig and Weiss 1971; Craig et al. 1975). A spate of recent experiments have used pain as an eliciting stimulus to study the neuroanatomical substrates of human empathy (see Bernhardt and Singer 2012). In a high-profile initial study, overlapping cortical activation (using functional magnetic resonance imaging) was observed in individuals who experienced pain first-hand or watched a loved one in pain (Singer et al. 2004). This shared activation pattern was seen in the rostral anterior cingulate

cortex and anterior insula, cortical areas associated with motivational-affective dimensions of pain (Rainville et al. 1997). Activation in these same regions can be induced via observation of pictures of people in pain (e.g., Botvininck et al. 2005; Godinho et al. 2006; Morrison et al. 2004). These findings suggest that empathy may occur without higher-order cognitive processes.

The fact that cortical activation in pain-associated regions can be elicited by observation of another's pain predicts that empathizing can alter one's own pain perception. This has been confirmed in a study in which viewing a video of a study confederate (i.e., an experimenter posing as a participant) undergoing pain testing lead participants to report more pain in response to a nociceptive stimulus, but only if they had previously formed a positive empathic bond with the confederate (Loggia et al. 2007). Both sensory and affective pain ratings were increased by empathy. The effects were observed even when the confederate received non-painful stimuli, suggesting that it is the empathy itself that alters pain perception, not merely the observation of pain behaviors.

2.3 Sex Differences in Social Effects on Pain

Sex differences can importantly affect how the social environment impacts pain-related behavior, especially in contexts involving stress (Taylor et al. 2000). Sex differences in pain behavior are well documented (Mogil 2012), and some research has focused on how pain response in women and men differ in various social environments. A number of studies have investigated the interaction between participant sex and the sex of the experimenter (Asiaksen et al. 2007; Feine et al. 1991; Gijsbers and Nicholson 2005; Kallai et al. 2004; Levine and De Simone 1991), but no consistent pattern has emerged. Jackson et al. (2005) found that during a cold pressor task, women but not men were more likely to focus on pain in conversation with the experimenter, and that this conversation correlated with lower pain thresholds. However, if the experimenter distracted female participants, reinterpreted the pain that female participants were experiencing, or encouraged women during the cold pressor task, mean female pain tolerance increased to match pain levels in men (Jackson et al. 2005).

Empathy for pain also exhibits robust sex differences, likely related to the higher trait empathy of women compared to men (Baron-Cohen and Wheelwright 2004; Hall 1978). For example, although both men and women exhibited empathy-related cortical activation after seeing study confederates in pain, only in men were those activations reduced if the confederates had played unfairly in an economic game played prior to pain testing (Singer et al. 2006). Sex differences have been demonstrated in empathy-induced event-related brain potentials (Han et al. 2008; Yang et al. 2009; Proverbio et al. 2009) and empathy-associated gray matter volume (Cheng et al. 2009). Finally, sex interacts with the influence of self-inflicted pain on pain perception in others, such that pain increased ratings of pain in video clips depicting pain expressions in men, but decreased ratings of pain in women (Coll et al. 2012).

3 Social Effects on Pain in Animals

Although the biopsychosocial model is now broadly accepted in medicine and pain research, it is commonly assumed that psychosocial aspects of pain can only be studied in human beings. Recent data from laboratory animal studies is beginning to challenge this assumption, and the rest of this chapter will be devoted to summarizing the nascent data in this field.

3.1 Contagion Effects

Empathy is a multidimensional construct that suffers from a lack of definitional consensus. Much of the debate focuses on whether empathy is a higher-order process that requires emotional or cognitive processing, and thus is distinctly different from its simpler counterparts, mimicry, and emotional contagion. De Waal (2008) likens empathy to a matryoshka, a Russian nesting doll. At its core are the precursors to empathy, which produce emotional contagion (when a subject's state results from the perception of an object's state); the doll's outer layers add more complexity and represent higher-order concepts such as sympathetic concern, perspective-taking, and prosocial behavior. The ability to share emotional states relies on a so-called perception–action mechanism (Preston and de Waal 2002) that includes mimicry and/or emotional contagion. Thus, emotional contagion is a form of empathy that can operate without the presence of evolved "theory of mind" (see Hatfield et al. 1993).

A variety of animals may be able to transmit emotional states to one another, especially those relating to stress. Rats use ultrasonic vocalizations and chemosignals to communicate stress, and are able to recognize and avoid the odor of a stressed rat (Valenta and Rigby 1968; Mackay-Sim and Laing 1981). In addition to producing avoidance, the social transfer of stress odors can modulate the pain sensitivity of a nonstressed conspecific. Naïve rats exposed to odors from stressed rats display significantly less pain behavior following injections of inflammatory agents; this contagious analgesia is opioid-mediated, being completely reversed by the opioid antagonist, naltrexone (Fanselow 1985). In another experiment, mice that witnessed other mice being attacked by biting flies displayed both analgesia and self-burying behavior when exposed 24 h later to flies whose biting mouth parts were removed (Kavaliers et al. 2001). The most obvious explanation of these findings is that rats and mice recognize the odor of a stressed conspecific and react with stress (and thus, stress-induced analgesia; Butler and Finn 2009) themselves. Perhaps most intriguingly, untreated cagemates of an oxytocin (OT)-treated rat were found to display hot-plate analgesia, an effect reversed by an OT antagonist (Agren et al. 1997b). The contagion mediated by OT is most likely driven by stress (Robinson et al. 2002) and occurs via olfaction (Agren et al. 1997b).

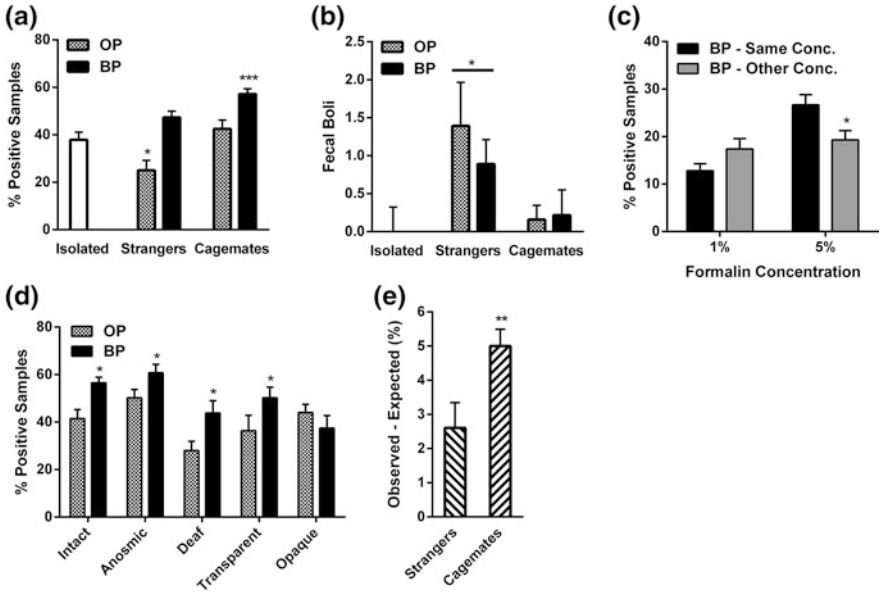

Fig. 1 Emotional contagion for pain in mice. Mice were tested for pain on the 0.9 % acetic acid abdominal constriction test (graphs **a**, **b**, **d**, **e**) or the formalin test (graph **c**) either alone (Isolated) or in dyads composed of strangers or cagemates where either one mouse was injected (one in pain; OP) or both mice were injected (both in pain; BP). **a** Increased pain behavior in cagemates (but not strangers) both injected with acetic acid. Note also a decrease in pain behavior in the Stranger-OP condition (see also Fig. 3). Bars represent mean ± SEM percentage of video samples containing abdominal constriction behavior. $*p < 0.05$, $***p < 0.001$ compared to Isolated. **b** Fecal boli (a measure of stress) deposited during pain testing shown in graph **a**. Increased numbers of fecal boli in stranger dyads suggest higher levels of stress in this condition. Bars represent mean ± SEM boli during the 30-min testing period. $*p < 0.05$ compared to Isolated. **c** Alteration in formalin (1 or 5 %) pain behavior caused by cotesting with a cagemate injected with the same concentration (Same Conc.) of formalin or the other concentration (Other Conc.). Pain behaviors were less extreme in the Other Conc. Condition, suggesting that pain sensitivity was affected bi-directionally by the pain levels of the other mouse. Bars represent mean ± SEM percentage of video samples containing hind paw licking behavior. **d** Blockade of pain contagion is only achieved by removing visual information via placement of an opaque (as opposed to transparent) Plexiglas barrier between the cagemate mice. Rendering the mice anosmic or deaf had no effect. Bars as in graph **a**. $p < 0.05$ compared to OP group. **e** Excess co-occurrence of pain behaviors over and above what would be predicted by chance in cagemate and stranger dyads, indicating synchronization of behavior. Bars represent mean ± SEM percentage of observed samples with pain behavior occurring in both mice compared to expected percentages calculated using joint probabilities. $**p < 0.01$ compared to Strangers. Adapted from Langford et al. (2006)

In addition to contagious analgesia, our lab has shown that mice also have the ability to transmit pain status between cagemates, resulting in contagious pain hypersensitivity; this occurs only if both mice in the dyad are in pain (Langford et al. 2006) (see Fig. 1). This phenomenon appears to be unique in that stress is not the mediating factor—stress levels are actually higher between dyads of strangers,

where no hypersensitivity is observed—and as such contagious pain hypersensitivity represents a very different form of contagion. In fact, mice experiencing inflammatory pain can bidirectionally modulate the severity of inflammatory pain behavior in a simultaneously tested cagemate; again, no effects are seen among strangers (Langford et al. 2006). Surprisingly, the transmitting sensory modality was vision, as placement of an opaque physical barrier between the mice was effective in blocking the phenomenon, but blockade of other sensory modalities including touch, olfaction, and audition were not. Mice (and rats and rabbits) are now known to display facial expressions of pain (Langford et al. 2010a; Sotocinal et al. 2011; Keating et al. 2012); these data suggest that visual stimuli, comprised of facial expressions alone or in combination with other body cues, may be primary drivers of pain contagion effects, although this has not yet been confirmed. In one experiment in this study (Langford et al. 2006), pain contagion was shown to transfer from one behavior and body part (abdominal constrictions) to another (hind paw withdrawal), suggesting that explicit "mirror neuron" theories of empathy (Gallese et al. 1996) may not account for this phenomenon. Of interest as well was the finding that pain behaviors (e.g., abdominal constrictions and hind paw licking) were synchronized in time over and above what would be expected by chance (Langford et al. 2006). Synchronization of autonomic indices has been demonstrated in humans during active empathy for negative emotions (Levenson and Ruef 1992). We note that empathy for pain in mice was wholly equivalent in both sexes, suggesting that sex differences in human empathy might have a sociocultural rather than a genetic basis.

A number of other contagious phenomena are known in animals, including tail hypothermia in rats (Agren et al. 1997a), core hyperthermia in mice (Thompson et al. 2003), contagious freezing behaviors (see Blanchard et al. 1991), and body posture mimicry (see Spinka 2012). Human infants mimic the facial expressions of their mothers (Pickens and Field 1993) and contagious distress quickly spreads among infants in nurseries (Geangu et al. 2010). Perhaps the most well-known contagious phenomenon is yawning. Yawn contagion has been reported to occur in humans, bonobos, chimpanzees, parrots, and dogs (Demuru and Palagi 2012; Romero et al. 2013; Miller et al. 2012; Massen et al. 2012) and susceptibility to contagious yawning is correlated with social familiarity (Romero et al. 2013; Demuru and Palagi 2012). Interestingly, individuals with autism spectrum disorders or high levels of schizotypal personality traits, which both severely effect social and communicative development, do not display contagious yawning (Senju et al. 2007; Platek et al. 2003).

3.2 Observational Fear Learning

Learning is not typically viewed as a form of emotional contagion, but the two share similar characteristics. While it is usually assumed that first-hand experience with a task is required to learn new information, studies of observational learning have shown that new information can be acquired through social facilitation and

imitation (see Olsson and Phelps 2007). Experiments exploring the neural circuitry underlying observational learning have primarily utilized classical fear conditioning paradigms, which involve an acutely painful stimulus (foot shock) and social interaction with "experienced" demonstrators (e.g., Knapska et al. 2006). In a recent high-profile finding, this observational learning in mice was related to social closeness, as freezing responses were higher in observer mice when demonstrator mice were socially related (e.g., siblings, mating partners), and impaired by the genetic deletion of $Ca_v1.2$ calcium channels in the mouse anterior cingulate cortex (Jeon et al. 2010). Social transmission of fear can be modified by genotype, with C57BL/6 mice acquiring the association much more readily than BALB/c mice (Chen et al. 2009), an inbred strain known to display low social approach behavior (Brodkin et al. 2004).

Aside from direct observational learning, chemosignals can directly modulate social fear-conditioned responses in mice. Bredy and Barad (2009) observed that exposure to a recently fear-conditioned familiar mouse impairs acquisition of conditioned fear and facilitates extinction of fear. The effect appeared to be entirely olfactory in nature because: (1) exposure to an olfactory chemosignal emitted by a recently fear-conditioned familiar mouse; and, (2) the putative stress-related anxiogenic pheromone, β-phenylethylamine, both mimicked the effect.

3.3 Effects of Housing and Social Reunion

When rodents are isolated (i.e., devoid of all social contact) their behavior is drastically altered, and pain behavior is no exception. For example, male rats housed in isolation autotomize their denervated limb following dorsal rhizotomy; autotomy behavior was almost completely prevented by co-housing with a female rat (Berman and Rodin 1982). A few studies have shown that social isolation decreases pain sensitivity and increases analgesic responding by enhancing μ-opioid activity (Becker et al. 2006; Coudereau et al. 1997; Puglisi-Allegra and Oliverio 1983). At the other end of the spectrum, crowding can affect pain sensitivity and opioid analgesia (Defeudis et al. 1976; Pilcher and Browne 1982).

An interesting study by Raber and Devor (2002) demonstrated that housing rats selectively bred for high autotomy (HA) in the neuroma model with rats selectively bred for low autotomy (LA) decreased the autotomy behavior of the HA rats and increased the autotomy behavior of the LA rats. The phenomenon, however, is likely not an example of contagion, since it did not depend on the autotomizing of the "demonstrator" rats. This phenomenon was mediated via olfaction, as it could be recapitulated with bedding from HA or LA rats. In a follow-up study, the same effect was demonstrated with low-autotomizing C58 inbred mice and high-autotomizing C3H/He inbred mice (Devor et al. 2007). Also in this study, a sex difference was noted: the C58 increase with C3H/He co-housing occurred in both sexes, whereas the C3H/He decrease with C58 co-housing was only observed in female mice.

The opioid system is not only altered by social isolation, as discussed above, but also by social reunion. Following a long period of separation, the reuniting of male mouse siblings was sufficient to decrease pain in an opioid-dependent manner (D'Amato 1998; D'Amato and Pavone 1993). The most important stimulus responsible for the analgesia among reunited siblings was physical affiliative contact, as there was a correlation between huddling behavior and pain sensitivity (D'Amato and Pavone 1996). Intriguingly, female siblings separated at weaning did not display any behavioral indices of recognizing their separated siblings, and did not have altered pain thresholds upon reunion. They did, however, recognize unrelated cagemates, as they showed opioid-mediated analgesia when reunited with them (D'Amato 1997).

3.4 Social Buffering

As in humans, laboratory animals demonstrate social buffering. Rats tested in groups of three demonstrate more approach/withdrawal (Rasmussen 1939) and less freezing behaviors (Davitz and Mason 1955) following foot shock compared to rats tested alone. In a modern study, the presence of a naïve rat blocked freezing of a test rat in response to foot shock, as well as expression of the immediate early gene, c-*fos*, in the stress-relevant paraventricular nucleus of the hypothalamus (Kiyokawa et al. 2004).

Responses to other (nonpainful) environmental threats are also modulated when in the presence of other animals. For example, a rat conditioned avoidance behavior from an anxiolytic stimulus is reduced in the presence of familiar others (Hall 1955; Baum 1969). Rats placed in novel environments exhibit lower levels of stress-related behaviors and plasma corticosterone when placed with a companion (Latane 1969; Leshem and Sherman 2006; Weijers and Weyers 1998). The social buffering effect was also observed when rats were paired with a physically separated (caged) rat (Latane 1969) or even an anaesthetized animal (Latane and Glass 1968), suggesting that physical interaction is not necessarily required for social buffering.

3.5 Prosocial Behavior

Through social communication and emotional contagion, animals may internalize the pain states of those around them. A small but growing set of observations suggests that animals will, in some cases, take actions that (whether deliberately or not) reduce observed suffering (even in unrelated individuals); however it remains unclear whether these actions serve to reduce the suffering of the other, or to reduce their own stress that accompanies the witnessing of suffering (Rice and Gainer 1962). For example, after conditioning, rats will actively press a lever to

reduce the suffering of another rat (Church 1959), an effect that was essentially replicated using pigeons (Watanabe and Ono 1986). Rats will increase grooming behaviors toward conspecifics that had recently received an electric shock (Knapska et al. 2010). We recently observed that (unaffected) mice demonstrate social approach behavior when a conspecific is in pain (Langford et al. 2010b) (see Fig. 2). This phenomenon was only observed in female mice and only in cagemate dyads; males and female strangers appear indifferent. Moreover, the degree of proximity significantly correlated with decreased pain behaviors in the affected mouse. That is, the social approach to pain appeared to produce analgesia (Langford et al. 2010b). Of note is the fact that social approach does not imply that the immediate environment surrounding an animal in pain is necessarily preferred; Watanabe (2012) also observed that mice approached pain, but later developed a conditioned place aversion to the compartment in which they briefly resided with the afflicted animal.

Although social approach to pain might be considered a true prosocial behavior, since the mouse "risks" exposure to the affected (and potentially infected) mouse, mere proximity is hardly a behavior requiring much sacrifice. Thus, a much more convincing demonstration of prosocial behavior in rodents was the recent observation that many (and especially female) rats will selectively and preferentially choose to free a trapped conspecific from a small restrainer, even when presented with a competing food reward, and even if the reward of the opportunity to play with the freed rat is prevented (Ben-Ami Bartal et al. 2011). This experiment did not, however, evaluate whether the frequency of liberation varied with familiarity status of the rats. In another experiment rats were found to be more willing to help an unknown conspecific if they had previously received help from that animal, which the authors interpreted as evidence that rats were capable of reciprocal altruism (Rutte and Taborsky 2007). The idea that these observations prove that rodents are capable of prosocial behavior remains controversial, however. Ants will release restrained nest-mates as well (Nowbahari et al. 2009), and presumably this occurs without emotional contagion or "intentionality" (Vasconcelos et al. 2012).

3.6 Social Stress and Pain

Like environmental stressors (e.g., restraint, forced swimming, foot shock), acute social stressors can also modulate pain behaviors in animals. The classic demonstration of this is so-called "defeat analgesia," whereupon following inter-male aggression in a resident-intruder paradigm, the losing intruder displays opioid-mediated analgesia (Miczek et al. 1982). Subsequent studies demonstrated defeat analgesia in rats, hamsters, and gerbils (Huhman et al. 1991; Rodgers and Hendrie 1983; Raab et al. 1985). Even without overt aggression, a social situation in which only one male is in pain can also produce stress-induced analgesia in the affected mouse, presumably caused by proximity to a potentially threatening (and healthy) conspecific (Langford et al. 2006, 2011) (Fig. 3). This analgesia occurred only

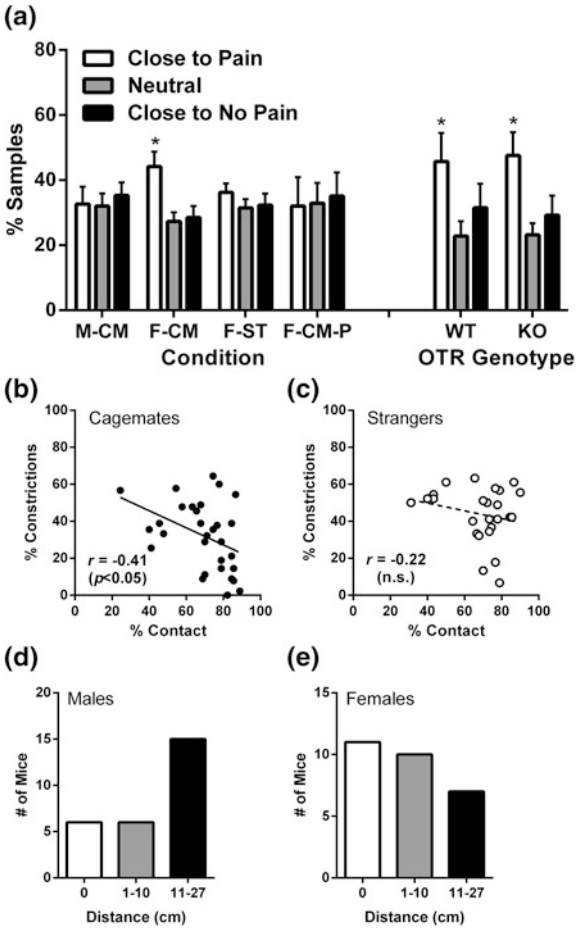

Fig. 2 Social approach to pain in familiar female mice. Mice were tested in a Plexiglas alleyway (77 × 5 cm) with either two (graph **a**) or one (graphs **b**, **c**, **d**, **e**) vertical metal bar "jails." One jailed mouse was injected intraperitoneally with 0.9 % acetic acid. (**a**) Positional choices of the "free" mouse in a paradigm where one jailed mouse was in pain and one jailed mouse was not. Triads were made up of same-sex males (M) or females (F), and all mice in the triad were either cagemates (CM) or strangers (ST). In one condition, the metal bars were replaced with a Plexiglas (P) barrier to prevent touching. In a separate experiment, female wildtype (WT) and oxytocin receptor (OTR) knockout (KO) mice were tested. Bars represent mean ± SEM percentage of samples in which the free mouse was located within 10 cm of the injected jailed mouse (Close to Pain), within 10 cm of the uninjected jailed mouse (Close to No Pain), or in the neutral area in between. $^*p < 0.05$ compared to other territories. **b**, **c**) Correlation between physical contact by the free mouse with an injected jailed mouse and writhing behavior of the jailed mouse. Mice in the dyad were same-sex cagemates (**b**) or same-sex strangers (**c**). Symbols represent percentage of samples in which the free mouse is touching the jail bars (x-axis) and percentage of samples featuring abdominal constrictions (y-axis). **d**, **e**) Sex differences in positional choices in free mice of both sexes in the one-jail paradigm shown in graphs **b**, **c**. Bars are frequency histograms of the resting positions of male (**d**) and female (**e**) mice in relation to the jail bars in cm. Adapted from Langford et al. (2011)

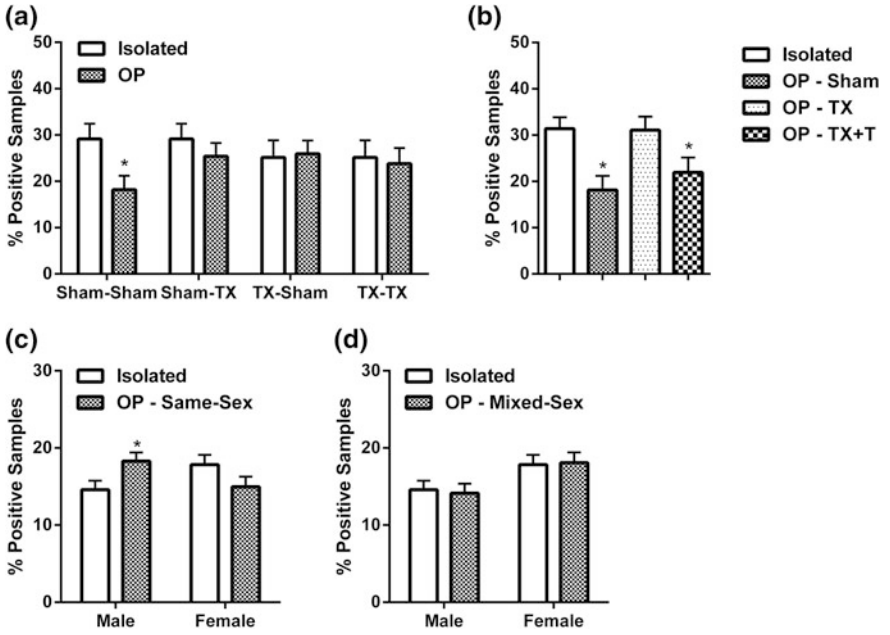

Fig. 3 Male-specific effects of pain-related social stress. Mice were tested for pain on the 0.9 % acetic acid abdominal constriction test either in isolation or in same-sex (graphs **a**, **b**, **c**) or mixed-sex (graph **d**) dyads where only one mouse was injected (one in pain; OP); all mice were strangers. In all graphs, bars represent mean ± SEM percentage of samples containing abdominal constriction behavior. **a** Stranger-related stress-induced analgesia in the OP condition is only observed when both mice in the dyad are gonadally intact (Sham–Sham). If either the injected mouse (*first position*) and/or the uninjected mouse (*second position*) are castrated (TX), no effect is observed. **b** Confirmation of the testosterone-dependence of stranger-OP analgesia in a separate experiment where the uninjected mouse was given sham surgery, castration, or castration plus testosterone propionate (TX + T) replacement. **c** Hyperalgesia rather than analgesia is observed in male but not female stranger-OP dyads when jail bars are interposed between the mice, reducing the threat level. **d** No effects on pain are seen in mixed-sex dyads. In all graphs, $^*p < 0.05$ compared to Isolated. Adapted from Langford et al. (2011)

when both stranger male mice in the dyad were gonadally intact, or gonadectomized but testosterone-replaced, and only when full contact was permitted between the mice. When only limited contact (through vertical metal bars) was allowed, reducing the threat level substantially, stress-induced *hyperalgesia* (Imbe et al. 2006) was observed in same-sex male dyads, but not same-sex female dyads or mixed dyads (Langford et al. 2011). This may be a similar phenomenon to Rodgers and Hendrie's (1983) observation of hyperalgesia in the resident winner of agonistic encounters in the resident-intruder paradigm.

In the only study of which we are aware examining the effect of *chronic* pain on social interactions, following chronic constriction injury, rats displayed less dominance behavior and increased submissive behaviors toward a cage intruder (Monassi et al. 2003).

3.7 Neurochemistry of Social Effects on Stress and Pain

Reviewing data in both the social attachment and stress literature, a viable theory exists to explain sex differences in social effects on pain in animal models. Taylor et al. (2000) conclude that OT and arginine vasopressin (AVP) form a common link between social attachment and stress response, and that sex differences exist as part of "fight-or-flight" (male) compared to "tend-and-befriend" (female) proclivities stemming from separate adaptive pressures. The neuropeptides OT and AVP are known to modulate social behaviors, including social bond formation, social approach behavior, associated affiliative behaviors, and physical contact (see Donaldson and Young 2009 for review). The effects of OT and AVP on social behavior appear to be sex specific: OT dictates approach behavior in female rodents, whereas AVP appears to regulate social approach behavior in male rodents (see Carter et al. 2009). The relevance of these neurochemical systems for pain is less clear. In our hands, social approach to pain in female mice remained unabated in OT receptor null mutant mice (Langford et al. 2010b). The role of OT itself cannot be excluded by this observation, however, as many of OT's behavioral effects are in fact mediated by the vasopressin-1A receptor (Schorscher-Petcu et al. 2010). The specific involvement of vasopressin-1A receptors in stress-induced analgesia in male but not female mice, and male but not female humans, has been demonstrated (Mogil et al. 2011), but not using social stimuli.

Obviously, the neurochemical basis of pain-relevant social behaviors is far more complex than currently understood. The study of the social neuroscience of pain in rodents will, of course, allow far more in-depth understanding of this neurochemistry than studies restricted to human subjects.

4 Conclusions

It would be folly to allege that social behaviors in rodents are in any way comparable to those of humans in their richness and complexity, but nor is it obvious that human social behaviors are qualitatively different than those of rodents. Similarly, there is no reason to believe that the genetics and neurochemistry underlying human social interactions is markedly different from that in rodents. We believe that the simple forms of social interaction that have already been demonstrated to modulate pain (e.g., contagion, social buffering, social stress) in rodents likely affect humans in similar ways, and with qualitatively similar neurophysiological determinants. Our laboratory has recently conducted translational experiments, in mice and university undergraduates (unpublished data), showing remarkable similarities in the effect of social manipulations on acute pain in both species. We believe that detailed understanding of the social neuroscience of pain, using experiments conducted in both mouse and man, is an eminently tractable goal.

