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Advances in Anatomy Embryology and Cell Biology

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Structure of Enteric Neurons

With 24 Figures and 2 Tables

Springer

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ISSN 0301-5556
ISBN-10 3-540-32871-8 Springer Berlin Heidelberg New York
ISBN-13 978-3-540-32871-1 Springer Berlin Heidelberg New York

Springer is a part of Springer Science+Business Media
springer.com

Printed in Germany

Editor: Simon Rallison, Heidelberg
Desk editor: Anne Clauss, Heidelberg
Production editor: Nadja Kroke, Leipzig
Cover design: design & production GmbH, Heidelberg
Typesetting: LE-TEX Jelonek, Schmidt & Vöckler GbR, Leipzig
Printed on acid-free paper SPIN 11685913 27/3150/YL – 5 4 3 2 1 0

This paper is dedicated to those human beings who permitted us to study the human enteric nervous system between October 2000 and October 2005.

Acknowledgements

I am very grateful to the following persons and institutions: Anita Hecht, Andrea Hilpert, Stephanie Link, Karin Löschner, Hedwig Symowski and Inge Zimmermann for excellent technical assistance; Tobias M. Lindig for performing the 3D-reconstructions; Hiroshi Kimura (Otsu Shiga, Japan) for providing us with the pChAT-antibody; Winfried L. Neuhuber (Erlangen, Germany) and John B. Furness (Melbourne, Australia) who gave valuable comments on the manuscript; Patricia Heron (Erlangen) for helping with the grammar; Gerhard Seitz and Barbara Blaser (Bamberg), Thomas Papadopoulos, Arno Dimmler, Roland Croner, Bertram Reingruber and Martin Rexer (Erlangen) as well as Holger Rupprecht and Daniel Ditterich (Fürth) for kind cooperation; and the Johannes and Frieda Marohn-Stiftung (Breh/99; Erlangen) and the Deutsche Forschungsgemeinschaft (BR 1815/3) for financial support.

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Abbreviations

BSA	Bovine serum albumin
CAB	Calbindin
CAR	Calretinin
CNS	Central nervous system
CuB	Cuprolinic blue
cChAT	Common choline acetyl transferase
CGRP	Calcitonin gene-related peptide
ChAT	Choline acetyl transferase
DAB	Diamino benzidine
DiI	1,1′-Didodecyl-3,3,3,3′-tetramethyl-indocarbocyanine perchlorate
DiO	3,3′-Dioctadecycloxacarbocyanine perchlorate
ENK	Enkephalin
ENS	Enteric nervous system
GABA	γ-Aminobutyric acid
GAL	Galanin
GFAP	Glial fibrillary acidic protein
HU	Anti Hu-protein
IPAN	Intrinsic primary afferent neuron
leuENK	Leucine enkephalin
metENK	Methionine enkephalin
NADPHd	Nicotinamide adenine dinucleotide phosphate diaphorase
NF	Neurofilament(s)
NMU	Neuromedin U
nNOS	Neuronal nitric oxide synthase
NPY	Neuropeptide Y
PBS	Phosphate-buffered saline
pChAT	Peripheral choline acetyl transferase
SOM	Somatostatin
SP	Substance P
TBS	Tris-buffered saline
3D	Three-dimensional
VIP	Vasoactive intestinal peptide

1 Introduction

> *The plexuses of Auerbach and Meissner are peculiar to the gut; they extend from the beginning of the unstriated portion of the oesophagus to the end of the rectum. They have usually been considered to belong to the sympathetic system, but it appears to me preferable to place them in a class by themselves. We may speak of them as forming the enteric nervous system.* (Langley 1900)

In this context, it is less important that Langley excluded the striated part of the oesophagus from his definition of the enteric nervous system (ENS). Much more remarkable seems to be that for Langley, a physiologist, structural reasons were the most decisive for taking the nervous system within the wall of the gastrointestinal tract as an entity unto itself. On the one hand, he argued that enteric nerve cells differ in their histological character from those in para- and prevertebral ganglia. On the other hand, there were few connections of enteric nerve plexuses with the central nervous system (CNS) through sympathetic or other autonomic nerves (which had already been described, however; Auerbach 1862). In his later, more famous monograph, he divided the autonomic nerves into three groups: sympathetic, parasympathetic and intestinal nerves (Langley 1921).

This division seems to be all the more modern considering that, during the following decades, many authors and textbooks moved away from this division. The significance of enteric neurons was reduced to that of postganglionic relay stations of vegetative nerves (Müller 1921; Lawrentjew 1929; Botár et al. 1942). In retrospect, this reduction is, amongst other things, even more surprising because functional as well as structural characteristics of the ENS, already partly known at that time, indicated a considerable autonomous character of gut functions. This autonomous character had by all means been identified with the intrinsic nerves (Bayliss and Starling 1899; Trendelenburg 1917).

In the 1970s, decades of stagnation of scientific research in this field came to an end. In particular, the introduction of immunohistochemistry revealed a (chemical) variety of enteric neurons which is unequalled in the remaining peripheral nervous system. The first monograph in this field carried the same title Langley used for this part of the nervous system in 1900: 'The enteric nervous system' (Furness and Costa 1987). A more up-to-date monograph is that of Furness (2006).

1.1 The Enteric Nervous System

The *ENS* is the nervous tissue embedded in the layers of the wall of the alimentary canal. It extends from the beginning of the oesophagus to the internal anal sphincter. It also includes nerve elements in the pancreas and the walls of gall bladder and bile ducts. The ENS consists of: (1) enteric neurons whose cell bodies lie within

wall of the gut, irrespective of the location of their axonal endings (some neurons project outside the gut); (2) axonal endings of extrinsic neurons (sympathetic and parasympathetic efferents as well as afferents whose cell bodies lie outside the above mentioned organs); and (3) enteric glial cells.

The *histological* description of the ENS is that of enteric plexuses. [Although linguistically incorrect, 'plexuses' is used as the plural form of 'plexus'.] These are nerve networks lying within the gut wall, of which there are two general types: (1) ganglionic plexuses contain clusters of neuronal cell bodies, denoted enteric ganglia, and interganglionic nerve fibre strands connecting the ganglia in various directions. Shapes of ganglia as well as the thickness and orientation of connecting strands determine the architecture of a given network; (2) non-ganglionic plexuses consist of nerve fibre strands commonly containing only axons and glia cells. The various plexuses are richly interconnected with each other. Thus, the (gut) ENS as a whole has a multi-layered tubular shape consisting of several interconnected network levels. The architecture of both ganglionic and aganglionic plexuses varies between species and shows interregional differences in the gut of a given species (e.g. Irwin 1931).

Concerning its *fine structure*, the enteric nervous tissue resembles more the central than the remaining peripheral nervous system. It contains only nerve and glia cells surrounded by a basement membrane but is devoid of connective tissue (although exceptions have been described by De Souza et al. 1988) and it is not entered by blood vessels. The border between nervous and other tissues has been considered to be a blood-plexus-barrier (Gershon and Bursztajn 1978), although other authors presented results contradicting this concept (Jacobs 1977; Gabella 1982). The fact that drugs that do not enter the CNS, such as hexamethonium, are effective in the ENS in vivo, indicates that any barrier between the blood and the ENS is not as secure as that of the CNS. The mean range of intercellular spaces is approximately 20 nm. There are numerous synapses on both somata and processes of neurons which frequently display asymmetric membrane specializations (Hager and Tafuri 1959; Baumgarten et al. 1970; Gabella 1972; Cook and Burnstock 1976a, 1976b; Wilson et al. 1981a, 1981b; Komuro et al. 1982). Up to nine different morphological types of neurons were described at the ultrastructural level (Cook and Burnstock 1976a). Correlations between light microscopically and (conventional-) ultrastructurally defined types were only exceptionally established e.g. in the case of type II neurons (Pompolo and Furness 1988; Song et al. 1995). Some more conclusions about synaptic connectivities of morpho-chemically defined types of neurons in the guinea-pig enteric circuits could be drawn by immunocytochemical studies e.g. on calbindin (CAB)-reactive type II neurons (Pompolo and Furness 1988), on γ-aminobutyric acid (GABA)-reactive type I neurons (Pompolo and Furness 1990), on serotonin-reactive type I neurons (Young and Furness 1995), on two functionally different, calretinin (CAR)-reactive type I neurons (Pompolo and Furness 1995) and on somatostatin (SOM)-reactive filamentous neurons (Portbury et al. 1995; Pompolo and Furness 1998). In these and other studies (e.g. Li et al. 1995; Li and Furness 2000; Portbury et al. 2001), close contacts between

immunoreactive, vesicle-containing boutons and nerve cell bodies lacking membrane specializations were recorded also and included in the discussion of synaptic connectivity between enteric neurons.

Enteric glia are different from other peripheral glia (e.g. Schwann cells), but they display greater resemblance to astrocytes of the CNS. Within ganglia, enteric glial cells outnumber enteric neurons and they display cell-to-cell-coupling. They have trophic and protective functions for enteric neurons, may be involved in neurotransmission and are suggested to be a link between the nervous and the immune system. Consequently, loss of glial cells or impairment of glial cell integrity is related to gut diseases. Most enteric glial cells are immunoreactive for S-100 or glial fibrillary acidic protein (GFAP) and there are results suggesting structural and chemical heterogeneity among enteric glial cells (Nada and Kawana 1988; Gershon and Rothman 1991; Hanani and Reichenbach 1994; Bush 2002; Cabarrocas et al. 2003; Jessen 2004; von Boyen et al. 2004; Rühl 2005).

Embryologically, the ENS, both its neurons and glia, derives from different regions of the neural crest (Young and Newgreen 2002; Burns and Le Douarin 2001). Its vagal part is the most prominent and most important source of enteric neurons and glia, from here all gastrointestinal regions are colonized. The sacral neural crest delivers, in addition to the vagal crest, precursors to the postumbilical gut. Gershon (1997) distinguished a third source, the truncal neural crest, which colonizes the rostral foregut (oesophagus, cardia).

1.2 Ganglionated Enteric Plexuses

1.2.1 Myenteric Plexus

The location of this network between the longitudinal and circular muscle layer is consistently described both throughout different species and regions. Thus, in contrast to the submucosal plexus, the original descriptions of Auerbach (1862, 1864) have only been extended but not restricted to subplexuses by later authors (e.g. Schabadasch 1930; Irwin 1931; Stöhr 1931). Auerbach distinguished primary nerve strands ('Maschenwerk 1 ter Ordnung') which ran in the longitudinal (oro-anal) direction and showed interconnections via transverse ganglia. Secondary strands ran circumferentially around the gut showing fine nerve fibres entering the circular muscle layer. Nerve fibres running parallel to the longitudinal musculature were also observed but were less prominent. Later authors (e.g. Stöhr 1931) introduced the term 'tertiary plexus'. Although this term was differently defined by some later authors, it is commonly used for nerve fibres branching off from primary and secondary strands which run irregularly within the meshwork of the primary strands. The architecture of this tertiary component of the myenteric plexus is partly dependent on the thickness of the longitudinal muscle layer. This network is regarded as the major source of innervation for this layer, at least in small animals (Furness and Costa 1987; Llewellyn-Smith et al. 1993; Furness et al. 2000b).

The myenteric plexus extends as a continuous ganglionated network from the beginning of the oesophagus to the internal anal sphincter. Direct connections between ganglia of the myenteric and the (external) submucosal plexus, interconnecting nerve strands, which cross the circular muscle layer have already been observed by Auerbach (1864), described in detail by Goniaew (1875) and later confirmed by Drasch (1881), Müller (1892) and others.

1.2.2 Submucosal Plexuses

Meissner (1857) was the first who unequivocally identified an intrinsic ganglionated nerve plexus within the gut wall, specifically that within the submucous layer. He recognized that this gut layer may be one of the regions of the human body with the highest density of nerves. He also noted that numerous nerve fibres of this plexus enter the muscle layer (he meant most probably the external muscle layer). Billroth (1858) observed that most components of the submucosal nerve networks were in closer proximity to the mucosa than the muscle. Although both authors studied larger mammals such as human, pig and cattle, neither differentiated the submucosal nerve networks into different plexus types. Drasch (1881) found that the submucosal plexus extends throughout the whole thickness of the submucosa with ganglia located at different levels.

In contrast to small laboratory animals (guinea-pig, mouse, rat) which display a single, monolayered submucosal plexus, larger species (e.g. horse, human, pig) have two or even three different submucosal plexus types. After the description of the myenteric plexus by Auerbach (1862, 1864), Henle (1871) described the *enteric* plexuses in a German textbook on neurology ('Nervenlehre') as consisting of an outer network and an inner network. Henle termed them 'Plexus myentericus externus' and 'Plexus myentericus internus' and he also referred to Auerbach and Meissner as the first describers of the two networks. The position of the 'Plexus myentericus externus' (Auerbach) was described by Henle (1871) as lying between the longitudinal and circular muscle layer and that of the 'Plexus myentericus internus' (Meissner) as lying on the outer surface of the muscularis mucosae. That is, the 'Plexus myentericus externus' of Henle (1871) corresponds to the myenteric, not to a submucosal plexus. The Russian histologist Schabadasch (1930), who gave the first clear description of two strikingly different *submucosal* plexus types (an outer and an inner one) in Macaques, obviously misunderstood Henle's text passage. Schabadasch used the following terms: for the outer submucosal plexus which lies close to the inner surface of the circular muscle and which was actually newly discovered by him: 'Plexus entericus internus (Henle)', for the inner submucosal plexus 'Plexus submucosus (Meissner)'. In the literature, this historical mistake led numerous authors to designate the outer submucosal plexus as Henle's plexus. Already Patzelt (1936) pointed to this mistake, Stach (1977b, 1979) suggested renaming the external submucosal plexus after Schabadasch.

A number of authors have described a third ('intermediate') submucosal plexus in various species including human (Gunn 1968; Hoyle and Burnstock 1989a, 1989b; Crowe et al. 1992; Dhatt and Buchan 1994). Consistent nomenclature based on morphological and chemical features of the different plexus types is required to avoid mistakes such as those described above. The most conclusive depiction, description and nomenclature for human submucosal plexuses was given by Wedel et al. (1999). These authors demonstrated three ganglionated plexuses in human colonic submucosa, distinguishable through different topography and network architecture: two submucosal networks were found close to each other in the outer part of the submucosa whereas the third was situated close to the muscularis mucosae.

The *plexus submucosus extremus* was located in the outermost portion of the submucous connective tissue, in close topographical association with the circular musculature. It contained single neurons and small ganglia. This plexus was originally described in rats and guinea-pigs (Stach 1972) and later was also found in other species including human, but not in the rabbit (Christensen and Rick 1987; Hoyle and Burnstock 1989b). The nerve fibre strands of this network ran parallel to each other and showed connecting strands running diagonally.

The *plexus submucosus externus* was observed in close proximity to the former network, although in a different plane of the outer portion of the submucosa and showed the widest meshing of the three submucosal plexuses. It contained the largest submucosal ganglia.

The *plexus submucosus internus* was found in close proximity to the muscularis mucosae, its ganglia displayed smaller diameters than those of the plexus submucosus externus.

In the pig, there exist various data indicating different functions of the different submucosal plexuses. These include differences in histology and vascularization (Stach 1977a, 1977b), fine structure (Mannl et al. 1986; Brehmer et al. 1994), immunohistochemical composition (Timmermans et al. 1990; Krammer et al. 1993a) and electrophysiological properties (Thomsen et al. 1997). Also for human, results of a number of studies indicate that the different submucosal plexuses have different functions and are not levels of a homogeneous network (Hoyle and Burnstock 1989b; Domoto et al. 1990; Crowe et al. 1992; Dhatt and Buchan 1994; Porter et al. 1999).

In contrast to the myenteric plexus which extends as a ganglionated network from the beginning of the oesophagus to the internal anal sphincter, submucosal ganglia are rare or even absent from the oesophagus and stomach (Furness 2006; Neuhuber et al. 2006).

1.3 Non-Ganglionated Enteric Plexuses

The following overview on these plexuses (listed from serosa to mucosa) that contain few, or in some cases no nerve cell bodies was adopted mainly from Furness and Costa (1987).

The *subserous plexus* is located between the serosa and the longitudinal musculature, its nerve bundles connect extrinsic with intrinsic nerves. In some regions, small ganglia occur near the mesenteric attachment or on the surfaces of the oesophagus and rectum.

The *longitudinal muscle plexus* consists of fine nerve bundles running parallel to the muscle bundles. In cases when the muscle layer is thin or in small laboratory animals, there is no prominent longitudinal muscle plexus, innervation of the longitudinal muscle layer is realized via the tertiary component of the myenteric plexus (Furness et al. 2000b).

The *circular muscle plexus* is oriented parallel to and located within the corresponding muscle layer.

The *deep muscular plexus* is a prominent aggregation of nerve fibres with the same origins (mainly the myenteric but also the submucosal plexus) and the same chemical coding as those of the circular muscle. This plexus separates a thin innermost layer of circular muscle cells from the major outer portion of this layer. It has been suggested that the innervation of the circular muscle is asymmetric, similar to that of arteries (Furness and Costa 1987).

The *plexus of the muscularis mucosae* is situated mainly at the external surface of the corresponding layer in small mammals and within the muscle in larger species.

The *mucosal plexus* can be subdivided into a subglandular, a periglandular and—in the small intestine—a villous plexus. It is very rich in nerve fibres and contains, in the human, solitary and aggregated nerve cell bodies (Wedel et al. 1999).

1.4 Morphological Classifications of Enteric Neurons

1.4.1 Dogiel's Classification

For several reasons, Dogiel's publications (1895a, 1895b, 1896, 1899) on the morphology of enteric neurons, which culminated in his paper dating from 1899, were pioneering. Firstly and simply, he was the first to classify different enteric neuron types. Secondly, he sought for structural features that would allow him to deduce functional assumptions, e.g. he followed the axonal course of some neurons to the musculature and thus concluded that they were motor neurons. Thirdly, he investigated a number of mammalian species and his three-type-classification was originally an interspecies approach. Fourthly, but actually rather accidentally, in his last paper (Dogiel 1899) he exclusively depicted intestinal enteric neurons of the two species which are in the focus of modern ENS research today, namely the guinea pig (which is today the best known and mostly used animal model in this field) and human (at whom the efforts of the medical aspect of research are directed). [Some gallbladder neurons he depicted in his last paper were derived from the dog.]

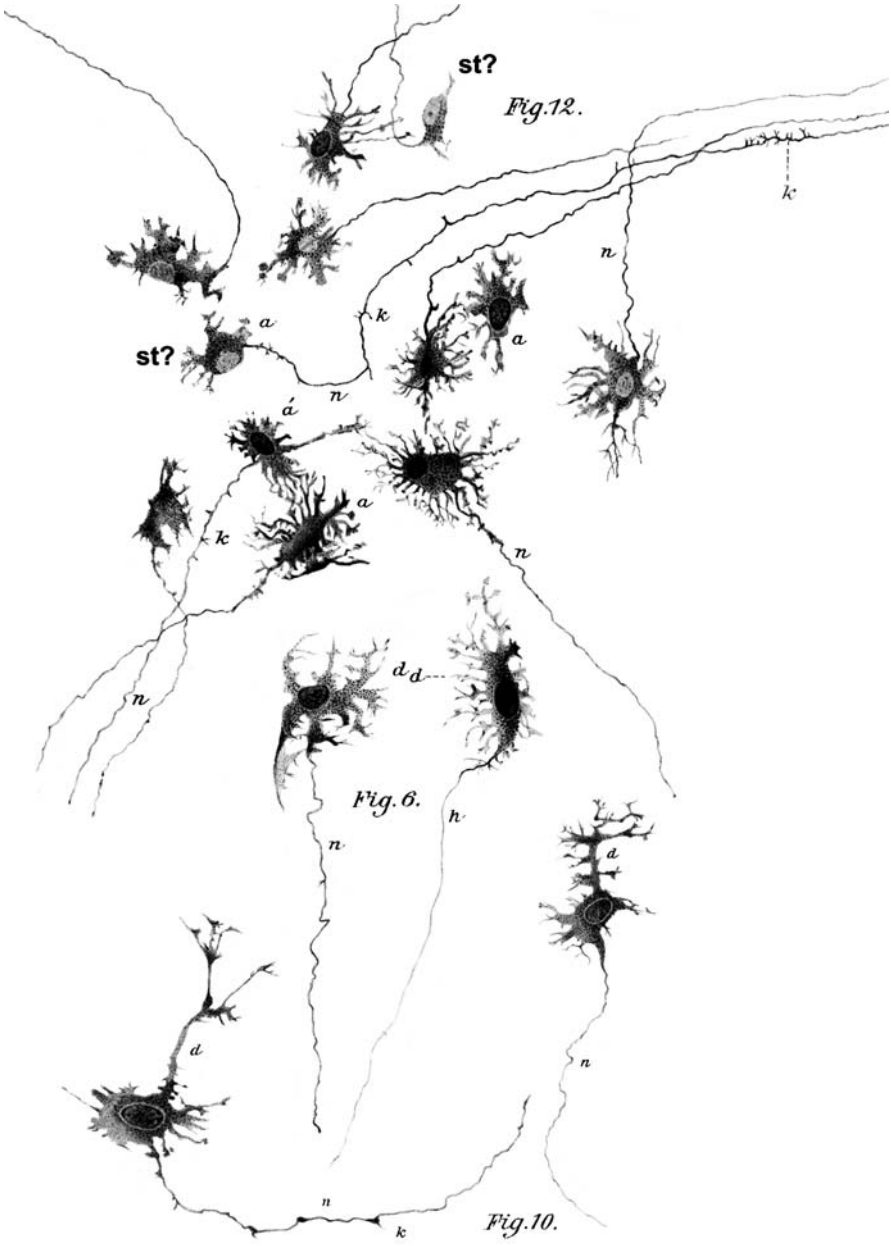
In the following, some characteristics of Dogiel's three neuron types are summarized because even today when numerous current ENS researchers refer to neuron morphology, they firstly (and frequently exclusively) use Dogiel's name, when in fact they are referring to the two-type-reduction of his classification system (see Sect. 1.4.2). Prior to this, it is important to mention that Dogiel applied an intravital methylene blue staining method and that the human gut segments he investigated derived from infants aged between 6 and 9 months. All neurons were described as having a number of dendrites and a single axon (the general view at that time). Dogiel's criteria for distinguishing the neuron types included the shapes and lengths of the dendrites as well as the shapes of the dendritic endings.

1.4.1.1 Type I Neurons

These neurons had stellar-shaped cell bodies with lengths ranging from 13 to 34 μm and widths between 8 and 21 μm. They were the most numerous neurons in the myenteric plexus, but were less common in the submucosal plexus. Type I neurons displayed between 4 and 20 short, flattened dendrites which divided several times into short, flat branches. The branching points were frequently broadened, the dendritic endings were sometimes blunt but mostly tapered, terminating as thin fibres. In some cases, all dendrites emerged from one pole of the soma, with the axon originating from the other (bipolar cells), whereas in other cases, all processes emanated from one pole (unipolar cells). Especially in the specimens derived from human infants, Dogiel observed neurons displaying one or two long, broad dendrite(s) in addition to numerous short dendrites. The axon ('Nervenfortsatz') mostly emerged with a cone shaped origin from the soma, and sometimes from one of the dendrites. This neural process was described as thin, sometimes varicose and frequently emitted thin collaterals. This latter point was very conspicuous in specimens derived from human infants. Dogiel was able to trace axons through several ganglia. Few of them ('einer oder der andere') he traced to the musculature, where they terminated in a fine network of fibres surrounding smooth muscle cells. Therefore, he generally considered type I neurons as motor neurons.

In Figs. 1 and 2, all human type I neurons (as explicitly marked by Dogiel or that can be deduced from his descriptions) are represented which have been depicted

Fig. 1 All human neurons marked or obviously classified as type I by Dogiel (1899) in his original Figs. 6, 10 and 12. Only those type I neurons were represented including Dogiel's original markings which were originally depicted with axons (*a*, type I neuron; *a'*, type I neuron with several short and a long dendrite; *d,* dendrite; *k*, axonal collaterals; *n,* axon). The two neurons below (Dogiel's Fig. 10) were reduced in size to 75%; they were originally depicted by Dogiel using a larger objective than all other type I neurons including those in our Fig. 2. The two neurons marked with 'st?' (by author) may correspond to stubby (type I) neurons whereas the other neurons may rather correspond to spiny (type I) neurons (see Sect. 4)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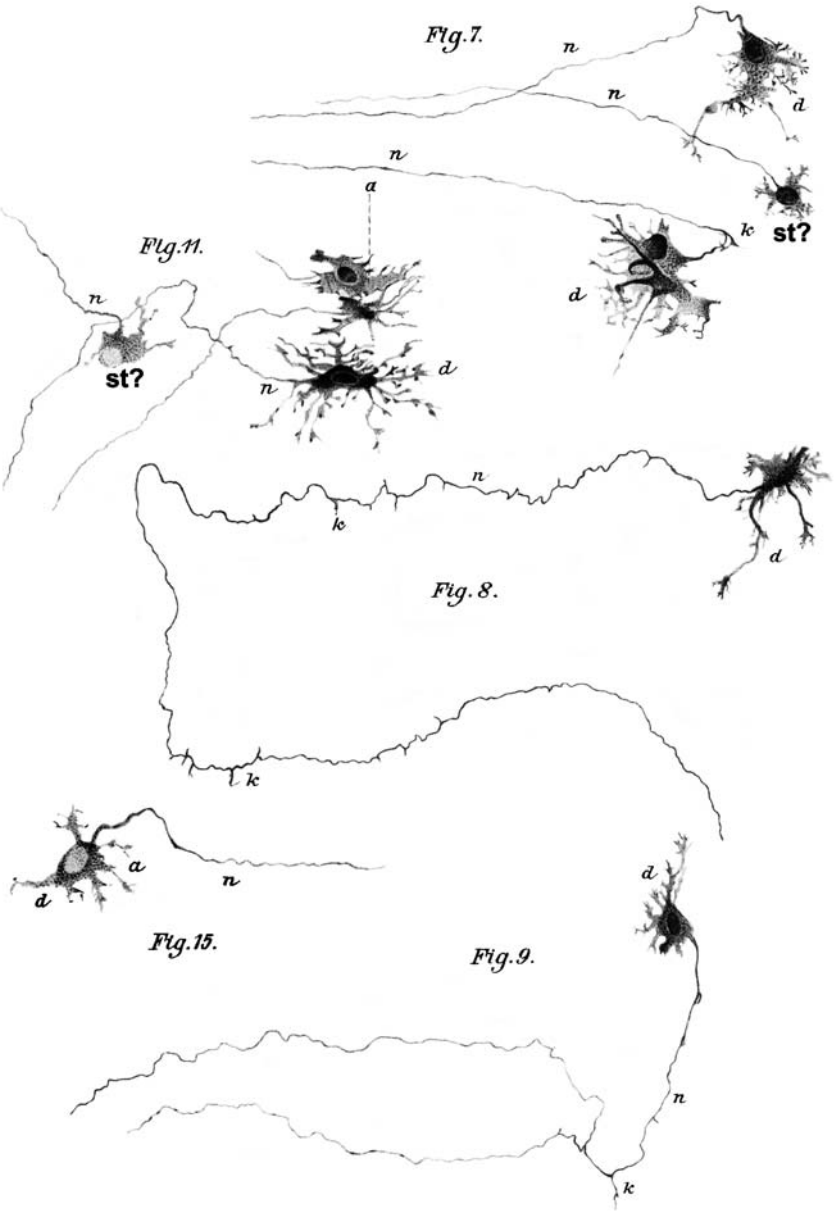


Fig. 2 All human neurons marked or obviously classified as type I by Dogiel (1899) in his original Figs. 7, 8, 9, 11 and 15. Only those type I neurons were represented including Dogiel's original markings which were originally depicted with axons (*a*, type I neuron; *d*, dendrite; *k*, axonal collaterals; *n*, axon). The two neurons marked with '*st?*' (by author) may correspond to stubby (type I) neurons whereas the other neurons may rather correspond to spiny (type I) neurons (see Sect. 4)

by Dogiel with their axons. Thus, for example, the neurons of Dogiel's Fig. 5 (1899) have been excluded.

1.4.1.2 Type II Neurons

These neurons were angular, stellar or spindle shaped, measuring 21–47 μm in length and 13–21 μm in width. They had three to ten dendrites and one axon. These cells were less numerous than the type I neurons in the myenteric ganglia but were more frequent in the submucosal plexus. After emerging from the soma, the processes (which Dogiel referred to as dendrites) were relatively thick. At some distance from the soma, some of them divided at acute angles into two to four thin branches. Finally, all processes, including their branches, left the ganglion of origin via interganglionic strands. Dogiel observed that some of these processes of myenteric type II neurons could be traced through the circular muscle bundles to the submucosa, whereas some of the dendrites of submucous type II neurons entered the mucosal plexus surrounding Lieberkühn's glands. Thus, Dogiel supposed type II neurons to be either secretomotor or sensory neurons but as he was of the opinion that the same type of neurons were also present in organs without glands, such as the heart and blood vessels, he favoured the sensory role of type II neurons. Both the dendrites and their branches were thin and their contours were smooth. In contrast to our recent view, Dogiel distinguished axons from dendrites in this type and described axons as processes emerging from a conus-shaped origin and displaying varicosities. Axon collaterals were shorter but displayed the same appearance as the corresponding dendritic branches. They ran through other ganglia but endings could not be observed.

[Ramón y Cajal did not distinguish between axons and dendrites in those enteric neurons which were obviously classified as type II by Dogiel and which displayed several long processes ('cellules à expansions longues', Ramón y Cajal 1911).]

In Fig. 3, all human type II neurons as depicted by Dogiel (1899) are represented.