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Neurobiology of Stress-Induced Hyperalgesia

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Abstract The intensity and severity of perceived pain does not correlate consistently with the degree of peripheral or central nervous system tissue damage or with the intensity of primary afferent or spinal nociceptive neurone activity. In this respect, the modulation of pain by emotion and context is now widely recognized. In particular, stress, fear and anxiety exert potent, but complex, modulatory influences on pain. Stress can either suppress pain (stress-induced analgesia) or exacerbate it (stress-induced hyperalgesia; SIH) depending on the nature, duration and intensity of the stressor. Herein, we review the methods and models used to study the phenomenon of SIH in rodents and humans and then present a detailed discussion of our current understanding of neural substrates and neurobiological mechanisms. The review provides perspectives and challenges for the current and future treatment of pain and the co-morbidity of pain with stress-related psychiatric disorders including anxiety and depression.

Keywords Pain · Stress · Anxiety · Rats · Mice · Humans · Neurobiological mechanisms

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Contents

1 Introduction

It is now widely acknowledged that the intensity and severity of perceived pain does not correlate consistently with the degree of peripheral or central nervous system (CNS) damage or the intensity of primary afferent or spinal nociceptive neurone activity. In this respect, the importance of context and the modulation of pain by emotion is now widely recognized. In particular, stress, fear and anxiety exert potent, but complex, modulatory influences on pain (Asmundson and Katz 2009; Rhudy and Meagher 2000, 2001b; Butler and Finn 2009; Ford and Finn 2008; Wiech and Tracey 2009). Research suggests that the interaction between emotion and degree of arousal can influence their impact on pain: positive emotions generally inhibit pain (regardless of arousal level), and negative emotions with low-to-moderate arousal enhance pain, whereas negative emotions with high arousal inhibit pain (de Wied and Verbaten 2001; Dougher 1979; Meagher et al. 2001; Rhudy and Meagher 2000, 2001a, 2003a, b). Hence, differential levels of valence and arousal may determine whether an aversive stimulus induces hyperalgesia or analgesia.

Over the past 20 years, there has been growing interest in the relationship and interaction between persistent pain and psychiatric disorders. Anxiety and depression share a complex reciprocal relationship with pain, whereby the perception of transient pain and the number of complaints about chronic pain are often greater in patients with these psychiatric disorders. Conversely, anxiety and depression are more prevalent in chronic pain patients (Asmundson and Katz 2009; Bair et al. 2003; Lieb et al. 2007; Dominick et al. 2012; Gambassi 2009). Clinical studies have shown that anxiety is associated with an increased frequency of chronic pain complaints (Asmundson and Katz 2009; Palermo and Drotar 1996; Atkinson et al. 1991; Dworkin et al. 1995; Lautenbacher et al. 1999; Kain et al. 2000). Correlational studies also indicate that anxiety is related to increased pain reports in subjects with high levels of trait anxiety (Dougher 1979; Malow et al. 1987). For example, research indicates that post-traumatic stress disorder (PTSD) is frequently accompanied by acute pain episodes as well as chronic musculoskeletal pain (Asmundson and Katz 2008; Otis et al. 2003; Shipherd et al. 2007). It is also the case that chronic pain patients are more likely to present with depression

(21.7 vs. 10.0 %) or anxiety disorders (35.1 vs. 18.1 %) when compared to the general population (McWilliams et al. 2003). Fishbain et al. (1986) found generalized anxiety disorder to be the most prevalent of the anxiety disorders in patients with chronic pain, seen in approximately 15 % of chronic pain subjects. Including generalized anxiety disorder, adjustment disorder with anxious mood, obsessive compulsive disorder (OCD), PTSD and agoraphobia, the prevalence of clinical anxiety among the population with chronic pain may be as high as 60 % (Fishbain et al. 1986). The co-occurrence of anxiety and/or depression with chronic pain amplifies the negative effects of each alone, often complicating treatment options and resulting in poor outcome (Asmundson and Katz 2008, 2009; Bair et al. 2003; Lieb et al. 2007).

In addition, stress has a major impact on pain perception. For example, stressful events worsen the symptoms perceived by patients suffering from chronic pain (Zautra et al. 2007; Zaza and Baine 2002; Fishbain et al. 2006; Conrad et al. 2007). Stress amplifies nociception in irritable bowel syndrome (IBS), headaches and abdominal pain (Bennett et al. 1998; Boey and Goh 2002; Alfven et al. 2008). Painful medical syndromes such as chronic shoulder/neck pain syndrome (Nilsen et al. 2007), the complex regional pain syndrome (Grande et al. 2004) and fibromyalgia (FM) (Van Houdenhove and Luyten 2006) are all strongly associated with, or precipitated by, stress.

The goal of this chapter is to provide a comprehensive review of research which has investigated the impact of stress on pain. The chapter deals with the phenomenon of stress-induced hyperalgesia (SIH), reviewing rodent and human models used to study this phenomenon and then discussing what is known about the neurobiological mechanisms mediating SIH. The chapter concludes with some perspectives on the implications and complications that stress presents for the current and future treatment of pain.

2 Stress-Induced Hyperalgesia

Stress/anxiety can enhance nociception and exacerbate pain in a phenomenon referred to herein as SIH. Evidence suggests that depending on the type of stress and pain model employed, stress/anxiety can enhance pain in rodents (Imbe et al. 2006; Bradesi et al. 2005; Vidal and Jacob 1986; Rivat et al. 2007; Quintero et al. 2000, 2003; Suarez-Roca et al. 2006a) and in humans (Schumacher and Velden 1984; Weisenberg et al. 1984; Dougher 1979; Cornwall and Donderi 1988; Al Absi and Rokke 1991; Williams and Rhudy 2007; Thompson et al. 2008; Rhudy and Meagher 2000; Rhudy et al. 2006). In general, more sustained but less intense stress in rodents (Imbe et al. 2006), or moderately arousing state/trait anxiety in humans (Carter et al. 2002; James and Hardardottir 2002; Rhudy and Meagher 2000), is often associated with enhanced pain through SIH. Less intense stress results in anxiety, which is a future-orientated emotion characterized by negative affect and apprehensive anticipation of potential threats (uncertain expectation)

(Rhudy and Meagher 2000). This uncertainty results in hypervigilance, uneasiness and somatic tension leading to increased alertness and scrutiny towards the environment. This in turn enhances sensory neurone receptivity, including sensitization of nociceptors, thus contributing to hyperalgesia. Moreover, anticipation of pain has been shown to activate brain regions in close proximity to brain regions activated by pain itself (Ploghaus et al. 1999).

In the absence of sufficient information as to the nature of a threat, or when the animal is not expecting an identifiable danger (e.g. being restrained), animals might have difficulty elaborating a defence response associated with analgesia. In this case, increased sensitivity to painful stimuli may represent a more adaptive response than analgesia, enabling the organism to detect any possible threat as early as possible. This hypothesis is in agreement with the general adaptive model of injury-related behaviour (Walters 1994), which suggests that when there is a high probability of injury, a fear state (active defensive response) is elicited that inhibits pain. In contrast, when the probability of injury is low, an anxiety state (a passive defensive response) and hyperalgesia result. The same principle was shown to apply in humans where anxiety/fear induced by an aversive event produces analgesia (Willer et al. 1981; Beecher 1969; Rhudy and Meagher 2003a), but anxiety occurring in the absence of knowledge regarding a forthcoming event is accompanied by hyperalgesia (Rhudy and Meagher 2000; Rhudy et al. 2006; Maier et al. 1982).

2.1 Rodent and Human Models of Stress-Induced Hyperalgesia

A number of animal models have been devised to study SIH (Table 1). Aversive stimuli or environments used to induce SIH include brief and mildly aversive, but innocuous, stimuli, for example novelty, vibration, air stress and holding (Vidal and Jacob 1982; Jorum 1988; Wagner et al. 2013). Other aversive stimuli are usually applied repeatedly over a prolonged period of time, with the intention of inducing a mild form of stress and hypervigilance. The use of stressors like forced swimming provides a second advantage: immobility induced by inescapable swim stress is thought to be a model of human depression (Porsolt et al. 1977), and patients with depression have increased pain sensitivity (Arnow et al. 2006). Thus, such models may have significant clinical validity.

Visceral hyperalgesia is commonly observed in patients with conditions such as IBS and interstitial cystitis. Psychological stress is widely believed to play a major role in the precipitation or exacerbation of IBS (Delvaux 1999). Colonic/bladder distention after a single or repeated stress induces visceral hyperalgesia (Bradesi et al. 2002, 2005; Ait-Belgnaoui et al. 2005; Robbins et al. 2007; Gue et al. 1997a; Toulouse et al. 2000).

Table 1 Summary of rodent and human models used to study SIH

	Stressor/source of anxiety	Painful stimulus	References
Rodents	Social threat/defeat	Formalin/radiant heat/acetic acid injection/mechanical stimuli	Andre et al. (2005), Langford et al. (2011), Marcinkiewcz et al. (2009), Rivat et al. (2010)
	Water avoidance	Colorectal distension/thermal and mechanical stimuli	Bradesi et al. (2005), Robbins et al. (2007), Schwetz et al. (2004), Larauche et al. (2008), Hong et al. (2009), Green et al. (2011), Chen et al. (2011)
	Restraint stress	Rectal distension/TF/hot tail immersion/TMJ formalin/HP	Bardin et al. (2009), Bradesi et al. (2002), Ohashi-Doi et al. (2010), Shen et al. (2010), Imbe et al. (2004), da Silva Torres et al. (2003b), Dhir et al. (2006), Gamaro et al. (1998), Gameiro et al. (2005), King et al. (2003, 2007)
	Holding/novelty	Tail shock/mechanical stimuli	Vidal and Jacob (1982), Rivat et al. (2007)
	Rotational stress	Formalin	Boccalon et al. (2006)
	Pre-natal stress (restraint)	Formalin	Butkevich and Vershinina (2001), Butkevich et al. (2006, 2007)
	Vibration	TF	(Devall et al. (2009), Jorum (1988)
	Foot or tail shock	Tail immersion/colorectal distension/TF/tail shock	Geerse et al. (2006), Tyler et al. (2007), King et al. (1999)
	Forced swim	Thermal stimuli, mechanical stimuli, formalin, HP, TF, carrageenan	Imbe et al. (2010), Quintero et al. (2000), Metz et al. (2001), Suarez-Roca et al. (2006a), Dhir and Kulkarni (2008), Abdelhamid et al. (2013), Fereidoni et al. (2007), Suaudeau and Costentin (2000), Suarez-Roca et al. (2008), Quintero et al. (2003)
	Noise stress	Bradykinin and mechanical stimulation	Khasar et al. (2005, 2009)
	Exposure to cold	Mechanical stimuli /foot shock/ capsaicin/Freund's adjuvant	(Satoh et al. (1992), Ohara et al. (1991), Fujisawa et al. (2008), Nasu et al. (2010), Omiya et al. (2000), Kawanishi et al. (1997), Okano et al. (1997)
	REM sleep deprivation	Mechanical stimulation	Wei et al. (2007)
	Air stress	Mechanical stimulation	Wagner et al. (2013)
	Immobilization	TF/formalin	Costa et al. (2005), Seo et al. (2006)

(continued)

Table 1 (continued)

	Stressor/source of anxiety	Painful stimulus	References
	Chronic mild stress	HP/formalin/mechanical stimuli/ Freund's adjuvant/SNL	Shi et al. (2010a, b)
	Maternal separation/deprivation	Colorectal distension/HP/acetone/ mechanical stimuli	Chung et al. (2007a, b), Zhang et al. (2008), (2009a, b), Burke et al. (2013); Wouters et al. (2012); van den Wijngaard et al. (2012)
	Whisker pad stimulation	Mechanical stimulation	(Reynolds et al. 2011)
Humans	Patients with mood disorders	Electrical pain/thermal stimuli/cold pressor pain	Adler and Gattaz (1993), Dworkin et al. (1995), Otto et al. (1989), Ward et al. (1982)
	Pre-operative state anxiety	Postoperative analgesic consumption	Kain et al. (2000), Wise et al. (1978); Scott et al. (1983)
	High and low trait anxiety	Thermal stimuli/mechanical stimuli	Thompson et al. (2008), Dougher (1979)
	Foot shock	Radiant heat/lower level of shock	Williams and Rhudy (2007)
	Threatening pictures	Immersing arms into hot water	Rhudy et al. (2006)
	Experimentally induced anxiety (pain-relevant negative information)	Radiant heat/cold pressor/foot shock/ischaemic arm pain/ mechanical pressure	Rhudy and Meagher (2000), (Al Absi and Rokke (1991), Weisenberg et al. (1984), Cornwall and Donderi (1988), Dougher (1979), Schumacher and Velden (1984), Benedetti et al. (2006)
	Trier social stress test	Quantitative sensory testing (control and fibromyalgia)	Crettaz et al. (2013)
	Experimental hypoglycaemia	Thermal quantitative sensory testing and thermal pain assessments	Gibbons et al. (2012)

TF tail-flick test; *HP* hot plate test; *TMJ* temporomandibular joint; *SNL* spinal nerve ligation

Studies of SIH in humans have largely focused on assessing pain responses in individuals with existing anxiety (trait) or experimentally induced anxiety (Table 1). To study whether pain-related anxiety specifically intensifies pain, volunteers have been exposed to laboratory-induced general anxiety or pain-specific anxiety through instructions/warnings (Weisenberg et al. 1984; Dougher 1979; Cornwall and Donderi 1988; Al Absi and Rokke 1991; Benedetti et al. 1997). Overall, these studies demonstrate that anxiety that is relevant to the painful stimulus typically exacerbates pain, while general anxiety that is not relevant to the painful stimulus can produce different effects, and often a reduction in pain. Some authors, instead of inducing anxiety in the laboratory, have classified healthy subjects into either high and low anxiety (trait anxiety) or high and low anxiety sensitivity (a fear of anxiety-related symptoms) using standard anxiety scales (such as Taylor's Manifest Anxiety Scale). The subjects were then assessed for response to painful stimulation (Thompson et al. 2008). Subjects with high anxiety showed enhanced pain responding compared to those with low anxiety.

A number of laboratory studies have examined pain responding in patients with mood disorders (Table 1). Indeed, studies on patients undergoing elective surgical procedures suggest that psychological variables such as pre-operative anxiety can predict the intensity of postoperative pain (Martinez-Urrutia 1975; Scott et al. 1983; Wise et al. 1978; Papaioannou et al. 2009; Vaughn et al. 2007; Ip et al. 2009; Kain et al. 2000). Although few human studies have investigated the effects of stressors per se on pain responding, one recent study exposed healthy female subjects or females with FM to the psychological stress of the Trier Social Stress Test, and subsequently assessed multiple sensory aspects of pain with the aid of quantitative sensory testing. Both healthy subjects and patients with FM showed stress-induced enhancement of pain sensitivity in response to thermal stimuli. Importantly, only the FM patients showed increased sensitivity in response to pressure pain (Crettaz et al. 2013). A second approach that has been used to study SIH in humans involves induction of hypoglycaemia (the stressor) with subsequent thermal quantitative sensory testing and thermal pain assessment (Gibbons et al. 2012).

Pain perception is variable across humans, and this is a possible confounding factor when studying the interaction between anxiety and pain. As ethical guidelines require that patients be told when they are in a "pain" experiment, experimental factors such as human attention and anticipation of a noxious stimulus can confound the results. Furthermore, though fear/anxiety may be induced experimentally, the subjects are reassured that no real danger would happen to them; this contrasts with animal studies, where subjects are almost certainly unaware of the nature, severity and potential consequences of the stimulus/threat they are about to experience.

A number of stressors used to produce SIH have also been used to produce stress-induced analgesia (SIA). One determining factor is the repetitiveness of the stressor. Repetitive stress favours the induction of hyperalgesia, not hypoalgesia, in rodents. Hence, repeated exposure to loud sound (Khasar et al. 2009), cold environment (Satoh et al. 1992), restraint (Gameiro et al. 2005) or swim stress

(Quintero et al. 2003) potentiates pain responding. In general, models that induce SIH involve chronic exposure to the stressor (days or weeks rather than minutes). This suggests that SIH results from more chronic psychological stress (whereas SIA results from acute/physical stress). Also, rat models of chronic pain, and not acute pain, tend to be associated with elevated pain behaviours in the presence of an aversive stimulus (Rivat et al. 2007). Furthermore, children who experience the chronic stress of recurrent abdominal pain display stress-induced hyperalgesia to the cold pressor test (Dufton et al. 2008). However, there are exceptions to the general finding that chronic stress exacerbates pain perception, while acute stress reduces it. Acute stress can produce hyperalgesia, and prolonged stress may evoke analgesia. For example, the earliest studies by Vidal and colleagues showed that acute exposure to emotionally arousing non-noxious stress, such as inescapable holding, novel environments or vibration, produced hyperalgesia, albeit in an immediate and transient fashion (Vidal and Jacob 1982, 1986; Jorum 1988). Also, chronic unpredictable stress can produce analgesia (Pinto-Ribeiro et al. 2004; Pignatiello et al. 1989).

Paradigms which use chronic stress result in hyperalgesia that usually lasts longer than that induced by acute stress, and this is consistent with human chronic pain that is associated with chronic anxiety-related disorders. For example, repeated cold stress facilitates the response to noxious stimuli for up to 3 days after the final exposure (Kawanishi et al. 1997; Omiya et al. 2000; Satoh et al. 1992; Hata et al. 1988; Okano et al. 1997). Other well-characterized paradigms of chronic stress and SIH include chronic restraint stress (da Silva Torres et al. 2003a, b; Imbe et al. 2004) and repeated forced swim stress (Quintero et al. 2000, 2003). Both induce long-lasting hyperalgesia, lasting up to 28 days after the cessation of stress.

The severity of the stressor may also differentially modulate pain sensitivity such that more severe stressors evoke SIA, whereas less severe stressors evoke SIH. Support for this proposal comes from a model of social threat in which acute, intense stress evoked by placing mice in close proximity to a stranger mouse produced analgesia (Langford et al. 2011). In contrast, reducing the severity of stress by limiting physical contact between mice produced SIH (Langford et al. 2011). In addition, exposure to very severe shock inhibits thermal pain reactivity in rats, whereas low-to-moderate intensity shock produces hyperalgesia (Table 1). Maier and colleagues showed that rats subjected to inescapable noxious shocks exhibited long-term analgesia, whereas rats receiving an identical number of shocks but which were able to escape did not show analgesia (Maier et al. 1982). In humans, the use of a brief and aversive mild shock (5 mA) that was only mildly painful produced hyperalgesia (Williams and Rhudy 2007). In contrast, higher-intensity shock (12 and 70 mA) produced analgesia (Williams and Rhudy 2007; Willer et al. 1981). Thus, repeated exposure to mild stress might create uncertainty about future threats. Increased alertness and early detection of threats, including nociceptive stimuli, would then seem to be an appropriate adaptive response.

The notion that severity of the aversive stimulus determines the direction of pain (hyperalgesia or analgesia) is consistent with theories of how attention

influences anxiety-/fear-related modulation of pain. Stimuli that are severe enough to take the attention away from the pain tend to reduce pain perception, while less intense stimuli increase the attention to the nociceptive stimulus, thereby enhancing pain perception (Arntz 1991; Arntz et al. 1994; Janssen and Arntz 1996). Whereas hyperalgesia may occur when the anticipatory anxiety is directed towards the pain itself (Benedetti et al. 1997; Sawamoto et al. 2000; Koyama et al. 2005; Keltner et al. 2006), analgesia may occur when anxiety shifts attention from the pain towards a stressor (Willer and Albe-Fessard 1980; Terman et al. 1986). However, this is not always the case as fear/anxiety irrelevant to pain can both enhance and suppress pain depending on the intensity of the fear/anxiety (Rhudy and Meagher 2000).

Anxiety-related modulation of pain may also be influenced by the type of nociceptive stimulus used to evoke pain responding. For example, while anxiety demonstrated no association with human pain intensity during cold stimulation, a significant hypersensitivity was found during heat stimulation (Thompson et al. 2008). Also, Bradesi et al. (2005) described a differential modulatory role of repeated psychological water avoidance stress on visceral versus somatic nociception in rats. Chronic water avoidance stress produced not only a transient somatic antinociceptive response, but also a sustained visceral hyperalgesia (Bradesi et al. 2005). Furthermore, restraint stress reduced the duration of lick/guard responses to thermal stimulation (analgesic effect) while also increasing sensitivity to thermal stimulation (hyperalgesic effect), as assessed by learned escape responses (King et al. 2003). In summary, the type of stressor, its intensity and duration, as well as the type of the pain model/test can influence both the direction of stress-induced pain modulation and the degree of analgesia or hyperalgesia. Indeed, a previous review of the literature suggests that the stress-regulatory circuit activated by a particular stressor is crucially dependent on stimulus attributes (Herman and Cullinan 1997).

The influence of genetic factors on anxiety-related hyperalgesia in rodents has been examined by assessing pain behaviour across inbred rat strains. The use of inbred strains makes it easier to dissociate genetic and environmental components of a trait because the animals within each strain are theoretically identical in their genotype. Using strains of rats with genetic differences in anxiety traits, studies have shown that the threshold for nociceptive response exhibits genetic variation (Gunter et al. 2000). Fecho and colleagues demonstrated significant strain differences in pain sensitivity between inbred Lewis and Fischer rats and outbred SD rats, all of which elicit different responses in tests of fear or anxiety (Fecho et al. 2005). At baseline, Fischer rats were the most sensitive to mechanical stimulation (with von Frey monofilament testing) and the least sensitive to noxious heat pain (the Hargreaves radiant heat test). Following intra-plantar administration of carrageenan, Lewis rats showed the least, and Fischer rats showed the highest, thermal hyperalgesia and mechanical allodynia/hyperalgesia (Fecho et al. 2005). In another study, Lewis rats displayed higher levels of avoidance of anxiogenic stimuli (Ramos et al. 1997) and enhanced hyperalgesia in the formalin test, as compared with spontaneously hypertensive rats (Ramos et al. 2002). The authors

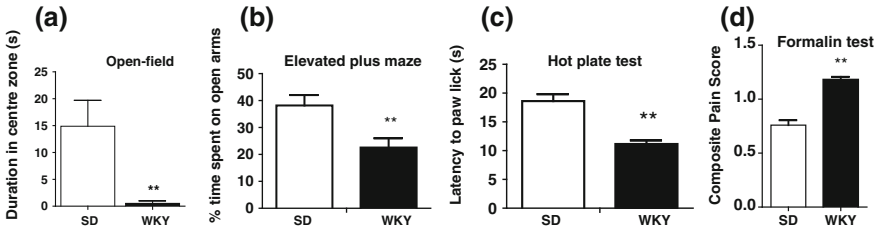

Fig. 1 Compared with Sprague–Dawley (SD) controls, Wistar-Kyoto (WKY) rats display an anxiogenic phenotype as evidenced by **a** the reduction in time spent in the centre zone of an open-field arena and **b** reduced time spent on the open arms of the elevated plus maze. **c** WKY rats demonstrate a reduction in the latency to hind paw lick in the hot plate test and **d** enhanced formalin-evoked nociceptive behaviour, indicating hyperalgesia to noxious thermal and chemical somatic stimuli. $^{**}P < 0.01$ versus SD. Data are means ± SEM (n = 12)

suggested that these strain differences reside in the gene *Tac1r*, a gene that encodes for the substance P receptor neurokinin (NK)1 receptor. The NK1 receptor influences both pain and anxiety (Ramos et al. 2002).

We and others have shown that Wistar-Kyoto (WKY) rats display a heightened level of anxiety-related behaviour in response to behavioural tests of stress and anxiety such as the acoustic startle, open field and elevated plus maze tests compared with other strains including Sprague–Dawley (SD), Wistar, spontaneously hypertensive and Fischer rats (Fig. 1) (Burke et al. 2010; Glowa and Hansen 1994; Paré 1992; Gentsch et al. 1987). WKY rats exhibit neurochemical differences in response to stress and anxiety tests compared with SD and Lewis rats (Burke et al. 2010; Pardon et al. 2002). Interestingly, WKY rats also exhibit reduced hot plate response latencies and enhanced formalin-evoked nociceptive behaviour, compared with SD rats (Fig. 1) (Burke et al. 2010; Rea et al. 2013). In addition, WKY rats exhibit an exaggerated visceromotor response to innocuous colorectal distention compared with low/moderate anxiety Fischer and SD rats (Gunter et al. 2000). In a peripheral nerve injury model, depression-like behaviour in WKY rats is associated with exacerbated mechanical allodynia compared to Wistar rats (Zeng et al. 2008). Moreover, chronic water avoidance stress resulted in augmented urinary bladder hyperalgesia in high-anxiety WKY rats but not in low/moderate anxiety SD rats, suggesting a genetic component in SIH (Robbins et al. 2007). Indeed, rats with high anxiety state show increased sensitivity to noxious colonic (Greenwood-Van Meerveld et al. 2005; O'Mahony et al. 2010; Gibney et al. 2010; Gunter et al. 2000) or urinary bladder (Robbins et al. 2007) distention and to somatic noxious stimuli (Burke et al. 2010; Zeng et al. 2008; Rea et al. 2013). To date, little is known about the genes associated with anxiety-pain co-morbidity. In patients with surgical pain, polymorphisms in three pre-specified pain-mood candidate genes (catechol-O-methyl transferase, serotonin transporter and brain-derived neurotrophic factor) were not associated with late postoperative change in mood or with a pain–gene interaction on mood (Max et al. 2006).

2.2 Neurobiological Mechanisms Mediating Exacerbation of Pain by Stress or Anxiety

2.2.1 The Role of Opioids in SIH

Long-lasting, delayed, swim-stress-induced thermal hyperalgesia was prevented by systemic administration of μ-opioid receptor antagonists, suggesting that endogenous opioid systems may, paradoxically, contribute to SIH (Suarez-Roca et al. 2006b). Pre-swim stress treatment with low doses of the μ-opioid receptor antagonist, naloxone, prevented the development of SIH, indicating that activation of μ-opioid receptors during forced swim stress might be required for the induction of hyperalgesia (Suarez-Roca et al. 2006b). Illness induced by lithium or lipopolysaccharides results in hyperalgesia in the rat formalin and tail-flick tests (Wiertelak et al. 1994), which is also blocked by the μ-opioid receptor antagonist, naloxone (McNally et al. 2000). In Long Evans rats, restraint stress-induced heat hyperalgesia was attenuated and enhanced following morphine and naloxone systemic administration, respectively. The authors suggested that the endogenous opioid system enhances stress-induced hyporeflexia and opposes stress-induced hyperalgesia (King et al. 2007). In contrast to the enhanced antinociception following administration of U-50488H (a κ-opioid receptor agonist) observed in repeated cold-stressed mice, the antinociception induced by DAMGO (μ-opioid receptor agonist) or morphine was reduced in these animals, suggesting that repeated cold-stressed mice are hyposensitive to supraspinal μ-opioid receptor-mediated antinociception, whereas antinociception through kappa-opioid receptors is increased (Omiya et al. 2000). In addition, repeated restraint decreased responses to morphine such a higher dose was required to illicit an antinociceptive effect in the rat temporomandibular joint formalin test (Gameiro et al. 2005). Opioid-mediated novelty-induced analgesia on tail-flick latencies (Siegfried et al. 1987) was not seen in repeatedly restrained rats (Torres et al. 2001), suggesting that repeated restraint stress induces an alteration in the nociceptive response, perhaps as a result of altered levels or release of endogenous opioids. In line with this idea, previous studies showed that chronic restraint stress modifies opioid activity (Drolet et al. 2001). For example, repeated restraint significantly decreased the density of opioid receptors in the spinal cord, frontal cortex and hippocampus (Dantas et al. 2005). Indeed, Omiya and colleagues showed that hypofunction of supraspinal μ-opioid receptors may explain the hyperalgesic effect of repeated cold stress in mice (Omiya et al. 2000).

Endogenous opioids are released in the CNS in response to noxious or aversive stimuli (Curtis et al. 2001). Frequent release of endogenous opioids as a consequence of repeated exposure to stressors could lead to over-activation and desensitization or downregulation of opioid receptors. Such a desensitization or downregulation may result in tolerance to the analgesic effects of endogenous opioids which, in turn, may underlie the hyperalgesia and reduced response to morphine observed after repeated swim stress. Though it is well known that

exogenous opioids induce analgesia, opioids can also produce hyperalgesia in rodents (Laulin et al. 1998, 2002) and in humans (Angst et al. 2003). When exposed to stress two weeks before pain or fentanyl, rats exhibited hyperalgesia (Rivat et al. 2007). Thus, exposure to increased levels of endogenous or exogenous opioids could have either analgesic or hyperalgesic effects on subsequent pain responding depending on the frequency, duration of exposure and prior experience. It is also worth noting that the activity of opioid receptors is strongly coupled to modulation of the inhibitory amino acid neurotransmitter GABA and the excitatory amino acid neurotransmitter glutamate (Christie et al. 2000). For example, tolerance to the analgesic effects of opioids is associated with hyperalgesia (Mayer et al. 1999) and increased activity of *N*-methyl-d-aspartate (NMDA) receptors (Trujillo and Akil 1991). Thus, alterations in opioid and NMDA receptor function could be implicated in the development and maintenance of SIH.

2.2.2 The Role of the Hypothalamo–Pituitary–Adrenal (HPA) Axis in SIH

Using a model of intermittent sound stress in rats, stress-induced enhancement of hyperalgesia required activity in the sympathoadrenal system (via release of adrenaline) and hypothalamo–pituitary–adrenal (HPA) axis (via release of corticosterone) (Khasar et al. 2008, 2009). Hyperalgesia in the tail-flick test following repeated forced swim stress was abolished in adrenalectomized rats (Fereidoni et al. 2007). Hypophysectomy potentiated inescapable restraint-induced hyperalgesia, but attenuated novelty-induced hyperalgesia (Vidal and Jacob 1982). However, dexamethasone, which is known to block the stress-induced release of adrenocorticotrophic hormone (ACTH) and endorphin from the anterior lobe of the pituitary, did not affect novelty-induced hyperalgesia. Instead, dexamethasone enhanced restraint-induced hyperalgesia (Vidal and Jacob 1982). Thus, hypophyseal factors, not affected by dexamethasone and originating from the pituitary, may participate in novelty-induced hyperalgesia, whereas analgesic mediators originating in the anterior pituitary (e.g. opioids) appear to counteract the holding-induced hyperalgesia.

Corticotrophin-releasing factor (CRF) is a hypothalamic peptide that stimulates the synthesis and release of ACTH and beta-endorphin from the pituitary. CRF interacts with CRF receptors, subtype 1 (CRF_1 receptor) and/or subtype 2 (CRF_2 receptor) (Bale and Vale 2004; Perrin and Vale 1999). CRF exerts hormonal actions in the periphery and also acts at supraspinal stress centres including the hypothalamus, amygdala, locus coeruleus, dorsal raphe nucleus and hippocampus. CRF plays an important role in stress-induced visceral hyperalgesia (Taché et al. 2004). Peripheral administration of the selective CRF_1 receptor antagonist, CP-154526, prior to water avoidance stress, prevented delayed stress-induced colonic hyperalgesia (Schwetz et al. 2004). In addition, systemic administration of the selective CRF_1 receptor antagonist, NBI 35965, abolished stress-induced visceral hyperalgesia (Million et al. 2003). In rats, central (i.c.v.) administration of

CRF mimics the effect of restraint stress in increasing the number of abdominal contractions to rectal distension (visceral hyperalgesia) (Gue et al. 1997b), and the hyperalgesic effect of such stress was blocked by i.c.v. administration of the CRF antagonist alpha-helical CRF9-41. CRF_1 receptors appear to contribute to stress-induced visceral hyperalgesia in a rat model of neonatal stress (Schwetz et al. 2005). Moreover, CRF_2 receptors might mediate stress-induced musculoskeletal hyperalgesia in mice exposed to forced swim stress (Abdelhamid et al. 2013).

In humans, peripherally administered CRF decreased pain perception thresholds and increased pain intensity ratings in response to rectal distension in healthy volunteers (Lembo et al. 1996). Another study in humans found that alpha-helical CRF significantly reduced the abdominal pain evoked by electrical stimulation in patients with IBS (Sagami et al. 2004).

2.2.3 The Role of GABA in SIH

Exposure to stress may regulate the stress response by changing GABAergic transmission in the CNS (Verkuyl et al. 2004, 2005). Stress may decrease the activity of the $GABA_A$ receptor complex, an effect mimicked by the in vivo administration of different inhibitors of GABAergic transmission and antagonized by anxiolytic benzodiazepines (Biggio et al. 1990).

Forced swim stress concomitantly produces behavioural hyperalgesia and reductions in spinal GABA release, both of which were prevented by pre-stress treatment with diazepam, a positive modulator of $GABA_A$ receptors (Suarez-Roca et al. 2008). The antihyperalgesic effect of diazepam was blocked by flumazenil, a selective antagonist of benzodiazepine-binding sites, suggesting the involvement of $GABA_A$ receptors. In the same experiment, pre-stress treatment with diazepam blocked pain-induced c-Fos expression in laminae I–VI of the ipsilateral lumbar dorsal horn in a flumazenil-sensitive manner. These findings suggest that a reduction in spinal $GABA_A$ receptor signalling underlies SIH. In addition, diazepam abolishes the transient thermal and mechanical hyperalgesia observed after brief exposure to non-noxious stressors such as a novel environment (Vidal and Jacob 1982) or 15-min exposure to restraint/vibration stress (Jorum 1988). However, diazepam did not change inescapable holding-induced hyperalgesia (Jorum 1988). Thus, the inhibitory effects of $GABA_A$ receptor stimulation on SIH appear to depend on the type of stressor. In research aimed at exploring the contribution of the dorsomedial hypothalamus to SIH, Heinricher and colleagues demonstrated that stimulation of the rat dorsomedial hypothalamus with the $GABA_A$ receptor antagonist bicuculline produced thermal and mechanical hyperalgesia, an effect associated with activation of ON-cells and suppression of OFF-cell firing in the rostral ventromedial medulla (RVM) (Martenson et al. 2009). Pharmacological blockade of ON-cell activity prevented behavioural hyperalgesia (Martenson et al. 2009). In subsequent work from this group, exposure of rats to air stress resulted in mechanical hyperalgesia which was blocked by pharmacological inactivation of the dorsomedial hypothalamus with the $GABA_A$ receptor agonist muscimol, or of

the RVM with the local anaesthetic lidocaine (Wagner et al. 2013). Despite significant cholecystokinin (CCK)-containing neuronal projections from the dorsomedial hypothalamus to the RVM, pharmacological blockade of CCK2 receptors in the RVM had no effect on SIH (Wagner et al. 2013).

2.2.4 The Role of Glutamate in SIH

Mechanical hypersensitivity induced by stressful REM sleep disturbance in a rat model of nerve ligation-induced neuropathic pain and sham controls was attenuated by spinal administration of an mGluR5 antagonist or an NMDA receptor antagonist (Wei et al. 2007). In nerve-injured animals, the antihyperalgesic effect was most pronounced with the mGluR5 antagonist. This work suggests a role for glutamate receptors in SIH and that mechanical hypersensitivity following the stress of REM sleep disturbance and peripheral nerve injury share a common spinal mechanism involving mGluR5 (Wei et al. 2007). Administration of the NMDA receptor antagonist, BN2572, prior to innocuous stress, completely prevented SIH in rats with prior pain and fentanyl experiences. This result suggests that sustained NMDA receptor blockade is required to counteract the activation of NMDA-dependent pronociceptive systems induced by innocuous stress in pain and prior opioid-experienced rats (Rivat et al. 2007). In addition, the NMDA receptor antagonist, ketamine, administered systemically at a dose that did not alter rat behaviour in the hot plate test in non-stressed rats, prevented and reversed SIH. This finding suggests that NMDA receptors contribute to both the initiation and maintenance of SIH (Suarez-Roca et al. 2006b).

Several studies have found that stressors such as restraint and forced swimming induce glutamate release in discrete brain regions including the hypothalamus and hippocampus (Engelmann et al. 2002; Fontella et al. 2004). Repeated cold stress markedly increased the capsaicin-evoked release of glutamate in the rat dorsal horn of the spinal cord (Okano et al. 1997). In addition, NMDA receptor antagonists prevent tolerance to morphine analgesia induced by repeated social defeat stress (Belozertseva and Bespalov 1998) and hyperalgesia induced by repeated administration of an opioid receptor agonist (Mao et al. 1998). The water avoidance model of visceral SIH was associated with decreased spinal expression of the glial glutamate transporter GLT1, the astrocytic marker glial fibrillary acidic protein (GFAP) and the Glu conversion enzyme glutamine synthetase, whereas expression of the glial glutamate transporter, GLAST, was upregulated (Bradesi et al. 2011). Moreover, visceral hyperalgesia was blocked by pharmacological inhibition of spinal NMDARs. The glial modulating agent propentofylline blocked this form of stress-induced visceral hyperalgesia (Bradesi et al. 2011). Evidence for SIH-related alterations in the supraspinal glutamatergic system comes from work showing that SIH resulting from chronic restraint stress is associated with a decrease in excitatory amino acid transporter 2 (EAAT2) protein levels, as well GFAP, in the PAG (Imbe et al. 2012).

2.2.5 The Role of Monoamines in SIH

Serotonin: Cold stress decreased the levels of both serotonin and 5-hydroxyindoleacetic acid in the hypothalamus, midbrain, thalamus, pons and medulla of rats (Hata et al. 1991). Cold stress induced hyperalgesia in mice which was suppressed by (1) the systemic administration of 5-hydroxytryptophan, a precursor of serotonin, and (2) L-3,4-dihydroxyphenylalanine, a precursor of catecholamines (Ohara et al. 1991). Administration of fluoxetine, a selective serotonin reuptake inhibitor, attenuated chronic restraint stress-induced hyperalgesia in the formalin test in rats (Gameiro et al. 2006). Fluoxetine also attenuated heat hyperalgesic and formalin-evoked nociceptive responding in rats exposed to unpredictable chronic mild stress (Shi et al. 2010b). In addition, forced swim-induced hyperalgesia was completely blocked by acute pre-treatment with tryptophan, a precursor of serotonin, and by long-term pre-treatment with clomipramine and fluoxetine, an effect not attributed to their analgesic properties (Quintero et al. 2000). Restraint SIH to thermal stimuli was associated with increased pERK immunoreactivity in neurons of the RVM, with three-quarters of these pERK-expressing neurons being serotonergic (Imbe et al. 2004). In the same study, levels of tryptophan hydroxylase were significantly increased in the RVM by chronic restraint stress. Taken together, these data suggest that the hyperalgesia after chronic/subchronic stress might be mediated by changes in central serotoninergic neurotransmission. Moreover, WKY rats exhibited hyperalgesia which was inversely correlated with serotonin and 5-HIAA levels in the hypothalamus (Burke et al. 2010). There is some evidence that 5-HT_{2B} receptors may mediate restraint stress-induced visceral hypersensitivity in mice (Ohashi-Doi et al. 2010).

The effect of swim stress on serotonin release in the brain is region-specific and bidirectional. Microdialysis studies in freely moving rats have shown increased serotonin release in several brain regions, especially in the raphe magnus, following short-lived forced swimming (Hellhammer et al. 1983; Adell et al. 1997; Ikeda and Nagatsu 1985). On the other hand, prolonged forced swimming (e.g. 30 min) diminished the efflux of serotonin in the amygdala and lateral septum (Kirby et al. 1995). Moreover, using the technique of in vivo microdialysis in rats, it was shown that swim stress decreased extracellular levels of serotonin in the ventral hippocampus and medial prefrontal cortex and increased serotonin release in the amygdala (Adell et al. 1997). Moreover, cold stress decreased the levels of both serotonin and 5-HIAA in the hypothalamus, thalamus, midbrain, pons and medulla (Hata et al. 1988). Current treatments for painful conditions such as temporomandibular disorders that show increased stress, depression, anxiety and somatization (Gatchel et al. 1996; Jones et al. 1997) utilize drugs, such as fluoxetine, that increase levels of serotonin (Stokes and Holtz 1997). Thus, changes in central serotonergic neurotransmission might explain, at least in part, the bidirectional changes in nociception (analgesia and hyperalgesia) seen after different stress conditions.

Noradrenaline (NA): Cerebral release of NA during stress has been implicated as a factor in the expression of anxiety (Tanaka et al. 2000). Anxiety, in turn, can enhance pain sensitivity in human subjects (Carter et al. 2002; Rhudy and Meagher

2003b). In addition, psychological stress induced by exposing rats to the sight, sound and odour of other rats being shocked was associated with increased NA turnover in the amygdala, hypothalamus and locus coeruleus (Tsuda et al. 1986). Clonidine, an alpha$_2$-adrenoceptor agonist and inhibitor of the synaptic release of NA, blocked vibration-induced hyperalgesia, indicating that enhanced noradrenergic system activity contributes to SIH (Jorum 1988). However, pre-treatment with milnacipran, a dual serotonin/NA uptake inhibitor, reversed repeated forced swim-stress-induced muscle hyperalgesia without modifying pre-stress muscle nociception. These data suggest that increased levels of central NA and/or serotonin reverses this form of SIH (Suarez-Roca et al. 2006a). Furthermore, chronic restraint SIH to thermal stimuli applied to the tail was associated with decreased pERK immun+oreactivity in the rat locus coeruleus, a major noradrenergic nucleus in the brain (Imbe et al. 2004).

Dopamine: At present, direct experimental evidence implicating a role of dopamine in SIH is lacking. However, exposure to acute stressors increases activation of dopaminergic neurons within the ventral tegmental area (VTA) (Bannon et al. 1983; Kalivas and Abhold 1987) and increases the release of dopamine in a number of brain regions, including the nucleus accumbens (Bertolucci-D'Angio et al. 1990; Wu et al. 1999). Chronic unavoidable stress decreased dopamine efflux in the nucleus accumbens shell (Gambarana et al. 1999). Indeed, chronic stress disrupts both serotonergic and dopaminergic function within the nucleus accumbens, the impact on dopamine outlasting that of serotonin (Wood 2004). Fibromyalgia, a clinical disorder characterized by disturbance of emotion and nociception, may be related to a hypodopaminergic state. A decrease in the concentration of dopamine metabolites (along with those of serotonin and norepinephrine) occurs in patients with FM (Legangneux et al. 2001; Russell et al. 1992). Further research is required to improve our understanding of the role of the dopaminergic system in SIH.

2.2.6 The Role of the Endocannabinoid System in SIH

Despite the well-established role of the endocannabinoid system in stress, anxiety and pain (Finn 2010), very few studies have investigated the role of endocannabinoids in SIH, with the only studies to date focusing on visceral hyperalgesia. Intra-peritoneal administration of the CB_1 receptor agonist arachidonyl-2-chloro ethylamine (ACEA) significantly diminished the enhanced visceromotor reflex to colorectal distention and also attenuated changes in electromyogram response in rats stressed by partial restraint (Shen et al. 2010). In contrast, the CB_1 receptor antagonist/inverse agonist rimonabant had the opposite effect (Shen et al. 2010). In the same study, stress upregulated CB_1 receptors in the colon. A study by Hong and co-workers showed that visceral motor response increased significantly in water-avoidance-stressed rats, indicating hyperalgesia (Hong et al. 2009). In the same experiment, levels of anandamide in the dorsal root ganglia (DRG) of stressed rats were increased, while CB_1 receptor expression was decreased and expression and phosphorylation of the transient potential vanilloid receptor 1

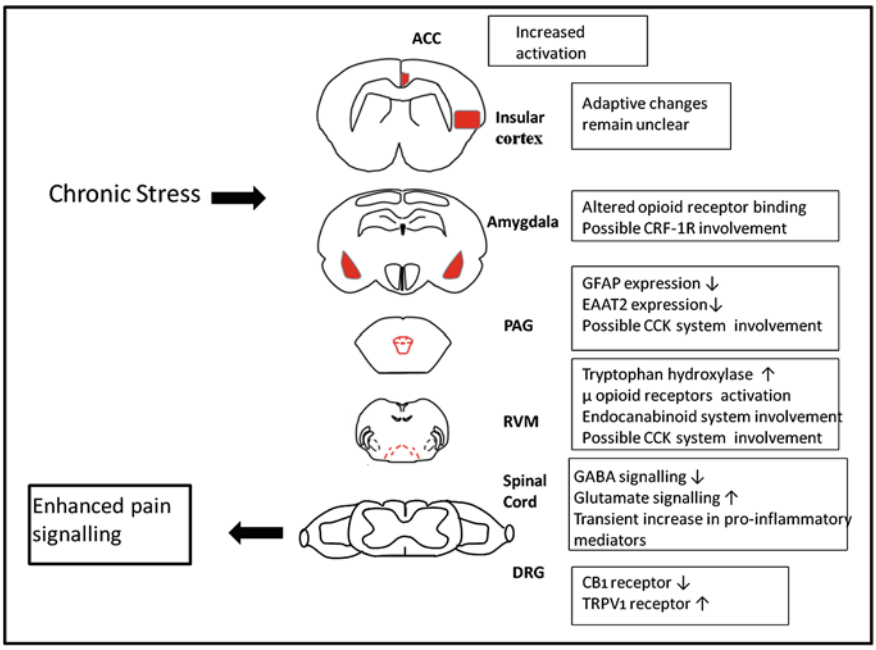

Fig. 2 Summary of some of the key sites and neurobiological mechanisms thought to mediate stress-induced hyperalgesia (SIH). Abbreviations: ACC (anterior cingulate cortex), PAG (periaqueductal grey), RVM (rostral ventromedial medulla), DRG (dorsal root ganglia), pERK (phosphorylated extracellular signal regulated kinase), 5-HT (5-hydroxytryptamine), NA (noradrenaline), GABA (gamma-aminobutyric acid), CRF-R1 (corticotrophin releasing factor receptor subtype 1), EAAT2 (excitatory amino acid transporter 2), CCK (cholecystokinin), TRPV1 (transient receptor potential vanilloid 1), GFAP (glial fibrillary acidic protein)

(TRPV1) was increased (Hong et al. 2009). Treatment of water-avoidance-stressed rats with the cannabinoid receptor agonist, WIN 55,212-2, or the TRPV1 antagonist, capsazepine, prevented the development of visceral hyperalgesia and blocked the upregulation of TRPV1 (Hong et al. 2009). These results suggest that endocannabinoid/endovanilloid signalling through CB_1 and TRPV1 has inhibitory and facilitatory effects, respectively, on stress-induced visceral hyperalgesia. A subsequent study from the same research group suggested a key role for corticosterone in the stress-induced alterations in CB_1 and TRPV1 expression (Hong et al. 2011). More work is needed to understand whether and how the endocannabinoid system is involved in other forms of SIH. Recent work from our laboratory has shown that the hyperalgesic behavioural response to intra-plantar injection of formalin in WKY rats compared with SD rats is associated with impaired pain-related mobilization of endocannabinoids and transcription of their synthesizing enzymes in the RVM (Rea et al. 2013). Pharmacological blockade of the CB_1 receptor potentiates the hyperalgesia in WKY rats, while inhibition of the endocannabinoid catabolizing enzyme, fatty acid amide hydrolase, attenuates the

hyperalgesia. The latter effect is mediated by CB_1 receptors in the RVM (Rea et al. 2013). These data suggest that impaired endocannabinoid signalling in the RVM underpins hyper-responsivity to noxious stimuli in a genetic background prone to heightened stress/affect.

Figure 2 provides a summary of the key neurobiological mechanisms that are believed to mediate SIH.

3 Conclusions and Implications for the Treatment of Pain

Clearly, the reciprocal interactions between stress and pain are highly complex and present both challenges and opportunities for the improved treatment of pain and its co-morbidity with stress-related psychiatric disorders. A thorough understanding of stress–pain interactions and the neurochemical and molecular mechanisms underpinning them (Fig. 2) could allow for targeting/harnessing of those mechanisms for therapeutic gain. Exacerbation of pain by stress and co-morbidity of pain with stress-related psychiatric disorders, including anxiety and depression, represent significant clinical challenges. Increased prevalence of catastrophizing in these patient groups adds further to the challenge. A multidisciplinary approach combining psychological interventions such as cognitive behavioural therapy with pharmacological treatment is likely to result in the best treatment outcomes for patients. Our ever-increasing understanding of overlap and interactions that exist between the neural substrates and neurochemical mechanisms that regulate pain and mood means that it may be possible to develop new drugs which can treat both pain and co-occurring anxiety/depression. Indeed, the current use of drugs such as pregabalin, amitriptyline and duloxetine for the treatment of both pain and anxiety/depression illustrates the close associations that exist between pain- and stress-related psychiatric disorders and suggests that novel drugs with improved efficacy and fewer adverse effects may eventually emerge from research focused on understanding stress–pain interactions.

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Part V
Plasticity and Chronic Pain

Endogenous Analgesia, Dependence, and Latent Pain Sensitization

Bradley K. Taylor and Gregory Corder

Abstract Endogenous activation of μ-opioid receptors (MORs) provides relief from acute pain. Recent studies have established that tissue inflammation produces latent pain sensitization (LS) that is masked by spinal MOR signaling for months, even after complete recovery from injury and re-establishment of normal pain thresholds. Disruption with MOR inverse agonists reinstates pain and precipitates cellular, somatic, and aversive signs of physical withdrawal; this phenomenon requires *N*-methyl-D-aspartate receptor-mediated activation of calcium-sensitive adenylyl cyclase type 1 (AC1). In this review, we present a new conceptual model of the transition from acute to chronic pain, based on the delicate balance between LS and endogenous analgesia that develops after painful tissue injury. First, injury activates pain pathways. Second, the spinal cord establishes MOR constitutive activity (MOR_{CA}) as it attempts to control pain. Third, over time, the body becomes dependent on MOR_{CA}, which paradoxically sensitizes pain pathways. Stress or injury escalates opposing inhibitory and excitatory influences on nociceptive processing as a pathological consequence of increased endogenous opioid tone. Pain begets MOR_{CA} begets pain vulnerability in a vicious cycle. The final result is a silent insidious state characterized by the escalation of two opposing excitatory and inhibitory influences on pain transmission: LS mediated by AC1 (which maintains the accelerator) and pain inhibition mediated by MOR_{CA} (which maintains the brake). This raises the prospect that opposing homeostatic interactions between MOR_{CA} analgesia and latent NMDAR–AC1-mediated pain sensitization creates a

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lasting vulnerability to develop chronic pain. Thus, chronic pain syndromes may result from a failure in constitutive signaling of spinal MORs and a loss of endogenous analgesic control. An overarching long-term therapeutic goal of future research is to alleviate chronic pain by either (a) facilitating endogenous opioid analgesia, thus restricting LS within a state of remission, or (b) extinguishing LS altogether.

Keywords Pain · Adenylyl cyclase · NMDA receptor · Latent sensitization · Opioid · Analgesia · Constitutive activity · Dependence · Addiction · Post-operative pain · Central sensitization · Stress

Contents

1 Introduction

Serious tissue injury causes the acute pain that we are all familiar with. For elusive reasons, acute pain accelerates toward a state of incapacitating chronic pain in hundreds of millions of people around the world. For example, back injuries lead to unrelenting, rampant low back pain that destroys quality of life. Unfortunately, adequate therapeutic strategies to stop chronic pain do not exist. Even the most powerful opiate pain killers such as morphine and oxycodone produce severe adverse effects including cognitive impairment and respiratory depression, as well as societal issues with drug diversion. Side effects aside, chronic pain responds poorly to treatment, despite considerable efforts toward the development of efficacious analgesic drugs. A fundamental but somewhat neglected long-term goal in the field is to better understand the ability of the central nervous system (CNS) to intrinsically inhibit the mechanisms that accelerate pain. In this chapter, we discuss a relatively new approach: to search for the mechanisms whereby mammals naturally recover from the pain of inflammation. This chapter focuses on the endogenous opioid system: during injury, opioid peptides are released at sites of pain modulation where they act at cognate receptors to put the brakes on the transmission of pain signals to the brain. Indeed, soon after tissue injury produces nociception, compensatory analgesic systems involving μ, δ, and/or κ opioid receptors are activated at multiple sites of pain modulation including the spinal cord and brain. This likely tempers the intensity of *acute* postoperative pain in humans (Levine et al. 1978). Here we describe recent data indicating that opioid receptors can acquire the potential to oppose *chronic* pain via a constitutive, ligand-independent activation mechanism. An understanding of the body's own pain defenses within the CNS should provide valuable insight into new strategies to prevent the transition from acute to chronic pain.

2 Opioid Receptors and Endogenous Analgesia

2.1 Opioid Receptors

Cutaneous noxious stimuli drive ascending pain transmission through the spinal release of glutamate and peptide neurotransmitters from presynaptic terminals of primary sensory neurons (Basbaum et al. 2009). Opioid receptors include the μ

(MOR), δ (DOR), and κ (KOR) types. Each is widely distributed throughout the nervous system, including key sites of pain modulation (Mansour et al. 1995; Erbs et al. 2014): in addition to expression in brain, peripheral nerve endings, and dorsal root ganglia (DRG), opioid receptors decorate the central terminals of primary afferent neurons and second-order neurons in the dorsal horn (DH) of the spinal cord (Besse et al. 1990; Kohno et al. 1999; Spike et al. 2002; Marker et al. 2005; Scherrer et al. 2009; Heinke et al. 2011). In specific, MORs and DORs produce their antinociceptive effects in molecularly and functionally distinct populations of sensory afferents terminating in the DH (Bardoni et al. 2014). This review will focus on the ability of these spinally located MORs to exert long-lasting inhibition of spinal pain transmission that is triggered by tissue injury.

Opioid receptor activation either by endogenous ligands or by exogenously administered agonists elicits powerful spinal antinociception (Yaksh 1987; Yaksh et al. 1988). MORs are a vital presynaptic target, and their activation leads to reduction of neurotransmitter (e.g., glutamate) release from the central terminals of primary afferent neurons (Jessell and Iversen 1977; Duggan and North 1983; Yaksh et al. 1988; Chang et al. 1989; Hori et al. 1992; Suarez-Roca and Maixner 1992; Glaum et al. 1994; Terman et al. 2001), ultimately leading to inhibition of spinal excitatory pain transduction (Yoshimura and North 1983). MORs are also an important postsynaptic target, as they are found in a population of mostly excitatory neurons in laminae I and II, where they inhibit the firing of action potentials, presumably leading to inhibition of nociceptive transmission to the brain (Willcockson et al. 1984; Jeftinija 1988; Schneider et al. 1998; Kohno et al. 1999; Aicher et al. 2000).

All opioid receptor subtypes are members of the heterotrimeric guanosine 5′-triphosphate-binding protein (G protein)-coupled receptor (GPCR) superfamily, Class A rhodopsin subfamily. Agonists dissociate $G\alpha_{i/o}$ which then inhibits adenylyl cyclase-mediated production of adenosine 3′,5′-cyclic monophosphate (cAMP), thus decreasing the opening of voltage-gated Ca^{2+} channels (VGCC) (Kohno et al. 1999; Kondo et al. 2005). The dissociated $G\alpha_{\beta\gamma}$ subunits promote the opening of G protein-coupled inwardly rectifying potassium channels (GIRKs) to further hyperpolarize the neuron (Fig. 1). This review will primarily focus on opioidergic inhibition/regulation of spinal adenylyl cyclases, specifically the calcium-sensitive adenylyl cyclase type 1.