1.4.1.3 Type III Neurons

These neurons were located both in central and peripheral parts of ganglia as well as within interganglionic strands. They had two to ten, in some cases even more dendrites which were rather thick at the point of their origin, but tapered further away from the cell bodies ('immer dünner und dünner'). The dendrites were longer than those of type I neurons. Although similar in shape to dendrites of type II neurons, the dendrites of type III neurons ended in thin, partly varicous filaments within the ganglion of origin. These filamentous endings formed pericellular baskets within the ganglia. The axonal origin was cone shaped, the axon smoothly contoured. From the initial trunk, between one and three dendritic side branches emerged. The axonal course could be followed through several ganglia

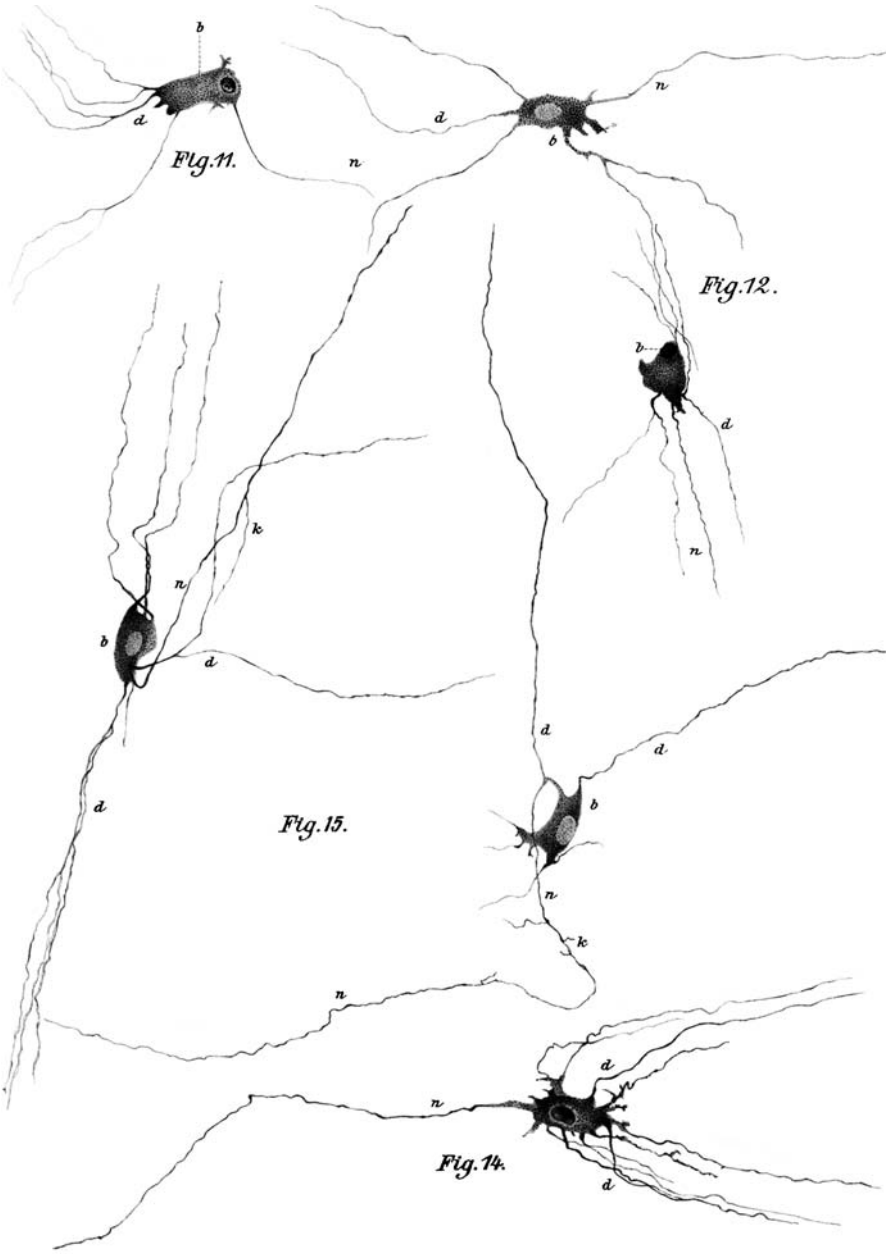


Fig. 3 All human neurons marked as type II by Dogiel (1899) in his original Figs. 11, 12, 14 and 15 (*b*, type II neuron; *d*, dendrite; *k*, collateral fibre; *n*, axon)

but endings could not be observed. Consequently, Dogiel did not speculate on the functional role of type III neurons.

No human and only two type III neurons from the guinea-pig were depicted by Dogiel (1899).

1.4.2 80 Years After Dogiel

Based on the works of Ramón y Cajal and formulated by Waldeyer (1891), the neuron theory ('Neuronenlehre') was founded as an alternative to the reticular, syncytial concept (see below) of the arrangement of the nervous tissue (reviewed in Kirsche 1984; Peters et al. 1991; Clarke and O'Malley 1996). When Langley (1900, 1921) introduced the name 'enteric nervous system' to characterize the peculiarity of the neuronal elements within the gut wall, the dispute between the proponents of the two rival theories was in full bloom and remarkably influenced the descriptions of enteric neuroarchitecture for more than half a century. Although Dogiel favoured the reticular concept of the nervous system, his classification was based on distinguishing neuronal processes into dendrites ('Protoplasmafortsätze') and axons ('Axencylinderfortsätze', 'Nervenfortsätze'). His attempts were the first to suggest a correlation between structural and functional features of enteric neurons. Very important for his functional conclusions were the observations that in some, although rare, instances axons of neurons could be followed to putative target tissues. Dogiel's (quite modern) concept was that different shapes of somata and dendrites correlate with different functions.

Prior to Dogiel, Ramón y Cajal (1893) suggested that enteric neurons have several long processes which cannot be distinguished into axons and dendrites. However, the following decades saw less striking progress in this field. A number of later authors abandoned Dogiel's distinction between dendrites and axons in type II neurons (La Villa 1898; Ramón y Cajal 1911; E. Müller 1921; van Esveld 1928; Oshima 1929; Harting 1931; Sokolowa 1931; Stöhr 1931; Okamura 1934) whereas others maintained this distinction (Koelliker 1896; Hill 1927; Rossi 1929; Iwanowa 1958). A further dispute arose as to the general feasibility and functional significance of a morphological classification of enteric neurons at all. This was declined by researchers until the recent past (Kuntz 1913, 1923; Johnson 1925; Michail and Karamanlidis 1967; Christensen 1988). The most important argument favouring the proponents of a functionally relevant morphological classification became the presence of *several long processes* in type II neurons and of *one long and several short processes* in other neurons. The validity of this distinction was sufficient for many authors (Hill 1927; Tiegs 1927; van Esveld 1928; Oshima 1929; Iwanow 1930; Harting 1931, Lawrentjew 1931; Kolossow and Sabussow 1932; Reiser 1932; Murat 1933; Cavazzana and Borsetto 1948; De Biscop 1949; Jabonero 1951, 1958; Stöhr 1952; Greving 1951b; Temesrékási 1955; Kolossow and Milochin 1963) and resulted in 'collapsing of the classifications' (Furness and Costa 1987) which furthermore frequently referred to only two types of neurons. The characteristic flat and lamel-

lar shape of the type I dendrites in various species ('lamellenförmig', Dogiel 1899; 'Dendritenlamellen', Lawrentjew 1929) became a facultative feature for the differentiation of type I neurons. Based on the shapes and lengths of their short processes, occasional subdivisions of the type I neurons had been proposed, but these merely underlined the general grouping into two types of enteric neurons (Hill 1927; Lawrentjew 1931; Greving 1951a, 1951b; Temesrékási 1955; Jabonero 1958).

Further reasons for the reduction of Dogiel's original classification were manifold. Many of the authors cited above were followers of the reticular concept of the nervous system. In an extreme view, the neuronal perikarya were regarded as cytoplasmatic and nucleated junctions of a complicated neural syncytium extending between the central nervous system and the target tissues. Consequently, some authors avoided the terms 'dendrite' and 'axon' since they would anyhow communicate with each other and described them rather as long and short 'processes' (Stöhr 1931; Reiser 1932). Others restricted their results to the 'proof' of anastomoses between dendrites of different neurons as demonstrated by drawings (Cole 1930).

In another view, the nerve cells within the gut were regarded as simple postganglionic neurons of sympathetic and parasympathetic preganglionic nerves. Important arguments for this view were deduced from the works of E. Müller (1921), Lawrentjew (1929, 1931), Sokolowa (1931) as well as Iljina and Lawrentjew (1932) who reported gradients of the distributions of type I and II neurons along the gastrointestinal tract and related these gradients to the segmental levels of parasympathetic and sympathetic preganglionic neurons, respectively. This was exaggerated by Botár et al. (1942) who moreover denied that structural differences between enteric neurons imply functional differences and that neurons in the gut establish local reflex arcs as was suggested by Dogiel. They obviously gave up the concept of the 'enteric nervous system' as defined by Langley.

Methodological factors influenced progress in this field also. In contrast to Dogiel most later authors did not use whole-mount specimens—they used sections. Some researchers recognized that the classical histological methods used (methylene blue technique, metal impregnations) which stain only subsets of neurons, may not demonstrate equivalent neuron populations in different species (Ottaviani 1940). The suggestion was made that unstained neurons may be functionally different from stained neurons (Schofield 1962).

Nevertheless, some new aspects were introduced during the decades between Dogiel and the 1970s. L.R. Müller (1912) distinguished two myenteric neuron types based on the topographical vicinity of their processes to the musculature. Besides Langley (1900), and in contrast with Dogiel, he was one of the first to suggest that ganglion cells in the gut are quite different from other autonomic neurons. He already recognized the absence of pericellular sheaths around enteric neurons, a feature that has been confirmed more recently by fine structural studies. Kuntz (1913, 1923) described terminations of nerve fibres ('synapses') on enteric neurons thus favouring the neuron theory of the nervous system. Iwanow (1930) found axons of type I neurons to be oriented in one direction and Temesrékási (1955) estimated lengths of axonal projections within the different ganglionated

plexuses. Ito (1936), as well as Ito and Nagahiro (1937), differentiated three classes of neurons based on their size and found that the eccentricity of nuclei is a frequent, non-pathological feature of enteric nerve cells. Small neurons were observed that did not belong to the types of Dogiel (Stöhr 1952; Gunn 1959, 1968) whereas Fehér and Vajda (1972) distinguished three types purely on the basis of cell soma size. Based on their staining intensity, enteric neurons were classified by Honjin et al. (1959), Michail and Karamanlidis (1967) and Sutherland (1967) into argentophobe and argentophile neurons. Schofield (1962) considered the number of processes to be relevant for functional properties, not their lengths and shapes.

Beginning from the late 1970s, the development of new techniques led to a dramatic increase of our knowledge of the diversity of enteric neurons. However, important reviews of that period neither mentioned any light microscopy-based classification of enteric neurons (Furness and Costa 1980) nor did they refer to Dogiel's types (Gershon and Erde 1981).

1.4.3 Stach's Classification in the Pig: General Remarks

Clear relationships between morphology and function are well established for CNS neurons, and these relationships provide a solid basis for CNS research. We are firmly convinced that different functions of enteric neurons will manifest themselves somehow in different structural features. To identify these differences, which are probably in part quite subtle, investigators require not only adequate visualization methodology, technical skill and observational scrutiny. In addition, the method of intellectual processing is crucial: does an observation serve to confirm the conventional hypothesis or is it its touchstone? Viewed after an interlude of decades, many obvious examples supporting the prior, worldly outlook can be found in the historical literature on the ENS (see Sect. 1.4.2). For many scientists of past decades, the syncytium theory of the nervous system, the reduction of the role 'of the intramural ganglion cells in the intestinal wall' to postganglionic relay cells of sympathetic and parasympathetic nerves, as well as the routine use of histological sections rather than whole-mount preparations seem to have provided irrefutable evidence for the old views. What was really observed or supposedly observed was then usually slotted into the incontrovertible theory (e.g. the reduced, two-type-classification of Dogiel) which also functioned as a conceptual basis. Only in the 1970s, the pressure brought to bear by new results, especially those gained by immunohistochemistry, become so strong that Langley's concept of the ENS had to be revived. But then the lack of advances in morphological concepts during the past decades took its revenge: with the now tremendously growing insight into the ENS, morphology was (and still is) quite often considered as an accessory, illustrating rather than a substantial contribution to advances in this field. At times, depictions of the *beautiful Stach neurons* were reviewed with admiration, but the often *exaggerated Stach types* were viewed with suspicion if not disparagement. The results presented in Sect. 3 refute these prejudices as we have now correlated

morphological with chemical differences. It is even more generally accepted that neurons that differ in their chemical characteristics also differ in function.

The most important difference between the classification system of Stach (1980–2000) and that of Dogiel (1896, 1899) was that the directions of axonal projections were not occasional, accessory observations but a main criterion for differentiating neurons into types. Already the renewed description of type I neurons (Stach 1980) was based on two main features: short dendrites with lamellar endings *and* orally running axons. In contrast, type III neurons (Stach 1982a) were described as neurons displaying long, slender, tapering dendrites *and* anally running axons. Type II neurons (Stach 1981) were non-dendritic *and* multiaxonal neurons with mainly circumferential and vertical axonal projections, i.e. the latter axons leave the myenteric plexus towards the submucosa. [In contrast to Dogiel (1899), Stach did not differentiate the processes of type II neurons into an axon and dendrites since all of them had the histological appearance of axons (see Sect. 3.3).] Stach was compelled to introduce a new type—IV—when he described neurons (Stach 1982b) with short, slender dendrites (unlike those of type III) which had tapering endings (unlike those of type I) *and* displayed an axon leaving the myenteric plexus (unlike types I and III) through interconnecting strands to the submucosa. These strands cross the circular musculature and connect the myenteric with the external submucosal plexus (Stach 1983; Brehmer et al. 1998). Further types were described in the years until 2000 (see Sect. 3).

A number of factors hampered an appreciable impact of Stach's classification on the advances made at the same time in the research on the ENS. Firstly, due to the political isolation of the former German Democratic Republic (East Germany), his publications were written mainly in German. Secondly (partly related to the first point), in the 1980s, simultaneously with the main publications of Stach, a number of modern neuroanatomical methods were applied in the field of the ENS. Stach's method of silver impregnation, resulting in brilliant depictions of neurons (as can be viewed in all of his publications) but, at the same time, being unreliable and ineffective (as cannot be seen in his publications but can be experienced by individual investigators) was antiquated in the eyes of most researchers in the field of the ENS. All the more because until that time the two-type-doctrine of enteric neurons was generally accepted, a need for further morphological research was (and partly still is) even occasionally rejected. This situation is in contrast to that in the CNS where a 'definite renaissance' of silver impregnation was seen (Jones 1984) and the need of morphological data on neurons has been stressed (Levitt 2001; van Pelt et al. 2001). Thirdly, the published results of Stach derived exclusively from the pig. This species is outside the focus of most research groups working in this field; notably it is seldom used for functional studies. Now we know that there are some general similarities between shapes of comparable neurons between different species but we also know that the shapes of corresponding neurons are not identical. Thus, especially for scientists working with the guinea pig as an animal model, the Stach classification of neuron types in the pig was considered as less significant.

During recent years, we shifted our focus to human enteric neurons and tried to emphasize the value of detailed morphological studies for modern neurogastroenterology. The first step in this direction was the last silver impregnation study published by Stach et al. (2000). Since that time, the successful application of immunohistochemical techniques for the visualization of the shapes of enteric neurons, with comparable quality to silver impregnation, enabled us to correlate structural with chemical features also of human enteric neurons. Nevertheless, on the one hand, it seems likely that without the morphological classification of enteric neurons in the pig, founded by Stach, our corresponding studies in human (Sect. 4) including the specification and extension of the three-type-classification of Dogiel would not have been possible. On the other hand, since we are able to stain human enteric neurons immunohistochemically in a quality comparable to silver impregnation, the main focus of our current research—the identification and definition of enteric neuron types—has been shifted from pig to human.

1.4.4 Classification in the Guinea Pig

The guinea pig is the best investigated animal model in the field of the ENS, its small intestine being the most extensively studied region. The results of numerous studies applying and combining various neuroscience methods (e.g. immunohistochemistry and immunocytochemistry, lesion, tracing and intracellular dye filling techniques, neurophysiological or pharmacological methods) led to an extensive classification system of enteric neurons which is the basis of our current understanding of enteric circuits (Costa et al. 1996; Furness et al. 2000a; Brookes 2001a, 2001b).

The first consistent attempt to reinvestigate and extend Dogiel's (1899) classification in the guinea pig was carried out using intracellular dye injections into 204 myenteric neurons of the mid-small intestine (Furness et al. 1988). Four shapes of neurons were distinguished: Dogiel type II neurons with several long processes, Dogiel type I neurons with lamellar dendrites, neurons with numerous filamentous dendrites and small neurons with few processes. Among the 204 neurons investigated, no Dogiel type III neurons were identified, although later a similarity between filamentous neurons and Dogiel type III neurons was suggested (Portbury et al. 1995; Furness 2006). The same spectrum of neurons was classified in the duodenum (Clerc et al. 1998a). Other studies by different groups described type III neurons both in myenteric and submucosal plexus (Kobayashi et al. 1984; Furness et al. 1985; Bornstein et al. 1986; Katayama et al. 1986; Steele et al. 1991). However, they hardly corresponded to the original descriptions given by Dogiel (Brehmer et al. 1999b). Using dye filling in combination with other methods, dendritic type II neurons (originally described in the pig, Stach 1989) could be later identified also in the myenteric plexus of the guinea pig small intestine (Bornstein et al. 1991b; Brookes et al. 1995).

Comprehensive studies on the chemical phenotypes of enteric neurons repeatedly led to the assumption that the Dogiel classification may not be sufficient for

describing the morphological and functional heterogeneity. As a result, small and large Dogiel type I neurons were distinguished as well as Dogiel type I neurons with lamellar dendrites, with club-like processes and with short filamentous processes (Furness et al. 1983b; Bornstein et al. 1984; Costa et al. 1996; Brookes 2001a). Some groups tried to incorporate criteria of Stach's classification in the pig for neurons in the guinea pig and classified neurons, e.g. as type IV (Lees et al. 1992; Furness et al. 2003).

In the guinea pig colon, attempts were made to adapt Dogiel's original types to results of combined morphochemical studies (Kobayashi et al. 1985) whereas others established a neuron classification specifically for this region (Messenger et al. 1994; Lomax et al. 1999). It has been suggested that the main difference in neuronal composition between small and large intestine is the existence of different sets of interneurons reflecting different physiological features (e.g. motility patterns) of the two intestinal regions (Lomax and Furness 2000; Furness 2006).

Also in the guinea pig stomach, a number of studies dealt with chemistry, projections and topography of neurons (Schemann 1992, Schemann et al. 1995; Brookes et al. 1998; Pfannkuche et al. 1998; Reiche and Schemann 1998; Michel et al. 2000; Schemann et al. 2001). Although most of these studies did not focus on structural features, it has been noted that Dogiel type II neurons seem to be absent from the gastric myenteric plexus (Schemann 1992; Brookes et al. 1998).

With the exception of classical observations (Lawrentjew 1929) there are no more recent investigations focussing on morphological phenotypes of myenteric neurons in the oesophagus (Neuhuber et al. 2006).

1.4.5 Classifications in Other Species

In species other than pig and guinea pig, classifications were based mainly on neurochemical characteristics. Attempts to correlate chemistry with morphology were made only sporadically.

In the rat, Schultzberg et al. (1980) and Sundler et al. (1993) have established chemical classification of enteric neuronal populations. Browning and Lees (1994, 1996) tried to correlate chemical and electrophysiological categories to the morphological types of Dogiel (1899) and Stach (1989). Mann et al. (1997, 1999) described shapes, chemistry and projections of Dogiel type II neurons which seem to be the intrinsic primary afferent neurons (IPANs) in this species.

In the mouse, chemistry and projection patterns of myenteric (Sang and Young 1996, 1998; Sang et al. 1997) and submucosal (Sang and Young 1998) neurons were investigated. Although Dogiel type I neurons were noted, no further morphological differentiation was attempted. In the mouse colon, Furness et al. (2004b) and Nurgali et al. (2004) presented electrophysiological, immunohistochemical and morphological findings indicating that Dogiel type II neurons are the IPANs also in the mouse.

Data about chemical coding and/or structural features of enteric neurons in other mammals is less systematic. Such studies were performed in dog (Daniel et al. 1985, 1987; Furness et al. 1990a, 1991; Wang et al. 1997), rabbit (Keast et al. 1987; Gábriel et al. 1997), opossum (Christensen 1988), horse (Pearson 1994), hamster (Toole et al. 1998) and sheep (Chiocchetti et al. 2004).

However, an important conclusion from the above studies is that interspecies differences hamper a direct transfer of knowledge from one species to another. There are some general similarities (e.g. ascending cholinergic and descending nitrergic pathways occur in all species) but there are a number of differences in detail. This is true for neurochemical properties and probably also for morphological features of enteric neurons (Sect. 5).

2 Material and Methods

Material handling and methods have been described previously in detail in the quoted references with two exceptions. First, immunocytochemical investigations of type V neurons (Sect. 2.2), second, post-mortem tracing by application of two different tracers (1,1′-didodecyl-3,3,3,3′-tetramethyl-indocarbocyanine perchlorate, DiI, and 3,3′-dioctadecycloxacarbocyanine perchlorate, DiO) into adjacent villi (Sect. 2.3).

Data of those patients from whom the figures in Sect. 4 originate are listed in Table 1.

Table 1 Data of those tumorous patients from whom the neurons depicted in Sect. 4 were derived

Age (years)	Sex	Segment	Oral/anal direction
18	Female	Ileum	Unknown
21	Female	Transverse colon	Known
28	Male	Duodenum	Unknown
41	Male	Duodenum	Known
42	Female	Duodenum	Known
53	Female	Duodenum	Known
54	Male	Sigmoid colon	Known
63	Female	Duodenum	Unknown
65	Female	Jejunum	Unknown
67	Male	Duodenum/jejunum	Unknown
69	Male	Jejunum	Known
70	Male	Descending colon	Known
73	Male	Ileum	Unknown
76	Female	Ileum	Known

2.1 Immunohistochemistry

(Brehmer et al. 2002a, 2002b, 2004a, 2004b, 2004c, 2005, 2006)

Human tissue samples originated from patients undergoing surgery for carcinoma (Table 1). Only tissue obtained from the non-tumour infiltrated borders of the resected gut segments were included, at least 5 cm from the tumour. The use of human tissues for these experiments was approved by the Ethical Committee of the University of Erlangen-Nuremberg. Intestinal segments were transported in physiological saline on ice (pH 7.3) to the laboratory. Upon arrival (1–6 h after resection), they were rinsed in Krebs–Ringer solution at room temperature.

Tissue samples were then transferred to Dulbecco's modified Eagle's medium (DME/F12-Ham, Sigma Chemical Company, St. Louis, MO, USA) containing 10 ml/l antibiotic-antimycotic (Sigma), 50 µg/ml gentamycin (Sigma), 2.5 µg/ml amphotericin B (Sigma), 10% foetal bovine serum (PAA Laboratories), 4 µM nicardipine and 2.1 mg/ml $NaHCO_3$, bubbled with 95% O_2 and 5% CO_2 at 37 °C for 2–5 h. Thereafter, they were incubated overnight in the same medium with 100 µM colchicine added to enhance peptide immunoreactivities in the neuronal somata. For fixation, samples were pinned out on a Sylgard-lined Petri dish and transferred to 4% formalin in 0.1M phosphate buffered saline (PBS; pH 7.4) at room temperature for 2–3 h. After several washes in 0.05 M Tris buffered saline (TBS; pH 7.4), longitudinal muscle/myenteric plexus whole-mounts were prepared.

Specimen incubations included the following steps: Preincubation for 2 h in 0.05 M TBS (pH 7.4) containing 1% bovine serum albumin (BSA), 0.5% Triton X-100, 0.05% thimerosal and 5% normal donkey serum. After rinsing in TBS for 10 min, they were incubated in a solution containing BSA, Triton X-100, thimerosal (see above) and the primary antibodies (Table 2) for 72 h (4 °C). After an overnight rinse in TBS at 4 °C, specimens were incubated in an equivalent solution as for the primary antibodies but with added secondary antibodies (Table 2; room temperature) followed by a rinse in TBS (overnight; 4 °C). To reduce lipofuscin induced autofluorescence, human whole-mounts were incubated in sodium acetate buffer (pH 5.0) containing 1 mM $CuSO_4$ for 60–90 min followed by a short rinse in distilled H_2O (Schnell et al. 1999; Brehmer et al. 2004a). Thereafter, whole-mounts were mounted with TBS-glycerol (1:1; pH 8.6). Whole-mounts incubated in solutions lacking primary antisera (negative controls) showed no staining.

Pig tissue samples (aged between 12 and 15 weeks) were obtained from a slaughterhouse. Duodenal samples were derived from the segment anal to the choledochoduodenal junction, jejunal samples from about 50 cm anal to the duodenojejunal flexure, ileal samples from about 1–1.5 m oral to the ileocecal orifice. Samples were transferred to the laboratory on ice-cold Krebs solution. Further treatment was similar to that described above, except the incubation temperature, which was adjusted to 38.5 °C, the body temperature of juvenile pigs.

Table 2 List of antibodies

Antigen	Host	Dilution	Source
Primary antisera			
Calbindin	Rabbit	1:1500	CB-38; Swant
Calcitonin gene-related peptide	Goat	1:200	1720-9007; Biotrend
Calcitonin gene-related peptide	Rabbit	1:100	11189; Progen
Calretinin	Goat	1:400	CG1; Swant
(common) Choline acetyl transferase	Goat	1:40	AB144P; Chemicon
Galanin	Rabbit	1:1000	T-4326; Bachem
Leu-enkephalin	Rabbit	1:200	4140-0204; Biotrend
Neurofilament	Mouse	1:100	0168; Beckman Coulter
Neurofilament 200	Mouse	1:200	N 0142; Sigma
Neuronal nitric oxide synthase	Guinea pig	1:1000	16059; Progen
Neuronal nitric oxide synthase	Rabbit	1:400	Dr. Mayer, University of Graz
Peripheral choline acetyl transferase	Rabbit	1:4000	Tooyama, Kimura (2000)
Somatostatin	Goat	1:100	7820; Santa Cruz Biotechnology
Somatostatin	Rabbit	1:100	A0566; DAKO
Substance P	Guinea pig	1:200	NB 300-187; Acris
Vasoactive intestinal peptide	Guinea pig	1:500	16071; Progen
Secondary antisera			
ALEXA Fluor 488, donkey anti-mouse		1:1000	A-21202; Mo Bi Tec
ALEXA Fluor 488, donkey anti-rabbit		1:1000	A-21206; Mo Bi Tec
ALEXA Fluor 555, donkey anti-goat		1:1000	A-21432; MoBiTec
ALEXA Fluor 555, donkey anti-rabbit		1:1000	A-31572; Mo Bi Tec
ALEXA, Fluor 647, donkey anti-goat		1:1000	A-21447; Mo Bi Tec
Cy3, donkey anti-goat		1:800	705-165-003; Dianova
Cy3, donkey anti-guinea-pig		1:1000	706-165-148; Dianova
Cy3, donkey anti-rabbit		1:1600	711-165-152; Dianova
Cy5, donkey anti-guinea-pig		1:100	706-175-148; Dianova
Cy5, donkey anti-rabbit		1:100	711-175-152; Dianova
Anti-mouse Ig, biotinylates		1:400	RPN 1001; Amersham
StreptABComplex/HRP		1:1000	K 0377; DAKO
Fab goat anti-rabbit IgG, GP-US		1:20	100.255; Biotrend

2.2 Immunocytochemistry

Ileal specimens from juvenile pigs (see above) were bubbled with carbogen in the above culture medium without colchicine for 2 h (38.5 °C). The fixation was carried out in a PBS solution containing 0.05% glutaraldehyde in addition to 4% paraformaldehyde. Samples were washed in 50% ethanol (four times for 10 min). After washing four times for 10 min with PBS, specimens were incubated in 1% sodiumborohydride in PBS for 1 h. Segments were then washed in PBS and stored overnight in PBS containing 0.05 % thimerosal (4 °C).

Longitudinal muscle/myenteric plexus whole-mounts were prepared; they were incubated in 40% methanol/0.15% H_2O_2 in distilled water (for blocking the endogenous peroxidase). Preincubation was carried out in TBS containing 1% BSA, 0.05% thimerosal and 5% normal donkey serum for 90 min at room temperature. Thereafter, specimens were incubated with primary antibodies against neurofilament(s) (NF; 1:100) and SOM (1:100; Table 2) in TBS containing 1% BSA, 0.05% thimerosal and 0.05% Triton-X 100 for 72 h (4 °C). Specimens were washed for 1 day in TBS (4 °C) and then incubated with biotinylated sheep anti-mouse IgG (1:400; Amersham) dissolved in the same solution as for primary antibodies for 4 h at room temperature. After washing for 1 day, they were incubated for 3 h at room temperature in avidin-biotin horseradish-peroxidase (1:1,000; Dako). Following a rinse in PBS, the diaminobenzidine (DAB) reaction was carried out. The specimens were washed in PBS, viewed under a stereomicroscope for morphological type V neurons, both for single cells and aggregates. These were trimmed into pieces of about 3×3 mm which were preincubated in TBS containing 1% BSA, 0.05% thimerosal, 1% gelatine and 10% normal goat serum, and then incubated in the same solution with added 1:20 gold-labelled (1 nm) goat anti-rabbit IgG (Biotrend) for 3 days at 4 °C. After washing in TBS (containing 1% BSA and 1% gelatine), they were postfixed in 2.5% glutaraldehyde, washed in PBS and treated in 1% OsO_4 for 1 h. Pieces were rinsed in distilled water and silver enhancement was carried out (Aurion). After washing in PBS, pieces were dehydrated through graded ethanol solutions and acetone and flatly embedded in epon (this method is an adaptation of that of Li and Furness 2000). Finally, sections were cut, stained with lead citrate for 15 min, and viewed under an EM 906 (Zeiss).