2.2 *Compensatory Development of Endogenous Analgesia*

Pain intensity and duration are regulated by numerous inhibitory systems, including spinally secreted opioid peptides and subsequent activation of opioid receptors (Basbaum and Fields 1984; Ossipov et al. 2010). During noxious stimulation or after severe tissue injury, opioid systems in the brain and spinal cord orchestrate an adaptive compensatory response to inhibit pain in humans under several circumstances. For example, opioidergic analgesia systems are thought to be activated

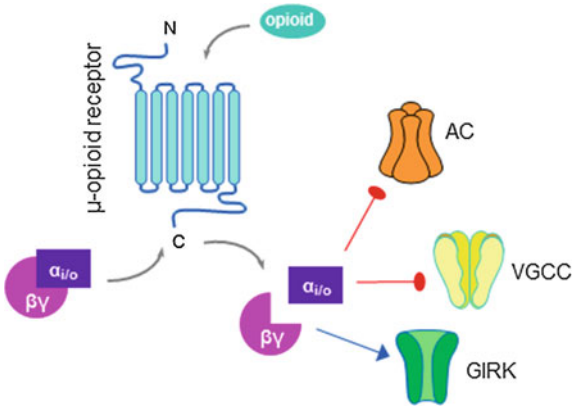

Fig. 1 Opioidergic signaling. Opioid agonists bind to the extracellular binding pocket of opioid receptors to activate intracellular inhibitory G proteins (Gαi/o—βγ). Dissociated G proteins can reduce neuronal excitation and/or neurotransmitter release via inhibition of adenylyl cyclases (*AC*), voltage-gated calcium channels (*VGCC*), and activation of inward-rectifying potassium channels (*GIRK*). *Red blunted lines* indicate inhibition, and *blue arrows* indicate activation

when a patient undergoes surgery, when a soldier is wounded in battle, or when a runner runs a marathon. Positron emission tomography studies in humans indicate that sustained pain causes the release of endogenous substances acting at MORs in the brain (Zubieta et al. 2001) (presumably to regulate the sensory and affective components of the pain experience). In rodents, repetitive or sustained noxious stimuli increase concentrations and/or release of opioid peptides (e.g., endorphins, enkephalins, dynorphins, and endomorphins) at sites of pain modulation within the rodent DH (Yaksh and Elde 1981; Basbaum and Fields 1984; Iadarola et al. 1986; Noguchi et al. 1992; Song and Marvizon 2003a; Ossipov et al. 2010), which can induce MOR activation (Tambeli et al. 2009), though this has yet to be shown in human spinal cord. Synaptic mechanisms of endogenous opioid inhibition in the DH include modulation of neuronal excitability and fine-tuning of glutamatergic nociceptive transmission at both presynaptic (Hori et al. 1992; Terman et al. 2001) and post-synaptic neurons (Willcockson et al. 1984; Jeftinija 1988; Aicher et al. 2000).

Numerous neuromodulatory systems other than the opioids promote endogenous analgesia (Millan 2002). For example, conditional knockdown of neuropeptide Y (NPY) before the induction of inflammation extended the time course of mechanical and heat hyperalgesia (Solway et al. 2011). Similarly, dual blockade of cannabinoid CB1 and CB2 receptor signaling prevented the resolution of postoperative allodynia (Alkaitis et al. 2010). These studies indicate that endogenous NPY and endocannabinoids hasten the resolution of inflammatory and neuropathic pain. Therefore, it is likely that endogenous MOR constitutive activity works in concert with numerous neuromodulatory systems to reduce nociception and aid in the resolution of pain.

2.3 Endogenous Mu Opioid Receptor Analgesia in the Dorsal Horn

The above-mentioned studies indicate that noxious stimulation recruits pain inhibitory opioidergic systems. When the stimulus produces physical tissue or nerve injury, this compensatory response can be quite long lasting. Thus, animal studies suggest that injury can increase opioid receptor density and opioid receptor intracellular signaling in the dorsal horn even after inflammatory hyperalgesia has subsided.

MOR expression in the superficial spinal cord progressively increases over days to weeks during chronic inflammation states and this is suggested to aid in the analgesic effectiveness of exogenously applied opiate drugs (Ji et al. 1995; Goff et al. 1998; Mousa et al. 2002). This can be long lasting: Autoradiography studies demonstrate that opioid receptor density remains elevated for almost 3 months in a complete Freund's adjuvant (CFA) model of rheumatoid polyarthritis (Calza et al. 2000). Furthermore, we recently reported that injury-induced MOR signaling does not necessarily dissipate over time, and can be maintained long enough to oppose *chronic pain*: (1) disruption of $G\alpha_{i/o}$ signaling with intrathecal pertussis toxin precipitated mechanical hyperalgesia in CFA-21d but not sham mice; (2) the antinociceptive effects (hotplate assay) of the MOR-selective agonist DAMGO were potentiated in the post-hyperalgesia state, 21 days after intraplantar injection of CFA; and (3) DAMGO-stimulated $GTP\gamma S^{35}$ binding in lumbar spinal cord sections was increased ($\uparrow E_{max}$) in CFA-21d mice compared to sham (Corder et al. 2013).

Augmentations in spinal MOR-G protein coupling lasted for at least 3 weeks after the injury, even though the initial bout of allodynia had resolved. This is consistent with earlier studies showing that noxious stimulation can induce spinal MOR activation that outlasts the pain stimulus that initiated it (Tambeli et al. 2009). Taken together, these findings demonstrate that injury increases MOR-G protein signal transduction in the DH that persists after the resolution of inflammatory hyperalgesia. Studies utilizing opioid receptor antagonists, discussed next, suggest that this long-lasting MOR signaling is poised to mediate endogenous antinociception. The remainder of this review discusses the evidence that this signaling can oppose the emergence and overt manifestations of chronic hyperalgesia.

2.4 Opioid Receptor Antagonists Increase Pain Intensity

By turning on opioid receptor systems, the body can temper acute postoperative pain, allowing the soldier to escape from the battlefield and the marathon runner to finish the race. This system is mimicked therapeutically when morphine is given to relieve various forms of pain, such as postoperative pain. On the other hand, one can predict that interruption of the opioid system would worsen the pain after surgery, traumatic injury, or pain during a marathon. This prediction is likely to be true, as indicated by numerous studies showing that opioid receptor antagonists can

increase pain intensity. For example, animal studies of ongoing or persistent inflammatory pain demonstrate that naloxone or naltrexone increases mechanical and/or heat hypersensitivity (Millan et al. 1987; Herz and Millan 1988; Hurley and Hammond 2000, 2001; Schepers et al. 2008a). Furthermore, as summarized in Table 1, human studies demonstrate that naloxone enhances hyperalgesia in the acute pain setting (Buchsbaum et al. 1977; Levine et al. 1979; Frid et al. 1981; Jungkunz et al. 1983; Anderson et al. 2002). This suggests that endogenous opioid analgesia provides an intrinsic braking mechanism that exerts inhibitory pain control soon after tissue injury (Ossipov et al. 2010). But whether opioid receptor signaling is maintained for sufficient duration to counter long-lasting nociceptive mechanisms remained a mystery long after the initial demonstration of endogenous opioid inhibition of acute pain. Can the endogenous opioid system persistently (or indefinitely) oppose persistent pain? This question remained difficult to answer for several reasons. First, ethical issues complicate opioid receptor antagonist studies in humans—one can imagine the difficulty in recruiting chronic pain patients who would submit to an intervention that would worsen their pain. Second, most animal models of chronic pain were developed to produce maximal allodynia and hyperalgesia, with the aim to evaluate the pain-reducing efficacy of pharmacological and other interventions. As a result, a ceiling effect would interfere with the ability to evaluate the ability of opioid receptor antagonist to further increase nociceptive responding. To get around this problem, a small number of studies have utilized tissue or nerve injury models that produce either a relatively low incidence or a

Table 1 Representative human studies describing the effect of naloxone on experimental and postoperative pain

Nociceptive model	Naloxone dose	*n*	Pain report	References
Electric shock	0.8 mg	5	No change	El-Sobky et al. (1976)
Electric shock	2 mg	21	↑ pain	Buchsbaum et al. (1977)
Electric shock plus cold water stressor	0.8 mg	32	↑ pain	Jungkunz et al. (1983)
Electric shock and thermal probe	20 mg	24	No change	Stacher et al. (1988)
Ischemia	2 mg	52	↑ pain	Frid et al. (1981)
Ischemia	2 mg	12	No change	Posner and Burke (1985)
Postoperative pain	9 mg	26	↑ pain	Levine et al. (1978)
Postoperative pain	0.4–10 mg	77	↑ pain	Levine et al. (1979)
Postoperative pain	4 mg	6	No change	Skjelbred and Lokken (1983)
Postoperative pain	10 mg	89	↑ pain	Gracely et al. (1983)
Subcutaneous capsaicin	140 μg/kg	20	No change	Benedetti et al. (1999)
Topical capsaicin	100 μg/kg	12	↑ pain	Anderson et al. (2002)
First-degree burn	21 μg/kg	22	No change	Werner et al. (2013)

Overall, these studies indicate that naloxone can increase pain, signifying the importance of endogenous opioid analgesic activity after injury

relatively low intensity of pain-like behavior. For example, naloxone produces allodynia in rodents with nerve injury that had initially failed to develop behavioral signs of neuropathic pain (Back et al. 2006). Also, opioids can mask the early incidence of pancreatic cancer pain (Sevcik et al. 2006). Third, endogenous opioid analgesia may mask the very existence of hyperalgesia, leading to the false impression that pain sensitization either 1. never developed after the injury (Back et al. 2006; De Felice et al. 2011) or 2. completely resolved after an initial bout of pain-like behavior. This latter idea can readily be tested with the administration of opioid receptor antagonists either during or after the apparent resolution of hyperalgesia (e.g., during the post-hyperalgesia state). For example, following the resolution of hyperalgesia after partial nerve ligation (Guan et al. 2010); or inflammation (Campillo et al. 2011; Corder et al. 2013), naloxone reinstated hypersensitivity. The remainder of this chapter focuses on this approach, which has helped us to understand in great detail the mechanisms that mask latent central sensitization, and will ultimately help in the search to find interventions that prevent or alleviate chronic pain.

3 Spinal Mechanisms of Persistent Pain

3.1 Central Sensitization

Chronic pain is determined by facilitatory mechanisms such as long-term potentiation of synaptic strength in DH neurons (Ikeda et al. 2006; Latremoliere and Woolf 2009; Ruscheweyh et al. 2011). Injury-induced spinal long-term potentiation may be a potential mechanism of a larger phenomenon that develops after severe tissue injury, known as central sensitization, which refers to an increased responsiveness of CNS nociceptive neurons to normal or subthreshold afferent input. Central sensitization is an NMDAR-dependent phenomenon that is widely believed to contribute to chronic pain states (Latremoliere and Woolf 2009). Less appreciated, however, are data suggesting that central sensitization can persist in the absence of behavioral signs of hypersensitivity (Reichling and Levine 2009; Asiedu et al. 2011), in a silent form often referred to as “latent sensitization” (LS).

3.2 Latent Sensitization

LS is important because it primes nociceptive systems such that, when inhibitory systems fail, a pain episode ensues (Le Roy et al. 2011; Corder et al. 2013). Thus, LS is a form of long-lasting pain vulnerability that develops after traumatic injury or stress, by which the organism may demonstrate greater susceptibility to a potentiated pain response upon subsequent injury or stressor (Aley et al. 2000; Rivat et al. 2002, 2007; Parada et al. 2003; Summer et al. 2007; Cabanero et al. 2009;

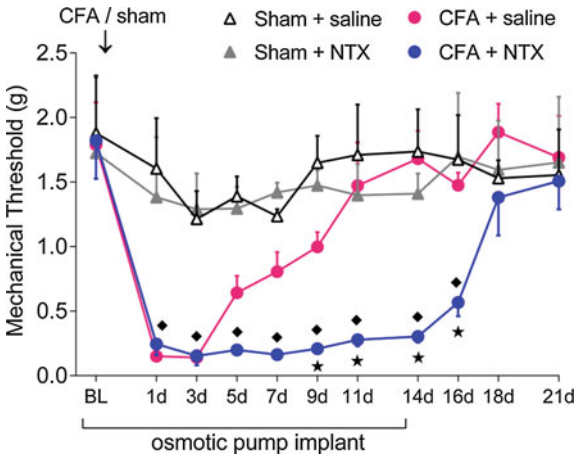

Fig. 2 Minipump infusion of NTX increases the duration of mechanical hyperalgesia, indicating that endogenous opioid receptor activity hastens the resolution of inflammatory pain. Changes in mechanical pain resolution after continual minipump infusion of saline or NTX (10 mg/kg/d) for 14d in sham and CFA mice ($n = 4–7$ per group). *Filled asterisk* $p < 0.05$ compared to CFA + saline group, *filled diamond* $p < 0.05$ compared to sham + NTX group. Adapted from Corder et al. 2013

Reichling and Levine 2009; Le Roy et al. 2011). For example, Price and colleagues recently reported that either intraplantar injection of interleukin-6 or plantar paw incision produced a transient acute hypersensitivity that was followed by a long-lasting sensitization of spinal nociceptive pathways (Asiedu et al. 2011). And, the lasting pain vulnerability promoted by LS can be manifested with pharmacological blockade of pain inhibitory systems (Campillo et al. 2011; Corder et al. 2013). For example, Fig. 2 illustrates that minipump infusion of naltrexone (NTX) prevents the resolution of inflammatory pain. These data indicate that NTX unmasks a LS in the spinal cord that persists beyond the resolution of pain and inflammation, reflective of hyperalgesic priming at the peripheral nervous system (Asiedu et al. 2011). In summary, LS represents a predisposition to relapse that may explain the episodic nature and vulnerability to stress that accompanies chronic pain syndromes in humans (Le Roy et al. 2011; Corder et al. 2013) and thus may reflect a critical mechanism responsible for the transition from acute to chronic pain (Rivat et al. 2007). LS has not yet been reported in humans (Pereira et al. 2013), and our understanding of mechanisms of CNS synaptic plasticity in LS was until recently limited to an involvement of NMDAR. Although the cellular mechanisms underlying LS are now emerging (see below and Corder et al.), its synaptic basis remains poorly understood and so this is the focus of intense ongoing investigations.

4 Opioid Receptor-Masked Sensitization

4.1 Endogenous Analgesia Maintains LS in a State of Remission

Until recently, the question as to whether opioid receptor activity persisted for long enough periods to dampen chronic pain remained unanswered. However, as predicted by long-lasting increases in opioid receptor density and signaling, endogenous opioid inhibition of acute nociception persists even after the initial signs of hyperalgesia have subsided (Yu et al. 1994; Guan et al. 2010; Joseph and Levine 2010; Campillo et al. 2011). This is demonstrated by the ability of opioid receptor antagonists to precipitate allodynia in the post-hyperalgesia state following tissue or nerve injury (Yu et al. 1994; Back et al. 2006; Sevcik et al. 2006; Guan et al. 2010; Campillo et al. 2011). In other words, LS outlasts the duration of the injury and can be revealed with opioid receptor inverse agonists that "rekindle" or "reinstate" hyperalgesia (Cabanero et al. 2009; Lian et al. 2010; Le Roy et al. 2011; Corder et al. 2013). For example, our laboratory produced cutaneous inflammation of one paw of the mouse and then allowed pain behavior to naturally subside over several days to weeks. While the animals were in this pain remission state, we disrupted intrinsic opioid receptor activity with inverse agonists. In addition to pain-like behaviors, we honed in on the spinal cord with a battery of physiological, biochemical, and molecular assays to determine effects on dorsal horn neurons that relay pain signals to the brain. As predicted, we found that inverse agonists reinstated pain-like behaviors and spinal neuron activation when administered months after the induction of inflammation (Fig. 3). This suggests that endogenous opioid analgesia silently continues long after an injury has healed and thus promotes the natural recovery of acute inflammatory pain. Thus, pain remission is maintained in part by opioid receptor activity that masks the pronociceptive components of LS.

4.2 Animal Models of Opioid Receptor-Masked Sensitization

Multiple MOR inverse agonists ((NTX, naloxone, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP), β-funaltrexamine (β-FNA)) reinstate hyperalgesia (Corder et al. 2013). These findings are consistent across pain models [CFA, plantar incision (Campillo et al. 2011; Corder et al. 2013), species [rats, mice], and multiple mouse strains [C57Bl/6, CD1 (Campillo et al. 2011; Le Roy et al. 2011; Corder et al. 2013)], and behavioral endpoints [mechanical hyperalgesia, thermal hyperalgesia conditioned place preference (CPP) measure of affective pain (Corder et al.), heat hyperalgesia, nocifensive behavior, mouse grimace scale of spontaneous pain (Corder et al. 2013)], and both sexes (Corder et al. 2013). Our core *pain reinstatement model* has already been replicated in several laboratories including C. Fairbanks (University of Minnesota), Z. Wang (University of Illinois), J. Mogil

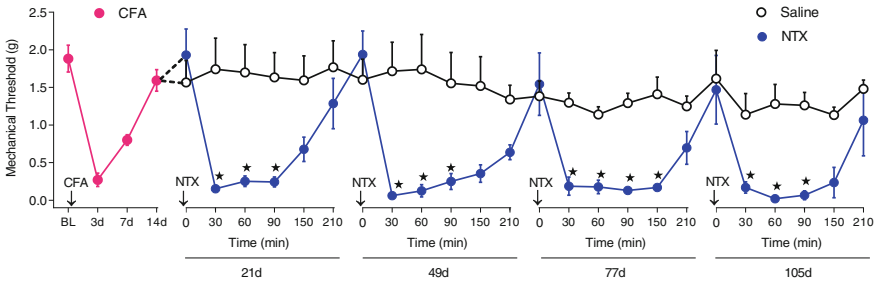

Fig. 3 NTX reinstates inflammatory pain even when administered 6 months after induction of inflammation. After baseline (*BL*) measurement of von Frey threshold, mechanical hypersensitivity was allowed to recover (*red circles*). Effect of repeated subcutaneous saline (*open circles*) or NTX (3 mg/kg, *blue circles*) injections on mechanical thresholds at 21, 49, 77, and 105d after CFA (n = 5–7). Adapted from Corder et al. 2013

(McGill University), and J. Marvizon (University of California, Los Angeles). Thus, opioid receptor-masked LS is long lasting, powerful, repeatable, and is likely relevant to a broad range of chronic pain subjects.

4.3 Multiple Opioid Receptors Can Mask LS

All three opioid receptors, MOR, DOR, and KOR, have been extensively investigated as potential therapeutic targets for disorders ranging from depression to severe pain (Lutz and Kieffer 2013). We focused on MOR, because the μ-selective inverse agonists CTOP and β-FNA reinstated hyperalgesia and spinal pain transmission in CFA 21d mice (Corder et al. 2013). These results do not, however, rule out DOR or KOR. Indeed, KORs might also maintain LS in a state of remission (Schepers et al. 2008b; Campillo et al. 2011). For example, the KOR-selective antagonist nor-binaltorphamine (norBNI) exacerbated CFA mechanical hyperalgesia at the injured paw and unmasked hyperalgesia at the contralateral paw. Also, norBNI reinstated mechanical hyperalgesia when injected weeks after plantar incision performed under remifentanil analgesia (Campillo et al. 2011). Alternatively, DOR may contribute to MOR or KOR function through heterodimerization (Gomes et al. 2000; Law et al. 2005; Yoo et al. 2014), so future studies should evaluate the effect of DOR antagonists as well.

4.4 Multiple Neurobiological Systems Can Mask LS

By and large, most of what we know about intrinsic masking of LS comes from interventions that disrupt endogenous opioid receptor systems. However, numerous other receptor systems likely maintain LS in remission; LS is also masked by

endocannabinoids (Alkaitis et al. 2010; Ji et al. 2011), alpha-2 adrenergic receptors (De Felice et al. 2011), and neuropeptide Y (NPY) receptor activity (Solway et al. 2011). For example, our laboratory recently used conditional NPY knockout mice and NPY Y1 and Y2 receptor antagonists to demonstrate that the endogenous NPY system in the DH is involved in repressing the LS induced by inflammation or nerve damage (Solway et al. 2011). So while this section focuses on opioid receptors, it should be kept in mind that they might work in concert with endocannabinoid, resolvin, neuropeptide Y, or other pain inhibitory systems to prevent the induction or hasten the resolution of chronic pain.

4.5 Long-Lasting Endogenous Analgesia Requires a Sensitized State

As indicated above, opioid mechanisms are thought to be recruited in response to intense, sustained nociceptive signaling. On the other hand, in wild-type uninjured mice, transient pain is not modulated by endogenous opioid tone, as opioid receptor antagonists such as NTX, naloxone, CTOP, and β-FNA do not change thermal or mechanical thresholds (Grevert and Goldstein 1978; Kern et al. 2008; Corder et al. 2013). This is consistent with observations in normal MOR knockout mice that do not show changes in acute nociceptive processing (Fuchs et al. 1999). Similar studies in normal human subjects also report no modulation of pain perception upon opioid antagonist treatment, suggesting little to no tonically active endogenous opioid signaling in the absence of tissue or nerve damage (El-Sobky et al. 1976; Schoell et al. 2010). Even in the setting of acute ongoing nociception, as is the case after the intraplantar injection of dilute formalin, naloxone has no effect (Taylor et al. 1997).

4.6 LS Develops Bilaterally

Mechanical sensitivity develops not only on the side of the body that receives tissue injury, but also on the contralateral side. This bilateral hyperalgesia is observed in numerous pain models (inflammation (Chillingworth et al. 2006), nerve injuries (Seltzer et al. 1990; Kim and Chung 1992), formalin (Aloisi et al. 1993), arthritis (Rees et al. 1996), sciatic inflammatory neuritis (Chacur et al. 2001), and muscle pain (Sluka et al. 2001, Ainsworth et al. 2006). Persistent inflammation increases the effectiveness of exogenously applied opioid agonists to inhibit contralateral nociceptive sensitivity in the rostroventral medulla (Hurley and Hammond 2000, 2001; Schepers et al. 2008a) and the spinal cord (Stanfa et al. 1992; Stanfa and Dickenson 1995). We now know that this extends to LS, because opioid inverse agonists produce contralateral hyperalgesia and spinal neuron sensitization (stimulus-evoked expression of phosphorylated extracellular signal-regulated kinase, pERK) when

delivered during the post-hyperalgesia state (Corder et al. 2013). Since connections between the two sides of the spinal cord are sparse at best, bilateral hyperalgesia suggests that the brain is involved in LS. Indeed, leading explanations for bilateral hyperalgesia include top-down facilitation involving descending facilitatory fibers from the brainstem (Koltzenburg et al. 1999; Tillu et al. 2008; Chai et al. 2012). Another possible mechanism involves communication of reactive spinal astrocytes through gap–junction networks across large distances (Hatashita et al. 2008; Gao and Ji 2010b). However, the lack of NTX-induced pERK in microglia and astrocytes suggests that glia are not activated upon loss of tonic MOR signaling (Corder et al. 2013).

Tissue injury produces long-lasting contralateral increases in MOR-G protein coupling, and β-funaltrexamine-induced decreases in basal MOR-G protein coupling (Corder et al. 2013). Thus, MOR is intricately involved in the regulation of contralateral LS. The presence of contralateral spinal MOR_{CA} and neural sensitization illustrates the spread of LS to areas of the CNS beyond those directly innervated by the injured tissue. If true, then loss of MOR_{CA} antinociception (e.g., during stress) could lead to the emergence of rampant chronic pain (Rivat et al. 2007; De Felice et al. 2011). Thus, MOR_{CA} might tonically repress widespread hyperalgesia.

4.7 Latent Sensitization Develops Across the Pain Neuraxis

LS is maintained within the periphery (Stein and Lang 2009; Guan et al. 2010) and local spinal circuitry (Ruscheweyh et al. 2011) and by descending facilitatory signals from brainstem nuclei (Porreca et al. 2002; De Felice et al. 2011). NTX is lipophilic and therefore readily crosses the blood–brain barrier (BBB), antagonizing opioid receptors at supraspinal, spinal, and peripheral sites. To address the site of action of endogenous opioid receptors, investigators have (1) evaluated local administration of low doses of NTX at the site of injury, at the level of the spinal cord using the intrathecal route, or into the brain; (2) used peripherally restricted receptor antagonists; or 3) evaluated neuronal activation at CNS sites of pain modulation.

4.7.1 Periphery

Peripheral mechanisms contribute to LS. For example, Levine and colleagues describe a large body of literature describing a long-lasting latent hyperresponsiveness within primary afferent nociceptors following local administration of the proinflammatory cytokine prostaglandin E_2, for which they have coined the term "hyperalgesic priming" (Hucho and Levine 2007; Reichling and Levine 2009; Ferrari et al. 2010; Joseph and Levine 2010; Green et al. 2011). In a partial L5 spinal nerve ligation model of neuropathic pain, Guan et al. waited 7–8 weeks for mechanical allodynia to subside and then injected either naloxone or naloxone methodide (a peripherally acting opioid receptor antagonist) (Guan et al. 2010).

Naloxone methiodide reinstated mechanical allodynia, suggesting a contribution of peripheral opioid receptors to LS in the setting of neuropathic pain. In an intraplantar CFA model of inflammatory pain, Stein and Lang waited 4–6 days and found that local injection of naloxone increased hyperalgesia (Stein and Lang 2009). In a similar model (using of lower amount of CFA), however, we reported that naltrexone methobromide, another opioid receptor antagonist with a quaternary amine that restricts BBB permeability, did not alter mechanical thresholds when injected 21 days after inflammation. Indeed, we found that tissue edema gradually resolved within 77 days after induction of inflammation (Corder et al. 2013). Beyond that time, we found that NTX reinstated hyperalgesia, suggesting that endogenous opioid signaling persists beyond tissue healing and the resolution of primary hyperalgesia. Thus, peripheral mechanisms may contribute to LS after nerve injury, but might be restricted to early timepoints after non-neural tissue injury. This prompted our focus on spinal cord and brain sites of action (Corder et al. 2013).

4.7.2 Spinal Cord Dorsal Horn

Opioids inhibit LTP in the DH (Terman et al. 2001; Benrath et al. 2004), and so we targeted the spinal cord with intrathecal administration of opioid receptor antagonists and inverse agonists. Either intrathecal NTX or NTX methobromide robustly reinstated mechanical hyperalgesia (Corder et al. 2013). This behavioral result was recapitulated with three molecular and neurophysiological markers of spinal nociceptive neuron activation to further localize opioid signaling and LS to the lumbar spinal cord: (1) Within the DH nociceptive circuitry, we visualized pERK. pERK is implicated in the spinal transduction of nociceptive signals and serves as a marker of central sensitization during pathological conditions (Ji et al. 1999, 2009). pERK is typically elevated following high-threshold (Aδ- or C-fiber) or noxious stimulation (Ji et al. 1999); importantly during the maintenance phase of chronic pain (>2 days post CFA), a low-threshold (Aβ-fiber) stimulation (Matsumoto et al. 2008) or light touch (Gao and Ji 2010a) can induce spinal pERK expression, suggestive of injury-induced central sensitization (Ji et al. 2003). We found that after pain resolution (21d), light touch was unable to effectively activate superficial DH neurons unless LS was disinhibited by NTX (Corder et al. 2013); (2) within spinal cord slices, we visualized intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in populations of DH neurons using Fura-2 labeling and wide-field fluorescence microscopy (Doolen et al. 2012). NTX administration to slices taken from post-hyperalgesia animals precipitated ↑ $[Ca^{2+}]_I$, indicating that LS survives in spinal nociceptive neurons within our slice preparation, and that opioid receptor activity tonically suppresses it (Corder et al. 2013); (3) NTX produced cAMP superactivation in the DH of post-hyperalgesia mice (Corder et al. 2013). Taken together, these data suggest that MOR actively represses signal amplification, similar to spinal GABAergic signaling (Baba et al. 2003; Torsney and MacDermott 2006; Torsney 2011). Our results

reveal the presence of sensitized spinal nociceptive neurons that outlast the resolution of hyperalgesia and remain under the control of endogenous opioid receptor inhibitory mechanisms (Corder et al. 2013).

5 Mu Opioid Receptor Constitutive Activity (MOR_{CA}) Inhibits LS

As demonstrated above, spinal MORs become tonically active after injury and continue to provide endogenous analgesia beyond wound healing (Corder et al. 2013). Receptor mechanisms driving tonic MOR signaling may involve either continuous opioid agonist stimulation or a unique, agonist-independent form of signaling termed constitutive activity (Costa and Herz 1989; Kenakin 2001, 2004; Seifert and Wenzel-Seifert 2002). Using a multidisciplinary approach involving physiological, genetic, and biochemical strategies, we demonstrated that the injury-induced tonic signaling state is facilitated by acquisition of constitutive activity. This MOR constitutive activity, which we term MOR_{CA}, continues to quiet nociceptive cells in the spinal cord, perhaps even in the absence of signals from opioid peptides. Here, we review the concept of constitutive activity and inverse agonism and then describe the evidence supporting the development of MOR_{CA} after repeated drug administration or cutaneous inflammation.

5.1 Classification of Opioid Receptor Antagonists

Morphine exerts actions such as pain reduction by binding to the extracellular binding domain of MORs, causing a conformational change in the receptor that then allows for G protein coupling and intracellular signal transduction. This positive efficacy of morphine broadly classifies this ligand as an *agonist*. A simple two-state model expounds that the receptor rests in an *inactive* conformation (Ri) and with the addition of an agonist (A), the receptor becomes active (Ra) and subsequently binds to a G protein (ARaG) (Fig. 4a). The actions of agonists can be blocked by addition of antagonists which prevent binding of the agonist to the receptor. This can occur through competition for the binding pocket or at an allosteric site. Regardless of mechanism, the definition of a true antagonist (i.e., a neutral antagonist) describes its action in terms of blocking an agonist without effecting receptor conformations that might alter signaling (i.e., antagonists do not have efficacy at a given receptor for either the active or inactive state). Thus, a neutral antagonist will not exert effects by itself, and so will not alter MOR_{CA}; rather, a neutral antagonist will block an agonist-induced ARaG state (Fig. 4b).

The term *inverse agonist* is used to describe ligands that display negative efficacy, a concept that requires a 3-state model (Fig. 4b). Negative efficacy is the idea

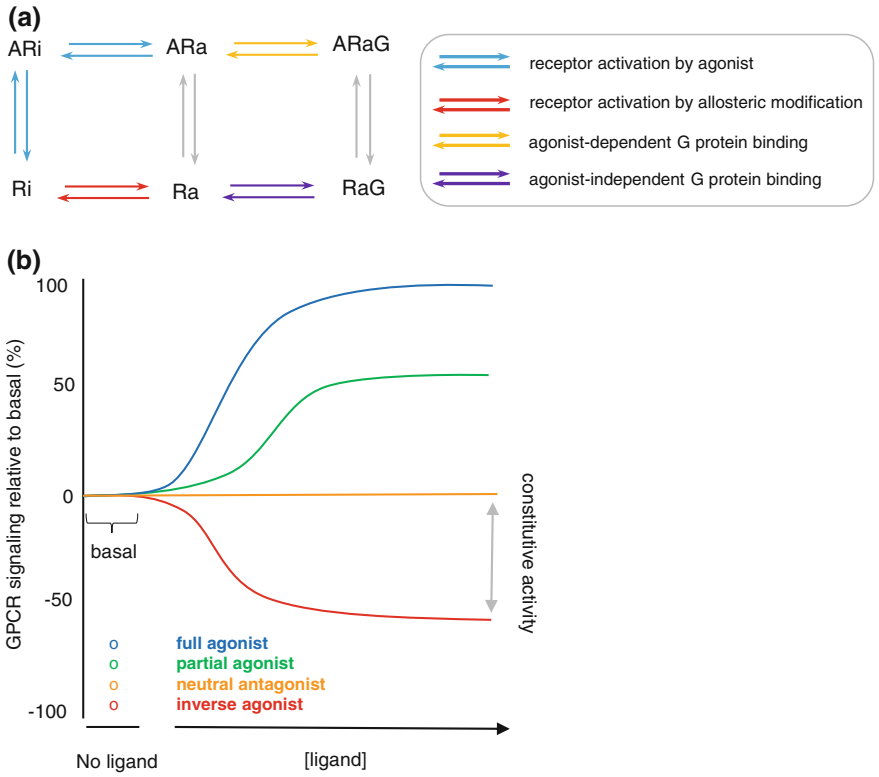

Fig. 4 Constitutive activity and inverse agonism. **a** The extended ternary complex model of GPCR signaling predicts that modulating the allosteric constant (*red arrows*) produces a spontaneously active receptor (*Ra*). Ra can then bind to G proteins (*G*) independent of agonist (*A*) facilitation (*pink arrows*). **b** Effect of various ligands on GPCR activity

that, upon binding to its receptor, a ligand can repress the spontaneous activity of the receptor (RaG) and promote the inactive state (Ri). Thus, inverse agonists characteristically reduce constitutive G protein coupling to receptors.

5.1.1 Naltrexone as an Inverse Agonist

A growing body of evidence indicates that opioid receptor ligands like NTX and CTOP have inverse agonist properties that increase following tissue injury (Corder et al. 2013) or chronic opioid exposure (Sadee et al. 2005). As illustrated in Table 2, cells obtained from whole animal models of opioid dependence, e.g., animals treated for several days with morphine, exhibit key characteristics of constitutive opioid receptor activity upon challenge with NTX (Wang et al. 2001a, 2004; Raehal et al. 2005; Sirohi et al. 2009; Lam et al. 2011; Navani et al. 2011). These results seem to outweigh the more discrepant results obtained from studies in cell culture

Table 2 In vivo studies in rodents subjected to chronic opioids or tissue injury which directly compare the ability of naltrexone and 6β-naltrexol to exert intrinsic activity and precipitate physical withdrawal

	In vivo mouse model	Robust Physical withdrawal?	Effect on basal GTPγS binding	Effect on cAMP	Comment	References
Naltrexone	Morphine dependence	Yes	↓	↑		Wang et al. (2001a)
	Morphine dependence	Yes	↓	↑		Wang et al. (2004)
	Morphine dependence	Yes	↓			Raehal et al. (2005)
	Morphine dependence	Yes				Sirohi et al. (2009)
	Morphine dependence	Yes				Navani et al. (2011)
	Latent pain sensitization	Yes	↓	↑	Hyperalgesia, neuron activation and cAMP	Corder et al. (2013)
6β-naltrexol	Morphine dependence	No				Wang et al. (2001a)
	Morphine dependence	No	No effect	No effect		Wang et al. (2004)
	Morphine dependence	No	No effect		↓ NTX-induced decrease in basal GTPγS binding	Raehal et al. (2005)
	Morphine dependence	No			↓ NTX-induced physical withdrawal	Sirohi et al. (2009)
	Morphine dependence	No			↓ NTX-induced physical withdrawal	Navani et al. (2011)
	Latent pain sensitization				↓ NTX-induced hyperalgesia, neuron activation, and cAMP	Corder et al. (2013)

All of these studies consistently indicate that naltrexone and 6β-naltrexol behave as an inverse agonist and a neutral antagonist, respectively. Thus, both chronic opioids and tissue injury generate constitutive activity at CNS opioid receptors

lines treated *in vitro* with opioid receptor agonists for a decidedly shorter treatment (overnight); these studies yield evidence both for (Sally et al. 2010) and against (Divin et al. 2009) intrinsic activity of NTX.

5.1.2 6β-Naltrexol Is a Neutral Antagonist

A large body of evidence indicates that 6β-naltrexol, a metabolite of NTX, exhibits key characteristics of a neutral antagonist when administered to cells obtained from opioid-dependent animals, e.g., little or no intrinsic activity (i.e., no effect) when administered by itself (Table 2). Furthermore, as expected for an opioid receptor neutral antagonist, 6β-naltrexol blocks the effects of inverse agonists (Wang et al. 1994, 2004, 2007; Bilsky et al. 1996; Kenakin 2001; Raehal et al. 2005; Sirohi et al. 2009; Sally et al. 2010; Lam et al. 2011; Navani et al. 2011) (Table 2). As described in more detail below, recent data in models of LS describe similar actions of 6β-naltrexol in vivo, for the first time, when administered to the whole animal (Corder et al. 2013).

5.2 Proposed Mechanisms of Constitutive Activity

As noted above, MOR signaling persists for several months after tissue damage. What mechanisms might maintain tonic signal transduction? Receptor signaling is initiated by either ligand binding or spontaneous acquisition of the active conformation. The former scenario is susceptible to antagonists that outcompete for the receptor binding pocket, while the latter case is blocked by inverse agonists with preferential affinity for the inactive state of the receptor and, therefore, reverse basal responses attributed to constitutive activity (Kenakin 2007). The mechanisms that initiate and maintain constitutive signaling after inflammation are unknown, but essential ideas come from the extended ternary complex model of GPCR signaling (Kenakin 2004). GPCRs exist in equilibrium of spontaneous Ra and Ri states, known as an *allosteric constant*. This constant represents a distinctive energy barrier for a given GPCR in a specific cellular environment which dictates the formation of spontaneous Ra states. Thus, lowering the allosteric constant (e.g., receptor point mutations, removal of Na^+ ions, loss of negative regulatory internalizing desensitizing proteins) increases the formation of Ra. In this paradigm, constitutive activity can be produced upon lowering of the energy barrier to spontaneously form the active state of the receptor. While the biophysical mechanisms of constitutive activity have yet to be fully understood, approximately 30 different studies using site-directed point mutations in the *oprm1* gene encoding MOR have found numerous amino acids that affect ligand binding, G protein coupling efficiency, and receptor phosphorylation and internalization, all of which increase MOR_{CA} (Chavkin et al. 2001). Importantly, genetic mutations are not necessary for constitutive activity to occur. Liu and Prather demonstrated that

prolonged exposure of morphine can induce constitutive activity that persists after agonist washout (Liu and Prather 2001), suggesting that posttranslational modifications to MOR or decreased interactions with negative regulatory proteins may induce constitutive activity. This might be achieved through posttranslational modification of MOR (e.g., hyperphosphorylation) (Wang et al. 1994) or a reduction in the association of the receptor with a negative regulatory protein, such as β-arrestin2 (Lam et al. 2011).

5.3 *Repeated Opiate Administration Increases MOR_{CA} in the Brain*

An intriguing hypothesis of drug addiction suggests that chronic opiates increase MOR constitutive activity to preserve physical and psychological dependence (Wang et al. 1994, 2004; Liu and Prather 2001; Shoblock and Maidment 2006; Meye et al. 2012), which is enhanced by the action of endogenously released enkephalins in the brain (Shoblock and Maidment 2007). Similarly, prolonged administration of exogenous opioid agonists, such as morphine or DAMGO, cause an increase in the number of constitutively active opioid receptors during the period of opioid dependence (Wang et al. 2001a; Sadee et al. 2005; Walker and Sterious 2005; Xu et al. 2007; Sally et al. 2010). Constitutive activity is experimentally observable as increased basal $GTP\gamma S^{35}$ binding, increased B_{max} of the agonist-responsive high-affinity $GTP\gamma S^{35}$ binding site, and suppressed forskolin-stimulated cAMP accumulation. While constitutive activity is usually difficult to detect outside of in vitro heterologous cell expression systems, as described above (Costa and Herz 1989; Liu and Prather 2001; Wang et al. 2007), MOR_{CA} has been observed in tissue obtained from the striatum (Wang et al. 2004; Shoblock and Maidment 2006) and the ventral tegmental area (Meye et al. 2012) of morphine-dependent mice. Inverse agonists decreased basal $GTP\gamma S^{35}$ binding, produced cAMP superactivation, and disinhibited GABAergic signaling, indicative of acquired state-dependent activity following chronic receptor stimulation. Although it remains unclear whether MOR_{CA} is important in disease states other than drug addiction, these studies provided the framework for experiments to determine whether MOR_{CA} operates in the setting of LS and chronic pain.

5.4 *Tissue Injury Establishes MOR_{CA} in the Spinal Cord*

The above suggests that if constitutive GPCR signaling is responsible for antihyperalgesia, then an opioid inverse agonist should produce hyperalgesia. We recently provided two major pieces of evidence indicating that injury establishes MOR_{CA} signaling in DH neurons and that MOR_{CA} analgesia maintains LS in a state of pain

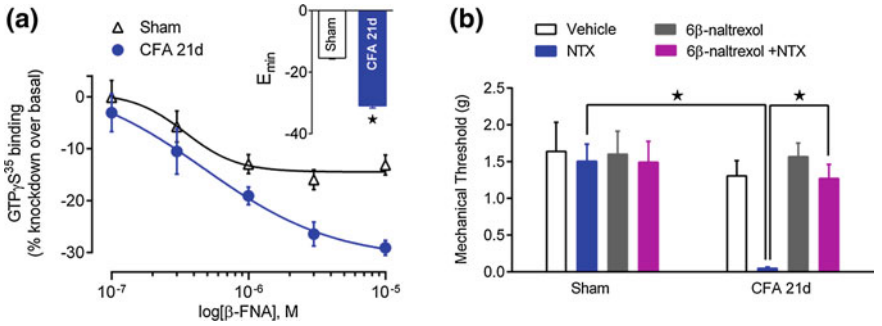

Fig. 5 Spinal μ-opioid receptors acquire constitutive activity (MOR_{CA}) after injury. **a** Dose–response effects of β-FNA intrathecal β-funaltrexamine (β-FNA) on basal GTPγS^{35} binding in lumbar dorsal horns of sham or CFA-21d mice; inset: group binding E_{max} (n = 7–9). **b** Effects of intrathecal administration of NTX (1 μg), 6β-naltrexol (10 μg), or coadministration of 6β-naltrexol + NTX in sham and post-hyperalgesia mice on mechanical hyperalgesia. *Filled asterisk* $p < 0.05$. Adapted from Corder et al. 2013

remission (Corder et al. 2013). First, GTPγS^{35} binding studies are essential to prove CA, and β-FNA, a putative inverse agonist at MOR (Liu et al. 2001), reduced constitutive GDP/GTPγS^{35} exchange in DH sections during the pain remission phase of LS in CFA-injured mice (Fig. 5a). Second, the MOR neutral antagonist 6-β-naltrexol prevented reinstatement of pain and $[Ca^{2+}]_i$ mobilization in spinal slices (Corder et al. 2013) induced by the MOR inverse agonist NTX (Fig. 5b). This is consistent with the expected blockade of the effect of an inverse agonist by a neutral antagonist. As noted above, a neutral antagonist is not expected to exert effects by itself and so as predicted, 6β-naltrexol failed to precipitate hyperalgesia or $\uparrow[Ca^{2+}]_i$. Thus, Corder et al. demonstrated in vivo acquisition of constitutive MOR signaling that naturally develops after injury, without chronic drug dosing (Sadee et al. 2005) or genetic manipulations (Chavkin et al. 2001).

One potential limitation of GDP/GTPγS^{35} exchange studies is the potential presence of trace endogenous opioid peptides. If true, then NTX, CTOP, and β-FNA might have behaved as antagonists to block the actions of opioid agonists. We concede the possibility of continual opioid release in the *ex vivo* spinal cord slice preparation following dissection. However, all spinal cord sections from injured and non-injured mice were treated identically. Therefore, any residual opioid content (as well as other conditions) was identical between the sections used for basal determinations or those incubated with or without β-funaltrexamine. We believe that the extensive buffer washing and incubation protocols were sufficient to remove any opioid peptides or other agonists from the extracellular space before incubation with GTPγS^{35} (Corder et al. 2013).

CA GPCRs are, like agonist-activated GPCRs, subject to internalization, phosphorylation, desensitization, and downregulation (Leurs et al. 1998). Interestingly, in DRG neurons, CA MORs recycle more rapidly through a caveolin-mediated mechanism, as compared to typical MORs which internalize through a clathrin-mediated

mechanism (Walwyn et al. 2007). Whether CFA produces lasting analgesia by increasing the recycling of CA MORs in DH, however, is difficult to determine because internalized CA MORs are too close to the membrane to detect by standard confocal microscopy (Walwyn et al. 2007). An alternative approach is to use flow cytometry to quantify surface receptor density.

The CA state of MOR is thought to depend on receptor modifications, including receptor interactions with accessory proteins or phosphorylation at numerous sites on MOR (Sadee et al. 2005, Connor and Traynor 2010; Mann et al. 2014), including constitutive phosphorylation at S363 in vivo (Illing et al. 2014). With the emergence of phosphosite-specific MOR antibodies and other pharmacological tools (Mann et al. 2014), future studies could determine whether CFA increases MOR phosphorylation or dephosphorylation that then contributes to MOR_{CA}.

5.5 Ligand-Dependent Tonic Activation of Opioid Receptors

Tissue injury produces long-lasting endogenous opioid inhibition of LS that is mediated by ligand-independent MOR_{CA} signaling. MOR_{CA} is maintained for sufficient duration to hasten the resolution of acute pain, oppose the development chronic pain, and/or temper the severity of chronic pain (Corder 2013). However, inverse agonists can block not only MOR_{CA}, but also MORs that are tonically activated by endogenous opioids. Thus, an emerging controversy is unfolding regarding whether MOR_{CA} operates alone or in concert with tonically activated MOR to generate long-lasting endogenous opioid analgesia after injury that then masks LS. Regardless, either mechanism can promote pain recovery, but can also lead to pathological consequences (i.e., dependence as discussed later) which can create a lasting susceptibility to develop chronic pain.

In support of a ligand-dependent mechanism, noxious input or tissue injury produces rapid increases in extracellular endogenous opioids within DH (Basbaum and Fields 1984; Noguchi et al. 1992; Song and Marvizon 2003b; Trafton and Basbaum 2004) including enkephalins (Yaksh and Elde 1981; Iadarola et al. 1986; Cesselin et al. 1989) and constitutive increases in prodynorphin products that lead to a modest tonic inhibition of heat nociception or acute inflammatory pain (Iadarola et al. 1988; Wang et al. 2001b, Campillo et al. 2011). Ligand-dependent signaling is an important consideration, but little is known about the significance of tonically activated MOR in models of chronic pain. Of interest, plantar incision increases dynorphin levels in the spinal cord that might then tonically inhibit pain (Campillo et al. 2011), and prodynorphin knockout mice exhibit exaggerated hyperalgesia after intraplantar formalin. These data suggest that dynorphin-mediated activation of kappa opioid receptors (KOR) contributes to a (modest) tonic pain inhibition during LS (Wang et al. 2001b). The contribution of ligand-dependent tonic activation of MOR to the silencing of LS, however, remains to be determined, for example, with measurements of opioid levels or interventions that disrupt opioid gene expression.

Evidence against a ligand-dependent mechanism is that 6β-naltrexol, acting as a neutral antagonist, failed to precipitate hyperalgesia or ↑$[Ca^{2+}]_I$ when administered during the post-hyperalgesia phase after inflammation (Corder et al. 2013). Moreover, blockade of NTX inverse effects by 6β-naltrexol, in all our behavioral and molecular assays, clearly argues that agonist-independent signaling is present in a population of spinal MORs. This is consistent with the expected blockade of the effect of an inverse agonist by a neutral antagonist.

Future studies with opioid peptide knockout mice or siRNA are needed to (1) ascertain the impact of endogenous opioids on LS; (2) determine whether the pain remission associated with MOR activation is driven by endogenous opioid release; and (3) distinguish hypotheses of MOR constitutive activity with tonic activation of MORs.

6 Molecular Mechanisms of Latent Sensitization

The following addresses the cellular signaling mechanisms that underlie the persistence of pathological nociceptive plasticity and LS.

6.1 NMDA Receptor (NMDAR)

It is well established that both the induction and maintenance phases of central sensitization require *N*-methyl-D-aspartate receptor (NMDAR)- and Ca^{2+}-dependent mechanisms (Latremoliere and Woolf 2009). Several studies support the contribution of NMDAR to LS as well. Indeed, either chronic environmental stress (Le Roy et al. 2011), injury (Rivat et al. 2002, 2007), or exposure to exogenous opioids has been suggested to generate an adaptive sensitization of pronociceptive NMDAR pathways through an unknown cellular mechanism. Similarly, as illustrated in Fig. 6, we reported that intrathecal NMDA-induced pain-like behaviors and ↑cAMP were potentiated in CFA-21d mice, indicating that injury increases NMDAR sensitivity (Corder et al. 2013). Furthermore, the NMDAR antagonist MK-801 attenuated NTX-induced reinstatement of spinal pERK activation, glutamate-evoked ↑$[Ca^{2+}]_I$, and hyperalgesia (Corder et al. 2013), indicating that NMDAR mechanisms play a critical role in mediating the manifestations of LS that is masked by opioid receptor activity.

The induction phase of CFA-hyperalgesia (<24 h after injury) is dependent on spinal NMDAR signaling, whereas the maintenance phase of hyperalgesia has NMDAR-independent components [Corder et al. 2013]. We reported that NTX does not increase hyperalgesia or glutamate-evoked spinal $[Ca^{2+}]_{intracellular}$ 24 h after CFA, suggesting that the endogenous opioidergic system does not impinge on the induction of CFA-hyperalgesia. In contrast, continuous infusion of NTX over the first 14d after CFA eliminated endogenous opioid analgesia, suggesting that the

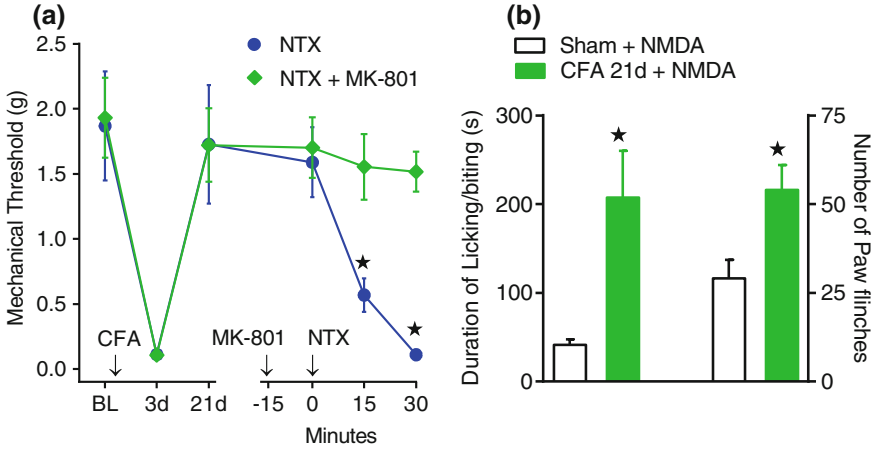

Fig. 6 NMDAR is required for NTX-induced pain reinstatement and is sensitized during the remission phase of latent pain sensitization. **a** Effect of intrathecal administration of the NMDAR antagonist, MK-801 (1 μg) on NTX-precipitated mechanical hyperalgesia (n = 5–10). **b** Effect of NMDA (i.t.; 3 pmol) on spontaneous nocifensive behaviors (*left*) and paw flinches (*right*) over 15 min in sham (n = 5) and CFA (n = 5) mice. *$p < 0.05$. Adapted from Corder et al. (2013)

opioidergic system opposes the maintenance of CFA-hyperalgesia. Taken together, our data suggest that the cellular mechanisms of the induction and maintenance of LS in our model are different.

NMDARs form heteromers of GluN1 (obligatory), GluN2A-D, and GluN3A-B subunits (Paoletti et al. 2013), with GluN1 and GluN2A-B shown to be critical to central sensitization and inflammatory hyperalgesia (Woolf and Costigan 1999; Woolf and Salter 2000; Guo et al. 2002; Abe et al. 2005; Gabra et al. 2007; Luo et al. 2008; Latremoliere and Woolf 2009). Future studies are needed to determine which of the key GluN2 subunits in DH are essential to LS.

6.2 NMDAR-Mediated Ca^{2+} Signaling

NMDAR-derived Ca^{2+} flux is necessary for ERK activation (Krapivinsky et al. 2003; Lever et al. 2003; Sindreu et al. 2007), specifically under pathological conditions or noxious stimulation (Ji 2004; Matsumoto et al. 2008). Furthermore, withdrawal from exogenous opioids results in synaptic LTP in spinal neurons and relies on post-synaptic NMDAR Ca^{2+} mobilization to mediate the hyperalgesia associated with physical withdrawal (Drdla et al. 2009). We found that pretreatment with intrathecal MK-801 resulted not only in prevention of NTX-induced hyperalgesia but also in loss of bilateral increases in dorsal horn pERK levels (Corder et al. 2013). Together, these results reveal that loss of tonic MOR signaling initiates an NMDAR—Ca^{2+}—pERK pathway to generate the reinstatement of pain.

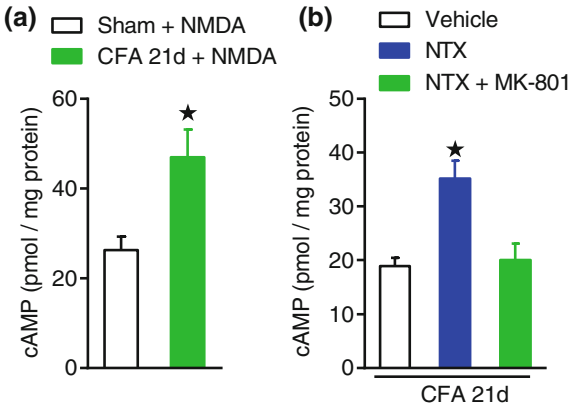

Fig. 7 Endogenous opioid withdrawal initiates NMDAR-dependent AC superactivation. **a** Effect of intrathecal administration of NMDA (i.t.; 3 pmol) on cAMP levels in sham (n = 5) and CFA (n = 5) mice, indicating a latent upregulation of an NMDAR-AC pathway during the remission phase of LS. **b** Effect of intrathecal administration of MK-801 (3 pmol) pretreatment on NTX-induced cAMP overshoot, indicating that NMDAR signaling contributes to AC superactivation during NTX-precipitated endogenous opioid withdrawal (n = 5 per group). *$p < 0.05$. Adapted from Corder et al. (2013)

6.3 NMDAR–cAMP Linkage

NMDAR-derived Ca^{2+} increases are linked to cAMP signaling pathways (Chetkovich and Sweatt 1993; Wong et al. 1999) necessary for LTP initiation (Wang and Zhuo 2002), opiate physical dependence (Zachariou et al. 2008), and spinal synaptic facilitation (Wei et al. 2006; Wang et al. 2011). Consistent with previous studies in brain showing that the Ca^{2+}-stimulated isoforms of adenylyl cyclase are activated by NMDARs (Chetkovich and Sweatt 1993), we reported and Fig. 7 illustrates that pretreatment with intrathecal MK-801 abolished the NTX-precipitated increases in cAMP (Corder et al. 2013). Moreover, direct activation of spinal NMDARs with intrathecal NMDA increased spontaneous nocifensive behaviors and spinal cAMP levels. Similarly, direct activation of the spinal AC system with intrathecal forskolin produced enhanced spontaneous nocifensive behaviors and intracellular cAMP. Thus, NMDAR signaling is directly linked to downstream cAMP signaling during opioid receptor inverse agonism.

6.4 Adenylyl Cyclase 1 (AC1)

Opioids produce their acute analgesic effects in part through inhibition of ACs. Of the 9 known isoforms, AC1 and AC8 are of particular interest, as they both couple NMDAR-induced cytosolic Ca^{2+} increases to cAMP signaling in the CNS

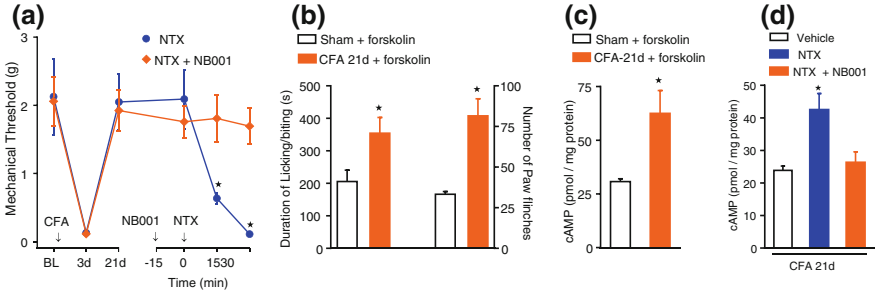

Fig. 8 Superactivation of spinal AC1 facilitates pain reinstatement and cellular opioid withdrawal. Effect of intrathecal administration of the AC1 antagonist, NB001 (1.5 μg), on NTX-precipitated mechanical hyperalgesia (**a**) and spinal cAMP content (**d**). Effect of the AC activator, forskolin (intrathecal, 1.5 μg (on spontaneous nocifensive behaviors (**b**) and paw flinches (**c**) over 15 min in sham and CFA mice, indicating AC1 superactivation during the remission phase of LS. $n = 6–9$. $^*p < 0.05$. Adapted from Corder et al. (2013)

(Wei et al. 2002). AC1 is expressed in the post-synaptic density of CNS neurons (Xia et al. 1991; Conti et al. 2007), in the brain and the superficial dorsal horn (Wei et al. 2002), and is subject to negative regulation by Gαi/o-coupled receptors in vivo (Nielsen et al. 1996). AC1 promotes phosphorylation and trafficking of AMPA receptors via protein kinase A (PKA) and/or protein kinase C zeta (PKMζ) (Lu et al. 2003; Li et al. 2010; Asiedu et al. 2011; Zhuo 2012), phosphorylates NMDARs via PKA—Src kinase (Xu et al. 2008; Qiu et al. 2013) and contributes to activity-dependent LTP in the spinal cord (Wang et al. 2011), and plays a prominent role in chronic pain (Wei et al. 2002). For example, as illustrated in Fig. 8, our recent data suggest that prolonged endogenous-mediated MOR signaling drives the upregulation of the spinal AC system in a manner that is analogous to opiate cellular dependence, thereby promoting the maintenance of LS (Corder et al. 2013). Thus, MOR blockade during the remission phase of LS with inverse agonists precipitates spinal cAMP overshoot (AC superactivation) and hyperalgesia (Corder et al. 2013). Furthermore, NTX reinstatement of hyperalgesia was lost in $AC1^{-/-}$ mice (Corder et al. 2013), and NB001, a selective AC1 inhibitor (Wang et al. 2011), prevented NTX-induced reinstatement of affective pain as well as hyperalgesia and cAMP overshoot (Corder et al. 2013). Also, pain-like behaviors and spinal cAMP elicited by the AC activator, forskolin, were potentiated (Corder et al. 2013), suggesting AC supersensitivity silently strengthens during the remission phase of LS. As illustrated schematically in Fig. 9, these data indicate that MOR_{CA} silences persistent AC1-mediated LS, and point to AC1 as a central component of LS that can be unleashed by MOR inverse agonism. Future studies are needed to determine whether AC1 expression itself is upregulated, and then which of the key effectors for cAMP, such as PKA or exchange proteins activated by cAMP (Epac), are essential to LS. Future studies are also needed to determine whether LS is a key pharmacological target for new analgesic drugs such as NB001, an AC1 blocker originally developed by Min Zhuo (Wang et al. 2011).