2.3 Double Labelling Post-Mortem Tracing

(Brehmer et al. 1997, 1999a and unpublished results)

Segments of pig small intestine were fixed under distension in 4% paraformaldehyde in PBS at room temperature for at least 2 h. Thereafter, pieces of the gut wall (about 2×2-cm areas from the region between the mesenteric and antimesenteric part of the circumference) were cut and the fluorescent tracers DiI and DiO (Molecular Probes, Eugene, OR, USA) were applied into two different mucosal villi using micromanipulator guided glass pipettes. The distance between the

two application points was less than 1 mm (Fig. 8). After incubation (5–6 months at 37 °C within the fixative), longitudinal muscle/myenteric plexus wholemounts were prepared. Thereafter, specimens were incubated in the above-mentioned fixative for 6–10 months at 37 °C (Baker and Reese 1993; Honig 1993). For evaluation of the specimens, submucosal as well as longitudinal muscle/myenteric plexus whole-mounts were prepared and viewed.

2.4
Image Acquisition of Immunofluorescent Specimens, Morphometry
(Brehmer et al. 2004a, 2004b, 2004c, 2005, 2006)

Using confocal laser scanning microscopy (Bio-Rad MRC 1000 attached to a Nikon diaphot 300, equipped with a krypton-argon laser, American Laser Corporation, Salt Lake City, UT, USA), extended focus images or single optical section images on the same focus plane were created in the ganglia by applying three different excitation wavelengths. The filter settings were 568 nm excitation/filter 605 DF322 (for Cy3, Alexa 555), 488 nm/522 DF32 (Cy2, Alexa 488) and 647 nm/680 DF322 (Cy5). Whether a 20× dry (numerical aperture 0.75) or a 40× oil immersion objective lens (numerical aperture 1.3) was used, the zoom factor was set between 1.0 and 2.0.

For investigation of lipofuscin autofluorescence only double labelling was carried out. One of the three excitation wavelengths (568 nm; Cy3) remained unoccupied by the corresponding secondary antibody, thus enabling observation of the uncontaminated lipofuscin autofluorescence.

Portraits of ganglia or neurons were prepared using Confocal Assistant 4.02, Adobe Photoshop CS 8.0.1 and CorelDraw 11. For counting reactive neurons in immunohistochemical studies, a transparent plastic sheet was placed over the computer screen and the three corresponding pictures were opened sequentially. Stained neurons were outlined with three differently coloured marker pens. Thereafter, all neurons on the sheet marked by one, two or three colours were counted.

For recording measurements of somata and dendritic fields, a digital camera system (Spot-RT-realtime, Visitron Systems, Munich, Germany) attached to a Leica Aristoplan microscope and SPOT advanced software (Version 3.5.6 for Windows, Diagnostic Instruments, USA) were used.

2.5
Three-Dimensional Reconstructions

A 40× oil immersion objective lens (numerical aperture 1.3) was used to collect the initial data sets of selected neurons, which were scanned at a resolution of 512×768 voxels using 0.6-µm intervals along the optical axis (z-series). Zoom factors varied between 1.5 and 2. Each imaged stack was resized to a power of 2, here 512×512 voxels (ImageJ 1.33), and deconvolved separately after axial intensity drop correction (z-drop) using the standard, non-blind deconvoluted from the amiraDeconv package (TGS, Inc.). Generally, the optical point spread function smears the image along

the optical axis about three times that in the x- and y-direction as shown by Turner et al. (1997, 2000). Since deconvolution compensates for the microscope's point spread function, a substantial spread effect is still noticeable. A compression procedure of 3 to 1 in the z-direction almost eliminates the residual spread effect along the optical axis. We performed this z-compression decreasing the voxel-depth by a factor of 3 once we applied an auto-adjustment for brightness and contrast to the whole volume followed by a conversion of the image stack into single images (ImageJ 1.33). The received single optical sections were loaded into Reconstruct 1.0.5.0 (Fiala 2005) and further processed with quantitative analyses regarding surface and volume parameters. Therefore, a semi-automated user-guided segmentation tracing the outline of the cell using Reconstruct's Wildfire region growing tool was carried out, saving the traces within the original data. Finally, the whole image stack was loaded into 3D Slicer software (Pieper et al. 2004) where an automated segmentation orientated on the previous traces was performed. Three dimensional surface models were generated using the ModelMaker module within Slicer and exported as high resolution TIF-files.

3
Chemical Coding of Stach's Neuron Types in the Pig

3.1
Preliminary Note: Cholinergic and Nitrergic Neurons

It has been shown previously that the commonly used antibodies against choline acetyl transferase (ChAT), which gave efficient results in labelling central cholinergic neurons, resulted in rather weak staining in the ENS of various species (Furness et al. 1983a; Schemann et al. 1993; Porter et al. 1996; Sang and Young 1998; Hens et al. 2000). Recently, a novel antibody against a peripheral form of ChAT has been characterized, the peripheral ChAT (pChAT; Tooyama and Kimura 2000) in contrast to the common ChAT (cChAT). The forms of ChAT differ in their mRNA; however, the pChAT protein displays regions which are suspected to be essential for its catalytic function. It has been demonstrated that the newly raised antibody against pChAT did not stain brain regions of known cholinergic nature but revealed a number of peripheral, including enteric, neurons (Tooyama and Kimura 2000; Nakajima et al. 2000). In the guinea pig ENS, Chiocchetti et al. (2003) found that one or the other form of ChAT is preferentially expressed in cholinergic neurons, i.e. submucosal CAR-reactive neurons are cChAT- but not pChAT-reactive whereas submucosal primary afferent neurons displayed strong pChAT but only weak or even absent cChAT reactivity. Our own results suggest that neither cChAT- nor pChAT-antibodies alone are sufficient for staining the whole cholinergic population in the pig (Sects. 3.2, 3.4, 3.7) and, that there are at least two morphologically defined populations (type III and VI neurons; Sects. 3.4, 3.7) displaying immunoreactivity for both one form of ChAT and neuronal nitric oxide synthase (nNOS).

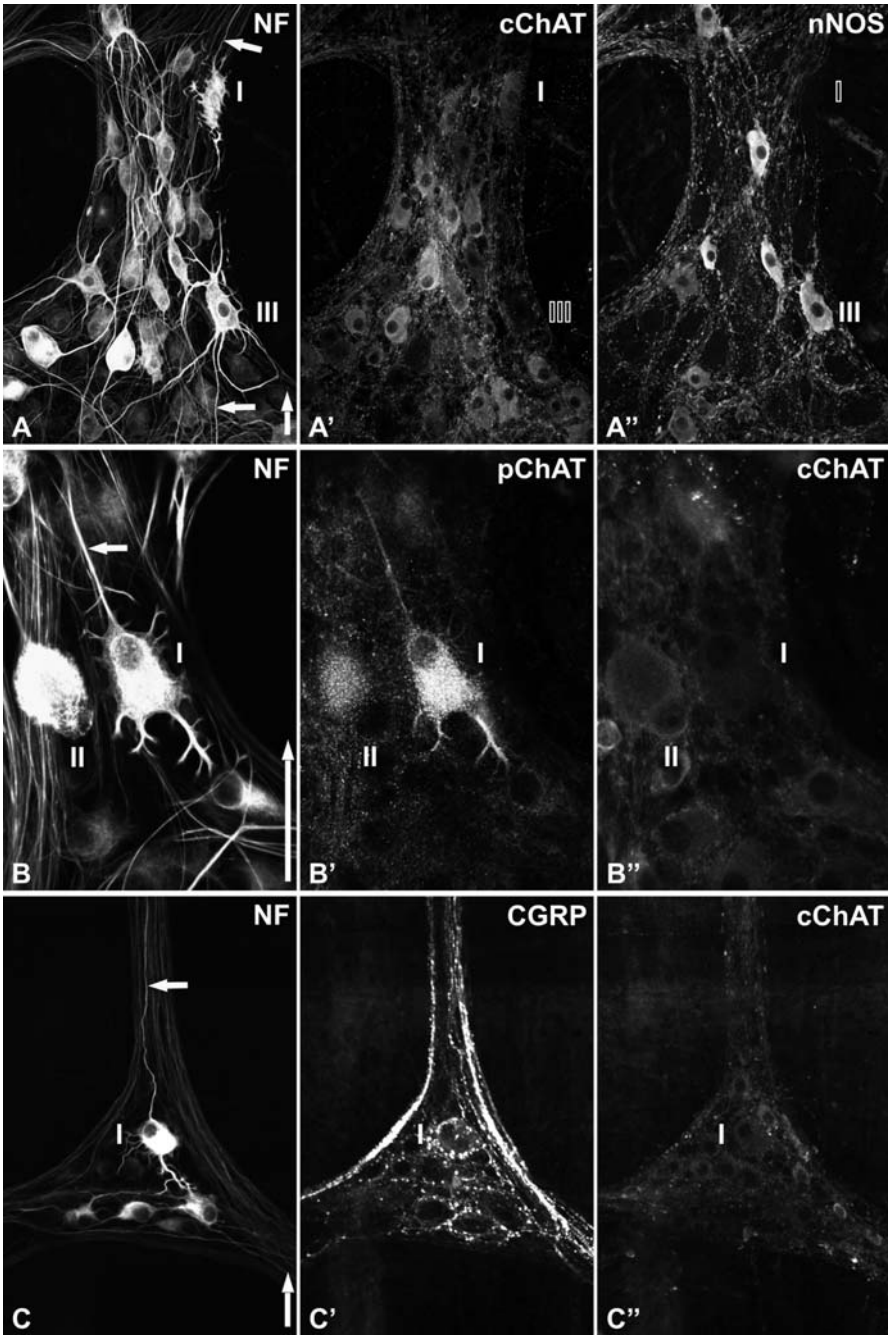
There are also different splice variants of nNOS (Saur et al. 2000); however, we have not yet been able to differentiate them immunohistochemically.

3.2 Type I Neurons

Stach (1980, 1989) described these neurons as multidendritic and uniaxonal. The majority of the axons of these neurons ran orally in the plane of the myenteric plexus as long as they could be followed, for up to 5 cm. The dendrites were mostly short, their endings frequently lamellar. This latter term, used only once by Dogiel in his last paper (1899), has been more strongly emphasized by Lawrentjew (1929), based on observations in the dog. With this morphological definition (short, lamellar dendritic/orally running axons), the population of Stach's type I neurons (in the pig) has quantitatively been narrowed down in contrast to Dogiel's type I neurons. This is an important distinction because we, in the following, meant Stach's type I neurons when dealing with the term type I (in the pig) whereas other authors have used this term for Dogiel's type I neurons (in various species). In the pig, a number of short dendritic, uniaxonal neurons were—by Stach and colleagues—not included in type I because of different dendritic shapes and axonal course (i.e. type IV neurons, Sect. 3.5).

In the small intestine of the pig, Stach type I neurons occur solely in the myenteric, but not in the submucosal plexus. In two different studies, using combined nicotinamide adenine dinucleotide phosphate diaphorase(NADPHd) reaction and silver impregnation (Brehmer and Stach 1997) as well as immunohistochemistry (Brehmer et al. 2004c), we found that Stach type I neurons were non-nitrergic (Figs. 8A, Fig. 6B). In the latter study, we demonstrated that these neurons may be cholinergic. In the pig ileal myenteric plexus, 52.5% of Stach type I neurons investigated displayed co-immunoreactivity for both forms of ChAT, 43.3% were positive only for pChAT whereas 4.2% were positive only for cChAT (Fig. 4B). We found no type I neurons simultaneously negative for both forms of ChAT. Further substances that could be demonstrated to be present in sub-

Fig. 4 A–C Type I neurons in the pig small intestine. **A** A myenteric ganglion of pig duodenum stained for neurofilaments (*NF*), common choline acetyl transferase (*cChAT*; **A'**) and neuronal nitric oxide synthase (*nNOS*; **A"**). The type I neuron with short, partly lamellar dendrites (*I*; *arrow* depicts axon) is positive for cChAT (**A'**) but negative for nNOS (**A"**). In contrast, the type III neuron with long, slender dendrites (*III*; *arrow* indicates axon) is negative for cChAT (**A'**) but positive for nNOS (**A"**). Note the different directions of axonal projection of the two marked neurons. **B** A myenteric type I neuron of pig ileum with short, partly lamellar dendrites (*I*; *arrow* indicates axon) is positive for both peripheral choline acetyl transferase (*pChAT*; **B'**) and cChAT (**B"**). A pseudouniaxonal type II neuron (*II*) is also positive for both enzymes. **C** A myenteric type I neuron of pig ileum (*I*; *arrow* indicates axon) is positive for calcitonin gene-related peptide (*CGRP;* **C'**) and cChAT (**C"**). *Arrowed scale bars* (50 μm) point orally in **A**, **B** and **C**


NF
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III
A
cChAT
I
III
A'
nNOS
I
III
A''
NF
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B
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II
B'
cChAT
I
II
B''
NF
I
C
CGRP
I
C'
cChAT
I
C''

populations of Stach type I neurons are enkephalin (ENK, 78%), substance P (SP, 24%) and calcitonin gene-related peptide (CGRP, 21%; Fig. 4C; all unpublished results).

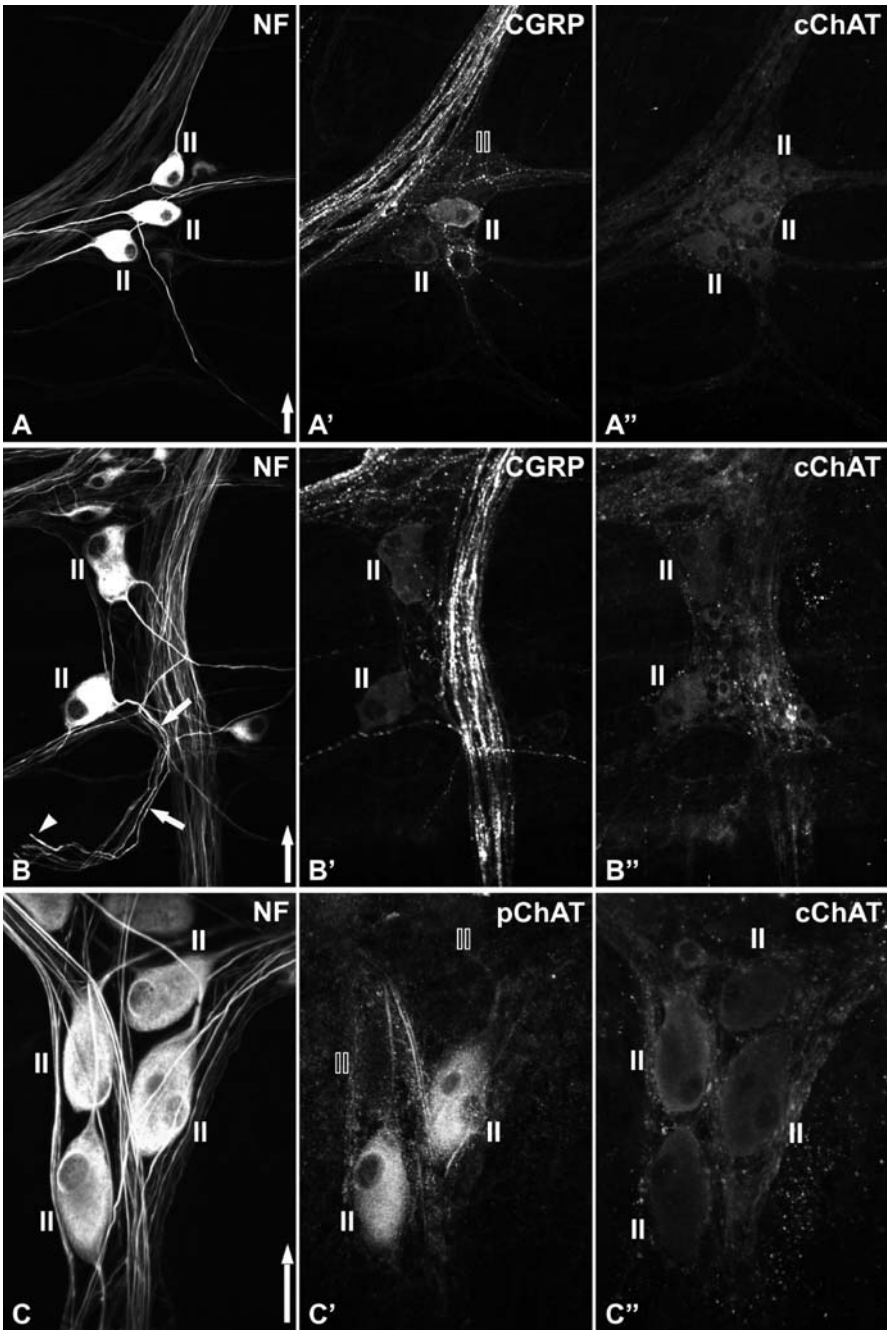
In two different approaches, we attempted to estimate the proportions of morphological neuron types in relation to the putative whole myenteric population. In a silver impregnation study (Brehmer and Stach 1998) we counted the neurons and the numbers of stained nucleoli of all (also those of unstained) neurons. In a later study (Brehmer et al. 2002a), we combined immunohistochemistry for NF with the histochemical cuprolinic blue (CuB) technique which is a presently accepted pan-neuronal marker (Phillips et al. 2004). The proportion of ileal Stach type I neurons ranged between 1.7% (Brehmer et al. 2002a) and 3.6% (Brehmer and Stach 1998). Although they are present also in other gastrointestinal regions, e.g. in the large intestine, this has not been thoroughly investigated.

ENK- and/or SP-positive type I neurons may be ascending inter- or (excitatory) motor neurons. Interestingly, Hens et al. (2002) found that ENK/SP co-reactive neurons project orally to the longitudinal musculature whereas ENK-positive (SP-negative) neurons project anally and SP-positive (ENK-negative) neurons have no polarized projection patterns. Cholinergic, SP-reactive neurons in the pig are regarded as excitatory circular muscle motor neurons (Timmermans et al. 2001; Brown and Timmermans 2004).

3.3
Type II Neurons

In contrast to Dogiel (1899), Stach (1981, 1989) did not differentiate the processes of type II neurons into one axon and several dendrites. In the small intestinal myenteric plexus of the pig, these neurons were defined as non-dendritic and multiaxonal neurons. They have smoothly contoured cell bodies and are located frequently at the borders of ganglia or, singly or in clusters, outside the main ganglia. All processes of type II neurons, including their branches, leave the ganglion of origin and display a uniform calibre as long as they can be traced (Fig. 4A). Consequently, based on their histological appearance and course beyond ganglionic boundaries, they were all regarded as axons (Stach 1981). This morphological view

Fig. 5 A–C Myenteric, non-dendritic type II neurons in the pig small intestine. **A** Three type II neurons (*II*) stained for neurofilaments (*NF*). All processes are axons. The two lower neurons are positive for calcitonin gene-related peptide (*CGRP*), the upper neuron is negative for CGRP (*empty II*; **A'**) whereas all three cells are positive for the common choline acetyl transferase (*cChAT*; **A"**). **B** Two type II neurons (*II*) triple-stained for NF (**B**), CGRP (**B'**) and cChAT (**B"**). Note that one axon of the lower type II neuron (marked by two axons) enters the disrupted interconnecting strand (*arrowhead*). **C** Of the four NF-reactive type II neurons in **A**, two are positive (*II*) and two are negative (*empty II*) for the peripheral choline acetyl transferase (*pChAT*; **C'**) whereas all neurons are positive for cChAT (**C"**; *II*). *Arrowed scale bars* (50 μm) point orally in **A**, **B** and **C**


NF
CGRP
cChAT
II
A
A'
A''
B
B'
B''
pChAT
C
C'
C''

has later been confirmed by electrophysiological experiments in type II neurons of the guinea pig (Hendriks et al. 1990; Furness et al. 2004a).

Principally, most of the axons of type II neurons run in two ways. Either, circumferentially in secondary strands of the myenteric plexus or, vertically in interconnecting strands which are directed to the submucosa and are disrupted in routine wholemounts which contain only the longitudinal musculature and the adhering myenteric plexus (Fig. 5B). In special whole-mounts, which additionally contained the circular muscle and the outermost part of the submucosa, we showed that these strands cross the circular muscle layer and join the external submucous plexus (Stach 1983; Brehmer et al. 1998). Thus, they are not a main source for nerve fibres to the circular muscle plexus. In a post-mortem tracing study, we applied DiI crystals into single villi of the pig small intestinal mucosa (Brehmer et al. 1999a). After incubation of several months, we found that within all three ganglionated plexuses, non-dendritic, multiaxonal type II neurons were labelled. In the myenteric plexus, one of their axons could frequently be followed from a disrupted interconnecting strand to the perikaryon whereas other axons of the same cell body entered a secondary strand and took a course parallel to the circular muscle bundles. Thus, type II neurons of all three plexuses project with one of their axons into mucosal villi.

The unique shape of type II neurons, i.e. the large, smooth cell bodies and the multiple long processes, together with their frequent extraganglionic myenteric position enabled Scheuermann et al. (1987) to identify type II neurons of the pig in a single staining immunohistochemical study for CGRP. Since that time, this peptide has been considered as marker peptide for pig type II neurons. This chemical coding has been completed by the demonstration of cChAT (Hens et al. 2000) and pChAT (Brehmer et al. 2004c) in type II neurons (Fig. 5A,B). The former was a combined tracing and immunohistochemical study corroborating the projection pattern (into mucosal villi) of type II neurons by their chemical identification, the latter showed that 94.2% of myenteric type II neurons contain both cChAT and pChAT whereas 5.8% contain only cChAT (Fig. 5C). However, it has been shown that at least two other neuron types in the pig displayed ChAT and CGRP reactivity (type I and V; Figs. 4C, Fig. 9C). Thus, this is not a specific chemical code of type II neurons. On the other hand, not all type II neurons are reactive for CGRP (Fig. 5A). Besides CGRP, some myenteric type II neurons also contain vasoactive intestinal peptide (VIP), some submucosal type II neurons were also shown to contain VIP, SP, galanin (GAL) or neuromedin U (NMU; Timmermans et al. 1989; Scheuermann et al. 1991). In contrast to some other morphological types which were hard to visualize by methods other than silver impregnation and NF-immunohistochemistry, type II neurons could also be represented by shape using various immunohistochemical markers including neuron-specific enolase and intermediate filaments (Scheuermann et al. 1989; Krammer and Kühnel 1992; Krammer et al. 1993b) as well as by the nicotinamide dinucleotide technique (Scheuermann and Stach 1983).

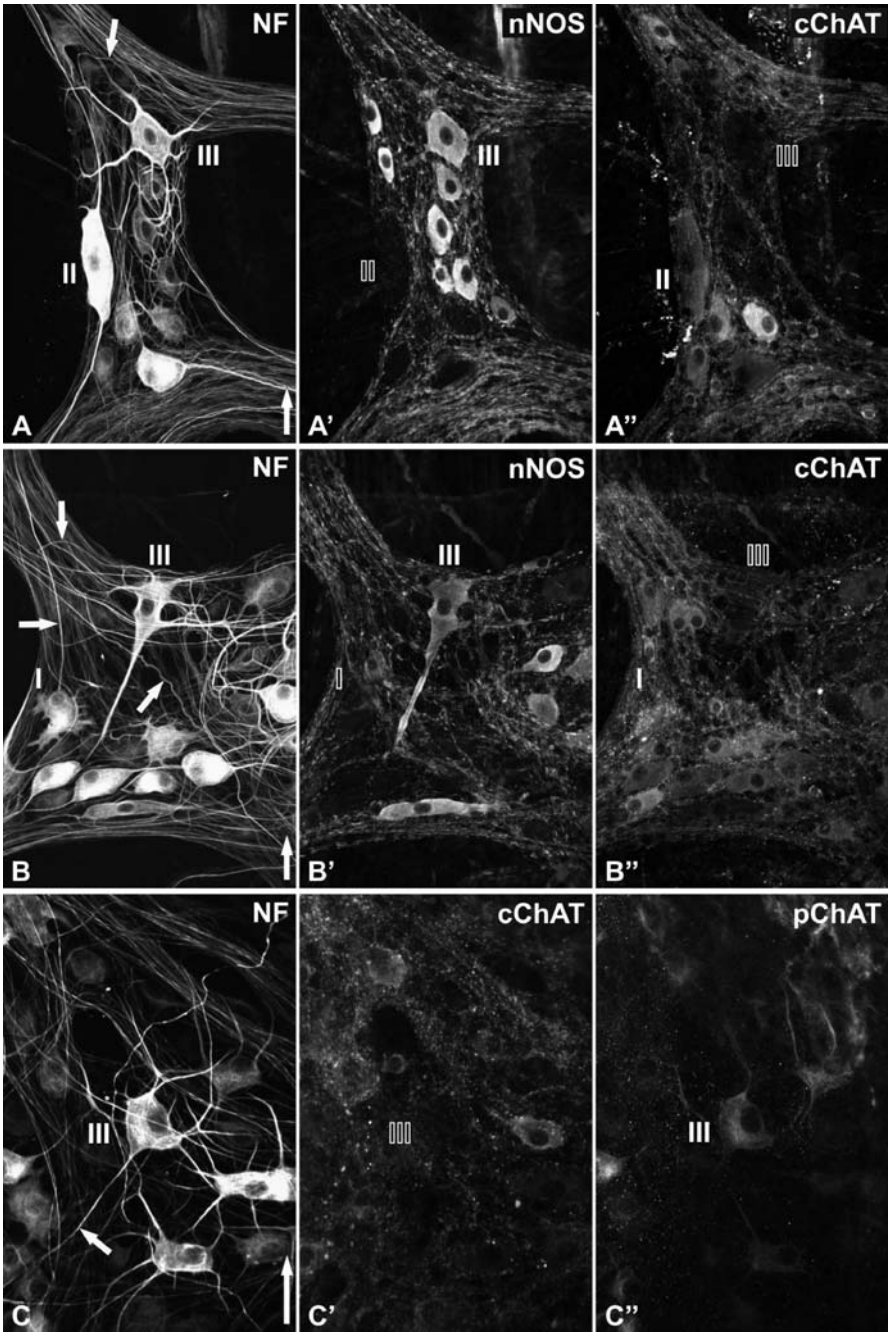
The proportion of type II neurons in relation to the whole ileal myenteric neuron number ranged between 6.5% (silver impregnation; Brehmer and Stach 1998) and 7.9% (NF-immunohistochemistry/CuB; Brehmer et al. 2002a).

Although there are interspecies differences concerning the chemical coding and electrophysiological properties between guinea pig, pig and other species (Cornelissen et al. 2000, 2001; Furness et al. 2004a, 2004b; Nurgali et al. 2004), type II neurons in the pig are regarded as IPANs (Timmermans et al. 2001; Brown and Timmermans 2004). These assumptions are based mainly on the morphology and projections of the neurons (Brehmer et al. 2004b).

3.4 Type III Neurons

Similar to type I neurons, these nerve cells are also multidendritic and uniaxonal (Stach 1982a). They have long, slender and branched dendrites. In the pig duodenum and jejunum, these neurons are very conspicuous in the myenteric and the external submucous plexus. In jejunal myenteric ganglia, the proportion of type III neurons ranged between 5.3% and 7% whereas in ileal ganglia no more than 0.5% could be found (Brehmer and Stach 1998). In myenteric ganglia, they are located in clusters within their central and anal parts. In the external submucosal ganglia, type III neuron cell clusters are typically located oppositely to groups of type II neurons (Stach 1977b). The majority of the axons of myenteric type III neurons ran anally within the myenteric plexus whereas those of the external submucosal type III neurons rather entered interconnecting strands to the myenteric plexus (Stach 1983; Brehmer et al. 1998). In contrast to the oral parts of the small intestine, the terminal ileum is almost devoid of typical type III neurons. Since our later NF-immunohistochemical studies in the pig were carried out mainly in the ileum, we did not refer to type III neurons here (Brehmer et al. 2002a, 2004c). In combined NADPHd-/silver impregnation studies (Brehmer and Stach 1997; Brehmer et al. 1998) we found that most but not all neurons displaying type III-like morphology were nitrergic (Brehmer and Stach 1997; Fig. 4A,B). This has been confirmed by unpublished results using triple immunohistochemistry. Furthermore, these experiments have shown that type III neurons are negative for cChAT but positive for pChAT (Figs. 6A–C, 4A, 14B). In this respect, they display similar chemical characteristics to type VI neurons (Sect. 3.7). However, both morphologically and

Fig. 6 A–C Myenteric type III neurons in the pig duodenum. **A** A neurofilament (*NF*)-reactive type III neuron with long, tapering dendrites (*III*; *arrow* indicates axon) is positive for neuronal nitric oxide synthase (*nNOS*; **A'**) but negative for the common choline acetyl transferase (*cChAT*; **A"**; *empty III*). In contrast, a non-dendritic type II neuron (*II* in **A**) is negative for nNOS (**A'**) but positive for cChAT (**A"**). **B** A type III neuron (*III*; the axon firstly runs to the left and turns into the anal direction; indicated by *two arrows*) is positive for nNOS (**B'**) and negative for cChAT (**B"**; *empty III*). In contrast, a type I neuron (I; *arrow* indicates axon) is negative for nNOS (**B'**; *empty I*) but positive for cChAT (**B"**; *I*). **C** A type III neuron (III; *arrow* indicates axon) is negative for cChAT (**C'**; *empty III*) but positive for the peripheral choline acetyl transferase (pChAT; **C'**). *Arrowed scale bars* (50 μm) point orally in **A**, **B** and **C**


NF
nNOS
cChAT
III
II
A
A'
A''
NF
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I
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B'
B''
NF
cChAT
pChAT
III
C
C'
C''

chemically, the population of type III neurons as presently defined, seems to be heterogeneous. Immunohistochemically, both serotonin (Scheuermann et al. 1991) and nNOS, but no co-localization of the substances could be demonstrated in type III-like neurons (Timmermans et al. 1994).