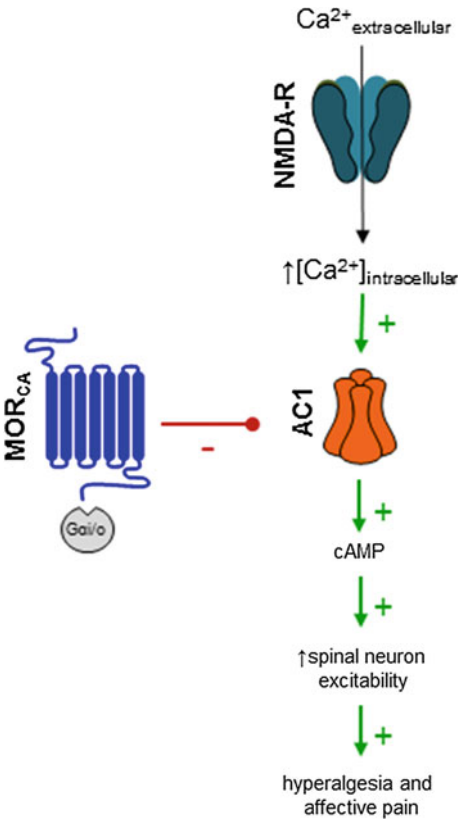

Fig. 9 Cellular signaling pathways underlying opioid receptor-masked latent sensitization. Spinal cord MOR signaling, through inhibitory $G_{ai/o}$ proteins, tonically represses AC1 production of cAMP, thereby reducing nociceptive signal transduction. Blockade of MOR results in the disinhibition of AC1, allowing for NMDAR-derived, Ca^{2+}-mediated activation, and downstream increases in signal transduction, neuron excitability, and ultimately, pain. Adapted from Corder et al. (2013)

7 Cellular Dependence on MOR_{CA}

As detailed above, administration of NTX during the post-hyperalgesia period of the CFA model activates not only spinal nociceptive transmission and pain, but also AC1. Chronic opiate exposure increases the expression of Ca^{2+}-sensitive ACs (Nestler and Aghajanian 1997; Christie 2008), particularly AC1 and AC8 (Lane-Ladd et al. 1997). During opioid withdrawal or opioid receptor antagonism, this upregulated AC1 population is disinhibited, producing a cAMP overshoot, or superactivation response (Zachariou et al. 2008; Mazei-Robison and Nestler 2012; Li et al. 2006).

7.1 Tissue Injury Triggers Cellular Dependence on MOR_{CA}

As noted above, during NTX-precipitated pain reinstatement, NMDAR-derived Ca^{2+} mobilization was potentiated, as was downstream activation of AC (reflected by enhanced responsiveness to forskolin) and associated cAMP overshoot in the spinal

cord (Corder et al. 2013). This AC supersensitivity following disruption of MOR is remarkably similar to that observed during withdrawal from opiate drugs. Further linking pain to withdrawal is the enhanced pronociceptive synaptic strength (Lu et al. 2003; Xu et al. 2008) that is observed following NMDAR-dependent spinal LTP at C-fiber synapses during withdrawal from exogenous opiates (Drdla et al. 2009).Thus, we propose a novel mechanism that determines the transition from acute to chronic pain: (1) Long-term MOR_{CA} inhibition of AC1-mediated central sensitization drives a counteradaptive, homeostatic increase in pronociceptive AC1 signaling cascades (Wei et al. 2002; Zhuo 2012), thereby paradoxically promoting the maintenance of LS; (2) injury initiates a dependence on MOR_{CA} that tonically prevents withdrawal hyperalgesia. Thus, anything that disrupts MOR_{CA} will trigger the transition to spinal cellular withdrawal (NMDA-mediated AC1 superactivation) and signs of chronic pain.

7.2 Cellular Opioid Withdrawal May Reflect de Novo NMDAR-Dependent LTP

Abrupt withdrawal from MOR_{CA} by an inverse agonist and the associated pain reinstatement could result from one of the several mechanisms: (1) disinhibition of tonically active primary afferent terminals; (2) disinhibition of AC1 superactivation in dorsal horn neurons; (3) potentiation of descending facilitatory signals from the brainstem; and/or (4) induction of *de novo* spinal NMDAR-AC1-dependent withdrawal. For several reasons, we favor the latter as a promising area of future investigation. First, exogenous opiate withdrawal in the spinal cord initiates *de novo* NMDAR-dependent LTP (Drdla et al. 2009; Zhou et al. 2010). Second, NMDAR signaling and post-synaptic spinal neuron Ca^{2+} rise are required in the induction, rather than maintenance, of spinal LTP and hyperalgesia (Weyerbacher et al. 2010; Sandkuhler and Gruber-Schoffnegger 2012). Third, AC1 is activated by NMDAR-derived Ca^{2+} and is necessary for LTP induction (Liauw et al. 2005; Wei et al. 2006). Fourth, intrathecal NMDA increased pain behaviors and spinal cAMP levels during the post-hyperalgesia state (Corder 2013), which suggests that spinal NMDAR–adenylyl cyclase signaling pathways are not occluded (saturated). Fifth, spinal NMDARs are not active prior to NTX-induced withdrawal, since intrathecal MK-801 alone neither increased mechanical threshold, reduced basal spinal intracellular calcium levels, decreased basal pERK expression, nor decreased basal cAMP levels when administered during the post-hyperalgesia state (Corder 2013).

8 Physical and Psychological Dependence on Endogenous Opioid Receptor Activity

8.1 Endogenous Opioid Dependence

Humans and other mammals develop tolerance and physical Dependence to exogenous opiate drugs, which can be termed *exogenous opiate dependence*. An emerging literature is suggesting that stress or dietary factors trigger the release of endorphins or enkephalins that then induce physical manifestations of *endogenous opioid dependence*. Physical (somatic) or psychological dependence can be revealed upon challenge with an opioid receptor antagonist. In the rodent, somatic indices of opiate withdrawal are similar to those encountered by an opiate drug addict experiencing withdrawal (Koob et al. 1992; Kest et al. 2002). For example, following repeated stress for 10 days in rats, naloxone produces whole-body tremors ("wet-dog" shakes) (Morley and Levine 1980). Also, following a stressful chronic schedule of warm water swimming, naloxone precipitates not only body tremors, but also piloerection and ptosis, suggesting that the body develops dependence on stress-induced activation of opioid receptors (Christie and Chesher 1982). Following excessive ingestion of sugar, naloxone triggers teeth chattering, forepaw flutters, head shaking, and anxiety, suggesting that endogenous dependence develops to sweet foods (Colantuoni et al. 2002). Following the chronic delivery of RB101, an enkephalinase inhibitor, naloxone produced forepaw tremor and decreased body weight, albeit to a mild degree, suggesting the elevated levels of endogenous enkephalin initiates endogenous dependence (Noble et al. 1994). Opioid blockade also elicits withdrawal signs in mice following heavy exposure to UV light (Fell et al. 2014). This is consistent with anecdotal evidence in humans that individuals who seek frequent UV light exposure develop dependence on reinforcing opioids, as indicated by the finding that 50 % of subjects (but 0 % of controls) exhibited withdrawal-like symptoms including nausea and jitteriness following administration of 5–25 mg of naltrexone (Kaur et al. 2006). Whether the mechanism of this endogenous opioid analgesia involves light-induced elevations of skin β-endorphin, as proposed (Fell et al. 2014), is uncertain and controversial. First, as expected for large molecules, evidence for the ability of β-endorphin to cross the blood–brain barrier, and thus reach the CNS, is sparse at best (Banks and Kastin 1990). Second, the chronic UV light conditions used by Fell et al. (Fell et al. 2014) were sufficient to trigger signs of tissue injury such as increases in local prostaglandin production. And as we describe next, tissue injury triggers the development of a powerful and well-characterized endogenous opioid physical dependence (Corder 2013).

8.2 Tissue Injury Triggers Physical Dependence

When administered during the post-hyperalgesia period after inflammatory insult, NTX produced numerous behaviors that reflected opioid withdrawal (Corder 2013). This is the first set of experiments to describe a physical dependence on endogenous opioid activity in the setting of persistent inflammation. Following NTX administration, mice exhibited jumping behavior (analogous to panic attacks and involuntary movement of the legs and arms observed in the addict when they stop taking opiate drugs), paw tremors, wet-dog shakes, increased locomotion, and importantly pain-like behaviors (Corder 2013).

Endogenous opioid physical dependence persists long after tissue healing, and even increases with the passing of months following the induction of inflammation (Corder et al. 2013). This indicates that physical dependence is not maintained by injury, but rather results from intensifying compensatory opioidergic neuroadaptations within the brain that could be either ligand dependent or ligand independent.

8.3 Tissue Injury Triggers Psychological Dependence

Pain comprises not only somatic and sensory (hyperalgesia) components, but also affective (aversiveness) components; the latter can be identified by changes in the rewarding property of analgesics and associated motivational behavior. In a CPP paradigm (Sufka 1994; King et al. 2009), the negative reinforcing capacity of intrathecal lidocaine (motivation to seek pain relief) demonstrates the presence of aversive pain 1d after inflammatory insult (He et al. 2012). This aversive component, as was the case with the numerous escape and somatomotor opioid withdrawal behaviors, was absent during the post-hyperalgesia period (21d after inflammatory insult), but could be triggered by naloxone (Corder et al. 2013).

8.4 Cellular, Physical, and Psychological Dependence Contribute to LS

In summary, endogenous activation of MOR initiates a compensatory process that can lead to a rebound effect when an antagonist/inverse agonist is given. This fits a common definition of "dependence," and dependence can indeed be transient. Overt evidence of dependence may be masked by the normal agonist effect of endogenous MOR activity. Pain-like behaviors following administration of inverse agonists reflect not only withdrawal from cellular dependence on MOR_{CA} in the spinal cord as described above, but also withdrawal from physical and psychological dependence on tonic MOR activity in the brain.

9 Conceptual Model, Conclusions, and Clinical Significance

9.1 Conceptual Model of the Delicate Balance Between LS, Dependence, and MOR_{CA} Analgesia

Based on our results, we suggest that injury produces a dynamic relationship between the systems that regulate analgesia and pain. As illustrated in Fig. 10, injury first activates pain pathways. Second, the spinal cord establishes MOR_{CA} as it attempts to control pain. Third, over time, the body becomes dependent on MOR_{CA}, which paradoxically sensitizes pain pathways. Pain begets MOR_{CA} begets pain vulnerability in a vicious cycle. The final result is a silent insidious state

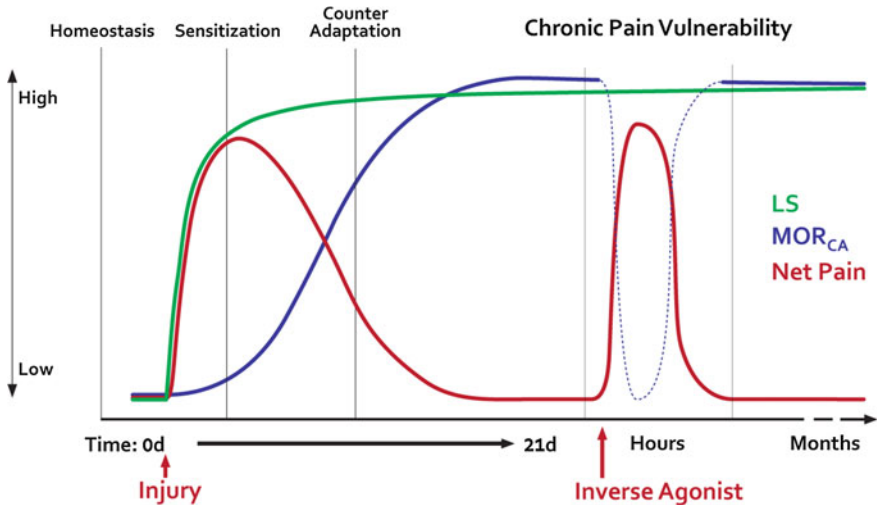

Fig. 10 Schematic summarizing the sequence of events that occur after injury, leading to a dynamic relationship between the systems that regulate analgesia and pain. Under naïve conditions, nociceptive and opioidergic systems have little to no activity, resting at a basal set point (homeostasis). Several changes occur following injury or strong nociceptive input. First, severe tissue injury triggers the development of an opioid receptor-masked LS that is driven by NMDA NR2 subunit and AC1/Epac1-mediated signaling (*Green line*). Second, a compensatory, counteradaptation—constitutively active μ-opioid receptor analgesia (*Blue line*, MOR_{CA})—enables the resolution of pain (*red line*) and thus maintains LS in a state of remission. The intensity and degree of sensory information transmitted through the nociceptive system returns to preinjury levels, while the opponent processes are potentiated but functionally cancel out one another. Third, if this balance is perturbed, e.g., upon inverse agonism of MOR_{CA} (*dotted line*), then blockade of tonic MOR signaling produces a superactivation of the NMDAR—AC1 pathways and LS can be visualized as pain reinstatement. Thus, pain is only detectable when endogenous analgesia is removed, leaving LS unchecked. Fourth, the body gradually becomes dependent on MOR_{CA}, which paradoxically sensitizes pain pathways. Pain begets MOR_{CA} begets pain in a vicious cycle. As a result, the nociceptive system becomes dependent on the counterbalancing signaling of tonic MOR. A recurring failure of MOR_{CA} (i.e., during stress) may reflect the transition from acute pain to a chronic state of pain vulnerability

characterized by the escalation of two opposing excitatory and inhibitory influences on pain transmission: silent pain mediated by AC1 which keeps the foot on the accelerator and pain inhibition mediated by MOR_{CA} (which keeps the foot on the brake). Indeed, stress (Rivat et al. 2007) or injury (Rivat et al. 2002) escalates opposing inhibitory and excitatory influences on nociceptive processing, as a pathological consequence of increased endogenous opioid tone. This raises the prospect that opposing homeostatic (or more precisely, allostatic) interactions between MOR_{CA} analgesia and latent NMDAR–AC1-mediated pain sensitization create a lasting vulnerability to develop chronic pain.

It is important to realize that the resolution of pain after an injury does not necessarily reflect a pathology-free condition. Instead, opposing interactions between MOR_{CA} analgesia and silent AC1-medicated pain create a lasting susceptibility to develop chronic pain. Indeed, a few clinical chronic pain studies have suggested that a transient upregulation of endogenous opioid analgesia, followed by opioid dysfunction, is associated with the onset of chronic pain (Bruehl et al. 2004, 2010). Using a motor vehicle analogy again, the fact that the "pain accelerator" (LS) and the "pain brake" (MOR_{CA}) are simultaneously pressed for long periods of time has several implications for vehicles (e.g., patients) where the brake pedal is stuck (e.g., severe trauma or major surgery). To keep the car moving, the accelerator pedal is always on, and the car ***depends*** on a constant brake, or else it will speed out of control (e.g., chronic pain). Future studies are needed to better understand the long-term consequences of simultaneous pressing of the accelerator and brake on pain. How can we prevent the brake pads from wearing out? Or can we replace them when they do?

9.2 Chronic Pain Vulnerability

We conclude that tissue injury promotes two opposing processes at pain modulatory synapses in DH: (1) tonic analgesic GPCR signaling such as MOR_{CA} that masks LS and (2) a pathological consequence of long-lasting tonic MOR_{CA} signaling that involves the upregulation in expression and function of NMDA receptor subunits and intracellular cAMP signaling. This drives a LS that persists in the post-hyperalgesia state and not only remains under the inhibitory control of MOR_{CA} but is also dependent on MOR_{CA}. Indeed, because LS and MOR_{CA} pathology spread beyond the injury site, a loss of MOR_{CA} analgesia could lead to the emergence of widespread, rampant chronic pain. This raises the prospect that the opposing interactions between MOR_{CA} and latent NMDAR–AC1 sensitization create a lasting susceptibility for the emergence of LS as a silent, insidious form of chronic pain. If true, then anything that can disrupt MOR_{CA} inhibitory systems would unleash the pain of endogenous opioid withdrawal, causing a pain episode to ensue. This has been modeled with inverse agonist challenge to MOR_{CA}, by which an ensuing reinstatement of pain reflects a process of spinal cellular withdrawal and AC1 superactivation to enhance neuronal excitability and pronociceptive synaptic

strength. Indeed, we propose that a long-term goal for future clinical studies is the development of a clinical test (e.g., naloxone challenge) for pain vulnerability. If LS exists, then naloxone should reveal this risk factor for pain relapse by reinstating secondary hyperalgesia.

9.3 Translational Relevance of LS and MOR_{CA}

The latent predisposition to relapse may explain the episodic nature and vulnerability to stressors that accompany chronic pain states in humans (Schweinhardt et al. 2008; Woolf 2011). But does injury trigger LS and long-term opioid analgesia in humans? Superficial cutaneous heat injury is a well-characterized and validated inflammatory model of human peripheral nociceptor sensitization, primary hyperalgesia (***PHA***), and SHA. It has been used extensively for pharmacodynamic research (Brennum et al. 2001; Werner et al. 2001, 2002a, b, Dirks et al. 2002a, b, 2003; Ravn et al. 2012, 2013). Sensitization is transient, reversible, and replicable (Werner et al. 2013). We recently reported that low-dose naloxone (0.021 mg/kg, i.v.), delivered following resolution of hyperalgesia after cutaneous heat injury, did not reinstate heat hyperalgesia (Pereira et al. 2013). Rather than limited tissue injury of the model or species differences, this negative result was likely due to insufficient blockade of endogenous opioid receptors, as higher doses of naloxone have been used to demonstrate reinstatement in humans (M.P. Pereira, J.B. Dahl, B.K. Taylor, M.U. Werner, unpublished results).

9.4 A New Approach to Prevent the Transition from Acute to Chronic Pain

Based on the emerging fields of endogenous opioid receptor analgesia and LS in the setting of injury, the overarching ***long-term therapeutic goal*** of future research is to alleviate chronic pain by either (a) facilitating endogenous opioid analgesia, thus restricting LS within a state of remission, or (b) extinguishing LS altogether. If LS is responsible for priming individuals to develop chronic pain, then clinically efficacious and novel treatments may stem from preventing its formation. Specifically, targeting the upregulation and/or signaling components of spinal AC1 may prove most beneficial. Treatments should be targeted to knockdown of AC1 (RNAi or other gene silencing technologies) or pharmacological inhibition of AC1 in order to erase persistent sensitization, while retaining the benefits of endogenous constitutive opioid signaling.

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Anatomical and Physiological Factors Contributing to Chronic Muscle Pain

Nicholas S. Gregory and Kathleen A. Sluka

Abstract Chronic muscle pain remains a significant source of suffering and disability despite the adoption of pharmacologic and physical therapies. Muscle pain is mediated by free nerve endings distributed through the muscle along arteries. These nerves project to the superficial dorsal horn and are transmitted primarily through the spinothalamic tract to several cortical and subcortical structures, some of which are more active during the processing of muscle pain than other painful conditions. Mechanical forces, ischemia, and inflammation are the primary stimuli for muscle pain, which is reflected in the array of peripheral receptors contributing to muscle pain-ASIC, P2X, and TRP channels. Sensitization of peripheral receptors and of central pain processing structures are both critical for the development and maintenance of chronic muscle pain. Further, variations in peripheral receptors and central structures contribute to the significantly greater prevalence of chronic muscle pain in females.

Keywords Pain · Musculoskeletal · Ion channel · Nociceptor · Hyperalgesia · ASIC · P2X · TRPV1 · Sex · Muscle

Contents

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1 Introduction

Chronic muscle pain affects between 11–24 % of the world's population with the majority of people experiencing musculoskeletal pain at some time in their life (Cimmino et al. 2011). Older, sedentary, unemployed, less well-educated individuals with anxiety are more likely to suffer from chronic muscle pain (Ahacic and Kåreholt 2010; Azevedo et al. 2012). Those who suffer from chronic muscle pain often report decreased productivity and a significant portion have had to change jobs or quit working entirely as a result of their pain (Miranda et al. 2010). In the U.S. alone, all forms of chronic pain are estimated to incur an economic burden of $500 billion annually.

The two primary diseases associated with chronic muscle pain are myofascial pain syndrome (MPS) and fibromyalgia (FM). Myofascial pain syndrome is characterized by regional muscle pain with areas of focal tenderness to mechanical pressure. These trigger points are palpable, taut masses typically found within the muscle belly. Affected muscles are stiff and contracted, which can put stresses on adjacent or antagonist muscles that lead to the development of secondary trigger points. MPS is often associated with anxiety and depression (Bennett 2007; Vázquez-Delgado et al. 2009). FM is the most extreme example of chronic muscle pain. Pain is widespread and, like MPS, patients often report areas of local tenderness to palpation. Along with pain, FM patients also report fatigue, depression, insomnia, and cognitive impairment (Mease 2005; Staud 2007). The combination of these symptoms, particularly pain and fatigue, leads FM patients to report significantly more disability and poorer physical fitness than other chronic pain conditions (Verbunt et al. 2008; Valkeinen et al. 2008). However, many other conditions, such as whiplash injury, neck pain, and chronic low back pain, also have subpopulations that can be considered as chronic muscle pain that is localized to one or two regions.

Treatment of chronic muscle pain has only been partially effective. Pharmacologic approaches have shown some usefulness (Mease 2005). Nonsteroidal anti-inflammatory drugs, some antidepressants (tricyclic antidepressants and serotonin-norepinephrine reuptake inhibitors), and some antiepileptics (gabapentin, pregabalin) have some benefit for pain and mixed effects on the attendant depression, insomnia, cognitive impairment, and fatigue (Mease 2005; Bennett 2007). Nonpharmacological approaches show some effectiveness in both MPS and FM. MPS patients benefit from gentle stretching of muscles, from massage of

trigger points to reduce stiffness, from exercises to improve strength and endurance, and from ergonomic adjustments to reduce muscle overuse (Bennett 2007; Thompson 2012). In FM, regular moderate exercise improves pain, fatigue, mood, and loss of sleep, though exercise can acutely exacerbate pain in patients that are unaccustomed to exercise (Staud 2007; Thompson 2012). Chronic muscle pain, with its substantial suffering and poor treatment options, remains a significant health burden worldwide.

The current chapter will review the peripheral mechanisms involved in chronic muscle pain. We will describe the anatomy of sensory fibers and their innervation, peripheral ion channels involved in transmitting nociceptive information from muscle, animal models of muscle pain, and changes that occur in nociceptors. We will also briefly touch on central pathways and sex differences in these models.

2 Anatomy

Sensory fibers innervating the muscle are classified into four groups based on size and myelination (Lloyd and Chang 1948). Group I and II fibers are large, myelinated fibers and play a role in proprioception. Group III and IV fibers are small myelinated and unmyelinated fibers, respectively, corresponding to Aδ and C fibers of the skin. Group III and IV, but not I and II, respond to application of noxious mechanical, thermal, and chemical stimuli to the muscle, indicating that they transmit nociceptive information from the muscle (Mense 1977; Kniffki et al. 1978; Pickar et al. 1994). The endings of group III and IV afferents are distributed throughout the muscle, terminating in extrafusal fibers, intrafusal fibers, connective tissue, fat, and the adventitia of both venules and arterioles. The majority of these nerves terminate as free endings in the adventitia of blood vessels, an ideal location for sampling blood for metabolites released as byproducts of muscle contraction (Stacey 1969).

Upon leaving the muscle, nociceptive and proprioceptive muscle afferents project to different layers of the spinal cord. Group I and II afferents project to deep layers of the dorsal horn (layers III–IV), while group III and IV afferents project to superficial layers (I, II, and the edge of III) (Mense and Craig 1988; Ling et al. 2003). Nociceptive muscle afferents then project to the brain via the spinothalamic tract (Foreman et al. 1979), where they terminate in the nucleus submedius, paraventricular nucleus anterior (PVA) (Kawakita et al. 1993; Min et al. 2011), and ventral posterolateral nucleus of the thalamus (Kniffki and Mizumura 1983). Muscle pain, like cutaneous pain, activates multiple regions of the brain—including the anterior cingulate cortex, primary and secondary somatosensory cortex, dorsolateral prefrontal cortex, and the insula (Peyron et al. 2000); however, muscle pain activates regions of the brain associated with emotional processing more strongly than skin pain: bilateral amygdala, caudate, orbitofrontal cortex, hippocampus, parahippocampus, and the superior temporal pole (Cheng et al. 2011; Takahashi et al. 2011). Further, muscle pain is associated

with activation of the periaqueductal gray and rostral ventromedial medulla, which have been implicated in modifying descending inhibitory pain pathways (Keay et al. 2000; Da Silva et al. 2010a).

3 Sensory Transduction

Muscle afferents are well suited for detecting the condition of muscle tissue. While skin afferents provide detailed information about the external environment and transmit pain-related information in response to external sources of injury, muscle afferents are sensitive to the capacity of the muscle to function as a force-generating organ with intermittent periods of high metabolic demand. Muscle afferents have poor spatial resolution as compared to skin, with single mechanically sensitive fibers innervating multiple receptive fields as far as 2 cm apart (Kumazawa and Mizumura 1977). This fits with the clinical description of muscle pain as diffuse and hard to localize. Yet, muscles are equipped with mechanically sensitive fibers specialized to detect innocuous pressure, noxious pressure, and graded forces of contraction (Mense and Meyer 1985). Patients often report mild muscle pain at rest that is acutely worsened by either pressure or use of injured muscle. In addition to mechanical forces, muscle afferents appear to be attuned to byproducts released during muscle contraction or accumulated under ischemic conditions, as ischemia itself is sufficient to elicit pain (Sacchetti et al. 1980). Thermal sensitive fibers, activated by cool or warm temperatures, are also present in muscle (Hertel et al. 1976; Kumazawa and Mizumura 1977), but muscles do not reach noxious levels of heat under physiological conditions (Saltin et al. 1968; Brooks et al. 1971).

Substantial changes occur in muscle during fatigue. The fibers of the muscle themselves are placed under significant strain, which correlates with the release of interstitial ATP (Li et al. 2003; Dessem et al. 2010). As the metabolic demands of muscle contraction increase, elevated oxygen uptake for aerobic respiration stimulates the production of reactive oxygen species (Delliaux et al. 2009) until the capacity for aerobic respiration is exceeded, at which point muscle begins accumulating lactic acid (Bangsbo et al. 1993). Norepinephrine concentration is also elevated in the interstitial fluid of the muscle during exercise (Li et al. 2005). Damage to muscle tissue during use plays an important role in muscle pain, as muscle activity that results in greater tissue damage produces significantly more pain than nondamaging activity (Faulkner et al. 1993; Proske and Morgan 2001; Gibson et al. 2009; Martin et al. 2009). This damage results in recruitment of neutrophils and macrophages (Malm et al. 2000; Tidball 2005) and the release of a wide range of factors including prostaglandin E2 (Karamouzis et al. 2001; Uchida et al. 2009), bradykinin (Murase et al. 2010), serotonin (Ernberg et al. 1999; Shah et al. 2005), and nerve growth factor (NGF) (Murase et al. 2010; Urai et al. 2013). Multiple targets for these molecules have been studied both in isolated cells and whole animals.

Acid Sensing Ion Channels. Acid sensing ion channels (ASICs) are nonselective cation channels activated by increases in extracellular proton concentrations (Waldmann et al. 1997b). Four genes code for six subunit types (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, ASIC4) with differential distributions across the central and peripheral nervous systems depending on the subunit (Waldmann et al. 1997a; Lingueglia et al. 1997; Waldmann 1998; Sherwood et al. 2012). It is particularly interesting that ASICs are found on fibers terminating in the tunica adventitia of muscular arteries, an ideal location for detecting protons released during muscle contraction (Molliver et al. 2005). A functional channel is composed of three subunits. In the case of muscle afferents, ASICs are formed as heterotrimers of the ASIC1a, ASIC2, and ASIC3 subunits. In DRG innervating muscle, ASICs are activated by pH 6.8–7.0 and demonstrate a rapidly desensitizing peak current followed by a smaller sustained current (Gautam and Benson 2013). Application of acidic solutions activates group IV afferents (Hoheisel et al. 2004) and produces enhanced pain behavior in animals (Sluka et al. 2001) and pain in humans (Frey Law et al. 2008). In animal models, $ASIC3^{-/-}$ mice do not develop hyperalgesia after repeated intramuscular acidic saline injection and show reduced secondary hyperalgesia after injection with an inflammatory compound (Sluka et al. 2003; Walder et al. 2011). $ASIC1a^{-/-}$ mice, on the other hand, do develop hyperalgesia with intramuscular acidic saline injections, but show reduced primary hyperalgesia in animals with muscle inflammation (Sluka et al. 2003; Walder et al. 2010). However, downregulation of ASIC3 in adult mice prevents the development of both primary and secondary hyperalgesia after muscle inflammation (Walder et al. 2011).

A number of factors can alter the function of ASICs. Expression of ASIC3 is enhanced under ischemic conditions and inflammation (Ikeuchi et al. 2008; Deval et al. 2008; Xing et al. 2011). Further, fatiguing muscle prior to treatment with acid or inflammatory compounds results in enhanced pain behavior in animals (Yokoyama et al. 2007; Sluka and Rasmussen 2010). Electrophysiological studies have shown lactate increases the sensitivity and magnitude of currents in ASIC1a and ASIC3 (Immke and McCleskey 2001). Finally, release of ATP can enhance ASIC currents through an interaction between ASIC3 and P2X5 (Birdsong et al. 2010). Thus, ASICs critically contribute to the development of muscle hyperalgesia, as they can be activated under a number of conditions that are related to inflammation and fatigue, and their selective knockdown or antagonism significantly reduces mechanical sensitivity in conditions known to produce muscle hyperalgesia.

Ionotropic Purine Receptors. The P2X family of ligand-gated ion channels is large and plays a role in diverse functions. Notably, P2X channels have been implicated in detecting stretch in the bladder (Sun and Chai 2004; Zagorodnyuk et al. 2007) and muscle afferents (Li and Sinoway 2002), as well as detection of hypoxia in the carotid bodies (Prasad et al. 2001; Campanucci et al. 2006) and lungs (Fu et al. 2004; De Proost et al. 2009; Syed et al. 2010). P2X receptors also contribute to a wide range of pain conditions including cardiac (Zhang et al. 2008; Wang et al. 2009), visceral (Xu et al. 2008), inflammatory (Oliveira et al. 2009;

Teixeira et al. 2010; Prado et al. 2013), neuropathic (Honore et al. 2002; Wang et al. 2003), and cancer (Kaan et al. 2010; Wu et al. 2012) pain. Application of P2X receptor agonists in the gastrocnemius (Reinöhl et al. 2003; Hanna and Kaufman 2004) and masseter (Connor et al. 2005) muscles activates group III and IV muscle afferents; similar treatments in animals (Shinoda et al. 2008) and humans (Mørk et al. 2003) are painful. The specific subtype of P2X receptor contributing to these effects is unclear. P2X3 is well studied and localizes to the DRG (Chen et al. 1995) as well as to the terminals of nerve fibers innervating the tunica adventitia of muscle afferents (Molliver et al. 2005). Eccentric muscle contractions, which are associated with muscle pain, increase P2X3 expression in muscle afferents (Dessem et al. 2010). Similarly, P2X4 and P2X5 are also found in the DRG (Xiang et al. 1998; Kobayashi et al. 2005) and studies of calcium imaging in cultured DRGs show that cells responding to physiologically relevant combinations of lactate, protons, and ATP are inhibited by an array of P2X antagonists in a pattern suggesting that both P2X4 and P2X5 contribute to activation of DRG (Light et al. 2008). Further, P2X5 is capable of physically interacting with and enhancing the proton-gated current through ASIC3, which is expressed in muscle afferents (Molliver et al. 2005; Birdsong et al. 2010). The contribution of these specific P2X subtypes to muscle pain has not yet been studied in behavioral experiments. In summary, purinergic receptors are sensitive to the ATP released during muscle activity and may contribute to fatigue-related muscle pain either directly, by generating an inward current through P2X3 or P2X4, and/or by enhancing the proton-gated current via P2X5-ASIC3 interactions.

Transient Receptor Potential Channels. The Transient Receptor Potential (TRP) family of ligand-gated ion channels contributes to many sensory modalities including cool (M8), noxious cold (A1), warm (TRPV1), noxious heat (TRPV2), acidic (TRPV1), inflammatory (TRPV1), and mechanical (TRPV4) stimuli. Among the TRP channels, TRPV1 is particularly well studied and has been implicated in detection of tissue ischemia (Wang et al. 2008; Zhong and Wang 2008; Xing et al. 2008, 2009) and contributes to the response of mechanically sensitive fibers, though TRPV1 itself does not appear to mediate mechanical transduction (Spencer et al. 2008; Inoue et al. 2009). Outside the muscle, TRPV1 also plays a role in a wide range of pain conditions, including neuropathic (Nakao et al. 2012; Wu et al. 2013), visceral (Sakurai et al. 2008; Matsumoto et al. 2012), skin (Barabas and Stucky 2013), and orofacial (Shinoda et al. 2011) pain. Within muscle, TRPV1 is important for mediating the exercise pressor reflex (Li et al. 2004; Kindig et al. 2005; Gao et al. 2007). TRPV1 is expressed on muscle cells themselves and contributes to adaptive responses to exercise, such has muscle hypertrophy (Ito et al. 2013) and improved endurance (Luo et al. 2012). Skeletal muscle afferents also express TRPV1 (Connor et al. 2005; Molliver et al. 2005; Tsukagoshi et al. 2006) and are excited by TRPV1 activation (Light et al. 2008; Jankowski et al. 2013). TRPV1 is activated by many diverse ligands including naturally occurring vanilloids (e.g., capsaicin, capisate, piperine), endogenous lipids (e.g., oxidized lineoleic acid metabolites, anandamide, N-oleoyldopamine, and N-arachidonoyldopamine) (Calixto et al. 2005; Green et al. 2013),

inflammatory mediators (Messeguer et al. 2006), protons (Tominaga and Tominaga 2005), and purine triphosphates (Lishko et al. 2007). TRP channels, however, have not been well studied in muscle pain. Administration of either TRPV1 or TRPA1 agonists produces mechanical hyperalgesia in animals (Ro et al. 2009). In animal models of muscle pain, antagonism of TRPV1 prevents mechanical hyperalgesia caused by eccentric contractions, but not by intramuscular injection of the inflammatory agent carrageenan (Fujii et al. 2008). Studies in knockout animals confirm the contribution of TRPV1 to the mechanical hyperalgesia caused by lengthening muscle contractions and also suggest that TRPV4 plays an important role in the generation of hyperalgesia (Ota et al. 2013). TRPV1 knockout mice also show decreased thermal hyperalgesia, but not mechanical hyperalgesia, in the carrageen muscle inflammation model (Walder et al. 2012). Thus, several members of the TRP family contribute to muscle hyperalgesia, likely by responding to distinct stimuli produced by tissue damage, inflammation, and muscle activity.

4 Models of Muscle Pain

Muscle pain has been studied using multiple approaches, both in terms of measuring and inducing pain. Muscle pain is commonly studied in the gastrocnemius (Hoheisel et al. 1993; Sluka et al. 2001) and masseter (Cairns et al. 2001; Dessem et al. 2010) muscles. Assessment of muscle pain is typically done by applying force-sensitive instruments to the muscle belly and observing the threshold at which a nocifensive behavior is triggered (Tillu et al. 2007; Khasar et al. 2009; Nasu et al. 2010).

Induction of muscle pain can be grouped into three broad categories—methods that rely on application of noxious compounds to muscle tissue, methods that use muscle activity, and methods that use stressful conditions to produce pain. Noxious substances including, but not limited to, acidic saline (Sluka et al. 2001), hypertonic saline (Ro et al. 2007), carrageenan (Diehl et al. 1988; Radhakrishnan et al. 2003), mustard oil (Han et al. 2008), complete freund's adjuvant (CFA) (Ambalavanar et al. 2007; Chacur et al. 2009), and PGE2 combined with carrageenan (Dina et al. 2008, 2011) are commonly used to produce muscle hypersensitivity. Of these, repeated acidic saline (pH 4 in 0.9 % saline) (Sluka et al. 2001), CFA (Ambalavanar et al. 2007; Chacur et al. 2009), carrageenan (3 % in 0.9 % saline) (Diehl et al. 1988; Radhakrishnan et al. 2003), and PGE2 (Dina et al. 2008, 2011) injections produce long-lasting enhanced pain behavior in animals. Notably, in each of these paradigms, the enhanced pain behaviors last substantially longer than inflammatory or histological changes in the affected muscle. Further, both the repeated acidic saline injections and single injection of carrageenan produced widespread muscle hypersensitivity in the untreated contralateral muscle, indicating that these methods of pain induction may recruit central mechanisms of sensitization (Coderre and Melzack 1985, 1987).

Fatiguing exercise has good face validity for the induction of muscle pain as many painful muscle conditions either result from damage during use (Gibson et al. 2009) or demonstrate exacerbation of pain with activity (Staud 2007). Eccentric contractions, in which a muscle lengthens during contraction, produce measurable tissue damage and inflammation and are associated with delayed onset muscle soreness (DOMS) (Proske and Morgan 2001). Animal models consist of stimulating muscle contractions with electrical pulses while applying a stronger mechanical force to the limb in the opposite direction, thus forcing the muscle to lengthen (Taguchi et al. 2005). This reflects the real-life condition of being handed an object and, finding that it is too heavy, easing the object to the ground. In this sense, eccentric contractions may serve as a model for muscle overuse or high intensity effort, which often results in temporary soreness. Another approach uses muscle activity to enhance muscle hypersensitivity to low-threshold insults. In this method, a subthreshold stimulus like 0.03 % carrageenan or pH 5 saline is injected into the muscle in combination with an exercise task. No changes in pain behavior are observed when these are given alone, but when combined with muscle activity, animals show robust, long-lasting and widespread mechanical hypersensitivity (Yokoyama et al. 2007; Sluka and Rasmussen 2010; Gregory et al. 2013). Further, a prior inflammatory stimulus, such as carrageenan, can prime the muscle to respond in an exaggerated way to a subsequent stimulus, such as PGE2 (Aley et al. 2000; Parada et al. 2003; Dina et al. 2008). Thus, multiple muscle insults can be combined to produce a long-lasting and widespread hyperalgesia that persists despite minimal tissue damage.

Finally, stress has been used to induce widespread muscle pain. Diseases of chronic muscle pain, including FM and MPS, are associated with anxiety and disturbances in stress response (Staud 2002; Martinez-Lavin 2007). Common methods for inducing stress are exposure to water (Green et al. 2011), unexpected blasts of noise (Strausbaugh et al. 2003; Khasar et al. 2005, 2009), and cooling of ambient temperature (Nasu et al. 2010). These approaches produce long-lasting mechanical hypersensitivity in the muscle and exaggerated responses to subsequent mild muscle insults (Alvarez et al. 2013).

5 Chronic Pain Mechanisms

Chronic pain has been defined in two ways—the duration of the pain and the underlying mechanisms for the pain. In the first case, any pain that exceeds some duration can be chronic pain and no assertions about the mechanisms of that pain are made. Levine and others suggest, however, that long-lasting (chronic) pain can be broken down into two types. The first type is the result of a repeated or persistent acute injury. When the source of acute pain is removed, the pain rapidly subsides. The second type may be initiated by some acute injury, but the pain continues even after the injury is resolved because of plastic changes in the

nociceptive system (Reichling et al. 2013). This second type of long-lasting pain includes diseases like fibromyalgia and is the most difficult to treat.

Peripheral Mechanisms. Peripheral sensitization involves changes in the function of the afferent terminals of nociceptive neurons. These changes can occur at the level of the receptor, intracellular signaling, or excitability of the cell. Likely, peripheral sensitization involves a synergistic combination of all three. Peripheral sensitization can occur after either inflammatory or noninflammatory events.

Prostaglandins are released in settings of tissue damage and can result in long-lasting muscle pain. In naive mice, application of PGE2 results in a short-lasting, acute pain; however, after treatment with carrageenan, PGE2 triggers a long-lasting mechanical hypersensitivity. The shorter-duration pain in naive animals is mediated by activation of protein kinase A by adenylyl cyclase and subsequent cAMP production (Reichling and Levine 2009). The longer-duration pain in carrageenan-treated animals, on the other hand, appears to be mediated by a change from Gs to Gi/o signaling downstream of PGE2 that requires protein kinase C epsilon (PKCε) (Khasar et al. 2008). In a process that requires the EP1 receptor, recruitment of PKCε leads to the sensitization of TRPV1 by activating phospholipase C and subsequently reducing concentrations of phosphatidylinositol 4,5-bisphosphate (PIP2), a phospholipid that normally inhibits TRPV1 function (Moriyama et al. 2005). PGE2 binding to another receptor, EP3, results in activation of protein kinase A (PKA) which in turn phosphorylates members of the P2X receptor family, among other targets. This enhances their response to ATP (Wang et al. 2007). Further, PGE2 application can enhance the response to acidic solutions (Rukwied et al. 2007).

Bradykinin is also released during inflammation and results in mechanical hypersensitivity (Murase et al. 2010). Bradykinin activates intracellular signaling pathways by binding to the B1 and B2 receptors. Several mitogen activated protein kinases (MAPKs) are activated by bradykinin binding to either of these receptors, including p38 and JNK. Further, B1 signaling can recruit PKCε, which may result in a similar activation of TRPV1, though this has not been tested directly (Meotti et al. 2012).

NGF is also released in the muscle during exercise (Murase et al. 2010; Urai et al. 2013) and after muscle incision (Wu et al. 2009). NGF is painful in both humans (Svensson et al. 2003; Deising et al. 2012) and animals (Woolf et al. 1994; Hathway and Fitzgerald 2006). NGF binds two receptors—TrkA and $p75^{NTR}$ (Eibl et al. 2012). NGF binding to TrkA activates the tyrosine kinase domain of TrkA, which triggers pathways involving ERK1/2, Akt, and PLCγ (Chao et al. 2006). NGF-enhanced pain in eccentric muscle contractions and ischemia is dependent on TRPV1, consistent with NGF's recruitment of PLC (Xing et al. 2009; Ota et al. 2013). Further, application of NGF to muscle can strengthen the synapses of primary afferent projections to the dorsal horn, though it is unclear if this is independent from or a consequence of enhanced sensitivity of receptors at the terminal (Lewin et al. 1992).

Noninflammatory mechanisms of peripheral sensitization are less well understood. Repeated acidic saline injections result in long-lasting muscle pain and are associated with lymphoplasmacytic infiltrates (Sluka et al. 2001), though these infiltrates do not appear to be necessary for mechanical hypersensitivity (Gregory et al. 2013). ASIC3 is necessary for long-lasting mechanical hypersensitivity in muscle (Sluka et al. 2003), but the role of ASIC3 in mediating this effect is unclear. Repeated acidic saline injections reduce substance P (SP) release from peripheral terminals. SP is typically considered an algesic substance, but signaling through the NK1 receptor at the terminal activates M-type potassium channels that reduce primary afferent excitability. Thus, by inhibiting SP, repeated acidic saline injections may enhance neuronal excitability through ASIC3 (Lin et al. 2011).

In the sound stress-induced muscle pain model, epinephrine is critical for maintaining the long-lasting mechanical hyperalgesia in rats (Khasar et al. 2009). Sustained exposure to epinephrine, either from stress or exogenous sources, switches intracellular signaling via EP receptors from Gs to Gi/o, resulting in activation of PCKε (Khasar et al. 2008). Prolonged exposure to epinephrine also triggers elevated expression of IL-6 and TNFR1 (Alvarez et al. 2013).

Peripheral sensitization is an important component of chronic muscle pain. Enhanced sensitivity of peripheral receptors may account for pain sensation in response to normally nonpainful stimuli. Further, the enhanced excitability may translate into more frequent action potential firing, which may set the stage for alterations in the central nervous system that can continue to enhance muscle pain and spread it beyond the affected tissue (Staud et al. 2009).

Central Mechanisms. Central sensitization refers to changes in the brain and spinal cord that result in enhanced nociceptive processing. The processes underlying central sensitization are not well understood, but central changes may begin with enhanced peripheral input (Staud et al. 2009). Manipulation of muscle with inflammatory substances or eccentric contractions not only produces peripheral sensitization, but also results in changes in the dorsal horn that correlate with nocifensive behavior (Mebane et al. 2003; Sluka et al. 2003; Taguchi et al. 2005; Chacur et al. 2009). Noxious stimulation of the muscle results in expansion of the receptive field for dorsal horn neurons (Hoheisel and Mense 1989; Hoheisel et al. 1993; Sluka et al. 2003; Chacur et al. 2009). Further, repeated injection of acidic saline into the muscle increases concentration of glutamate and aspartate in the spinal cord (Skyba et al. 2005) and phosphorylation of the NR1 subunit of the NMDA receptor (Bement and Sluka 2007). Antagonism of either NMDA or non-NMDA type glutamate receptors reverses mechanical hyperalgesia after acidic saline injection (Skyba et al. 2002). Microglial activation in the spinal cord has been shown in an inflammatory model of muscle pain and inhibition of microglia with minocycline reverses behavioral measures of pain (Chacur et al. 2009).

In the acid-saline model, removal of afferent input does not alter the widespread hyperalgesia once fully developed; supporting the idea that hyperalgesia in this model is maintained by changes in the central nervous system (Sluka et al. 2001). However, in other models like the hyperalgesic priming model, blockade of PCKε in primary afferent fibers reversed the hyperalgesia. Thus, while nociceptive input

is clearly necessary for initiation of muscle pain, changes in peripheral nociceptors and/or changes in central pathways could maintain the hyperalgesia. Whether plasticity in peripheral or central pathways plays a predominant role may depend on the animal model and likely mimics the diversity observed in clinical populations.

Several subcortical structures have been implicated in chronic muscle pain. Descending input from the vagus nucleus modifies baseline nociceptive thresholds and the effects of bradykinin on muscle pain (Khasar et al. 2003b, c). The RVM shows increased staining of phosphorylated NR1 subunit of the NMDA receptor after repeated acidic saline injection (Da Silva et al. 2010a). The hyperalgesia caused by repeated acidic saline injections is absent in animals lacking the NR1 subunit and is reversed by injection of NMDA receptor antagonists or ropivicaine into the RVM (Tillu et al. 2007; Da Silva et al. 2010a, b; Sluka et al. 2012). Further, acidic saline injection into the muscle increased staining of phosphorylated ERK in the central nucleus of the amygdala, piriform cortex, paraventricular hypothalamic nucleus, and anterior nucleus of the paraventricular thalamus, and the intensity of the pERK signal correlated with muscle pain. Interestingly, inhibition of ERK in the anterior nucleus of the paraventricular thalamus was sufficient to prevent long-lasting muscle pain (Chen et al. 2010; Min et al. 2011).

The contribution of cortical structures to chronic muscle pain is less well understood. A functional MRI study found that when female fibromyalgia patients were given an intramuscular solution of protons and PGE2, more regions of the cortex—specifically, the left anterior insula ipsilateral to treatment—were activated as compared to healthy controls (Diers et al. 2011). A structural analysis of the brains of female fibromyalgia patients found a greater striatal gray matter volume in fibromyalgia patients as compared to healthy controls (Schmidt-Wilcke et al. 2007). The significance of these patterns is unclear. More work must be done to understand potential contributions of cortical structures to chronic muscle pain.

6 Sex Differences in Chronic Muscle Pain

While substantial sex differences have been observed in diseases of chronic muscle pain, pain mechanism research has primarily focused on males. Most chronic muscle pain conditions are more common in women, with fibromyalgia affecting up to ten times more women than men, depending on the diagnostic criteria. In muscle, a study of eccentric exercise in humans found no significant sex differences in the levels of myoglobin, TNFα, IL1β, and nitric oxide, suggesting that the muscles of males and females are equally prone to damage under strenuous conditions (Dannecker et al. 2012). Instead of at the muscle, sex differences in muscle pain appear to be mediated by variations in the sensitivity of peripheral terminals and processing in central structures. Peripheral injection of NMDA into the masseter muscle evoked greater and longer-lasting pain in human females (Cairns et al. 2001; Castrillon et al. 2012). In animals, this same treatment evoked

more intense nociceptor firing (Cairns et al. 2001). This NMDA effect is modulated by peripheral treatment with estrogen, suggesting that estrogen modifies NMDA receptors, either directly or indirectly. A similar effect has been found in the epinephrine-induced muscle pain model, with greater mechanical sensitivity observed in female rats. Estrogen contributes to sex differences by enhancing signaling through PKCε and PKA in nociceptive neurons (Dina et al. 2001; Hucho et al. 2006). Thus, circulating or local estrogen may contribute to sex differences in pain by directly modifying the sensitivity of nociceptors through multiple mechanisms.

Sexual dimorphism in the central processing of pain has not been well studied. However, central processing may be an important link in the development of chronic, widespread muscle pain. Specifically, low-intensity muscle insults (local fatigue and muscle insult), separated by time or distance, produce hyperalgesia that is greater, longer-lasting, and more widespread in female mice when compared to male mice. These data suggest sex differences in the way stimuli are processed centrally may increase the risk for developing widespread pain in female mice. In this model, sex differences are explained neither by changes in the RVM, an area of the brainstem implicated in exercise-enhanced pain, nor by circulating estrogen (Tillu et al. 2007; Da Silva et al. 2010a; Gregory et al. 2013). In contrast, whole-body-fatigue-induced pain results in enhanced hyperalgesia in female mice that is dependent on circulating estrogen (Sluka and Rasmussen 2010). Thus, different models may show different mechanisms underlying sexual dimorphism. Greater excitability of dorsal horn neurons in female rats may contribute to such sex differences (Ji et al. 2012). Further, sexual dimorphism in the autonomic system may also contribute, as females differ substantially in their response to vagotomy and medullectomy in both bradykinin- and epinephrine-evoked muscle pain (Khasar et al. 2003c). Although the source of this dimorphism remains unclear, gonadectomy of juvenile animals does not reverse this effect (Khasar et al. 2003a). Whether sex differences in the exercise- or epinephrine-enhanced pain are due to organizational effects of sex hormones during development, or some other mechanism, requires further examination (Greenspan et al. 2007).

7 Conclusion

Chronic muscle pain remains an important, but poorly understood clinical issue. Muscle pain is mediated by nerve endings specialized for the detection of ischemia, mechanical forces, and tissue damage within muscle tissue. These peripheral nerves transmit nociceptive information to anatomically distinct regions of the spinal cord and brain, which may contribute to the substantial differences in the experience of muscle pain as compared to skin pain. Further, activation of these distinct regions may contribute to the affective symptoms seen in some diseases associated with chronic muscle pain. The mechanisms underlying the development and maintenance of chronic muscle pain are still poorly understood. Figure 1 shows a schematic diagram underlying the multiple factors that can contribute to

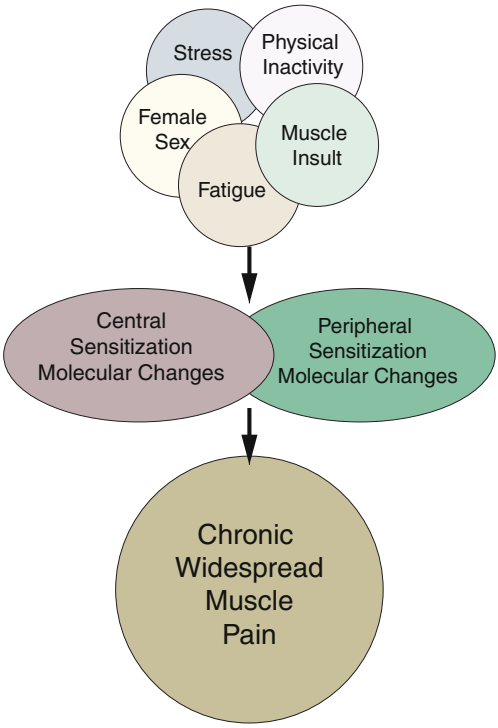

Fig. 1 Schematic diagram outline factors that contribute to the development of widespread and long-lasting muscle pain. We propose that combination of multiple stressors result in peripheral and/or central sensitization to produce widespread and long-lasting muscle pain. These stressors can include multiple muscle insults separated in time and space, or they include physical inactivity combine with muscle insult, fatigue combined with muscle insult, or stress combined with muscle insult. Further sex influences the development of chronic muscle pain with females more likely to develop chronic muscle pain. Molecular and cellular changes can occur anywhere along the nociceptive pathways from the nociceptor to the cortical neuron and are likely involved in driving the chronic muscle pain and hyperalgesia. Lastly, the peripheral and central pathways can interact with each other to result in enhanced chronic pain

the development of chronic muscle pain. Development of chronic muscle pain is influenced not only by muscle insults, but also by activity level, stress, fatigue, and sex. These factors can result in molecular and cellular changes in both nociceptors and in central neurons that result in sensitization to maintain the pain. Furthermore, peripheral and central pathways can interact to further enhance the observed molecular and cellular changes and enhance pain. Thus, chronic muscle pain involves peripheral modification of receptors as well as alterations of nociceptive neurons and central processing. Sex differences are similarly not well understood. While sex hormones in the adult may contribute to some of the difference, they clearly are not the only factor. Further studies are needed to better understand both the transition to chronic pain and the sex differences in chronic muscle pain.

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Acute and Chronic Pain in Children

Gareth J. Hathway

Abstract Pain in neonates and children differs to that in adults. One of the many challenges associated with the diagnosis and management of pain in early life is that neonates are non-verbal and therefore incapable of communicating their pain effectively to their caregivers. Early life pain is characterised by lowered thermal and mechanical thresholds, and exaggerated and often inappropriate behavioural reactions to pain. These differing behavioural reactions are underpinned by increased excitability/decreased inhibition within the spinal dorsal horn. This itself is the result of immaturity in the anatomical expression of key neurotransmitters and neuromodulators within spinal pain circuits, as well as decreased inhibitory input to these circuits from brainstem centres, and an immature relationship between neuronal and non-neuronal cells which affects pain response. These differences between early and adult pain impact upon not just acute reactions to pain, but also the incidence, severity and duration of chronic pain. In this chapter, chronic pain in childhood is discussed, as are the structural and functional differences that underpin differences in acute pain processing between adults and children. The ability of pain that occurs in early life to alter life-long pain responding is also addressed.

Keywords Neonate · Inflammation · Microglia · Rostroventral medulla · Periaqueductal grey · Neuropathic · Spinal cord · Dorsal horn · GABA · Glycine · Life-long pain · Descending control

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1 Introduction

Neonates respond to their environment differently to adults, and this is particularly true when it comes to the detection and interpretation of somatosensory information. Children react to painful stimuli in an exaggerated manner. The magnitude of the stimulus necessary for it to be interpreted as painful is significantly lower than is the case in adults, and the behavioural responses that such stimuli provoke are larger, uncoordinated and often inappropriate (Fitzgerald 2005). Pain is subserved by complex networks of specialised neuronal and non-neuronal cells within the central and peripheral nervous systems which react to external and internal stimuli. Those stimuli determined to be potentially or actually capable of damaging somatic tissue will provoke a behavioural response. This normally well-adapted neuronal system is not hard-wired at birth: it is adaptable and subject to manipulation either by injury, drugs or disease (Fitzgerald 2005). The way in which we process pain as adults is shaped by our experiences early in life, and early life pain can significantly alter somatosensory processing in adulthood. In this chapter, the many facets of pain processing that are different in early life and the ways these impact upon integrated physiological responses to noxious insult will be discussed. The chapter will also examine how pain in early life modulates the maturation of the CNS and leads to altered pain-responding in adults.