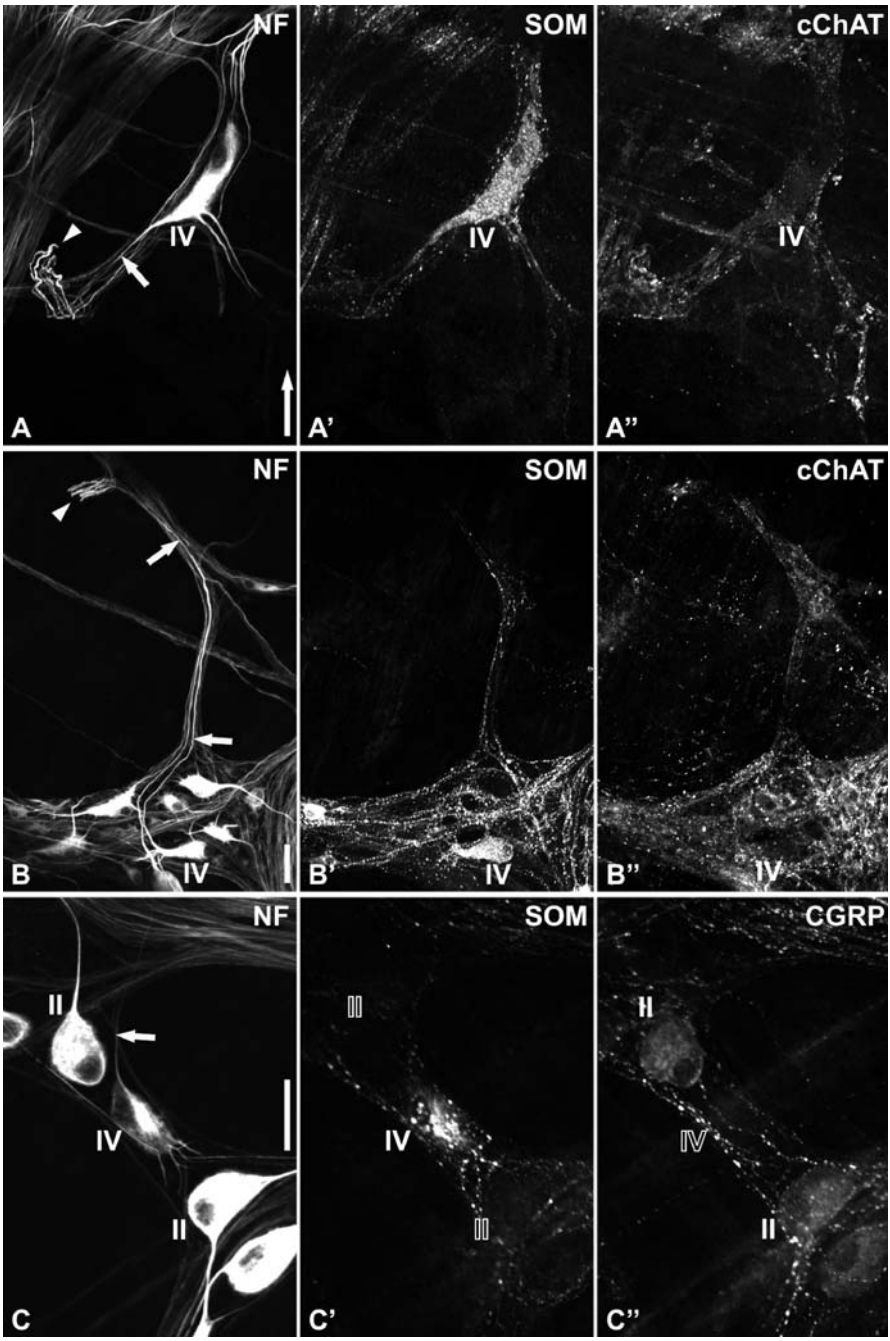
Besides type III neurons projecting longitudinally (mostly anally) within the myenteric plexus, Timmermans et al. (1993) demonstrated intestinofugal neurons with type III morphology projecting to prevertebral ganglia. These were partly immunoreactive for serotonin.

Among neurons with type III morphology, there may be both descending inter- and motor neurons (Brown and Timmermans 2004). As mentioned above, further combined morpho-chemical studies are needed to decipher subpopulations of neurons which are presently classified as type III.

3.5 Type IV Neurons

These neurons were defined as multidendritic, uniaxonal neurons (as are type I and III neurons), displaying mainly short dendrites (as type I neurons; for quantitative explanation of the term 'short' see Sect. 3.6) with tip endings (as type III neurons) and axons running into disrupted interconnecting strands (Fig. 5A,B) indicating a vertical projection pattern (which is different from other myenteric, dendritic neuron types hitherto described; Stach 1982b, 1989). Furthermore, the dendritic origins were not evenly distributed around the circumference of the somata (Fig. 7A–C). This feature has been termed a 'polar' dendritic emergence, in contrast to the circumferential or 'radial' emergence pattern of the dendrites of type I or III neurons. The position of the nucleus within the soma of type IV neurons was, in contrast to that of type I and III neurons, typically eccentric. Already in the 1930s, it was observed that the position of enteric neuronal nuclei are frequently eccentric and it has been supposed that this is a non-pathological feature (Ito 1936; Ito and Nagahiro 1937). Stach (1982b) described this feature as typical for special neuron types (see also type V neurons: Sect. 3.6). The frequency of type IV neurons in the upper small intestine is between 1.9% and 2.2%, in the ileum 3.2% (silver impregna-

Fig. 7 A–C Myenteric type IV neurons in the pig ileum. **A** A type IV neuron with few short, not branched, tapering dendrites (*IV*). The axon (*arrow*) enters a disrupted interconnecting strand (*arrowhead*). The neuron is co-immunoreactive for both somatostatin (*SOM*; **A'**) and the common choline acetyl transferase (*cChAT*; **A"**). **B** A type IV neuron is co-reactive for both SOM (**B'**) and cChAT (**B"**; weak reactivity, only detectable in the part of the soma from which the axon emerges). In **B**, the axon (indicated by *two arrows*) runs for several hundred micrometres before entering a disrupted interconnecting strand (*arrowhead*). **C** A type IV neuron (*arrow* indicates axon) is positive for SOM (**C'**) but negative for calcitonin gene-related peptide (*CGRP*; **C"**; *empty IV)*. In contrast, two type II neurons are negative for SOM (**C'**; *empty II*) but positive for calcitonin gene-related peptide (*CGRP*; **C"**). *Scale bars* indicate 50 μm, the *arrowed bar* in **A** is oriented orally


NF
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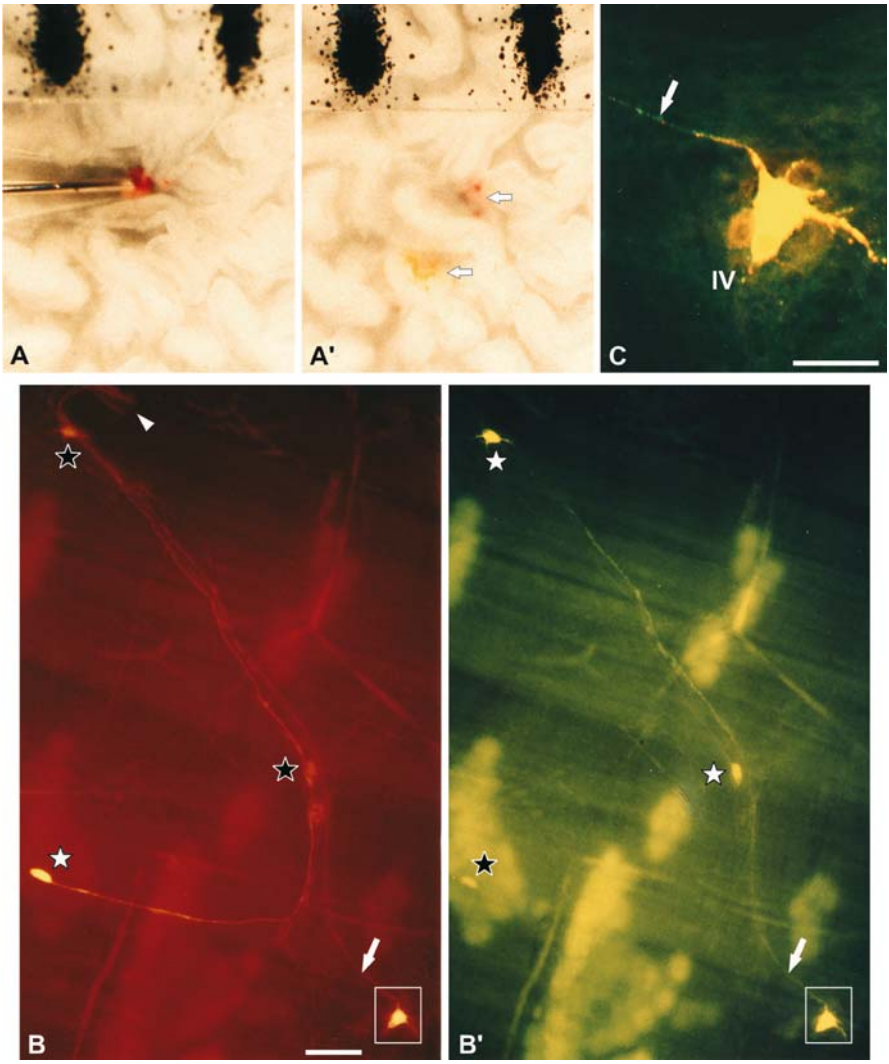


Fig. 8 A–C Application of a DiI crystal (**A**) and situation after the application of both DiI (*red*) and DiO (*yellow*; both indicated by *two arrows* in **A'**) into different villi (distance between two bars = 1 mm; light microscopic illumination). An area of the myenteric plexus excited for DiI (**B**) and DiO (**B'**) as viewed under conventional fluorescence microscopy. Neurons marked with *filled asterisks* are specifically labelled but can be detected using the other excitation wavelength as well (*empty asterisks*). Using confocal microscopy, these neurons could be demonstrated by one excitation wavelength only, these were only singly labelled. The neuron within the box (*arrow* indicates axon) in **B** and **B'** displayed specific fluorescence in both excitation wave lengths. This neuron is represented with a merged *colour* (*yellow* in **C**; *arrow* indicates axon). Morphologically, this is a type IV neuron (*arrowhead* in **B** indicates disrupted interconnecting strand). *Scale bar* in **B** is 50 μm, *scale bar* in **C** is 100 μm

tion; Brehmer and Stach 1998). The values for the ileum, applying NF immunohistochemistry combined with CuB, were between 1.9% and 2.4% (Brehmer et al. 2002a).

In the special whole-mounts mentioned above, axons of type IV neurons ran together with those of type II neurons through interconnecting strands. In a post-mortem DiI tracing study, we showed that both myenteric type II and type IV neurons project into mucosal villi (Brehmer et al. 1999a). By applying two differently fluorescent tracers (DiI and DiO) into two adjacent villi of fixed intestinal segments, some myenteric type IV neurons were found to be labelled by both tracers (Fig. 9; Brehmer 2000). Thus, it seems likely, that axons of type IV neurons divide and supply more than one mucosal villus. This may be indicative of a structurally coded basis for the coordinated control of adjacent mucosal areas. Similar observations made by using different methodical approaches have been made in the guinea pig (Furness et al. 1985; Song et al. 1992).

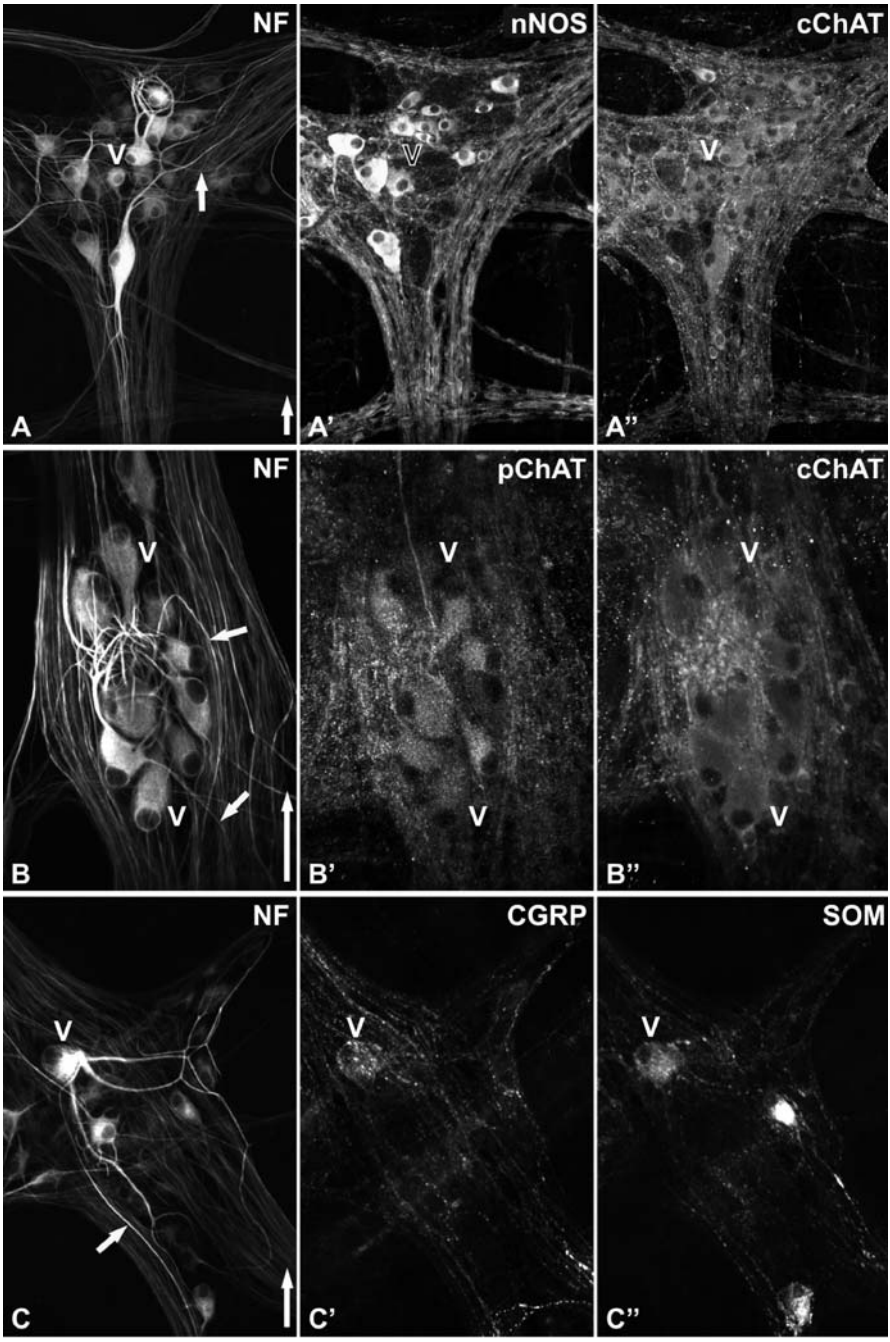
As to their chemical coding, Hens et al. (2000), by applying supravital tracing with subsequent immunohistochemistry, demonstrated that myenteric neurons resembling type IV neurons showed immunoreactivity for SOM and cChAT (Fig. 7A,B). They are, in contrast to type II and V neurons, negative for CGRP (Fig. 7C). Additionally, we found that type IV neurons express, almost without exception, co-reactivity for both pChAT and cChAT (Brehmer et al. 2004c). Besides that, they are non-nitrergic (Brehmer and Stach 1997; Brehmer et al. 2004c).

In addition to pig type II neurons, type IV neurons are the only known population projecting from the myenteric plexus to the mucosa (Brehmer et al. 1999a; Hens et al. 2000). In contrast to putative intrinsic primary afferent type II neurons, cholinergic, SOM-reactive type IV neurons may have a secretomotor function (Timmermans et al. 2001; Brown and Timmermans 2004). This is the case for type IV neurons in the guinea pig small intestine (Furness et al. 2003).

3.6 Type V Neurons

This very peculiar neuron population exists in two forms, as single type V neurons and as aggregate type V neurons (Stach 1985, 1989). Similar to type I, III and IV neu-

Fig. 9 A–C Myenteric type V neurons in the pig ileum. **A** The marking (*V*) indicates one of two neurons of a type V aggregate, the other neuron is less well stained with neurofilaments (*NF*) and is situated above and to the right of the marked type V neuron (*arrow* indicates axon). The marked neuron has two long and branched dendrites surrounding another neuron and is immunonegative for neuronal nitric oxide synthase (*nNOS*; **A'**; *empty V*) but positive for common choline acetyl transferase (*cChAT*; **A"**). **B** A type V aggregate consisting of nine neurons (the upper and most lower neuron is marked with *V*; two of the axons are marked with *arrows*). All neurons are positive for both the peripheral and the common choline acetyl transferase (*pChAT*, *cChAT*; **B'**, **B"**). **C** A single type V neuron with three long, branched dendrites (*V*; *arrow* indicates axon) is co-immunoreactive for both calcitonin gene-related peptide (*CGRP*;**C'**) and somatostatin (*SOM*; **C"**). *Arrowed scale bars* (50 μm) point orally in **A**, **B** and **C**


NF
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NF
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C"

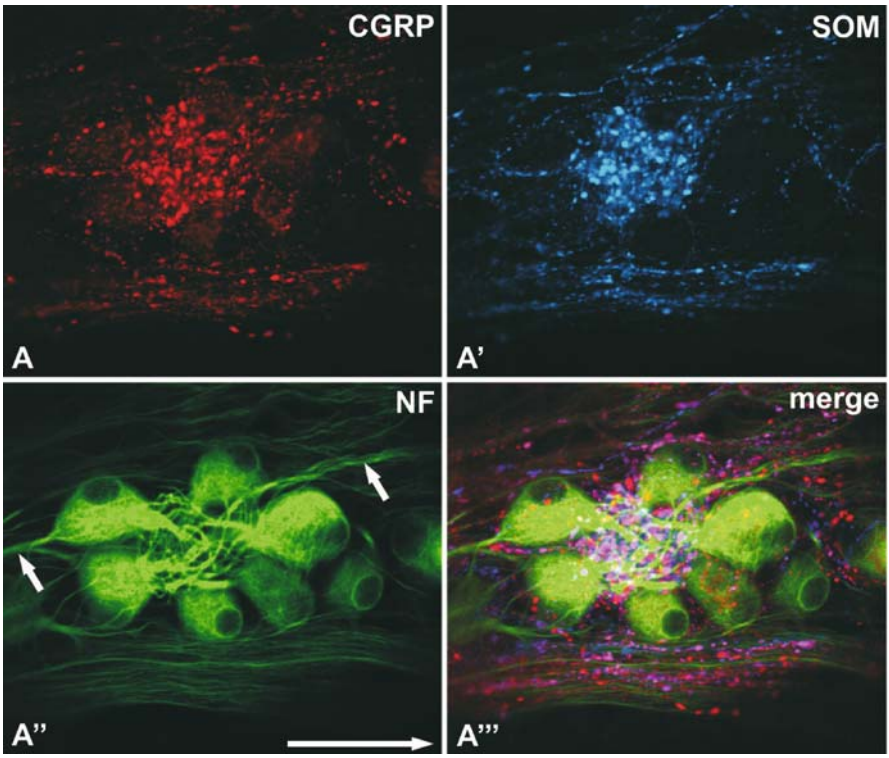
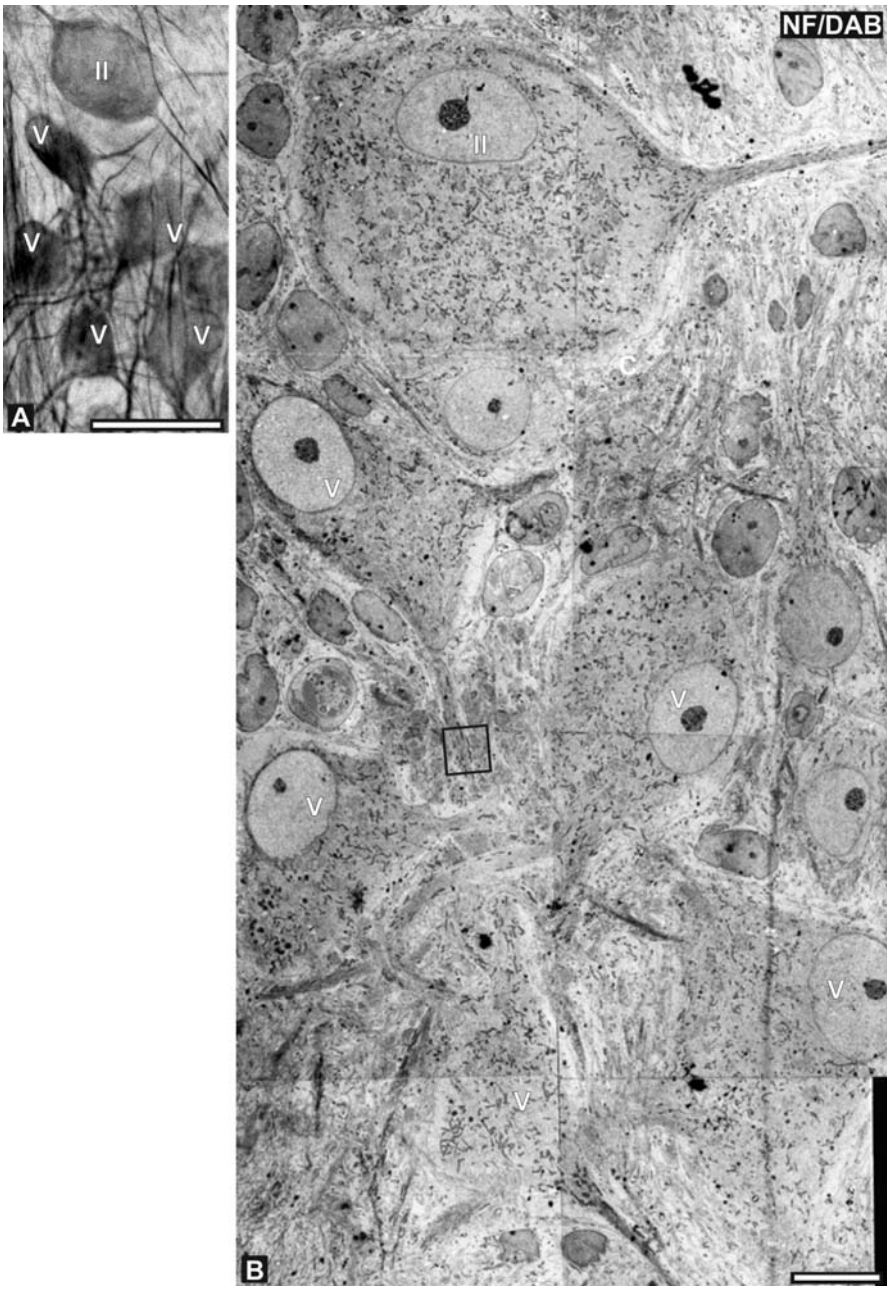


Fig. 10 Chemical coding of boutons apposing the dendritic tangle of a type V neuron aggregate. All four images are single optical sections through the aggregate. The boutons are co-immunoreactive for both calcitonin gene-related peptide (*CGRP*; *red* in **A**) and somatostatin (*SOM*; *blue* in **A'**). Since the material was not treated with colchicine prior to fixation, the reactivity for CGRP and SOM within the somata is weak or even absent. In **A''**, the neurofilament (*NF*)-stained neurons and their dendritic tangle are seen. In the merged picture (**A'''**), the close topographical correlation between the numerous boutons (*mixed colour: purple*) and the dendritic tangle is obvious. *Arrowed scale bar* (50 μm) points orally

rons, they have been defined as multidendritic and uniaxonal. However, frequently the 'multi'dendritic appearance is reduced to very few, sometimes only one, albeit very long and extensively branched dendrite(s) (Fig. 14A,C). The axonal projection pattern of type V neurons is mainly directed anally. They display, similar to type IV neurons, a polar arrangement of their dendrites and an eccentric nucleus. Their

Fig. 11 A,B A type V neuron aggregate consisting of five neurons, stained for neurofilaments (*NF*) and visualized by DAB reaction, has been trimmed under the light microscope (5× V in **A**; a type II neuron is additionally marked) and subsequently viewed under the electron microscope (5× V in **B**; the type II neuron is additionally marked). The *box* indicates the area which is magnified in Fig. 12A. *Scale bar* in **A** is 50 μm, *scale bar* in **B** is 10 μm


II
V
V
V
V
V
A
NF/DAB
II
V
V
V
V
V
B

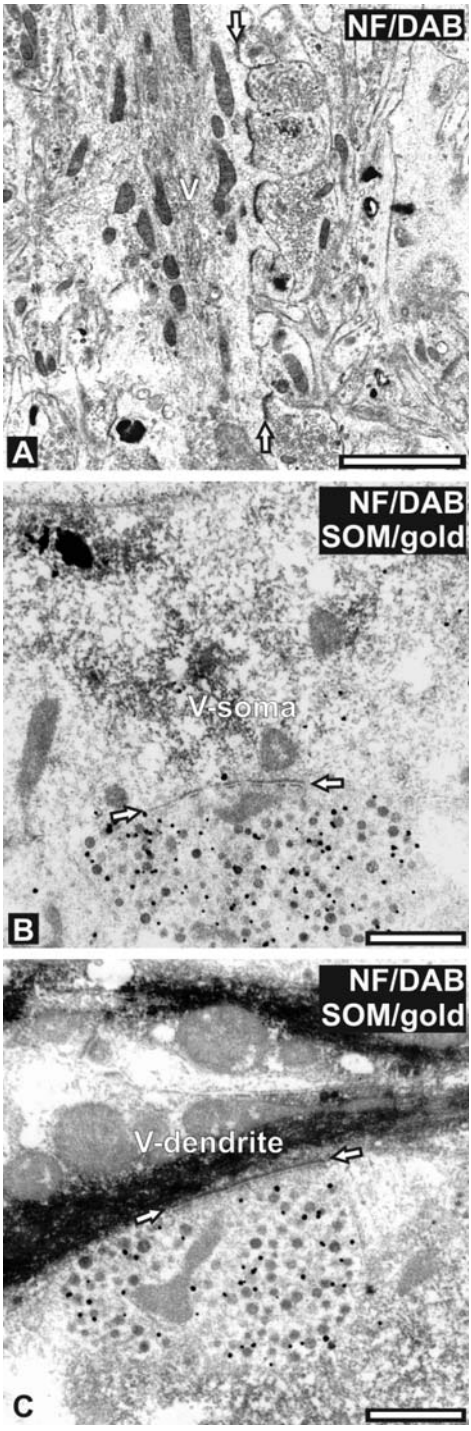
preferred location along the pig small intestine is the ileum. Here, their proportion has been estimated to be 2.3% (silver impregnation; Brehmer and Stach 1989) and 1.1% (NF-immunohistochemistry/CuB; Brehmer et al. 2002a). In contrast, in the jejunum their frequency was between 0.4% and 0.7% (Brehmer and Stach 1998).

A very conspicuous morphological feature of ileal myenteric ganglia are the type V neuron aggregates (Figs. 9B, 10, 11). These are accumulations of 2–12 (seldom even more) type V neurons. Their dendrites extend mostly towards the centre of the aggregates where they form tangles, whereas the somata, typically with peripherally displaced nuclei, are located circumferentially around the tangles. Among their other morphological characteristics, single type V neurons have some similarities to both type IV and type II neurons.

In a morphometric study, we attempted to provide objective information on the differences in dendritic architecture between type IV and single type V neurons (Brehmer and Beleites 1996). Ileal myenteric neurons displaying a polar emergence pattern of dendrites and an eccentric position of the nucleus, were divided into two groups based on their axonal course (type IV into interconnecting strands leaving the myenteric plexus, type V anally within the myenteric plexus). Using a computer-aided morphometric program, we estimated somal areas and diameters as well as the following dendritic parameters: numbers of primary dendrites, single dendritic lengths, numbers of dendritic branching points and numbers of terminal segments. We found that the longest dendrite of a type V neuron (the 'prominent' dendrite) including all of its branches, is many times longer than the corresponding somal diameter whereas, in type IV neurons, the longest dendrite is at most twice the somal diameter. In the pig, single type V neurons may have two or three prominent dendrites. Furthermore, we observed that, according to the single dendritic lengths which we arranged in histograms, dendrites of type V neurons displayed two frequency peaks. We assumed that type V neurons may have two populations of dendrites, a feature probably important for their functional role.

Due to their few but long processes and their smoothly contoured somal outlines, type V and type II neurons display some similarities in shape. This similarity in shape is historically illustrated in both human, by Cavazzana and Borsetto (1948: Fig. 21), and pig, by Gunn (1968: Fig. 15), who depicted type V neurons but misidentified them as type II neurons. The morphological decision of whether

Fig. 12 A–C Synapses on type V neurons. **A** This is the magnified view of the box in Fig. 11B. There is a dendrite of a type V neuron which is synaptically contacted by several boutons (*zone between the two arrows*). **B** A bouton immunoreactive for somatostatin (*SOM*) visualized by colloidal gold is in synaptic contact with a type V neuronal soma (identified by double staining for neurofilaments (*NF*)/diamino benzidine (*DAB*) reaction). The postsynaptic membrane is thicker than the presynaptic one (*zone between the two arrows*). **C** A bouton immunoreactive for SOM visualized by colloidal gold is in synaptic contact with a type V dendrite (identified by double staining for NF/DAB). The postsynaptic membrane is thicker than the presynaptic one (*zone between the two arrows*). *Scale bar* in **A** is 2 μm, *scale bars* in **B**, **C** are 1 μm


NF/DAB
V
A
NF/DAB
SOM/gold
V-soma
B
NF/DAB
SOM/gold
V-dendrite
C

a process is an axon or a dendrite is thus crucial for the differentiation of type II and type V neurons.

The significance of detailed morpho-chemical analyses has been emphasized by two multilabel immunohistochemical studies. Previously, all CGRP-immunoreactive neurons in the pig were considered to represent non-dendritic type II neurons due to their smoothly contoured cell bodies (Scheuermann et al. 1987; Hens et al. 2000). Using combined CGRP/NF/cChAT immunohistochemistry, we found that CGRP/NF/cChAT-co-reactive neurons with smoothly contoured outlines and few, long processes could morphologically be subdivided into type II (non-dendritic, multiaxonal neurons projecting circumferentially and vertically) and type V neurons (dendritic, uniaxonal neurons projecting anally; Figs. 9, 5, 7). From this virtual discrepancy, i.e. morphological diversity versus chemical similarity, we postulated the need for a more precise chemical analysis of these two neuron types (Brehmer et al. 2002a). This subsequent immunohistochemical analysis demonstrated that CGRP is co-localized with SOM exclusively in myenteric type V but not in type II neurons in pig ileum. Additionally, both methionine- and leucineENK (metENK, leuENK) as well as both cChAT and pChAT but not nNOS have been found in type V neurons (Fig. 9A,B; Brehmer et al. 2002b, 2004c).

We found numerous close appositions of boutons on perikarya and dendrites of both single (Brehmer et al. 2002b) and aggregate type V neurons (Fig. 7). These boutons displayed immunoreactivities for the same marker combinations as did the somata (here we demonstrated CGRP/SOM). Consequently, we tried to identify synapses and the chemical coding of their presynaptic terminals on type V neurons. As a first step, NF/DAB-stained wholemounts were prepared. Thereafter, type V aggregates or single neurons, respectively, were located, trimmed, photographed and embedded for electron microscopy. Numerous synapses displaying membrane specializations were seen within the dendritic tangles of type V neuron aggregates (Figs. 10, 11A). Presynaptic clear and dense core vesicles were seen in the presynaptic boutons. In most cases, the postsynaptic membrane was thicker (Fig. 12B,C) whereas, in some cases, no difference in thickness between pre- and postsynaptic membranes could be observed. As a second step, we tried to identify the chemical nature of the presynaptic boutons. Wholemounts were double-stained for NF/DAB and SOM or CGRP, respectively, with colloidal gold as second antibody. Gold-labelled, presynaptic boutons containing SOM and apposing NF/DAB-positive type V neuronal perikarya and dendrites could be demonstrated (Fig. 12B,C). Likewise, CGRP-immunopositive presynaptic boutons were found. Thus, type V neurons are contacted synaptically by boutons displaying the same chemical code as the neurons themselves.

From the above, it is concluded that type V neurons may act as descending, cholinergic interneurons. We have argued that they may be equivalent to the ChAT/SOM descending interneurons of the guinea pig (Brehmer et al. 2004b). However, the possibility that submucosal non-type V or prevertebral neurons displaying the same chemical code as myenteric type V neurons (CGRP/SOM) may synaptically contact myenteric type V neurons cannot be excluded at present.

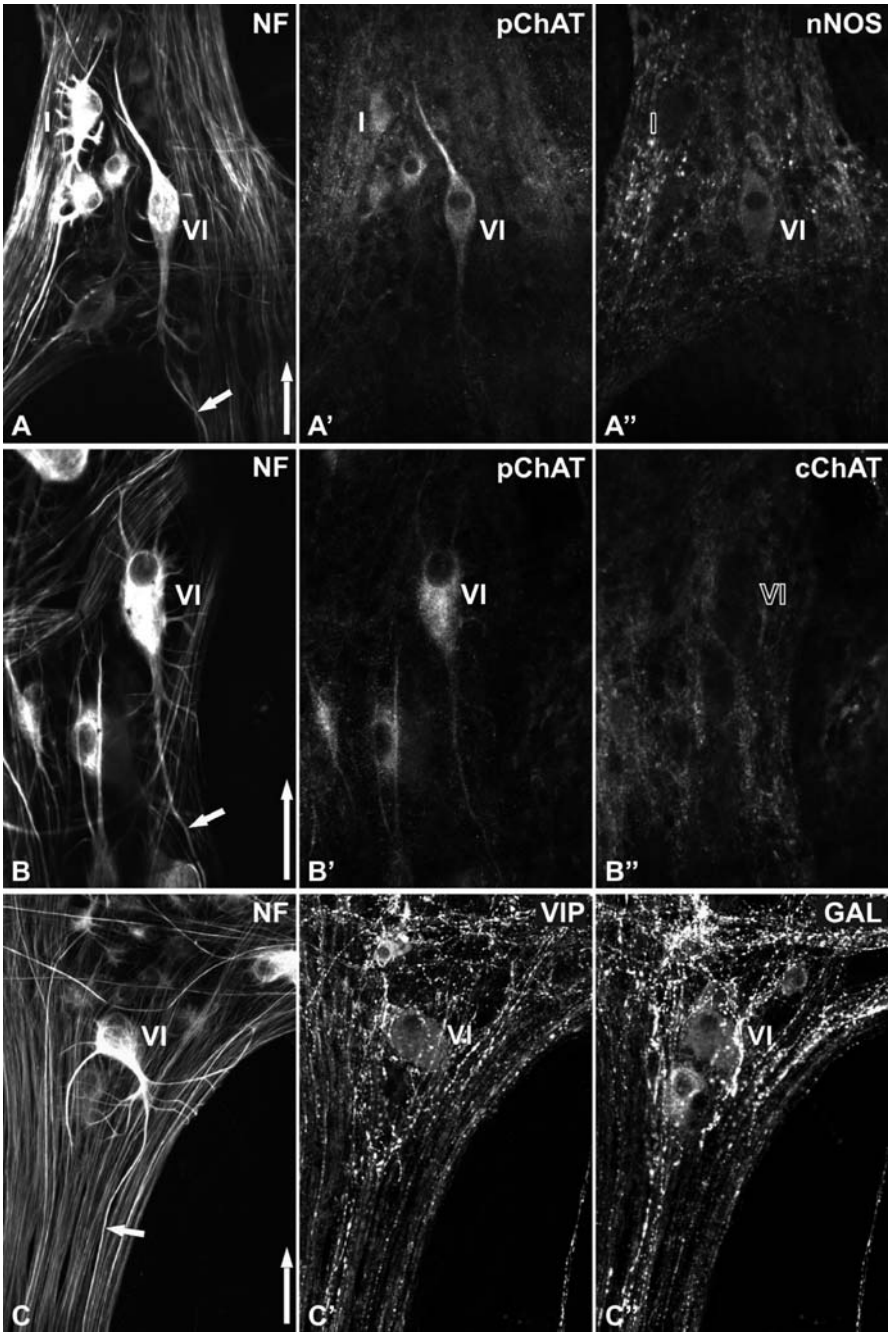
3.7 Type VI Neurons

The morphological hallmark of this neurons is, in addition to the somal dendrites, the so-called axonal dendrites which arise from the prominent axon hillock and the proximal axonal segment of these neurons (Stach 1989). Thus, they are multidendritic and uniaxonal neurons. Most of the axons of myenteric type VI neurons project anally in the plane of the myenteric plexus (Fig. 12). These neurons are also present in the external submucous plexus from where the axons enter interconnecting strands towards the myenteric plexus, but their further course is not known. The dendrites are of medium length, partly branched with tip endings. These neurons are mainly present in the ileum, their proportion in this segment is between 1.3% (Brehmer et al. 2002a) and 2% (Brehmer and Stach 1998). In the jejunum, their frequency is lower (between 0.4% and 0.7%; Brehmer and Stach 1998).

Type VI neurons are a nitrergic population as has been demonstrated with both NADPHd reactivity combined with silver impregnation and immunohistochemistry (Brehmer and Stach 1997; Brehmer et al. 1998, 2004c). Interestingly, they were immunonegative for cChAT but 95% of them were positive for pChAT (Brehmer et al. 2004c; Fig. 13A,B). Furthermore, about 90% of nNOS-reactive type VI neurons are positive for VIP and about 70% of VIP-positive type VI neurons were co-reactive for GAL (Fig. 13C; unpublished results).

Applying two surgical techniques leading to hypertrophy of the external muscle coat, we investigated morphometrically somal sizes of neuron types I, II, IV, V and VI (Brehmer et al. 2000). The only population displaying marked somal enlargement which could be related to zones of muscular hypertrophy unequivocally was the type VI neurons. In a subsequent study (Brehmer et al. 2001) we compared dendritic parameters of type VI neurons (projecting mainly anally) with those of type IV neurons (projecting vertically to the mucosa; Sect. 3.5) within mechanically stressed ileum of pigs. In contrast to type IV neurons which were unchanged throughout, type VI neurons displayed increased dendritic parameters correlated with sites of muscular hypertrophy and type VI somal hypertrophy. These parameters included total dendritic lengths, numbers of dendrites, numbers of branching points and numbers of dendritic endings. In conclusion, as these neurons were located in hypertrophied ileal segments and projected mostly

Fig. 13 A–C Myenteric type VI neurons in pig ileum. **A** A type VI neuron with characteristic axonal dendrites (emerging between the soma marked with *VI* and the axon marked with *arrow*) is co-reactive for both peripheral choline acetyl transferase (*pChAT*; **A'**) and neuronal nitric oxide synthase (*nNOS*; **A"**). In contrast, a type I neuron (*I*) is positive for pChAT (**A'**) but negative for nNOS (**A"**; *empty I*). **B** A type VI neuron (*VI*; *arrow* indicates axon) is reactive for pChAT (**B'**) but non-reactive for the common choline acetyl transferase (*cChAT*; **B"**; *empty VI*). **C** A type VI neuron (*arrow* indicates axon) is co-reactive for both vasoactive intestinal peptide (*VIP*; **C'**) and galanin (*GAL*; **C"**). *Arrowed scale bars* (50 μm) point orally


NF
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C'
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anally onto the region of mechanical or functional stenosis, respectively, it may be suggested that these neurons have received signals from the changed segment which led them to enhanced activity, maybe as inhibitory muscle motor neurons or descending interneurons. This enhanced activity may have caused their somal and dendritic hypertrophy reflecting structural plasticity. However, it was important for us to note that the type-specific features (most importantly the axonal dendrites) were preserved; thus, hypertrophied type VI neurons could unequivocally be distinguished from other neuron types.

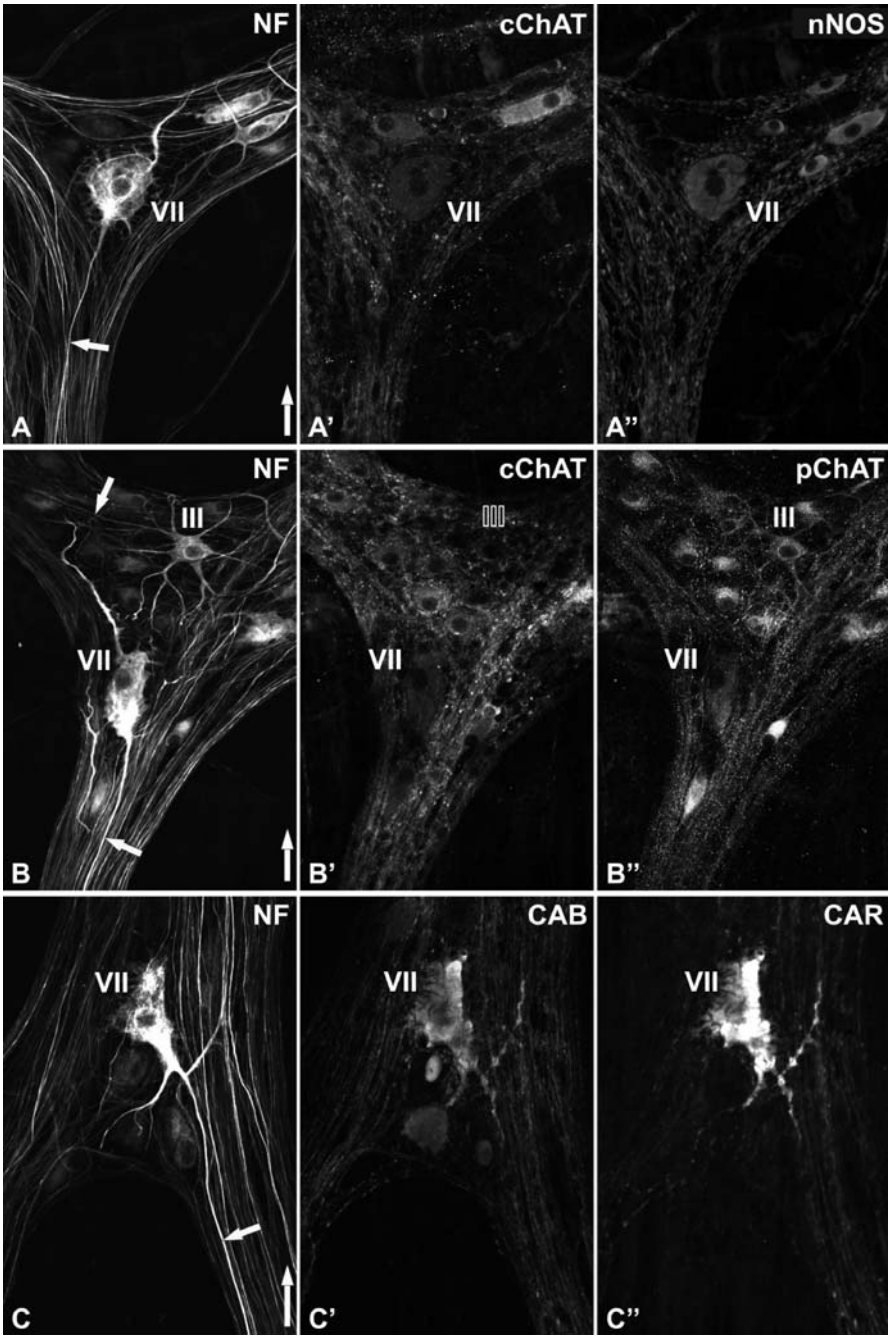
Pig type VI neurons, reactive for both nNOS and a cholinergic marker (pChAT), chemically resemble subpopulations of both human and guinea pig (each displaying reactivity for cChAT and nNOS), presumed descending interneurons (Furness et al. 2000a; Porter et al. 2002). Thus, type VI may be a population of descending interneurons in the pig.

3.8 Type VII Neurons

These dendritic, uniaxonal neurons have been originally described in material from human, pig and dog (Stach et al. 2000). Type VII neurons are a very peculiar population, i.e. they are restricted to the duodenum and proximal jejunum and represent a very small population (about 0.4% in NF/CuB double-stained whole-mounts; unpublished results). The somata of these neurons are commonly larger than those of the surrounding other types (e.g. types I, II, III) and display frequently frayed outlines as visualized by staining for the cytoskeletal marker NF. This is in contrast to the outlines of the somata of other neuron types which are smoothly contoured between the origins of processes. The distinctive morphological feature is one or few main dendrites which emerge frequently near the axonal origin ('axonpolar' position of main dendrites; Fig. 13). There are frequently numerous other dendrites but they are very short and inconspicuous. The axons of type VII neurons are very prominent and can frequently be followed to the edge of the whole-mount. In about 90% of cases, they run anally, the remainder orally.

Our previously unpublished observations on their chemical coding indicate that 89% of type VII neurons are co-immunoreactive for cChAT and nNOS (Fig. 14A), while pChAT could only be found in a minority of neurons (about 25%–30%;

Fig. 14 A–C Myenteric type VII neurons in pig duodenum. **A** A type VII neuron displaying one main dendrite (*arrow* indicates axon) is co-reactive for both common choline acetyl transferase (*cChAT*; **A'**) and neuronal nitric oxide synthase (*nNOS*; **A"**). **B** A type VII neuron displaying two main dendrites (*arrow* indicates axon) is co-immunoreactive for both cChAT (**B'**) and the peripheral choline acetyl transferase (*pChAT*; **B"**). In contrast, a type III neuron is negative for cChAT (**B'**; *empty III*) but positive for pChAT (**B"**). **C** A type VII neuron with two main dendrites (*arrow* indicates axon) is co-reactive for both calbindin (*CAB*; **C"**) and calretinin (*CAR*; **C"**). *Arrowed scale bars* (50 μm) point orally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VII
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A'
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NF
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VII
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NF
CAB
CAR
VII
VII
VII
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Fig. 14B). Furthermore, ENK, VIP (but not GAL), CAB and CAR were present in a majority of type VII neurons (Fig. 14C).

There are no further data on type VII neurons in the literature. However, their topographically restricted occurrence (duodenum, upper jejunum) argues for a specialized function of this intestinal segment. Differences in motor patterns of various human gut regions have been described (Kellow et al. 1986).

3.9
Dendritic Type II Neurons, Mini Neurons, Giant Neurons

Apart from casual observations, these neuron populations have not yet been thoroughly investigated in our NF studies. Descriptions of dendritic type II and giant neurons have been based primarily on silver impregnated whole-mounts (Stach 1989; Stach et al. 2000) whereas the populations of mini neurons were defined mainly by their immunohistochemical coding (Stach 1989; Timmermans et al. 1997).

Dendritic type II neurons are, in contrast to non-dendritic type II neurons as described above, dendritic and multiaxonal neurons. In the pig, their predominant occurrence is the submucosal plexus, mainly those of the large but also of the small intestine. Their axonal projection pattern in the pig is not known but, at least partly, they may project to the mucosa. It seems that their chemical coding in the pig is similar to the non-dendritic type II neurons, i.e. they contain CGRP but not SOM (Fig. 15).

Mini neurons are small neurons displaying frequently short, scarcely stained dendritic trees. Several populations have been defined immunohistochemically.

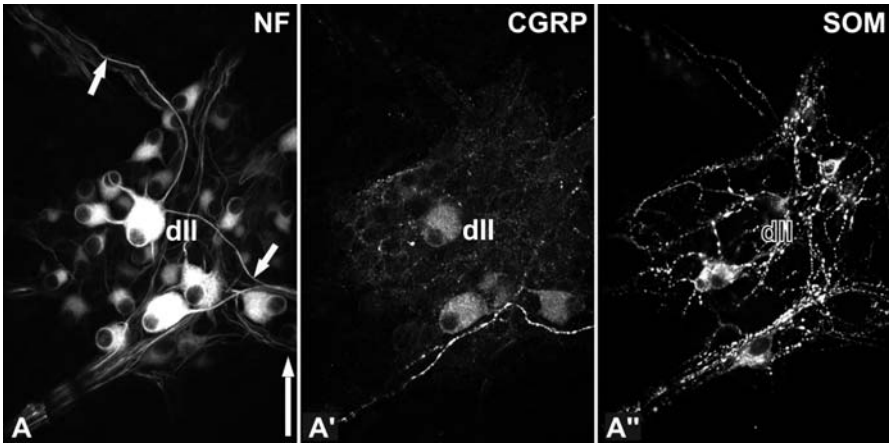


Fig. 15 A ganglion of the inner submucous plexus with a marked, neurofilament (*NF*)-reactive dendritic type II neuron (*dII*; *two arrow* indicate two axons) displaying several short, non-branched dendrites with tip endings. The neuron is reactive for calcitonin gene-related peptide (*CGRP*; **A'**) but negative for somatostatin (*SOM*; **A"**; *empty dII*)

Firstly, VIP/GAL-immunoreactive neurons which are probably cholinergic and, at least in the inner submucosal plexus, non-nitrergic (Stach 1989; Timmermans et al. 1997). They are located in all three ganglionated plexuses of the pig small intestine, most frequently in the inner submucosal plexus. In view of the morphological type VI neurons displaying VIP/GAL/nNOS co-localization, it has to be rechecked whether both populations occur separately in the myenteric (and, likely, also the external submucosal) plexus. Secondly, cholinergic NMU/SP-immunoreactive neurons are present mainly in the inner submucosal plexus (Timmermans et al. 1989). Thirdly, GABA-reactive neurons, mainly in the inner submucosal plexus, were about 40% were coreactive for SP (Wu et al. 1998). Fourthly, small nitrergic neurons present mainly in the myenteric and outer submucosal but only sparsely in the inner submucosal plexus (Brehmer and Stach 1997; Brehmer et al. 1998; Van Ginneken et al. 1998). The first three populations may be integrated in mucosal and/or vascular regulation whereas the latter may rather be involved in motor processes.

Giant neurons are, by number, surely the smallest population known so far. They have been found exclusively in the most oral part of the duodenum (up to several centimetres beyond the pyloric sphincter) in human, pig and dog. They have long, branched, tapering dendrites and an axon running mainly anally in pig and dog (in human also longitudinally but the direction was unknown). Their function is unclear.

4
Morphological Neuron Types and Their Chemical Coding in the Human

Our only silver impregnation study dealing with the morphology of human enteric neurons (Stach et al. 2000) was carried out on a relatively small sample in comparison to the material available in animal studies. An important parameter of the classification in the pig ENS, the oro-anal orientation of axonal projections, could not be determined in this study. Nevertheless, we tried to find equivalences of neurons between human and animals based on morphological grounds. Equivalent neuron populations in different species fulfil corresponding roles (e.g. primary afferent, interneuronal, motorneuronal) within their respective enteric circuits. In some cases, our later immunohistochemical studies confirmed our original suggestions of equivalences (e.g. type II). In other instances, we had to correct them (e.g. type I). Finally, in some cases we have not yet achieved conclusive results (e.g. type IV).

4.1
Nomenclature Used for Human Enteric Neurons

Any nomenclature has to consider both historical aspects and actual requirements. In detail, inclusion of these two factors into a conclusive classification system is difficult.

In our silver impregnation study (Stach et al. 2000), we found numerous uniaxonal neurons displaying relatively short, spiny dendrites. These resembled both Dogiel type I neurons (one axon, several short dendrites) and, in some respect, Stach type I neurons in the pig (except the absence of marked lamellar dendritic endings and the unknown direction of their axonal projection). These 'spiny' neurons were termed 'type I' neurons (Stach et al. 2000).

Later, in an immunohistochemical study (Brehmer et al. 2004a), we found that these spiny 'type I' neurons projected anally and were nitrergic. Both features were in striking contrast to the pig type I neurons namely, the latter project orally and are non-nitrergic (Sect. 3.2). Thus, these neurons fulfilled criteria for Dogiel type I but hardly for Stach type I neurons.

In the same study, we identified smaller, somewhat inconspicuous NF-positive, nNOS-negative neurons with very short, 'stubby' dendrites whose axons ran frequently in the oral direction (Brehmer et al. 2004a; these neurons were obviously not impregnated in the above silver impregnation study). These human 'stubby' neurons were more similar to Stach type I neurons in the pig (oral axonal course, non-nitrergic phenotype) than to the human spiny 'type I' neurons. Thus, the 'stubby' neurons displayed criteria of both Stach's and Dogiel's type I neurons. However, the term type I was already 'occupied' by the spiny type I neurons in human (Stach et al. 2000).

We assume that Dogiel has added both our recent stubby and spiny neurons to his type I neurons (see Figs. 1, 2). Based on our current knowledge, it is essential to designate different structures with different names. Below, we give an overview on the neuron types and explain why we have used their terms. We start with the best-defined populations.

Type II neurons (Sect. 4.2) are the *non-dendritic*, pseudouni- or multiaxonal neurons. This definition considers criteria given by Dogiel (1899) and Stach et al. (2000) and is, to our knowledge, generally accepted.

Stubby (type I) neurons (Sect. 4.3) and *spiny (type I) neurons* (Sect. 4.4) are different populations which resemble each other in their pure outward shape namely, they are uniaxonal and have relatively short dendrites. Their structure corresponds to that of Dogiel type I neurons. Thus, this term means a fundamental category which does not allow any specific functional suggestion. In our opinion, the situation is similar to that of pyramidal neurons which differ between layers and regions in the cerebral cortex as to their dendritic architecture, synaptic input, axonal projections etc.

Type V neurons (Sect. 4.5) frequently display a single stem-like process from which both the axon and dendritic branches emerge. They were first described in the pig (Stach 1989) and later in human (Stach et al. 2000).

Type III neurons (Sect. 4.6) are uniaxonal neurons displaying comparatively long, branched dendrites (Dogiel 1899; Stach et al. 2000).

Dendritic type II neurons (Sect. 4.7) are pseudouni- or multiaxonal neurons with slender, branched dendrites. They were first described in the pig (Stach 1989) and later in the human (Stach et al. 2000).

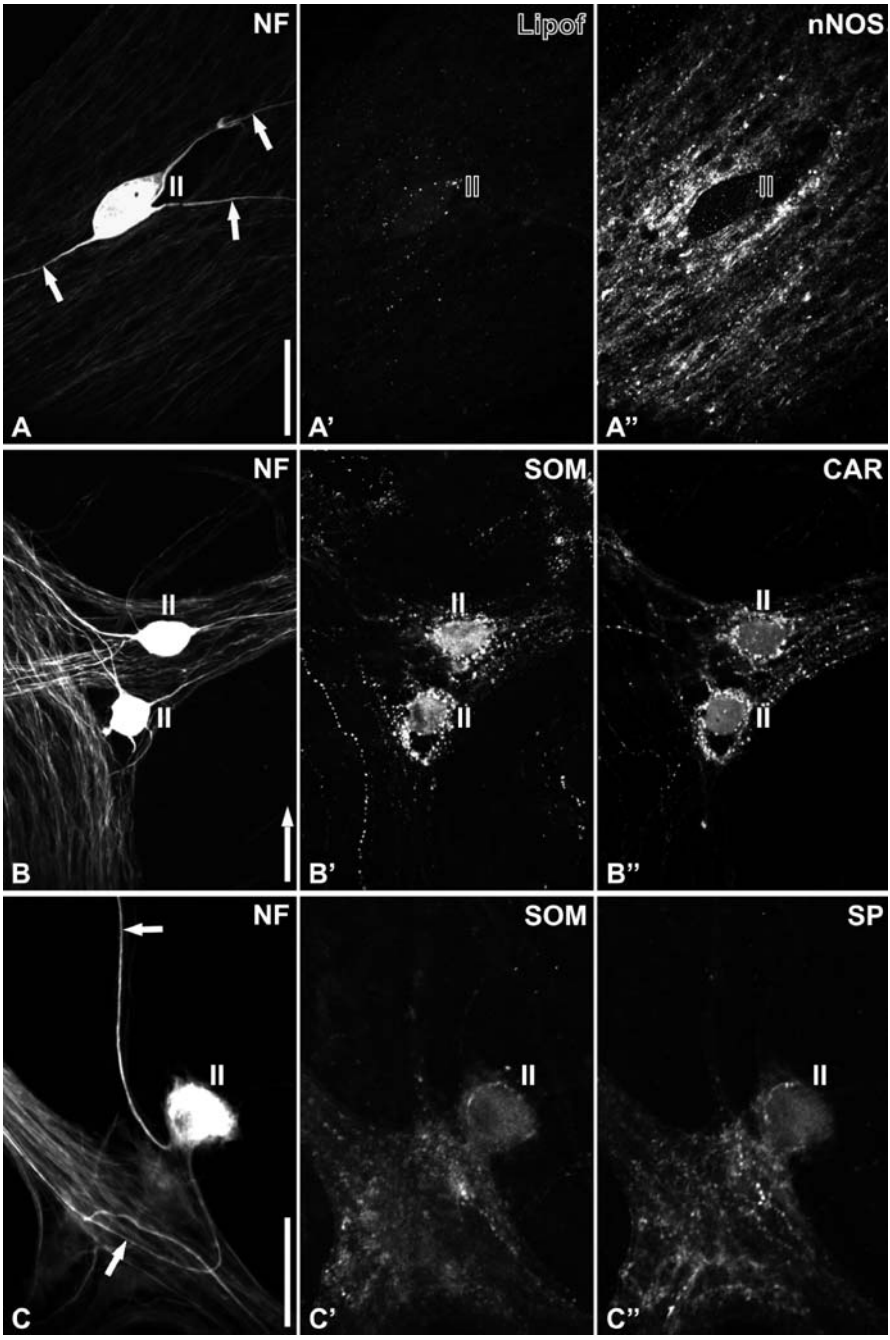
4.2
Type II Neurons
(Dogiel 1899; Stach et al. 2000; Brehmer et al. 2004a, 2004b)

As already described in the pig, these neurons were multiaxonal and mostly non-dendritic also in human (Fig. 16). Their cell bodies were located within ganglia or outside them within secondary strands. They had one (bifurcating) to six long processes which appeared histologically to be axons, i.e. they left the ganglion of origin and displayed a uniform calibre as long as they could be traced. After ramification, both branches preserved this axonal character. The axons run both circumferentially, within secondary strands of the myenteric plexus, and vertically, i.e. they entered disrupted interconnecting strands leading towards the submucosal/mucosal layers. We suggest that at least a part of the latter axons run from the myenteric plexus at least up to the submucosa (see below).

Although these neurons were in general non-dendritic, their somata sometimes did not display the smooth contours as described in other species. There was a considerable number of type II neurons displaying somata of a rough or even rugged surface. Partly, somal extensions had the appearance of short dendrites. Irregular or 'multiangular' surfaces of originally smooth somata of sensory neurons have been described as age-related changes (Kiss 1933; Scharf 1958). It is also possible that the overnight incubations in culture medium containing colchicine may have produced these shapes. The dendrite-like processes were interpreted as non-obligatory, variable features of human myenteric type II neurons. Similar features were also demonstrated in guinea pig and mouse type II neurons (Furness et al. 1988; Nurgali et al. 2004). These dendrite-like structures are morphologically quite different from those of the dendritic type II neurons (Sect. 4.7). As to their lipofuscin pigmentation pattern, they displayed fine, less intensely autofluorescent granules (Fig. 16A'). We found both ganglia containing up to 10 type II neurons and ganglia without any type II neuron. They were also found on ganglionic boundaries and, mostly singly, within interganglionic or secondary strands.