2 Peripheral Nervous System

Pain is usually triggered by damage to tissue and it is therefore necessary to discuss the development of the primary sensory neurons that innervate tissue. These cells sense our physical environment and are specialised to detect either innocuous or noxious stimuli. Primary afferent sensory neurons have cell bodies

that lie within the dorsal root ganglia (DRG). The DRG are accumulations of neuronal cell bodies and supporting cells that lie in a paravertebral chain parallel to the spine. Primary afferent cells have both central and peripheral terminals innervating the spinal dorsal horn (SDH) and the target tissue respectively. These sensory fibres are broadly classified as either A- or C-type. A-type primary afferent fibres are further divided into the Aα, Aβ or Aδ subtypes. Of particular relevance to the processing of somatosensory information are Aβ fibres. These large diameter fibres are thickly myelinated and usually convey non-noxious tactile information to the dorsal horn (DH) of the spinal cord. Aδ fibres have smaller diameters and are more thinly myelinated than Aβ fibres, as demonstrated by a decreased action potential conduction velocity (see Fitzgerald 2005). These fibres convey both innocuous and noxious information and thus can be classified as nociceptors. Further sub-divisions of Aδ fibres are made by some authors based on thermal activation thresholds (Meyer et al. 2006). C-fibres are true nociceptors, that is, cells which are specialised to detect noxious stimuli, although they are also implicated in non-nociceptive sensations such as itch (Jennings and Fitzgerald 1996). C-fibres can be further sub-divided into peptidergic and non-peptidergic based on the expression of the neurotransmitters Substance P and Calcitonin-Gene Related Peptide (CGRP).

Primary afferent fibres are derived from neural crest cells in the dorsal part of the neural tube (Woolf and Ma 2007). Both A and C types of neurons are specified at an early stage of embryonic neurodevelopment, with this process being controlled by a family of specific transcription factors, particularly nerve growth factor (NGF) and glial cell-line derived neurotrophic factor (GDNF) (Fitzgerald 2005). In rodents, all sensory neurons are born in two "waves" covering a period from embryonic day 12–15, with the C cells being born in the second wave and with future peptidergic C cells being born prior to the non-peptidergic cells (Hall et al. 1997; Jackman and Fitzgerald 2000). Both central and peripheral processes are immediately sent out from all cell types, with C cells lagging behind A cells in this process. Once these processes reach their target destination, connections become functional and are capable of conveying nociceptive information.

Sensory neurogenesis is under the control of key genes and also, perhaps fundamentally, is regulated by access to neurotrophic factors. The key neurotrophic factor involved in this process is NGF. This neurotrophin not only determines the fate of nociceptors but also governs cell survival in the embryonic period (Davies 2000; Fitzgerald 2005) and its influence persists postnatally. NGF determines the physiological properties of nociceptors (Ritter et al. 1991; Lewin and Mendell 1994) and influences whether C-fibres express CGRP and Substance P (Davies 2000; Hall et al. 2001). Fundamentally, NGF regulates the density with which sensory neurons innervate their distal target, with increased NGF concentration leading to hyperinnervation of target tissue (Davis et al. 1997). NGF also regulates the outgrowth of axons (Markus et al. 2002).

The expression of the TrkA receptor which mediates the actions of NGF is down-regulated in a subset of C-fibres, with these neurons becoming increasingly dependent on GDNF which mediates its actions via the Ret receptor (Bennett et al.

1996; Molliver et al. 1997). These two subsets of nociceptors go on to become peptidergic (TrkA positive) and non-peptidergic subtypes of C-fibres.

The central terminals of C-fibres enter the DH just before birth in the rat and they terminate in a precise and somatotopic pattern within laminae I and II. Within the DH, non-peptidergic terminals can be detected prenatally, their density increasing over the first 10 postnatal days. Peptidergic fibres enter the DH later (Braz et al. 2005). The terminals of these immature C-fibres are functional at birth, but neurotransmitter release, and therefore the functional implications of activating them, are different. Neurotransmitter release from primary afferent terminals and the ability of primary afferent depolarisation to evoke changes in excitability of intrinsic DH neurons that they form synaptic contact with are weak and do not mature until the end of the second postnatal week. This contrasts with the ability of A-fibres which, when stimulated prenatally, evoke robust action potentials in DH neurons (Fitzgerald and Jennings 1999; Baccei et al. 2003). Although functionally mature, the central termination pattern of Aβ fibres within the DH is very different in the early postnatal period. In the adult these fibres terminate in lamina V; however, early in life, they also share a termination with C-fibres in the superficial DH (laminae I-II). From E15, Aβ fibres penetrate the DH in a somatotopic manner (Mirnics and Koerber 1995a, b) and make synaptic contact with DH neurons (Coggeshall et al. 1996; Fitzgerald et al. 1994). These superficial Aβ terminals slowly withdraw over the first three postnatal weeks (Fitzgerald et al. 1994), with this process being dependent upon activity within the neurons (Beggs et al. 2002). The functional implications of this immature Aβ termination is that the low threshold fibres are able to access DH neurons within laminae I and II that process noxious information and thereby, stimuli of non-noxious intensity may be processed in the same way as noxious inputs. However, the extent and significance of this is subject to debate (Woodbury and Koerber 2003), yet it is known that Aβ stimulation at P3 increases c-Fos (an immediate early gene indicative of neuronal activity) expression in laminae I and II but not at P21 (Jennings and Fitzgerald 1996), with sensitisation of DH neurons to subsequent stimulation (Jennings and Fitzgerald 1998). Moreover, poly-synaptic Aβ inputs to neonatal GABAergic neurons are found in the DH in early life (Daniele and MacDermott 2009).

3 Spinal Dorsal Horn

Reflex behaviours in young animals and humans are exaggerated compared to mature subjects. Although there are differences in the primary afferent innervation of the DH in early life, there are also differences in the intrinsic properties of the DH. Generally, this is thought to reflect a lack of inhibitory neurotransmission within the DH leading to increased excitability of spinal pain circuits and more subtle changes in the expression of receptors for excitatory neurotransmitters (Fitzgerald 2005). GABA and glycine are the main inhibitory neurotransmitters within the DH and are released from interneurons as well as descending

projections from the brainstem (Kato et al. 2006). Within the most superficial laminae of the DH (SDH) the number of GABAergic neurons peaks within the first two postnatal weeks (Dougherty et al. 2009; Ma et al. 1992; Schaffner et al. 1993). Glycinergic neurons are a subset of GABAergic neurons throughout the embryonic and postnatal periods (Todd and Sullivan 1990) with the two neurotransmitters being stored and released from the same synaptic vesicles (Jonas et al. 1998). Glycinergic mini-IPSCs are absent within the SDH until the second postnatal week, yet robust responses to exogenously applied glycine can be evoked (Baccei and Fitzgerald 2004). This indicates that although functional receptors may be present, glycinergic neurons are absent until this developmental timepoint (Baccei and Fitzgerald 2004). From P8, mixed GABA and glycinergic mIPSCs can be detected in lamina I-II, but by P21 this is replaced by pure glycine receptor-mediated mIPSCs within lamina I and largely GABAergic potentials within lamina II (Baccei and Fitzgerald 2004).

This developmental reorganisation of inhibitory signalling in the DH leads to a net increase in DH excitability and to younger subjects displaying reflex behaviours which lack coordination. Reflexes, once initiated, may continue for a protracted period even after the stimulus has ceased (Ekholm 1967; Fitzgerald et al. 1988). Lower mechanical and thermal thresholds to evoke activity within intrinsic DH neurons are observed in early life compared with adulthood (Fitzgerald 2005). Moreover, receptive fields for limb withdrawal are larger in neonates than in adults, with stimuli evoking a limb withdrawal that is not always appropriate for the location of the stimulus (Holmberg and Schouenborg 1996a, b).

This inherent increase in spinal excitability as a result of this immature inhibitory signalling in early life is attributable to underlying immaturity in the SDH and the way in which sensory information is processed. In early life, there is likely a lack of fine-tuning of sensory information, which itself is caused by a lack of synaptic inhibition within spinal cord pain circuits. This is exacerbated by the presence of A-fibre terminals within the superficial DH (laminae I and II) of neonates that further increase excitability. This A-fibre induced excitation has a critical role in the maturation of nociceptive circuits. Newborn animals have a high error rate in the direction of reflex withdrawals made in response to noxious stimuli. Neonates are just as likely to move the affected limb toward the stimulus as they are to move away. This aberrant behavioural response matures over the first 3 postnatal weeks (Waldenstrom et al. 2003). However, if low intensity innocuous inputs to the limb or tail are blocked with local anaesthetics, then this postnatal tuning is prevented and inappropriate behaviours remain. Postnatal tuning of nociceptive circuits depends upon the development of appropriate excitatory and inhibitory signalling within the DH, and the expression of neurotransmitters and their receptors changes throughout the postnatal period.

4 Descending Control of Spinal Excitability

Descending inputs from the brainstem control nociceptive transmission at the DH (Ren and Dubner 2002; Pitkanen et al. 1998; Gebhart 2004; Tillu et al. 2007; Bee and Dickenson 2008; Zhang et al. 2009; Basbaum and Fields 1984; Fields and Basbaum 1978; Fields and Heinricher 1989; Hellman and Mason 2012; Hathway et al. 2009a, 2012). Rodent brainstem nuclei differentiate between E11 and E16, and become identifiable by E18 (Altman and Bayer 1984). Axons from these sites project to the spinal cord well before birth (Cabana and Martin 1984; Leong 1983; Fitzgerald 1987), but the point at which these axons and their collaterals establish synaptic contact with target cells is currently unknown.

For some time it was thought that supraspinal sites played very little, if any, role in controlling spinal excitability in early life (Fitzgerald and Koltzenburg 1986; van Praag and Frenk 1991). Electrical stimulation of the dorsolateral funiculus (DLF), the spinal tract which conveys descending axons from the brainstem, fails to inhibit Descending cell firing in response to C-fibre activation before P14 (Fitzgerald and Koltzenburg 1986). By the end of the third postnatal week, adult-like responses can be seen. The DLF conveys axons which emanate from the nuclei of the rostroventral medulla (RVM) which themselves receive dense projections from the midbrain periaqueductal grey (PAG). Similar to the effect of DLF stimulation in early life, electrical stimulation of the PAG is unable to inhibit DH cell firing in response to Aδ and C-fibre activation in the early postnatal period (van Praag and Frenk 1991). PAG-mediated inhibition only emerges from P21. Both the Fitzgerald and Koltzenburg and the van Praag and Frenk studies led to a period of reduced interest in the role of the brain in controlling spinal pain processing in early life. Neither study, however, investigated the ability of supraspinal sites to facilitate spinal pain processing. Descending fibres which emanate from the RVM are known to powerfully and bi-directionally control spinal excitability (Zhuo and Gebhart 1997, 2002). We have shown that in early life, electrically stimulating the RVM prior to P21 is unable to inhibit spinal reflex excitability. Prior to this age, electrical stimulation of the RVM over a wide range of amplitudes is only able to facilitate, that is, increase reflex size and decrease mechanical thresholds (Hathway et al. 2009a); descending inhibition is lacking. The RVM therefore contributes to the enhanced excitability seen in DH pain circuits in the neonatal period and plays an important role in subsequent responses to noxious stimulation in later life. The maturation of descending pain control systems is dependent upon the action of endogenous opioid peptides during a critical period in the fourth postnatal week (Hathway et al. 2012), and pharmacological interventions which influence the opioidergic systems within the CNS before this developmental epoch can significantly affect the maturational profile of descending control systems (Hathway et al. 2012). The relevance of these sites to adult pain processing is considerable in terms of both normal and pathological functioning.

5 Long-Term Pain in Early Life

This article has so far discussed acute nociception in early life which in turn has direct relevance to longer-term, what might be classified as chronic, pain states in the neonatal and preadolescent period. The remainder of this chapter will summarise the effects of chronic pain with an origin in disease, and long-term pain as a result of surgical interventions, on the organism in early life and the long-term consequences on the processing of noxious information throughout life.

Within neonatal intensive care units in developed countries, it has become standard procedure to attempt to resuscitate and offer long-term perinatal care for infants born prematurely, even as young as 24 weeks post-conception. This policy has been justified with increasing survival rates for these neonates (Dani et al. 2009). However, the prematurity of these children frequently results in surgical interventions being required to correct life-threatening defects. Prematurity itself can have longer term effects upon cognitive and behavioural development (Bhutta et al. 2002; Marlow et al. 2005). Early neonatal care is characterised by painful tissue damaging stimulation, such as repeated heel prick incisions to collect blood necessary for on-going care and which can be required for months. These procedures create an adverse sensory environment in which the neonatal nervous system is developing (see above) and there is increasing evidence that this can lead to long-term, subtle changes in sensory processing later in life (Johnson et al. 2009).

The increasing evidence that exposure to pain in early infancy leads to long-term changes in pain sensitivity in later life (Fitzgerald and Walker 2009) led researchers to hypothesise that the neonatal intensive care experience alters the development of central pain pathways and changes the neuronal responses to noxious stimulation in the infant brain. It is plausible that by the time a premature infant reaches term-age their early life experience may alter the central nervous system processing of noxious events. There has been significant research into both the acute and long-term effects of neonatal injury and in the next section I will discuss the impact of neonatal injury upon adult pain processing.

6 Long-Term Effects of Inflammatory Pain in Early Life

The pain associated with inflammation, be that as a result of the administration of an inflammogenic molecule or as the result of physical trauma (e.g. surgical injury with resulting tissue inflammation), is probably the most commonly observed pain syndrome associated with early life. Plantar hindpaw incision is a well established post-operative pain model that is widely used in adult rodents and has also been used in neonatal animals (Brennan 2005; Brennan et al. 1996, 2005; Walker et al. 2009b; Beggs et al. 2002). This animal model has the advantage of being highly reproducible, impacting upon the same relative area of the hindpaw regardless of

the age, and therefore the size, of the animal. In this model, surgical incisions through the skin and fascia of the plantar hindpaw, along with elevation and longitudinal incisions of the plantaris muscle followed by suturing, produces behavioural mechanical hyperalgesia (Walker et al. 2009b; Ririe et al. 2003) in neonatal rodents from the age of P3 through to P17. The duration of this hyperalgesia in the neonate is shorter than that seen in the adult with the pain resolving in 48–72 h in neonates whereas it extends beyond 7 days in adults (P54) (Walker et al. 2009b). The reasons for this shorter duration are not known but may reflect aspects of the developmental processes which are taking place in the neonatal CNS. This acute, idiosyncratic hyperalgesic response to injury in neonates is not specific to surgical skin wounding. Neonatal responses to formalin, a potently painful stimulus, are more intense and last for longer than in adults (Guy and Abbott 1992; Teng and Abbott 1998), with immature rodents displaying less organised and specific pain behaviours than more mature animals. This probably reflects developmental immaturity in spinal pain pathways (see above). In adult rodents, formalin injection provokes a bi-phasic behavioural response with the first phase being relatively short-lived, reflecting peripheral sensitisation and the second, longer phase reflecting alterations in spinal excitability. The second phase is absent in neonatal rodents and this coincides with a lack of descending inhibition from brainstem nuclei (Guy and Abbott 1992; Hathway et al. 2009a). Mustard oil, which is a potent activator of C-fibres via the TRPA1 receptor (Bandell et al. 2004), produces both primary and secondary hyperalgesia in neonates and adults (Walker et al. 2007). However, in neonates, responses to mustard oil are smaller when compared to those in juvenile and adult rodents (Walker et al. 2007; Jiang and Gebhart 1998). This lack of potency in younger ages reflects an immaturity in the properties of C-fibres and the synaptic connectivity of C-fibres within the DH in younger ages (Jennings and Fitzgerald 1998).

Neonatal injuries have been experimentally and clinically associated with alterations in the response to future injury and the long-term processing of sensory information. Severe inflammation associated with the potent inflammogen Complete Freund's adjuvant (CFA, purified bacterial cell wall which produces inflammation via the Toll-like Receptor 4) injected subcutaneously in the hindpaw in the neonatal period has been associated with increased responses to further inflammatory stimuli (formalin and capsaicin) administered later in life (Hohmann et al. 2005; Ruda et al. 2000). The degree of the initial inflammation associated with injection of CFA was quite high when compared to adults; yet more moderate neonatal inflammation following the administration of a different inflammogen, carrageenan, was also able to alter sensory processing later in life. Ren et al. (2004) demonstrated elegantly that unilateral administration of carrageenan to a hindpaw between P3 and P14 caused basal global hypoalgesia in response to both mechanical and thermal stimuli. Yet when re-inflamed with the same inflammogen in the same paw, these animals exhibited an increased hyperalgesic response when compared to animals that had initially been challenged with saline in the neonatal period. These data indicate a segmental impact of neonatal inflammation that affects the development of spinal nociceptive circuits. Subsequently, Walker et al.

(2009b) suggested a critical period for the development of this enhanced hyperalgesic response following neonatal injury. Using the plantar hindpaw incision model, they demonstrated that initially injuring the hindpaw of rodents between P3 and P6 was necessary for the enhanced response to re-injury 2 weeks later. These critical periods in the development of spinal pain processing have also been previously observed with inflammatory stimuli and surgeries (Ruda et al. 2000; Wang et al. 2004; Sternberg et al. 2005).

The presence of these critical periods suggests that injury in early life triggers specific forms of synaptic plasticity that are developmentally regulated. Walker et al. (2009b), via the use of a long-term sciatic nerve block, demonstrated that primary afferent activity is required. The efficacy of primary afferent block in preventing these plastic changes indicates that abnormal or altered primary afferent activity initiates these processes. This may be because of altered innervation of target tissue by the primary afferent fibre (Moss et al. 2005). Alternatively, it could be the result of changes in the expression of specific genes which change the biophysical properties of the primary afferent membrane, for example the insertion of increased numbers of TRPV1 receptors with the result of a decreased threshold for activation (Ji et al. 2002). Additionally, it should be remembered that these critical periods exist in a timeframe when normal and significant developmental processes are taking place within the DH.

As an example of the long-term consequences of neonatal incision on the properties of key components of the nociceptive circuit, Ririe et al. (2008) showed that hindpaw incision caused a significant change in the resting membrane potential of large diameter sensory neurons that persists beyond the time in which behavioural sensitivity is displayed. This change in resting membrane potential made these neurons more excitable and therefore potentially more able to contribute to changes in synaptic efficacy within the DH. This is but one example of a biophysical consequence of early life injury which has the potential to have long-term consequences. More recently, Beggs et al. (2012) showed that early life injury "primes" non-neuronal cells, namely microglia. Microglia are key cells within the CNS that contribute to the initiation of chronic pain states in animal models (Beggs and Salter 2010; Tsuda et al. 2005; Ji and Suter 2007). Previously, we have shown that microglial responses to pain differ with age (Moss et al. 2007; Hathway et al. 2009b) and Beggs and colleagues have shown that neonatal skin incision enhances the spatial distribution of microglia and alters their morphology. Furthermore, inhibition of microglia with minocycline prevented re-injury induced hyperalgesia. Therefore, the long-term changes in the maturation of the neuronal components of the pain pathway, at least within the DH, are subject to modification by non-neuronal cells and these interactions have the capacity to alter sensory functioning throughout life. Additionally, increased nociceptor activity drove these changes in the non-neuronal cell phenotype. A feedback loop is therefore established whereby increased nociceptive input to the DH via nociceptors induces changes in non-neuronal cell phenotype, which itself can alter sensory afferent properties and intrinsic DH cell responses to subsequent stimulation with an increase in DH excitation.

7 Neuropathic Pain in Early Life and the Consequences of Neonatal Neuropathic Injury on Long-Term Nociception

In adults, chronic neuropathic pain is a significant cause of long-term disability, which is often difficult to both diagnose and treat, being refractory to many currently prescribed analgesics (Dworkin 2002; Harden and Cohen 2003). In neonates and children, neuropathic pain is very rare and it is more often diagnosed in adolescents (Chalkiadis 2001). As an example, obstetric complications during birth can result in avulsion of the brachial plexus. This injury in adults is extremely painful (Berman et al. 1998; Parry 1984), however in neonates it rarely results in pain (Anand and Birch 2002). This lack of neuropathic pain in children does not present a clinical challenge since it is desirable; however, understanding its neurobiological basis is important for the treatment of chronic pain in adults. The first comprehensive study of neuropathic pain in the neonatal period came from Howard et al. (2005) who demonstrated that neither spinal nerve ligation nor chronic constriction injury, both widely utilised experimental models of neuropathic pain, were able to evoke behavioural hypersensitivity in young rodents. Neuropathic pain was not recognisable until 33 days of age, a stage reflective of adolescence. This observation, whilst notable, lacked any mechanistic basis. It was not until studies were performed that were inspired by advances within adult pain neurobiology that a more mechanistic understanding of the lack of neuropathic pain in neonates and juveniles could be proposed.

Since the late 1990s it has increasingly become appreciated that chronic pain syndromes can be partially attributed to interactions of neurons with non-neuronal cells, as has been mentioned above. Principally, spinal microglial cells are known to be central to the pathogenesis of neuropathic pain (Colburn et al. 1997; Stuesse et al. 2000; Coull et al. 2005; Tsuda et al. 2005; Zhuang et al. 2005). Work emanating from the laboratory of Professor Maria Fitzgerald at University College, London, focussed on the lack of neuropathic pain in neonates by investigating the involvement of non-neuronal, neuroimmune cells in the maturing spinal cord. Moss et al. (2007) demonstrated that although both the glutamate receptor agonist NMDA or the inflammogen lipopolysaccharide could activate resident microglia within the spinal cord of 10-day old rodents, spared nerve injury could not. Furthermore, the intrathecal injection of cultured and ATP-activated (pro-inflammatory) microglia into the spinal cord of this age group of rats failed to provoke a mechanical allodynia which is seen when the same manipulation is attempted in adults. This study therefore highlighted substantial differences in the way in which neurons and microglia communicate in the neonatal DH. Subsequently, this group illustrated that it is not the neuropathic *injury* per se that is required for microglial activation. A further study (Hathway et al. 2009b) sought to address what factor(s) were required for microglial activation in both adult and neonatal DH. This study found that electrical stimulation of primary afferent fibres at sufficient intensity to activate C-fibres was able to activate microglia in adults in

the absence of injury to the sensory neurons and establish a state of central sensitisation in these animals as indexed by a significant mechanical allodynia that persisted for 48 h. The same stimulation protocol applied to P10 rats, however, failed to evoke alterations in the activation state of the microglia. Adult C-fibre activation was accompanied by DH increases in IL-6 and Mcp-1 at 3 h and Mmp3, CSF-1 and CD163 at 24 and 48 h following stimulation whereas levels of these pro-inflammatory molecules were unaffected in neonates. A further study by Costigan et al. (2009) compared gene expression patterns in the DH of adult and neonatal rodents following spared nerve injury. This study showed 148 differentially regulated genes in adult but not young rodents that are known to be involved in microglial and T-cell biology. This paper was the first illustration of a role for T-lymphocytes in pain processing and it clearly demonstrated an increased infiltration of the DH by these normally hemopoietic cells into adult spinal cord but again not in neonates. Both microglia and T-cells are able to synthesise and release interferon-γ in the adult spinal cord but not in the neonate and this study showed that full neuropathic-like mechanical allodynia can only be produced when this cytokine is expressed.

These interactions between neuronal and non-neuronal cells are not restricted to the spinal DH. Vega-Avelaira et al. (2009) showed that within the DRG of adult rodents, spared nerve injury resulted in clustering of macrophages around the cell bodies of damaged large A-type neurons but not C-fibres. This did not occur in neonatal DRG. In the same study, high throughput analysis of mRNA from DRG of adults and neonatal rodents showed that there were 206 specific genes regulated in adults and only 3 specifically regulated in neonates. These data further expanded our understanding of the lack of neuropathic pain in neonates and suggest that largely it is due to a different interaction between neurons and non-neuronal cells.

However, despite the above evidence, clear distinctions between adult and neonatal responses to neuropathic injury are not as simple as they may appear. Further work by Vega-Avelaira et al. (2012) extended the observation period post-neonatal neuropathic injury into adolescence and early adulthood, something which previous studies (Howard et al. 2005) had not done. This work has shown that although no immediate behavioural hypersensitivity following spared nerve injury is seen in neonatal rodents, 21 days after surgery mechanical withdrawal thresholds are 2-fold lower in injured animals when compared to sham controls for the hindpaw ipsilateral to injury. At 28-days post-surgery, withdrawal thresholds are 1.7-fold lower. Thermal thresholds are however unchanged. This delayed hyperalgesia is accompanied by a delayed glial activation with microglia showing morphological changes associated with central sensitisation 21-days following injury but not before this time point. This is also accompanied by an increase in levels of glial acidic fibrillary protein (GFAP), a marker for astrocytes. These data therefore suggest that neonatal neuropathic injury is able to prime neuroimmune responses so that when the spinal cord has reached a mature state the neuropathic phenotype is unmasked.

8 Clinical Observations and Significance of Early Injury

As already stated, the postnatal period is a neurodevelopmental period characterised by significant refinements in the structure and function of the peripheral and central nervous systems. Consequently, there are critical periods in which aberrant neural activity can alter normal development (Hensch 2004). Within other sensory modalities such as vision (Bourne 2010), it has been appreciated that the highly plastic nature of the brain in early life can alter sensory perception throughout life. Most studies into the effects of aberrant signalling during critical periods rely on loss of function (e.g. monocular blinding)(Blakemore et al. 1978). Within the context of pain, we have to consider loss of function, and perhaps more prescient to clinical situations, enhanced function following noxious stimulation and how this affects sensory development. Neonates who require intensive care and/or surgery, especially babies born significantly pre-term, will be subject to a diverse range of both noxious and non-noxious stimuli which have a significant effect on sensory input and this will, in turn, affect developmental and plastic processes within the developing pain pathway. The ability of neonatal pain to impact on longer term sensory processing was first appreciated by Grunau et al. (1994a, b) who reported lowered pain sensitivities in toddlers subjected to neonatal noxious insult and who were born pre-term when compared to full-term controls. Hermann et al. (2006) reported that repeated painful procedures in the neonatal period had long-term effects on sensory processing that extended beyond infancy. In fact, this was true regardless of whether the children were born at term or prematurely, however, pre-term children were more likely to see alterations in sensory processing. Subsequently, Walker et al. (2009a) confirmed this observation in a retrospective study of a separate cohort of term and pre-term children. These long-term changes in sensory processing seem to be mostly restricted to thermal sensory processing. Walker et al. (2009a) found no effect of early life injury upon mechanical processing which, they suggested, was evidence for early injury affecting C-fibre-mediated processes within the more mature CNS. These changes in sensory processing are not related to alterations in cognitive ability, but reflect true changes in the neurons that underpin sensory processing and the way that they are connected. A neurological explanation for the phenomenon of developmental hypoalgesia is incomplete. In laboratory animals, global hypoalgesia following inflammatory and surgical wounding has been observed before (Ren et al. 2004; Walker et al. 2009b) and the involvement of primary afferent fibres, at least for the induction of these changes, is acknowledged (Walker et al. 2009b). However, the way in which the pain pathway is altered at the cellular or network levels is currently unknown and is the subject of research. The fact that early life pain induces global changes in sensory thresholds suggests either a change throughout the CNS or within specific brain regions and nuclei which can effect changes across spinal segments and dermatomes. Further work is required to fully elucidate these mechanisms.

9 Concluding Remarks

Pain is a complex phenomenon. This is especially true in the clinical context of early life where the complexities of the sensory and affective response to noxious stimulation are enhanced by immature and highly plastic developmental processes taking place throughout the peripheral and central nervous systems. This review has considered the normal developmental processes that take place in pain centres during early life and mapped these onto clinical and laboratory observations of the consequences of both inflammatory and neuropathic pain that occurs in the neonatal and juvenile period. Although more research is required before we adequately understand normal neurodevelopment in the DH and brain during the postnatal period, it is becoming increasingly clear that pain in early life can alter sensory processing for a considerable time in the life of the affected individual. Furthermore, these alterations are not restricted to the injured tissue but extend globally across the whole body. The emerging data highlight the importance of the interactions between neuronal and non-neuronal cells in mediating these processes and further work is required to refine our understanding of this, as well as the role of supraspinal sites in establishing pain responses.

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