We have demonstrated a number of substances in morphologically defined human type II neurons. A majority of them displayed immunoreactivity for CAR, SOM and SP (Fig. 16B,C) whereas CAB and CGRP were present in only a minority of

Fig. 16 A–C Human non-dendritic, multiaxonal type II neurons. **A** A neurofilament (*NF*)-positive type II neuron (*II*) with three axons (*arrows*). In **A'**, no immunostaining was applied; very fine granular, isolated autofluorescent lipofuscin granula can be observed (*empty II*). The neuron is negative for neuronal nitric oxide synthase (*nNOS*; **A"**, *empty II*) (duodenum, 28-year-old patient). **B** Two NF-stained type II neurons are co-immunoreactive for both somatostatin (*SOM*; **B'**) and calretinin (*CAR*; **B"**) (descending colon, 70-year-old patient). **C** A NF-stained type II neuron with two axons (*arrows*) is co-immunoreactive for both SOM (**C'**) and substance P (*SP*; **C"**) (jejunum, 65-year-old patient). *Scale bars* are 50 µm, *arrowed scale bar* in **B** points orally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type II neurons. These results concur with most findings of earlier studies by other groups. Timmermans et al. (1992) as well as Dhatt and Buchan (1994) demonstrated CGRP in only a few human myenteric neurons. Besides some type II, there are also some other neurons positive for this peptide. CAR has been demonstrated to be co-localized with SP in myenteric neurons of human small intestine (Walters et al. 1993) whereas co-localization of SOM and SP was demonstrated by Accili et al. (1995) and Hens et al. (2001) but not by Dhatt and Buchan (1994). Wattchow et al. (1997) found CAR-reactive somata in the myenteric plexus as well as CAR-reactive nerve fibres in both submucosa and mucosa of human colon. These authors suggested that at least a proportion of myenteric CAR neurons may have vertical projections. Thus, there are some literature data which support our suggestion that human myenteric type II neurons, most of them reactive for SOM, SP and CAR, project into the mucosa. Hens et al. (2001) were able to demonstrate myenteric neurons labelled from the human small intestinal mucosa, some of which were reactive for both SOM and SP. These authors did not use cytoskeletal markers but described some of the traced neurons to have a multidendritic appearance whereas others revealed smooth cell bodies and were reactive for SP.

From these indications, we suggest that type II neurons are the human IPANs (Sect. 5.2.1)

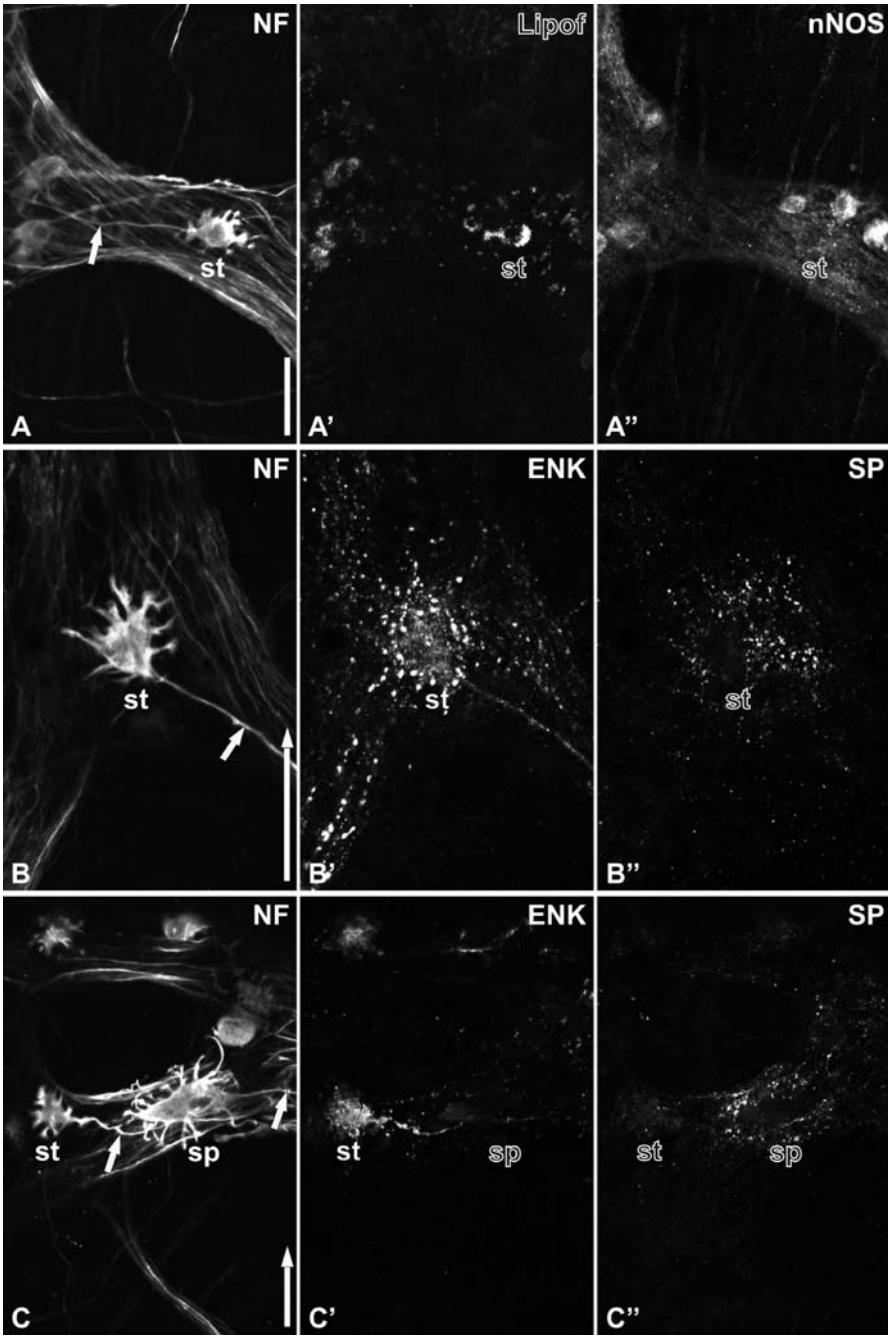
4.3 Stubby (Type I) Neurons

(Brehmer et al. 2004a, 2005)

These neurons displayed relatively small somata, one axon and had short, stubby, partly lamellar dendrites which were mostly not branched (Fig. 17).

The somata were flattened in the myenteric plane. Stubby neurons were clearly smaller than type II neurons. The mean values of somal areas of stubby neurons were between 259 (±47; sigmoid colon of a male patient aged 54 years) and 487 μm^2 (±113; ileum of a female patient aged 77 years) whereas those of type II neurons were between 633 (±108) and 972 μm^2 (±228; measurements of type II and stubby neurons were from the same patients). Stubby neurons tended to display the coarse,

Fig. 17 A–C Human stubby neurons. **A** A neurofilament (*NF*)-stained neuron (*st*; *arrow* indicates axon) with short, stubby dendrites. It displays the coarse, aggregated pattern of lipofuscin pigmentation (*empty st* in **A'**) and is non-reactive for neuronal nitric oxide synthase (*nNOS*; **A"**; *empty st*) (duodenum, 63-year-old-patient). **B** A NF-stained stubby neuron (*st*; *arrow* indicates axon) with short, scarcely branched dendrites displaying partly lamellar endings. It is co-reactive for leu-enkephalin (*ENK*; **B'**; *st*) but negative for substance P (*SP*; **B"**; *empty st*) (sigmoid colon, 54-year-old patient). **C** Two NF-stained neurons, the one on the left is a stubby neuron (*st*; *arrow* indicates axon), that on the right is a spiny neuron (*sp*; *arrow* indicates axon). The stubby neuron is reactive for ENK (**C'**; *st*), in contrast, the spiny neuron is negative (*empty sp*). Both neurons are negative for SP (**C"**; *empty st*, *empty sp*) (ileum, 76-year-old patient). *Scale bars* are 50 μm, *arrowed scale bars* in **B**, **C** point orally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intensely fluorescent pattern of lipofuscin pigmentation (Fig. 17A'). In some stubby neurons the pigment granules surrounded the nucleus forming a closed circle, in others they occupied only a part of the soma sometimes additionally occupying the proximal part of a dendrite.

The dendrites generally emerged from the circumference of the soma but only rarely from the inner (circular muscle) or outer (longitudinal muscle) surfaces (Figs. 19, 20). The ratios of mean dendritic field area per mean somal area of stubby neurons ranged between 2.0 and 2.6 in the small and between 2.2 and 2.8 in the large intestine. Both values reflect their relatively short dendrites in both the small and large intestines.

Their axons were relatively thin and they left the ganglion of origin within primary strands frequently in the oral direction. They could mostly be followed beyond the next ganglion but seldom beyond a second ganglion. We found that 50.4% of axons of stubby neurons projected orally, while 29.4% ran anally. The axons of the remaining 20.2% could either not be followed after leaving the ganglion of origin or ran circumferentially within primary strands and no oral/anal turn was observable. Only exceptionally, have we observed axons of stubby neurons entering secondary strands of the myenteric plexus.

As to their chemical coding, we found that the majority of leuENK-positive myenteric neurons had the NF morphology of stubby neurons (97.5%; Fig. 17B,C). The proportion of leuENK-immunoreactive neurons related to the putative whole myenteric neuronal population (as visualized by immunoreactivity for anti HU-protein, HU) was between 5.9% in the small and 8.3% in the large intestine. Of the leuENK-positive, stubby neurons, only 8.7% were simultaneously SP-immunoreactive. There were also a number of leuENK-negative, stubby neurons which have not yet been estimated by number. Both leuENK-positive and -negative stubby neurons were frequently surrounded by intensely fluorescent leuENK-positive boutons (Fig. 17B'). Similar observations were made using antibodies against metENK, but this has not yet been evaluated quantitatively.

Earlier reports have described ENK-immunoreactive neurons in the human myenteric plexus (Kapadia and Kapadia 1986; Dhatt and Buchan 1994; Krantis et al. 1998). At least some of them may be the source of ENK-positive fibres within the external musculature (Kapadia and Kapadia 1986; Lolova et al. 1986; Wattchow et al. 1988), suggesting that these neurons are muscle motor neurons. However, apart from rare exceptions, we did not observe axons of leuENK-positive/stubby neurons running into the secondary strands of the myenteric plexus which may the principal gateway for innervation of the (circular) musculature also in human. On the other hand, it may be that motor axons run first within the primary strands for some distance and then leave them to run into the secondary strands. Such axonal courses were already observed by Dogiel (1899). In the guinea pig, ENK-immunoreactive neurons of one population are circular muscle motor neurons (Costa et al. 1996; Furness et al. 2000a), while other ENK neurons are ascending interneurons (Brookes et al. 1997). As revealed by NF staining, the latter authors described these neurons as small, uniaxonal Dogiel type I neurons with short, usu-

ally lamellar dendrites. This morphological description is quite similar to that of the stubby neurons. Since we demonstrated leuENK-positive boutons surrounding several leuENK-reactive, stubby neurons, it seems not unlikely that at least some of the ENK-reactive neurons in the human are interneurons.

As to the co-localization of ENK and SP in a minority of stubby neurons, the latter peptide has been demonstrated in ascending circular muscle motor neurons of the human colon (Wattchow et al. 1997). Wattchow et al. (1988) showed partial coexistence of SP and ENK in external muscle fibres of human intestine and suggested that these fibres were excitatory. We cannot answer the question of whether ENK-reactive, stubby neurons with and without additional SP reactivity are also functionally different or are rather chemically variable subtypes of a common population. Both ENK- (Lolova et al. 1986; De Laet et al. 1989; Johanson et al. 1991; Munakata et al. 1997; Porter et al. 1998) and SP-reactive neurons (Porter et al. 1998; Neunlist et al. 2003) were shown to participate in plastic changes in pathological conditions. ENK/SP-co-reactive structures will have to be further evaluated in future studies as will the leuENK-negative, stubby neurons. Interestingly, in the pig myenteric plexus, there is a difference in projection pattern between ENK-positive/SP-negative neurons (mainly descending) and ENK/SP-co-reactive neurons (mainly ascending; Hens et al. 2002).

Some of the neurons depicted by Dogiel are somewhat similar to our stubby neurons (Figs. 1, 2). However, it should be noted that Dogiel (1899) described and depicted the dendrites of his type I neurons to bear secondary and tertiary branches. Since most of the dendrites of our stubby neurons were not branched it seems that Dogiel (1899) may have focused on other than stubby neurons (probably spiny neurons; Sect. 4.4) when establishing his type I population.

Based on their projection pattern and chemical coding, we conclude that stubby neurons may be either ascending motor or interneurons (Sects. 5.2.2. and 5.2.3).

4.4
Spiny (Type I) Neurons
(Brehmer et al. 2004a, 2006)

These neurons displayed, in the small intestine, somewhat larger somata than stubby neurons (Fig. 17C; although this has not yet been analysed statistically). They had mostly spiny, less frequently also lamellar dendrites which were partly branched and one axon (Fig. 19).

Their somata were mostly smaller than those of type II neurons, except in the duodenum of a patient aged 42 years. In this wholemount, the somal areas of spiny neurons were 607 μm^2 (±114), those of type II neurons 641 μm^2 (±153). The remaining values showed significant differences in each wholemount, ranging between 487 μm^2 (±127) and 701 μm^2 (±166) for spiny neurons and between 630 μm^2 (±135) and 914 μm^2 (±172) for type II neurons.

Their dendrites emanated radially from the perikaryon, some of them branched, partly multiple, but in general the side branches were short. The tips of dendrites

were mostly spiny or thorny although some were also club-shaped or lamellar. Their origins were scattered not only along the somal circumference but also over the whole somal surface including the upper and lower sides. Frequently, whole cells had a hedgehog-like appearance. These characteristics were more pronounced in the small intestine. In the large intestine, most of the spiny neurons possessed shorter dendrites, which had partly spiny, partly slender, tapering endings (Figs. 20, 18). Infrequently, they were lamellar in shape. The ratio somal area/dendritic field area was between 3.7 and 4.6 in the small intestine which reflects the longer dendrites of spiny neurons in contrast to stubby neurons (Fig. 19). However, in the colon, the ratio was between 2.5 and 2.8 thus, there was no difference to the ratio in comparison to stubby neurons (2.2 to 2.8; Fig. 20).

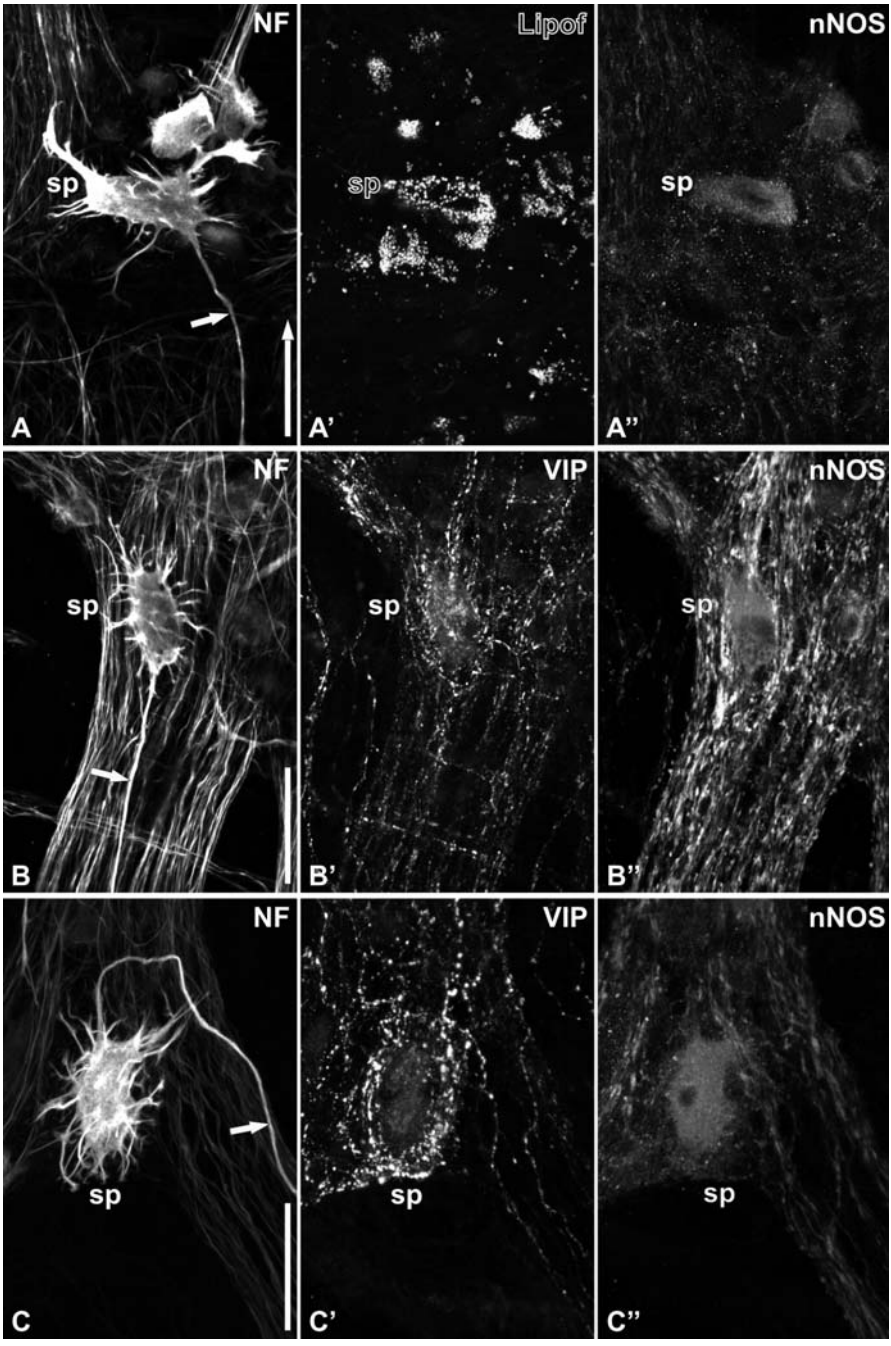
Spiny neurons had thick, prominent axons which could be easily followed within primary strands of the plexus and through other ganglia. In most cases, they emerged as direct anal projections, sometimes they emanated orally, reverting to an anal course after a short run. We found that, of the axons which could be followed beyond the next neighbouring ganglion, about 95% ran anally and 5% orally.

While almost all VIP/nNOS-positive neurons were NF-reactive spiny neurons and all spiny neurons displayed nNOS reactivity, not all spiny neurons displayed simultaneous VIP co-reactivity. This proportion of neurons needs to be investigated further.

The proportion of VIP/nNOS-co-reactive neurons in relation to the number of HU-positive neurons ranged between 5.8% (small intestine) and 17.5% (large intestine). The suitability of HU as a pan-neuronal marker has been proposed for enteric neurons derived from both laboratory animals (Karaosmanoglu et al. 1996; Phillips et al. 2004) and human (Ganns et al. 2006). The only earlier study presenting com-

Fig. 18 A–C Human spiny neurons. **A** A neurofilament (*NF*)-stained spiny neuron (*sp*; *arrow* indicates axon) displays the coarse, aggregated lipofuscin pigmentation pattern (**A'**; *empty sp*) and is reactive for neuronal nitric oxide synthase (*nNOS*; **A"**; *sp*) (jejunum, 69-year-old patient). **B** A NF-stained spiny neuron (*sp*; *arrow* indicates axon) is co-reactive for both vasoactive intestinal peptide (*VIP*; **B'**; *sp*) and nNOS (**B"**; *sp*) (duodenum/jejunum, 67-year-old patient). **C** A NF-stained spiny neuron (*sp*; *arrow* indicates axon) is co-reactive for both VIP (**C'**; *sp*) and nNOS (**C"**; *sp*) (ileum, 73-year-old patient). *Scale bars* are 50 μm, *arrowed scale bar* in **A** points orally

Fig. 19 A,B 3D reconstruction of human spiny and stubby neurons from small intestine. **A** A spiny neuron (*sp*; *arrow* indicates axon) is immunonegative for leu-enkephalin (*ENK*; **A'**; *empty sp*) but positive for vasoactive intestinal peptide (*VIP*; **A"**). **A"'** The neurofilament (*NF*)-stained neuron after 3D reconstruction. **B** A stubby neuron (*st*; *arrow* indicates axon) is immunopositive for leu-enkephalin (*ENK*; **B'**) but negative for vasoactive intestinal peptide (*VIP*; **B"**; *empty st*). **B"'** The NF-stained neuron after 3D reconstruction (both neurons from duodenum, 42-year-old patient). The spiny neuron in **A"'** has been enlarged after reconstruction thus equalizing the difference in original magnifications between the neurons in **A** and **B**. The difference in size between the two neurons in **A"'** and **B"'** is realistic. *Arrowed scale bars* (50 μm) point orally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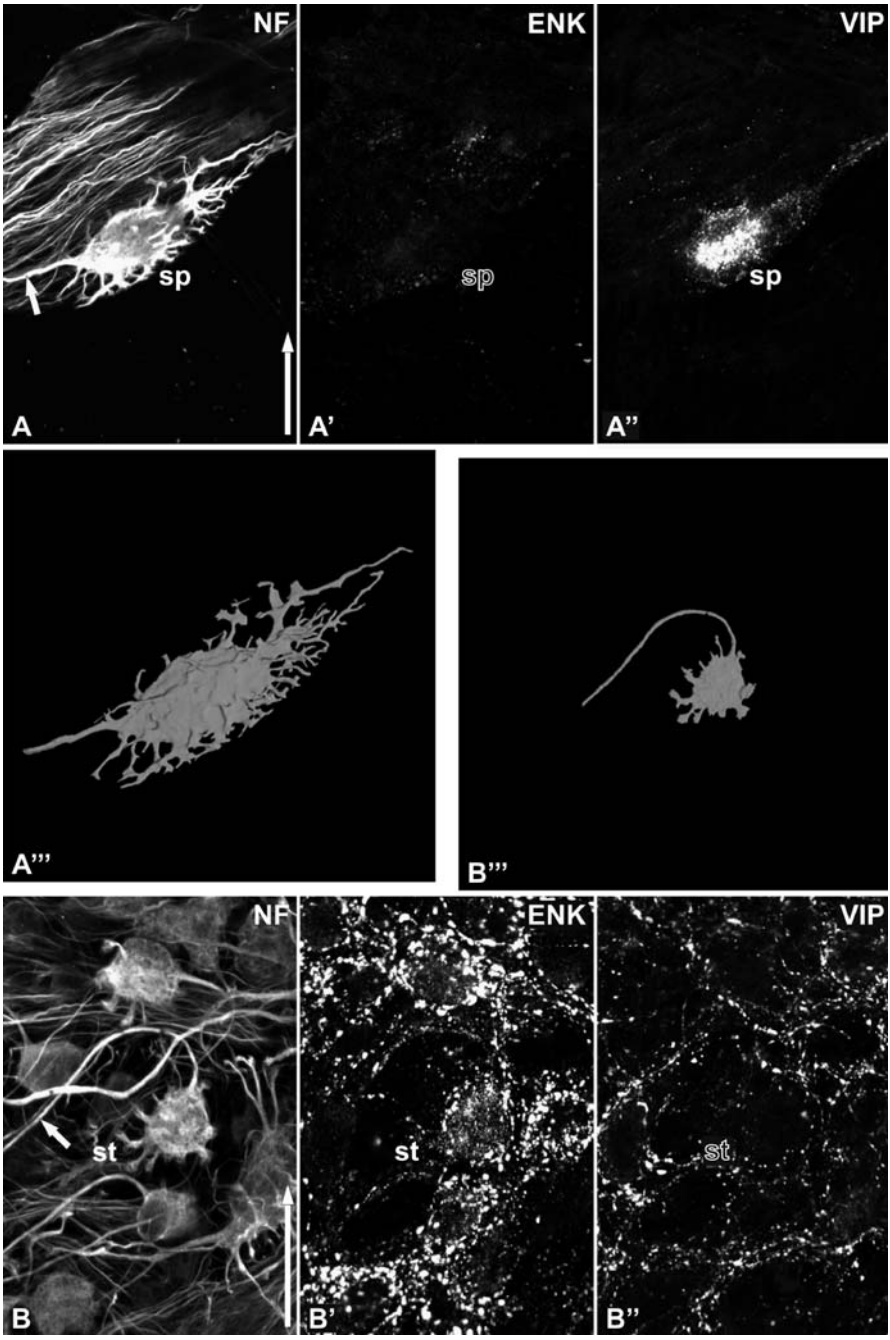
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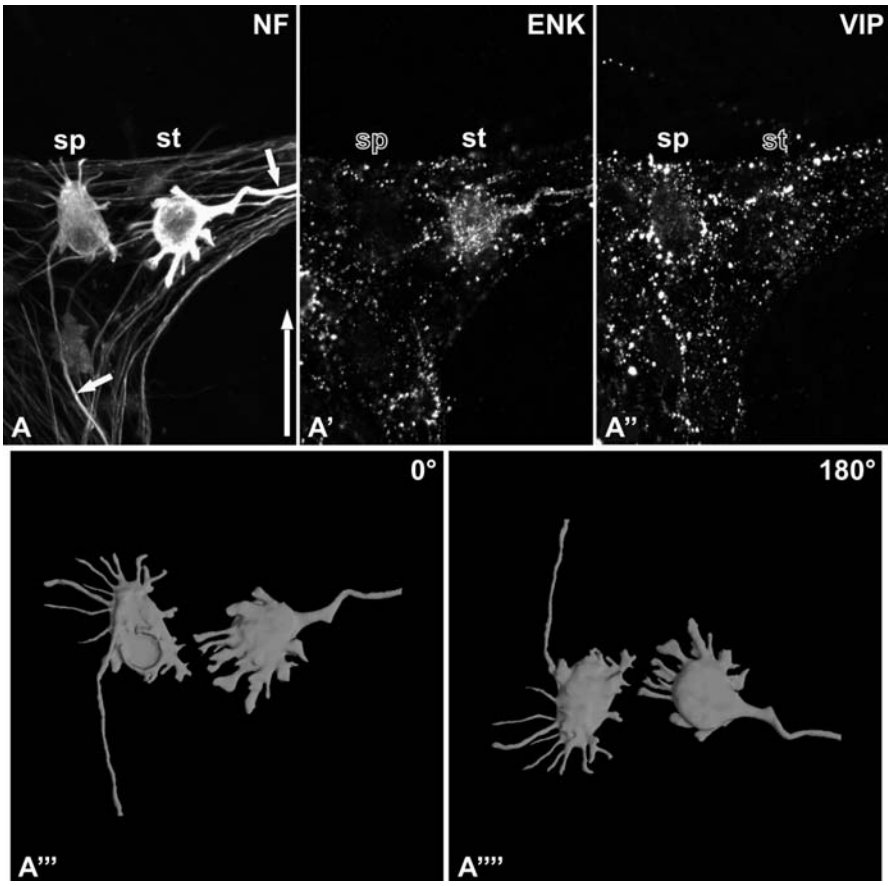

Fig. 20 3D reconstruction of human spiny and stubby neurons from large intestine. **A** A spiny neuron (*sp*; *arrow* indicates axon) is negative for leu-enkephalin (*ENK*; *empty sp* in **A'**) but positive for vasoactive intestinal peptide (*VIP*; **A"**) whereas a stubby neuron (*st*; *arrow* indicates axon) is positive for ENK (**A'**) but negative for VIP (**A"**; *empty st*). Both neurons are 3D reconstructed in (**A"'**) and (**A""**). In the latter, they are rotated around the transverse axis of the image and so they are seen from the reverse side. Note that the quantitative differences between spiny and stubby neurons as observed in the small intestine (soma size, dendritic lengths; see Fig. 19) are scarcely observable in the large intestine. However, the difference in dendritic shape (stubby versus spiny) is clearly visible (transverse colon, 21-year-old patient). *Arrowed scale bar* (50 μm) points orally

parable values in relation to the putative whole myenteric neuronal population was that of Pimont et al. (2003). These authors estimated the proportion of nNOS/VIP neurons in the human gastric fundus to be 7.2%±6%. Both Keränen et al. (1995), by using NADPHdiaphorase histochemistry instead of nNOS immunohistochemistry, and Porter et al. (1997), by applying combined DiI tracing for circular muscle motor neurons and subsequent immunohistochemistry, found neurons displaying

this chemical code in the human colonic myenteric plexus. Neither related the proportions of VIP/nNOS-neurons to the whole neuronal population.

In contrast, many more studies on human material demonstrated either nNOS reactivity (alternatively, NADPHdiaphorase activity) or VIP immunoreactivity. Proportions of neurons with nNOS reactivity or NADPHdiaphorase activity (without simultaneous demonstration of VIP) have been given by Wester et al. (1999): 40% in neonates and 25%–30% in 15-year-old juveniles. Belai and Burnstock (1999), in small intestinal samples, found between 20% and 56% in control adult and aged human subjects whereas Pimont et al. (2003) estimated 40.8%±4.5% in gastric fundus. Our values ranged between 29.4% (duodenum) and 42% (sigmoid colon). The population of nNOS-reactive neurons is commonly referred to as nitrergic although, it is not yet clear which of the three different nNOS proteins described by Saur et al. (2000) is actually present at the site of nNOS-immunoreactivity. This may be functionally significant.

As yet, we could not recognize a clear correlation between age, segment and proportion of nitrergic neurons. In rats, Phillips et al. (2003) found that age-related cell loss occurred preferentially in non-nitrergic populations indicating that nitric oxide may play a role in neuronal survival (Keilhoff et al. 2002; Sandgren et al. 2003). In contrast, Wu et al. (2003) showed that age-related proportional changes in the neuronal composition of the rat oesophagus enteric ganglia display strain-dependent differences. In Wistar rats, due to a more prominent non-nitrergic cell loss, a relative increase in number of nitrergic neurons with age could be observed, whereas in Sprague-Dawley rats, no difference between the dimension of cell loss between nitrergic and non-nitrergic neurons occurred.

The proportion of human myenteric VIP-positive neurons (without simultaneous demonstration of nNOS/NADPHd) has been given by Dhatt and Buchan (1994) in the ileum as 5% of all neurons, by Pimont et al. (2003) in the gastric fundus as 19.6±6.9%, by Singaram et al. (1995) in the ascending colon as 39.7%, by Neunlist et al. (2003) in the colon as 6.9% and by Anlauf et al. (2003) in small and large intestine counts as varying between 11% and 14%. Our values of VIP-positive neurons (irrespective of their nNOS co-reactivity) lay between 7.1% (jejunum) and 22.4% (sigmoid colon). It is obvious that results of various studies differ. On the one hand, this may be due to different methodological approaches (different staining techniques, quantification procedures). On the other hand, it may be that numbers and proportions of (enteric) neurons may range more significantly in humans (especially elderly) than in laboratory animals, the latter being kept under standardized conditions. Furthermore, it is established that VIP not only acts as a neurotransmitter but also plays a role in neuroprotection, growth regulation and functions as an anti-inflammatory agent (Ekblad and Bauer 2004). Several authors investigated the numbers and proportions of VIP-positive neurons in various pathological conditions. Singaram et al. (1995) found a reduced number of VIP neurons in constipated patients whereas demonstration of increased numbers was given by Sjolund et al. (1997) in patients with slow transit constipation and by Belai et al. (1997) in segments with Crohn disease. On the other hand, Neunlist et al.

(2003) could not demonstrate significant alterations between control segments (of non-inflamed intestine) and segments from both inflamed and non-inflamed regions of gut from patients suffering from ulcerative colitis. Thus, the proportion of VIP neurons seems to depend on a number of factors. Besides that, it is not clear which subpopulations of neurons are normally VIP-negative but express VIP immunoreactivity under various pathological conditions. It may be possible that nitrergic neurons upregulate their VIP expression but also that non-nitrergic neurons change their chemical phenotype.

From the above, we suggest that spiny neurons may be descending motor or interneurons (Sects. 5.2.2 and 5.2.3).

4.5
Type V Neurons

(Stach et al. 2000; Brehmer et al. 2004a, 2004b)

Similar to type V neurons in pig, their (very likely) counterparts in human occur as single cells and in aggregates. In contrast to the pig where they are prominent in the ileum, they represent a conspicuous population in human duodenum and jejunum (Figs. 21, 24A–C). Here, both single and aggregated neurons were observed. However, the aggregates were not as conspicuous and tightly packed as known from the pig ileum.

Most usually, type V neurons displayed a relatively small (in relation to, e.g. type II neurons), always smoothly contoured soma (in contrast to type II neurons; see Sect. 4.2) with one single stem-like process from which both the very long and branched dendrites with tapering endings and the one axon emerged (Fig. 21). These morphological features closely resemble the general structure of invertebrate neurons i.e. the soma is set off from all processes (Ramón y Cajal 1911; Strausfeld 1976; Brown 1991). The single stem process of invertebrate neurons has also been called ‘cell body fibre’, ‘neurite’ or ‘segmento intercalar indiferente’ by these and other authors. The resemblance with invertebrate neurons described for human type V neurons is even more pronounced than in pig type V neurons. In this species, the axon has frequently a separate somal origin, in addition to one or few dendrites emerging from the soma. However, in some human type V neurons, the axons directly emerge from the soma, also two or three dendrites—instead of a single stem-like process—can emerge from the soma.

This resemblance in shape between type V and invertebrate neurons may not be pure coincidence. Data and suggestions from quite different scientific fields point to a possible common evolutionary root of recent invertebrates and the gut including its ENS (Romer 1972; Hahn and Bishop 2001; Shimizu et al. 2004).

Type V neurons could be found both within ganglia and within interganglionic strands. They mostly displayed a fine granular, less intensely autofluorescent pattern of lipofuscin pigmentation in their soma.

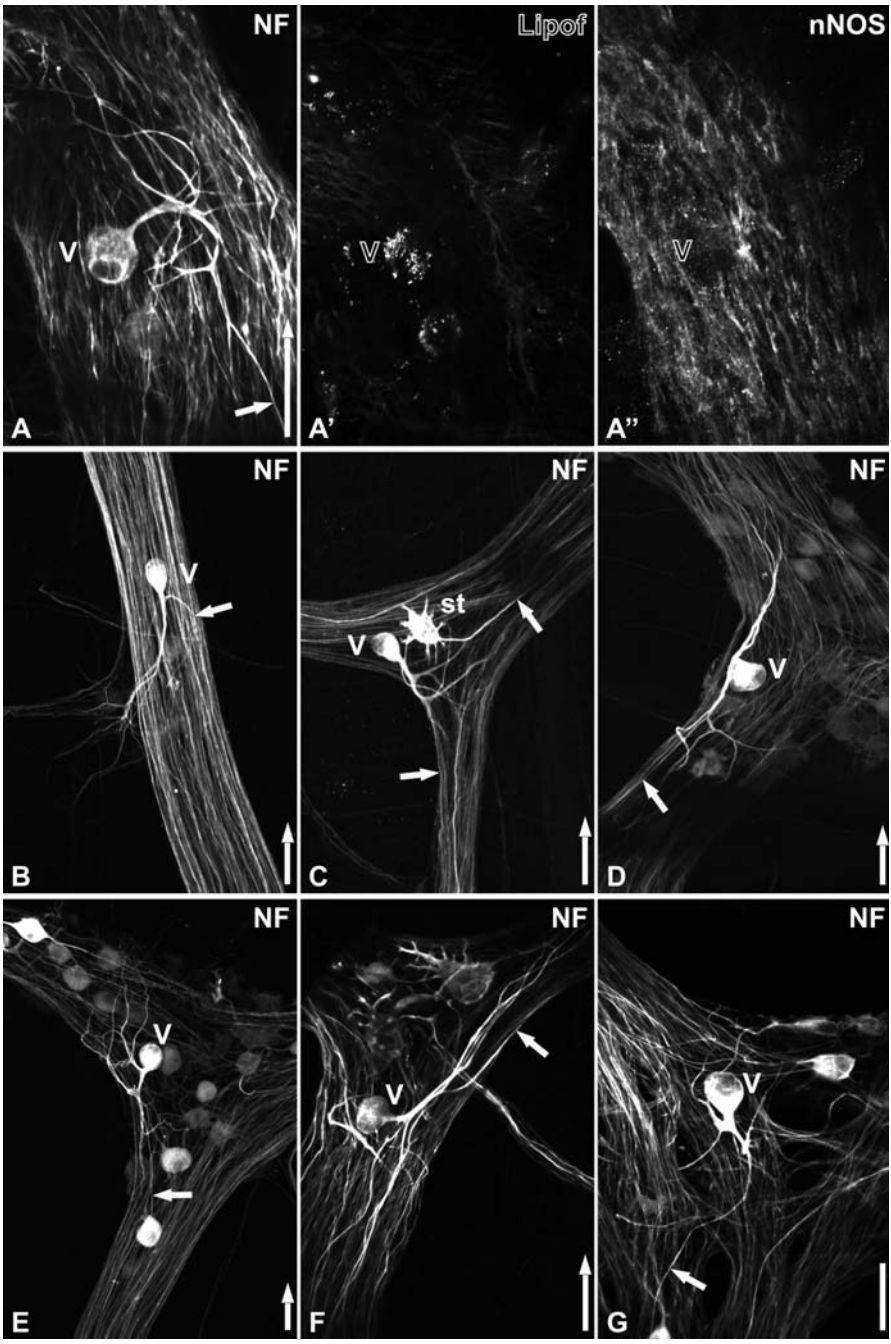
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Fig. 21 A–G Human type V neurons. **A** A human type V neuron (*V*) with the characteristic single stem-like process from which dendritic arborizations and the axon (*arrow*) emerge. The neuron displays the fine granular pattern of autofluorescent lipofuscin pigmentation (**A'**; *empty V*) and is non-reactive for neuronal nitric oxide synthase (*nNOS*; **A"**; *empty V*) (jejunum, 69-year-old patient). **B–G** Type V neurons (*arrows* indicate axons; *st*, stubby neuron in **C**). **B–E** Duodenum, 53-year-old patient; **F** duodenum, 41-year-old patient; **G** ileum, 18-year-old patient. *Scale bars* are 50 μm; *arrowed scale bars* in **A–F** point orally

The axons were thin and could seldom be followed beyond the next ganglion, let alone the ganglion beyond that. In most cases they were directed anally within the primary strands of the myenteric plexus.

As to their chemical coding, the only definite characteristic known as yet is that they are non-nitrergic (Fig. 21A). In a comparative study of type II versus type V (Brehmer et al. 2004b), we found that 18.6% of type V neurons contained SOM, 2.7% contained CAR and 0.5% displayed SP immunoreactivity. Thus, type V neurons differed distinctly from type II neurons, whose majority displayed co-reactivity for all combinations of the three markers. Unpublished observations showed that a minority of type V neurons contained also ENK. However, apart from NF we did not find any other immunohistochemical marker which stained a majority of type V neurons.

We suggest that, due to their similar morphology, type V neurons in human and pig are equivalent neurons and that their possible counterparts in guinea pig may be the cholinergic, SOM immunoreactive descending interneurons with long branched dendrites (Portbury et al. 1995; Song et al. 1997b; Pompolo and Furness 1998). In all three species, they occur singly or in groups. This is very conspicuous in pig (Stach 1985, 1989; Brehmer et al. 2002b) and in guinea pig (Portbury et al., 1995; Song et al., 1997b) and less conspicuous in human. However, the typical tangling of dendrites as observed in the pig can also be found in human type V aggregates.

4.6
Type III Neurons

(Stach et al. 2000; Brehmer et al. 2004a, 2004b)

These neurons had a single axon and several long, mostly slender and branched dendrites which emerged radially from their soma (Fig. 24). Their position in the myenteric plexus was frequently outside the ganglia, lying mostly singly beside primary strands, partly close to emerging smaller interganglionic or even secondary strands (Fig. 22B,C,H,K). Nevertheless, their axons did not enter the secondary strands but ran within the primary strands. A preferred oral/anal orientation of the axonal course could not yet be observed.

Similar to type II and type V neurons, type III neurons displayed a fine granular, less intensely autofluorescent pattern.

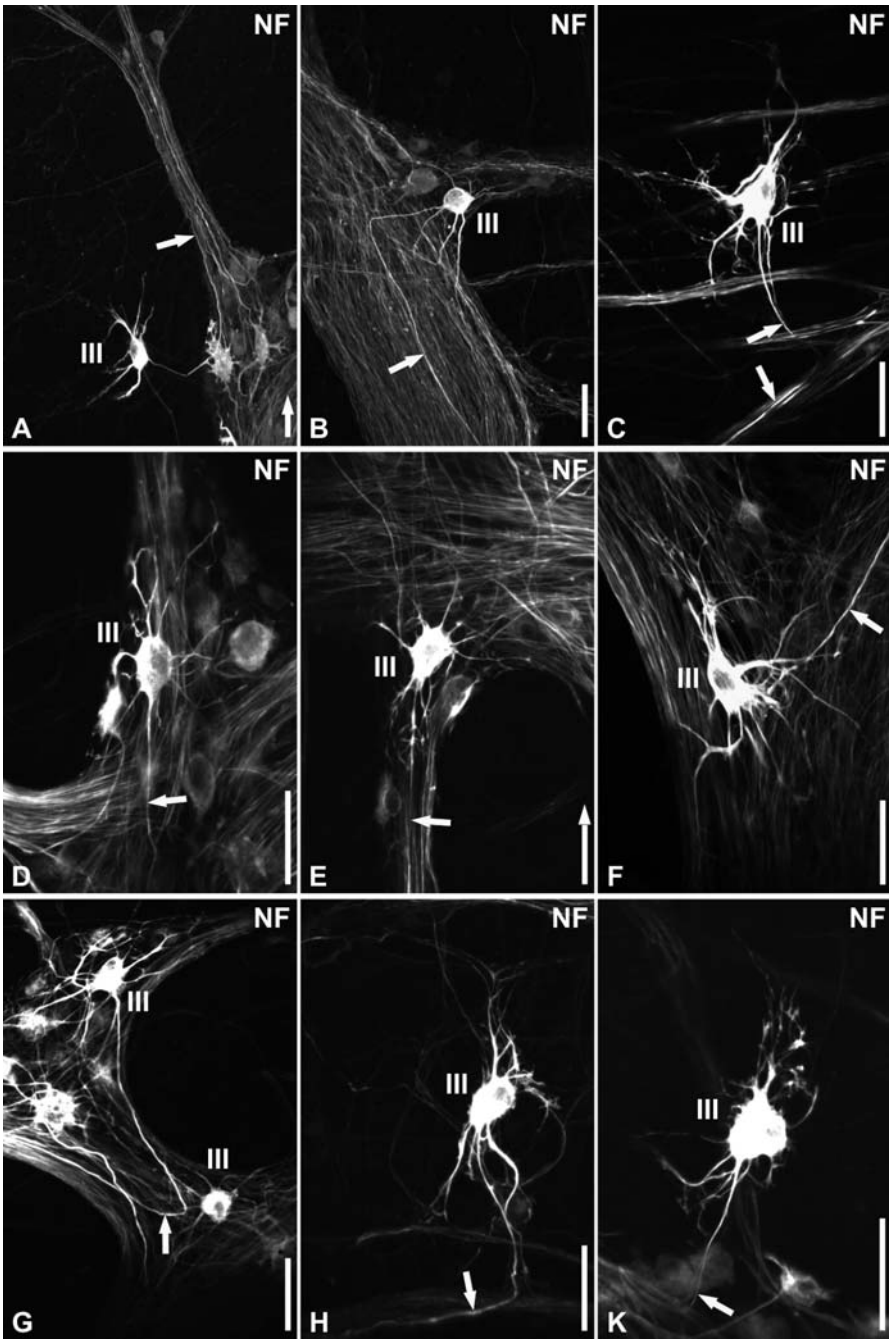


Fig. 22 A–K Human type III neurons. **A–K** Type III neurons displaying long, partly branched dendrites with mainly tapering endings which generally emerge radially from their somata (*arrows* indicate axons). **A** duodenum, 53-year-old patient; **B** duodenum, 28-year-old patient; **C, D, F–K** ileum, 18-year-old patient; **E** duodenum, 41-year-old patient. *Scale bars* are 50 μm; *arrowed scale bars* in **A** and **E** point orally

At present, we don't know whether this is a homogeneous population. Their dendritic shape and branching pattern is somewhat different from neuron to neuron. A common chemical feature is their non-nitrergic phenotype. Type III neurons were conspicuous when we tried to define the specific chemical coding of type II neurons. In some but not all specimens, several type III neurons displayed the same combinations of markers as did type II neurons (SOM/CAR, SOM/SP or SP/CAR, respectively). However, a number of type III neurons in these specimens were not co-reactive but this proportion has not yet been investigated in detail. The fact that type III neurons displaying the same chemical code as type II neurons could be found in only some of our specimens may point to a chemical plasticity in this population.

4.7
Dendritic Type II Neurons
(Stach et al. 2000; unpublished observations)

Apart from some single examples in our NF-stained specimens, we have no further information on this population beyond that described in the above paper. These neurons have one immediately branching or two or three axons and a number of slender, branched and tapering dendrites (pseudouni-, bi- or triaxonal; Fig. 22). All neurons observed in NF/nNOS-stained specimens were non-nitrergic. In contrast to the pig where they occur mainly in the submucosal plexus, in human they were found in the myenteric plexus. This topographical occurrence is similar to the guinea pig, were myenteric dendritic type II neurons have been characterized as primary afferent neurons, partly with long anal projections (Bornstein et al. 1991b; Brookes et al. 1995).

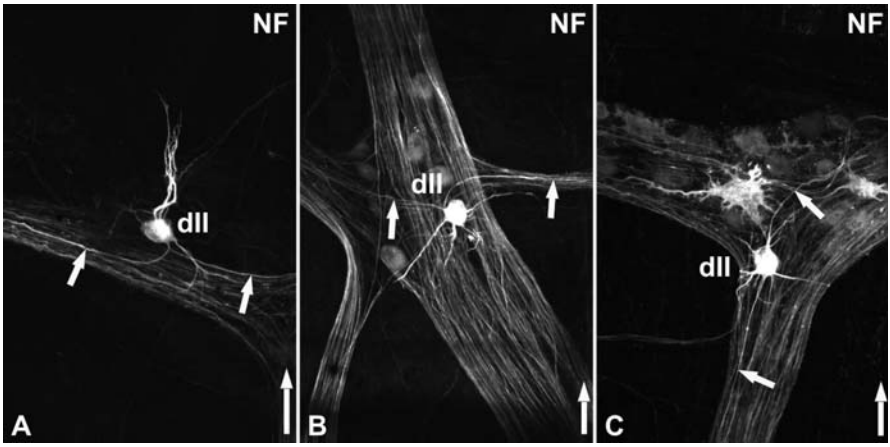


Fig. 23 A–C Human dendritic type II neurons. **A–C** Dendritic type II neurons (*dII*) displaying long, slender dendrites and are pseudouniaxonal (**A**, **B**; *arrows* indicate axons) or biaxonal (**C**; *arrows* indicate axons) (duodenum, 53-year-old patient). *Arrowed scale bars* are 50 μm

Fig. 24 A–D Myenteric ganglia from human duodenum (28-year-old patient). **A**, **B** Marked neurons are type II (*II*), type V (*V*) and spiny neurons (*sp*). **C**, **D** Marked cells (with corresponding *Roman letters*) include type II, III and V neurons. Furthermore, there are some neurons with main dendrites (*empty arrowheads*; *arrows* in **D** indicate their axons). *Scale bars* are 100 μm

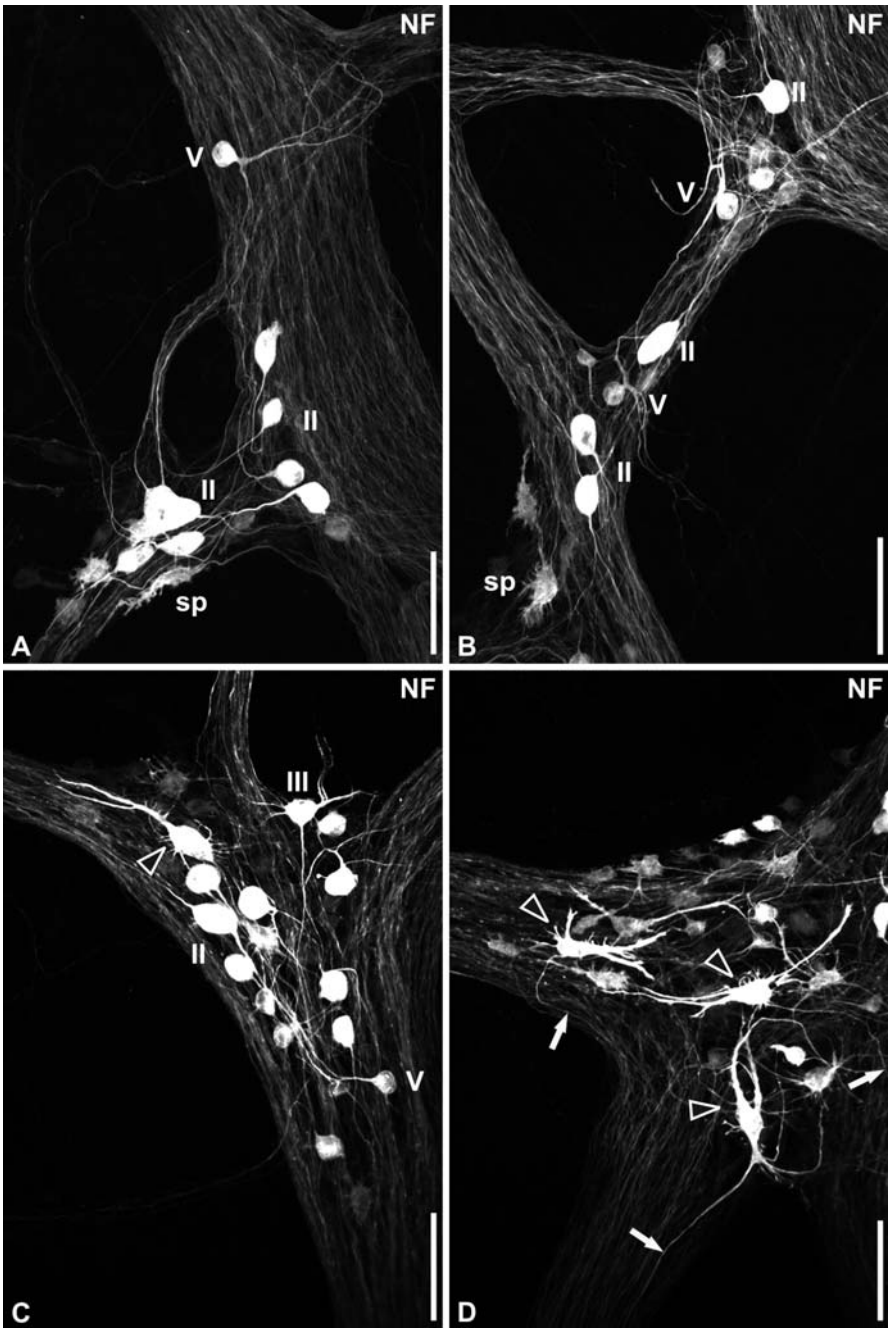
4.8
Spiny Neurons with Main Dendrites or Human Type VII Neurons?
(Stach et al. 2000; Brehmer et al. 2006)

Exclusively in the duodenum and upper jejunum, we found neurons which displayed, in addition to commonly shaped spiny dendrites, very long main dendrites (Fig. 23C,D). These main dendrites had very short, spiny side branches. Apart from these prominent main dendrites and their exclusive topographical occurrence which is restricted to the duodenum and oral jejunum, we have, at present, no hint to place them in their own category (equivalent to the pig and dog: type VII in human?). Also their chemical code hitherto defined (VIP/nNOS/NF) does not argue for a separate type. Presently, we regard the main dendrites as a region-specific feature of spiny neurons rather than these neurons as a region-specific type. Further studies are required to solve this problem.

5
Discussion

5.1
What Does NF Immunohistochemistry Achieve?

Classifications of both silver impregnation and NF immunostaining (Sects. 3 and 4) refer to only subpopulations of neurons, namely those containing NF (Vickers and Costa 1992). Both methods visualize only one—although prominent—part of the cytoskeleton of those neurons which contain NF. In the present classifications of human and pig enteric neurons, neither neurons that do not contain NF (Eaker and Sallustio 1994; Eaker 1997; Ganns et al. 2006) nor the shapes of the membrane-covered surfaces of NF-immunoreactive neurons are considered. Furthermore, both silver impregnation and NF immunostaining are unable to visualize the axonal morphology distant from the soma, i.e. axonal branching and terminal axonal fields. These were, mostly, visualized by intracellular dye injections into single cells and resulted in the representations of the 'axonal morphology' of guinea pig IPANs (Bornstein et al. 1991b; Clerc et al. 1998b), myenteric inter- or motor neurons in small (Furness et al. 1989, 2000b; Bornstein et al. 1991a) or large intestine (Lomax et al. 1999), of submucosal neurons (Song et al. 1997a) or of IPANs in the mouse (Nurgali et al. 2004).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5.2 General Remarks on Equivalent Neurons of Different Species

The ENS of the guinea pig consists of three main neuronal elements for intrinsic coordination of major gastrointestinal functions: (1) intrinsic primary afferent (sensory) neurons which are the first links of neuronal reflex arcs and can be activated by chemical and/or mechanical stimuli; (2) several types of interneurons projecting anally, orally or intestinofugally; and (3) various types of effector neurons innervating the muscle layers of both gut wall and intrinsic blood vessels, the mucosa and probably also immune cells. It is likely that this general division into three functional groups of neurons is valid throughout species, and that equivalent neuron populations fulfil corresponding roles within their respective enteric circuits.

Application of various neuroscience methods and method combinations (e.g. immunohistochemistry, immunocytochemistry, lesion, tracing and intracellular dye filling techniques, neurophysiological and pharmacological approaches) resulted in the establishment of 14 different enteric neuron types in the guinea pig small intestine (Costa et al. 1996; Furness et al. 2000a). Due to fragmentary data derived from species other than guinea pig, knowledge on equivalences of enteric neuron types between species is speculative. There are some general principles of enteric neuronal organization e.g. ascending cholinergic and descending nitrergic pathways (Timmermans et al. 1997; Reiche and Schemann 1998) or acetylcholine and SP as transmitters for excitatory as well as nNOS and VIP as inhibitory transmitters for muscle innervation (Wood et al. 1999). However, in detail, the chemical coding is well known to show species differences (Gershon et al. 1994). Moreover, studies in human specimens displayed changes of chemical codes in pathological conditions (Camilleri 2001), even beyond the gut regions directly affected by the disease (Schneider et al. 2001). Thus, for interspecies comparisons, the chemical coding of neurons is only of limited value.

Interspecies comparisons based on morphological descriptions have to consider the method of visualization. Visualization of, e.g. a cytoskeletal protein may result in quite a different depiction of the same neuron than would intracellular dye filling or immunostaining for a neuroactive substance. Thus, caution is required when comparing results obtained in the same species but with different methods. In case of myenteric type II neurons in human small intestine, we have argued that morphology is an important link when trying to decipher equivalences of neuron populations between different species. In the end, the main argument of researchers suggesting neuronal subtypes to be IPANs in species other than guinea pig is derived from morphology: neurons with smooth cell bodies and several long processes, suspected to be Dogiel type II neurons, were and are suggested to be sensory. Another positive example for the value of detailed morphological analyses of enteric neurons may be the type V neurons. Although in human (in contrast to the pig, Brehmer et al. 2002b) we have not yet found specific markers for their chemical characterization (apart from NF), their structural features are so unique that it seems obvious to suggest a common functional role in both species.

As mentioned above, in case of human stubby (type I) versus spiny (type I) neurons (Stach et al. 2000; Brehmer et al. 2004a, 2005, 2006), we have experienced that the pure outward shape of neurons is an insufficient criterion for suggesting interspecies equivalences. The combined knowledge of as many as possible criteria (morphological, including synapses received and projection to targets, chemical etc.) is needed to deduce from experimental results the position of a given neuronal population within its enteric circuit. Another example in this context is the type III neurons. In pig small intestine, the Stach type III neurons are a morphologically well-defined population with a characteristic dendritic tree, axonal projection pattern and topographical distribution along the gut, between different plexuses and within ganglia. In contrast, human type III neurons are a less prominent population. Apart from the pure presence of 'neurons with long, slender and branched dendrites' in human myenteric ganglia (described but not depicted from human gut by Dogiel 1899) and their non-nitrergic phenotype (in contrast to most of the typical type III neurons in the pig), our knowledge on this population is hardly beyond that of Dogiel.

In the CNS, it is well known that neuron types, e.g. pyramidal cells of the cerebral cortex, are equivalent elements throughout phylogenetic and ontogenetic development. Although they are subjected to adaptive processes influencing and changing various morphological aspects, principal structural features remain stable and allow their definition as 'pyramidal cells' across species boundaries (Ramón y Cajal 1911; Marín-Padilla 1998). Also the first classification of enteric neurons by Dogiel (1899) was an 'interspecies' approach. However, it is well known from both central and peripheral neurons that equivalent neurons of different species alone does not rule out somewhat differently sized and shaped dendritic arbors. Dendritic shapes are the result of a number of quite different, both activity-dependent and -independent influences, e.g. neurotrophic factors during embryonic development, phylogenetic level, species size, size of innervation target, synaptic activity etc. (Ramón y Cajal 1911; Purves and Lichtmann 1985; Purves et al. 1986; Wong and Ghosh 2002).

Thus, there is yet a lot of work to do until we will be able to put the human morphological enteric neuron types into 'classes' (Brookes 2001a) categorized by their functional role and to suggest their equivalences with corresponding populations in laboratory animals such as the guinea pig.

5.3 Putative Functional Categories of Human Enteric Neurons

5.3.1 Human Intrinsic Primary Afferent Neurons

In pig (Stach 1981, 1989; Brehmer et al. 1999a; Hens et al. 2000), guinea pig (Furness et al. 1990b), rat (Mann et al. 1997), human (Stach et al. 2000, Brehmer et al. 2004b), mouse (Nurgali et al. 2004) and sheep (Chiocchetti et al. 2004) there are myenteric neurons displaying type II morphology. That is, they are principally non-

dendritic but pseudouni- or multiaxonal. They project, one the one hand, within the myenteric plexus, mainly circumferentially, and, on the other hand, vertically, through interconnecting strands to submucosal and, probably also in human, mucosal layers. Immunohistochemically, these neurons reveal general similarities (e.g. their cholinergic, non-nitrergic phenotype) but also differences. In the pig they contain both forms of ChAT, CGRP and in some cases SP (Scheuermann et al. 1987; Hens et al. 2000; Brehmer et al. 2002a), in the guinea pig they show immunoreactivities for ChAT, CAB, tachykinins and the neurokinin receptor 3 (Furness et al. 2000a), in human they are non-nitrergic and the vast majority contain SOM, SP and CAR (Brehmer et al. 2004a, 2004b). CGRP, also present in some type II neurons (Brehmer et al. 2004b), was suggested to be present in sensory neurons (Timmermans et al. 1992; Foxx-Orenstein et al. 1996).

Also electrophysiologically, interspecies differences were demonstrated. In guinea pig and mouse, the slow after-hyperpolarization phenomenon is regarded as typical for morphological type II neurons (Furness et al. 2004a; Nurgali et al. 2004) whereas in those of the pig it is less marked (Cornelissen et al. 2000) and its general presence in human myenteric neurons is at least questionable (Brookes et al. 1987). Also the synaptic properties of type II neurons differ between guinea pig (Furness et al. 2004a) and pig (Cornelissen et al. 2001). It seems not unlikely that these morphologically equivalent neurons in different species have equivalent functions, namely that of human IPANs (Furness et al. 2004a). At present, we have only few, unpublished data on human submucosal type II neurons. This has to be further investigated.

5.3.2 Human Interneurons

Combined tracing and immunohistochemical studies provided the most profound basis for identification of these neurons in human gut since their results combined at least two independent features, i.e. axonal projection and chemistry. Wattchow et al. (1995), using DiI tracing from the myenteric plexus, found uniaxonal neurons projecting up to almost 7 cm anally (descending interneurons) and up to almost 4cm orally (ascending interneurons). The vast majority of them were equipped with short, lamellar dendrites and were classified as Dogiel type I neurons (Wattchow et al. 1995). Fifty-one per cent of the proposed descending interneurons in the large intestine were found to be immunoreactive for VIP but not for tachykinin (Wattchow et al. 1997). Porter et al. (2002) found ChAT to be present in most ascending interneurons, only a small minority contained nNOS. In contrast, 23% of orally projecting interneurons were reactive for tachykinins, only 2% for VIP (Wattchow et al. 1997). Porter et al. (2002) found nNOS-positive, ChAT-positive and ChAT/nNOS-co-reactive descending interneurons in the colon. Wattchow et al. (1997) also demonstrated CAR in 23% of proposed descending interneurons and in 3% of proposed ascending interneurons. In human gastric fundus, similar groups of chemically coded neurons with differences in their relative proportions

were found (Pimont et al. 2003), those containing both ChAT and nNOS may be interneurons also in the stomach.

We suggest that our spiny (type I) neurons (Brehmer et al. 2004a, 2006), immunoreactive for VIP/nNOS/NF, may be descending interneurons corresponding to VIP-reactive neurons demonstrated by Wattchow et al. (1997) since their axons were observed to run mostly anally, not infrequently for distances more than 1cm without leaving the myenteric plexus. However, an inhibitory motor neuronal function cannot be excluded. Stubby (type I) neurons reactive for ENK and NF, some co-reactive for SP (Brehmer et al. 2004a, 2005), may be either ascending interneurons, corresponding with the population of tachykinin-immunoreactive neurons by Wattchow et al. (1997) or (excitatory) motor neurons.

Further studies suggested GABA, partly co-localized with NADPHd (Nichols et al. 1995; Krantis et al. 1998) to be involved in interneuronal circuits. Anlauf et al. (2003) found three major groups of chemical phenotypes, cholinergic, catecholaminergic and nonadrenergic/non-cholinergic neurons. They argued for an interneuronal role of subpopulations of cholinergic neurons. Human cholinergic neurons displayed extensive co-immunoreactivity for VIP (Anlauf et al. 2003; Schneider et al. 2001). Serotonin, a candidate for interneuronal transmission also in human, has been demonstrated in myenteric and submucosal nerve cell bodies (Wakabayashi et al. 1989). Anlauf et al. (2003) demonstrated serotonin-positive fibres in both myenteric ganglia and musculature. An interneuronal role of neuropeptide Y (NPY) has been discussed by Peaire et al. (1997) who demonstrated the NPY receptor subtype Y1 on nitrergic neurons in both the myenteric and submucosal plexus.

5.3.3
Human Muscle Motor Neurons

Similar to interneurons, combined tracing and immunohistochemical studies were the most significant studies for identification of these populations in human intestinal samples. Wattchow et al. (1995) found myenteric neurons mostly of Dogiel type I morphology with short projections (maximum 16mm) to both the circular (small and large intestines) and the longitudinal musculature (small intestine). Most of the orally projecting circular muscle motor neurons contained tachykinins (Wattchow et al. 1997) and ChAT (Porter et al. 1997) whereas most anally directed muscle motor neurons were positive for VIP (Wattchow et al. 1997) and nNOS, and were partly coreactive for both markers (Porter et al. 1997). A (excitatory) motorneuronal function cannot be excluded for our stubby (type I) neurons since Wattchow et al. (1988) found nerve fibres reactive for SP and/or ENK in the external muscle of human intestine. Similarly, spiny (type I) neurons may be also inhibitory motor neurons. Proposed circular muscle motor neurons were also found in submucosal ganglia, partly also displaying reactivity for nNOS and VIP (Porter et al. 1999). The presence of VIP in myenteric nitrergic neurons as well as in circular muscle has also been shown by Keränen et al. (1995).

Haem oxygenase is the enzyme for production of carbon monoxide. Neurons immunoreactive for this enzyme, some of them co-reactive for nNOS, may be involved in inhibitory muscle innervation (Miller et al. 2001). Another co-existing substance possibly involved in inhibitory neurotransmission to the muscle may be adenosine triphosphate (Belai and Burnstock 2000). A further peptide suggested to be involved in (excitatory) motor processes is GAL. It has been demonstrated in both myenteric and submucosal neurons (Hoyle and Burnstock 1989b), its action on motility has been investigated by Bálint et al. (2001). Adenosine receptors have been found on a number of human enteric neurons including VIP- and SP-positive neurons in both the myenteric and submucosal plexus (Christofi et al. 2001).

Neurotransmission from enteric excitatory and inhibitory motor neurons to the gut musculature is considered to be primarily via the interstitial cells of Cajal (Ward and Sanders 2001; Huizinga et al. 2004). These act as pacemakers of gut motor activity, transmit nerve signals to smooth muscle and are sensors of mechanical activity (Thuneberg 1982; Huizinga et al. 2004). Signal transmission between interstitial and muscle cells is likely realized via gap junctions (Daniel 2004).

5.3.4 Other Human Effector Neurons

In contrast to the pig (type IV neurons), we were not yet able—apart from single, occasional observations—to identify a human myenteric neuron population projecting through interconnecting strands towards the submucosa, additionally to type II neurons. However, such neurons seem to exist. Hens et al. (2001), by tracing from mucosal villi, found five chemical phenotypes including SOM, SP, SOM/SP, VIP and neurons non-reactive for each of these markers. Most of the neurons were multidendritic whereas a minority was suggested to be of type II morphology. Domoto et al. (1990) found mucosal projections of submucous neurons containing VIP. Similar to the myenteric plexus, an overlap between VIP and ChAT was demonstrated in the human submucosa (Schneider et al. 2001). Besides SP and VIP, Crowe et al. (1992) found also leuENK-, SOM- and serotonin-immunoreactive submucous neurons. Some ENK- (Ferri et al. 1982; Keast et al. 1985) and also NPY-reactive fibres (Keast et al. 1985) were found in human intestinal mucosa.

5.4 Plasticity

Although the gastrointestinal tract harbours the largest number of neurons outside the CNS and is, in this context, not smaller than the spinal cord (Furness and Costa 1987), our knowledge on diseases related to defects in the innervation of the gut is rather limited. As a consequence, a number of gastrointestinal disorders is referred to as 'functional' (Thompson et al. 1999; Rasquin-Weber et al. 1999) because of the absence of morphological abnormalities of gut tissues including the ENS (Goyal and Hirano 1996). This situation, namely the lack of understanding of enteric cir-

cuits and its possible disturbances, may be comparable to that prior to understanding the correlation between structure, chemistry and function of motor areas in the brain: at that time Parkinson tremor and other disorders were called 'functional' (Kopin 1993; Keppel Hesselink 1996; Yanagisawa 1996; Wood et al. 1999). Nevertheless, neurogastroenterology as a new subspeciality of gastroenterology has been established and the gut is considered as a 'neurological organ' (Holzer et al. 2001).

An important question to be answered prior to designate the change of a parameter as pathological is to characterize age-related, non-pathological alterations (Wade 2002). Ageing per se seems not to change functional parameters towards a pathological situation which implies that there is a 'functional reserve' of the gastrointestinal tract (Firth and Prather 2002). However, there are some features which may point to specific age-related changes in both laboratory animals and humans and which may potentially affect gastrointestinal functions. These include, e.g. decrease of numbers of enteric neurons, preservation of nitrergic rather than cholinergic neurons and, that intrinsic sensory neurons may be among the 'age-labile' neurons (Santer and Baker 1988; Santer 1994; Phillips et al. 2003; Wade and Cowen 2004; Abalo et al. 2005).

Apart from neuron loss, it is known from central neurons that there are age-related alterations of dendritic morphology (Coleman and Flood 1987; Works et al. 2004). These changes are species and region specific. Morphometric investigations on dentate granule cells of hippocampus of rhesus monkeys showed that, during ageing, distal dendritic branching decreased whereas proximal dendritic branching increased (Luebke and Rosene 2003). This example highlights firstly, the need of detailed morphological knowledge of types of neurons as a basis for revealing type-related changes and secondly, that age-related plastic changes of dendritic morphology are a complex event. In the ENS, age-related plastic changes of dendritic morphology of special neuron types are fully unknown, which is not surprising since the knowledge on the dendritic morphology of distinct human neuron types is still fragmentary.

Another age-related feature in postmitotic cells such as (enteric) neurons is the inevitable accumulation of the 'age pigment' lipofuscin, already in neurons of comparatively young human patients (Brehmer et al. 2004a). It is not clear what the biological consequences of the different pigmentation patterns found in human enteric neurons are. It would be interesting to know if nitrergic neurons in the rat also show the coarse pattern of pigmentation as demonstrated, e.g. in human spiny neurons. If so, it would mean that the consequence of an intense accumulation of lipofuscin in neurons may not be cell death or, in turn, neurons unable to accumulate great amounts of lipofuscin (e.g. human type II neurons) may be prone to cell death.

For decades, the search for neuropathological changes in gut segments affected by inflammation, tumour or Hirschsprung disease was done using classical histological methods (Masson 1921–1930, cited by Reiser 1932; Rieder 1935; Miyake and Oda 1938; De Biscop 1949). These authors noted, among other things, degenerative changes of neurons including general changes on their nuclei, cytoplasm

and processes. More recently, also neuropathological correlates of functional gastrointestinal disorders and changes in specific inflammation states (the latter being reviewed in Geboes and Collins 1998) were searched for. Besides changes in nerve cell and plexus architectures described already earlier, also numbers of enteric neurons and their changes were considered (Smith 1972; Schuffler et al. 1978, 1985; Schuffler and Jonak 1982; Krishnamurty et al. 1985; Yoshida et al. 1988; Chinn and Schuffler 1988; Geboes and Collins 1998; Wedel et al. 2002).

Since, in the guinea pig, the importance of chemical coding for the identification of enteric neurons has been recognized, a number of studies dealt with the neurochemical characterization of human enteric neurons and their alterations in diseased states (reviewed in: Goyal and Hirano 1996; Giaroni et al. 1999; Holzer et al. 2001; Schemann and Neunlist 2004). There are only very few anomalies in which the causal connection between neuropathy and clinical symptoms is obvious or very likely, e.g. in Hirschsprung disease where the segmental absence of enteric neurons causes spastic contraction of the musculature (De Giorgio et al. 2004) or in infantile hypertrophic pyloric stenosis where a lack of nNOS in pyloric tissue and subsequent lack of nitric oxide, an important inhibitory neurotransmitter, is obviously responsible for the inability of the pyloric sphincter to relax (Vanderwinden et al. 1992). There is growing evidence that changes in the ENS due to inflammation affect most if not all elements of enteric circuits (Mawe et al. 2004; Lomax et al. 2005).

Recent classifications on neuropathies are based on numerous single, relatively unstructured assessments of tissue biopsies and provide only a limited insight into structure–function relationships (De Giorgio and Camilleri 2004), both under physiological and pathophysiological conditions. Novel classifications of neuropathies not only require a more quantitative, systematic assessment of tissue samples but have to be based on a more precise knowledge of the morphological and immunohistochemical starting points of healthy human enteric neurons in different age classes from which pathological alterations may begin to develop. One general difficulty to do this (as we have tried and will try) is, that the access to adequate human tissue samples is generally restricted. The resulting main problem in defining standard values is to have to do this, unlike in laboratory animals, under non-standard conditions. Tissue samples considered to be 'healthy' derived mostly from patients suffering from tumours although both earlier and more recent authors have tried to characterize changes of enteric neurons and plexuses in tumorous patients (e.g. Rieder 1935), even when the tumour was outside the gastrointestinal tract (Chinn and Schuffler 1988). To repeat, the basic requirement for defining pathological alterations of enteric neurons is the detailed knowledge of their healthy morphology. Although it was not her intention, Smith (1972) demonstrated this necessity when searching for neuropathological correlates in a case of intestinal pseudo-obstruction: she depicted a neuron which was 'swollen and has lost its dendrites' (Smith 1972: Fig. 10.3 on page 53), this was, in fact, an unequivocal type II neuron without any obvious morphological alterations.

Concerning our efforts to classify human enteric neurons, the role of dendritic morphology is, at present, only a diagnostic one, not more than it was at the

time of Dogiel and Ramón y Cajal. There is growing evidence that dendrites are an important neurocellular component which integrates a major proportion of afferent information and influences the way and direction of signal processing, whether onto the soma or, conversely, onto dendritic endings for signal transfer to neighbouring neurons (Euler and Denk 2001; Wong and Ghosh 2002; Häusser and Mel 2003). 'The great diversity of dendritic morphology ... (probably) corresponds to an equally large variety of dendritic mechanisms and functions' (Euler and Denk 2001). There is no other possible first step to understand these connections between structure and function in enteric neurons than to decipher the principles and dynamics of their shapes, i.e. to define their typical and significant structural features in consideration of plastic changes caused by influences of development including ageing processes and/or disease.

6 Summary

The ENS contains numerous different neuron populations which belong to three main groups, primary afferent neurons, interneurons and effector neurons. The most extensive knowledge on the different enteric neuron types is derived from studies in the guinea pig. A significant obstacle for the transfer of this knowledge to putative equivalent enteric neurons of other species, including human, is species differences as to their morphological, chemical, physiological etc. phenotypes. Modern morphological classifications are based on the work of the Russian histologist Dogiel. Since the late 1970s, refined morphological classifications of enteric neurons beyond Dogiel have been attempted mainly in two species, the pig and the guinea pig. These reflect the immunohistochemical diversity of enteric neurons more precisely but are far from being complete. In this paper, we follow two aims. First, we have presented an overview on the chemical coding of the morphological neuron types described by Stach in the pig intestine. In doing so, we have pointed out the difference between the definitions of type I neurons given by Dogiel and Stach. Second, we have attempted to provide a basis for the morpho-chemical classification of human enteric neurons as revealed by their immunoreactivity for NFs and several neuroactive substances or related markers. According to results from guinea pig, where there is functional evidence, human morphological type II neurons (non-dendritic, multiaxonal; co-reactive for NF, CAR, SOM, SP) seem to be the intrinsic primary afferent neurons. This conclusion is based primarily on structural equivalence. Human ENK-positive, stubby (type I) neurons may be ascending inter- or motorneurons. In contrast, nitrergic, VIP-reactive spiny (type I) neurons may be descending inter- or motor neurons. Further, morphologically defined human neuron types, i.e. type III, type V and dendritic type II neurons, are non-nitrergic but could not be chemically defined as yet. Future investigations of morpho-chemical characteristics of human enteric neurons including also other cytoskeletal markers will provide a broader basis for neurohistopathological diagnostics of gut diseases.

References

Abalo R, Rivera AJ, Vera G, Martin I (2005) Ileal myenteric plexus in aged guinea-pigs: loss of structure and calretinin-immunoreactive neurones. Neurogastroenterol Motil 17:123–132

Accili EA, Dhatt N, Buchan AMJ (1995) Neural somatostatin, vasoactive intestinal polypeptide and substance P in canine and human jejunum. Neurosci Lett 185:37–40

Anlauf M, Schäfer MK-H, Eiden L, Weihe E (2003) Chemical coding of the human gastrointestinal nervous system: cholinergic, VIPergic, and catecholaminergic phenotypes. J Comp Neurol 459:90–111

Auerbach L (1862) Ueber einen Plexus myentericus, einen bisher unbekannten ganglionervösen Apparat im Darmkanal der Wirbelthiere. Morgenstern, Breslau, pp 1–13

Auerbach L (1864) Fernere vorläufige Mittheilung über den Nervenapparat des Darmes. Arch Pathol Anat Physiol 30:457–460

Baker GE, Reese BE (1993) Using confocal laser scanning microscopy to investigate the organization and development of neuronal projections labeled with DiI. In: Matsumoto B (ed) Cell biological applications of confocal microscopy. (Methods in cell biology, Vol. 38). Academic Press, New York, pp 325–344

Bálint A, Fehér É, Kisfalvi I Jr, Mate M, Zelles T, Vízi ES, Varga G (2001) Functional and immunocytochemical evidence that galanin is a physiological regulator of human jejunal motility. J Physiol (Paris) 95:129–135

Baumgarten HG, Holstein A-F, Owman Ch (1970) Auerbach's plexus of mammals and man: Electron microscopic identification of three different types of neuronal processes in myenteric ganglia of the large intestine from rhesus monkeys, guinea-pigs and man. Z Zellforsch 106:376–397

Bayliss WM, Starling EH (1899) The movements and innervation of the small intestine. J Physiol (Lond) 24:99–143

Belai A, Burnstock G (2000) Pattern of distribution and co-localization of NOS and ATP in the myenteric plexus of human fetal stomach and intestine. Neuroreport 11:5–8

Belai A, Boulos PB, Robson T, Burnstock G (1997) Neurochemical coding in the small intestine of patients with Crohn's disease. Gut 40:767–774

Belai A, Burnstock G (1999) Distribution and colocalization of nitric oxide synthase and calretinin in myenteric neurons of developing, aging, and Crohn's disease human small intestine. Dig Dis Sci 44:1579–1587

Billroth T (1858) Einige Beobachtungen über das ausgedehnte Vorkommen von Nervenanastomosen im Tractus intestinalis. Arch Anat Physiol (Leipzig): 148-158

Bornstein JC, Costa M, Furness JB, Lees GM (1984) Electrophysiology and enkephalin immunoreactivity of identified myenteric plexus neurones of guinea-pig small intestine. J Physiol (Lond) 351:313–325

Bornstein JC, Costa M, Furness JB (1986) Synaptic inputs to immunohistochemically identified neurones in the submucous plexus of the guinea-pig small intestine. J Physiol (Lond) 381:465–482

Bornstein JC, Furness JB, Smith TK, Trussell DC (1991a) Synaptic responses evoked by mechanical stimulation of the mucosa in morphologically characterized myenteric neurons of the guinea-pig ileum. J Neurosci 11:505–518

Bornstein JC, Hendricks R, Furness JB, Trussell DC (1991b) Ramifications of the axons of AH-neurons injected with the intracellular marker biocytin in the myenteric plexus of the guinea pig small intestine. J Comp Neurol 314:437–451

Botár J, Battancs L, Becker A (1942) Die Nervenzellen des Dünndarms. Anat Anz 93:138–149

Brehmer A (2000) Morphologie von Neuronentypen im Enterischen Nervensystem. Shaker, Aachen

Brehmer A, Beleites B (1996) Myenteric neurons with different projections have different dendritic tree patterns: a morphometric study in the pig ileum. J Auton Nerv Syst 61:43–50

Brehmer A, Stach W (1997) Morphological classification of NADPHd-positive and -negative myenteric neurons in the porcine small intestine. Cell Tissue Res 287:127–134

Brehmer A, Stach W (1998) Regional structural differences in the neuronal composition of myenteric ganglia along the pig small intestine. Anat Rec 250:109–116

Brehmer A, Stach W, Addicks K (1994) Fine structural distinction between ganglia of the outer and inner submucosal plexus in porcine small intestine. Acta Anat 151:188–193

Brehmer A, Schrödl F, Neuhuber W (1997) Mucosal innervation of the pig small intestine. A preliminary report. Biog Amines 13:251–257

Brehmer A, Stach W, Krammer H-J, Neuhuber W (1998) Distribution, morphology and projections of nitrergic and non-nitrergic submucosal neurons in the pig small intestine. Histochem Cell Biol 109:87–94

Brehmer A, Schrödl F, Neuhuber W, Hens J, Timmermans J-P (1999a) Comparison of enteric neuronal morphology as demonstrated by DiI-tracing under different tissue handling conditions. Anat Embryol 199:57–62

Brehmer A, Schrödl F, Neuhuber W (1999b) Morphological classifications of enteric neurons — 100 years after Dogiel. Anat Embryol 200:125–135

Brehmer A, Göbel D, Frieser M, Graf M, Radespiel-Tröger M, Neuhuber W (2000) Experimental hypertrophy of myenteric neurones in the pig: a morphometric study. Neurogastroenterol Motil 12:155–162

Brehmer A, Frieser M, Graf M, Radespiel-Tröger M, Göbel D, Neuhuber W (2001) Dendritic hypertrophy of Stach type VI neurons within experimentally altered ileum of pigs. Auton Neurosci 89:31–37

Brehmer A, Schrödl F, Neuhuber W (2002a) Morphological phenotyping of enteric neurons using neurofilament immunohistochemistry renders chemical phenotyping more precise in porcine ileum. Histochem Cell Biol 117:257–263

Brehmer A, Schrödl F, Neuhuber W (2002b) Correlated morphological and chemical phenotyping in myenteric type V neurons of porcine ileum. J Comp Neurol 453:1–9

Brehmer A, Blaser B, Seitz G, Schrödl F, Neuhuber W (2004a) Pattern of lipofuscin pigmentation in nitrergic and non-nitrergic, neurofilament immunoreactive myenteric neuron types of human small intestine. Histochem Cell Biol 121:13–20

Brehmer A, Croner R, Dimmler A, Papadopoulos T, Schrödl F, Neuhuber W (2004b) Immunohistochemical characterization of putative primary afferent (sensory) myenteric neurons in human small intestine. Auton Neurosci 112:49–59

Brehmer A, Schrödl F, Neuhuber W, Tooyama I, Kimura H (2004c) Co-expression pattern of neuronal nitric oxide synthase and two variants of choline acetyltransferase in myenteric neurons of porcine ileum. J Chem Neuroanat 27:33–41

Brehmer A, Lindig TM, Schrödl F, Neuhuber W, Ditterich D, Rexer M, Rupprecht H (2005) Morphology of enkephalin-immunoreactive myenteric neurons in the human gut. Histochem Cell Biol 123:131–138

Brehmer A, Schrödl F, Neuhuber W (2006) Morphology of VIP/nNOS-immunoreactive myenteric neurons in the human gut. Histochem Cell Biol 125:557–565

Brookes SJH (2001a) Classes of enteric nerve cells in the guinea-pig small intestine. Anat Rec 262:58–70

Brookes SJH (2001b) Retrograde tracing of enteric neuronal pathways. Neurogastroenterol Motil 13:1–18

Brookes SJH, Ewart WR, Wingate DL (1987) Intracellular recordings from myenteric neurones in the human colon. J Physiol (Lond) 390:305–318

Brookes SJH, Song Z-M, Ramsay GA, Costa M (1995) Long aboral projections of Dogiel type II, AH neurons within the myenteric plexus of the guinea pig small intestine. J Neurosci 15:4013–4022

Brookes SJH, Meedeniya ACB, Jobling P, Costa M (1997) Orally projecting interneurones in the guinea-pig small intestine. J Physiol 505:473–491

Brookes SJH, Hennig G, Schemann M (1998) Identification of motor neurons to the circular muscle of the guinea pig gastric corpus. J Comp Neurol 397:268–280

Brown AG (1991) Nerve cells and nervous systems. Springer, London

Brown DR, Timmermans J-P (2004) Lessons from the porcine enteric nervous system. Neurogastroenterol Motil 16 (Suppl 1):50–54

Browning KN, Lees GM (1994) Reappraisal of the innervation of rat intestine by vasoactive intestinal polypeptide and neuropeptide Y-immunoreactive neurons. Neuroscience 62:1257–1266

Browning KN, Lees GM (1996) Myenteric neurons of the rat descending colon: electrophysiological and correlated morphological properties. Neuroscience 73:1029–1047

Burns AJ, Le Douarin NM (2001) Enteric nervous system development: analysis of the selective developmental potentialities of vagal and sacral neural crest cells using quail-chick chimeras. Anat Rec 262:16–28

Bush TG (2002) Enteric glial cells. An upstream target for induction of necrotizing enterocolitis and Crohn's disease? Bioessays 24:130–140

Cabarrocas J, Savidge TC, Liblau RS (2003) Role of enteric glial cells in inflammatory bowel disease. Glia 41:81–93

Camilleri M (2001) Enteric nervous system disorders: genetic and molecular insights for the neurogastroenterologist. Neurogastroenterol Motil 13:277–295

Cavazzana P, Borsetto PL (1948) Recherches sur l'aspect microscopique des plexus nerveux intramuraux et sur les modifications morphologiques de leurs neurones dans les divers traits de l'intestin humain pendant la vie. Acta Anat 5:17–41

Chinn JS, Schuffler MD (1988) Paraneoplastic visceral neuropathy as a cause of severe gastrointestinal motor dysfunction. Gastroenterology 95:1279–1286

Chiocchetti R, Poole DP, Kimura H, Aimi Y, Robbins HL, Castelucci P, Furness JB (2003) Evidence that two forms of choline acetyltransferase are differentially expressed in subclasses of enteric neurons. Cell Tissue Res 311:11–22

Chiocchetti R, Grandis A, Bombardi C, Clavenzani P, Costerbosa LG, Lucchi ML, Furness JB (2004) Characterisation of neurons expressing calbindin immunoreactivity in the ileum of the unweaned and mature sheep. Cell Tissue Res 318:289–303

Christensen J (1988) The forms of argyrophilic ganglion cells in the myenteric plexus throughout the gastrointestinal tract of the opossum. J Auton Nerv Syst 24:251–260
Christensen J, Rick GA (1987) Intrinsic nerves in the mammalian colon: confirmation of a plexus at the circular muscle-submucosal interface. J Auton Nerv Syst 21:223–231
Christofi FL, Zhang H, Yu J-G, Guzman J, Xue J, Kim M, Wang Y-Z, Cooke HJ (2001) Differential gene expression of adenosine A1, A2a, A2b, and A3 receptors in the human enteric nervous system. J Comp Neurol 439:46–64
Clarke E, O'Malley CD (1996) The human brain and spinal cord. A historical study illustrated by writings from antiquity to the twentieth century. Norman Publishing, San Francisco
Clerc N, Furness JB, Bornstein JC, Kunze WAA (1998a) Correlation of electrophysiological and morphological characteristics of myenteric neurons of the duodenum in the guinea-pig. Neuroscience 82:899–914
Clerc N, Furness JB, Li ZS, Bornstein JC, Kunze WAA (1998b) Morphological and immunohistochemical identification of neurons and their targets in the guinea-pig duodenum. Neuroscience 86:679–694
Cole EC (1930) Anastomosing neurons in the myenteric plexus of the human sigmoid flexure. J Comp Neurol 50:209–215
Coleman PD, Flood DG (1987) Neuron numbers and dendritic extent in normal aging and Alzheimer's disease. Neurobiol Aging 8:521–545
Cook RD, Burnstock G (1976a) The ultrastructure of Auerbach's plexus in the guinea-pig. I. Neuronal elements. J Neurocytol 5:171–194
Cook RD, Burnstock G (1976b) The ultrastructure of Auerbach's plexus in the guinea-pig. II. Non-neuronal elements. J Neurocytol 5:195–206
Costa M, Brookes SJH, Steele PA, Gibbins I, Burcher E, Kandiah CJ (1996) Neurochemical classification of myenteric neurons in the guinea-pig ileum. Neuroscience 75:949–967
Cornelissen W, De Laet A, Kroese ABA, Van Bogaert P-P, Scheuermann DW, Timmermans J-P (2000) Electrophysiological features of morphological Dogiel type II neurons in the myenteric plexus of pig small intestine. J Neurophysiol 84:102–111
Cornelissen W, De Laet A, Kroese ABA, Van Bogaert P-P, Scheuermann DW, Timmermans J-P (2001) Excitatory synaptic inputs on myenteric Dogiel type II neurones of the pig ileum. J Comp Neurol 432:137–154
Crowe R, Kamm MA, Burnstock G, Lennard-Jones JE (1992) Peptide-containing neurons in different regions of the submucous plexus of human sigmoid colon. Gastroenterology 102:461–467
Daniel EE (2004) Communication between interstitial cells of Cajal and gastrointestinal muscle. Neurogastroenterol Motil 16 (Suppl 1):118–122
Daniel EE, Costa M, Furness JB, Keast JR (1985) Peptide neurons in the canine small intestine. J Comp Neurol 237:227–238
Daniel EE, Furness JB, Costa M, Belbeck L (1987) The projections of chemically identified nerve fibres in canine ileum. Cell Tissue Res 247:377–384
De Biscop G (1949) Über pathologische Veränderungen am Auerbachschen Plexus bei Megacolon. Z Zellforsch 34:141–159
De Giorgio R, Camilleri M (2004) Human enteric neuropathies: morphology and molecular pathology. Neurogastroenterol Motil 16:515–531
De Giorgio R, Guerrini S, Barbara G, Cremon C, Stanghellini V, Corinaldesi R (2004) New insights into human enteric neuropathies. Neurogastroenterol Motil 16 (Suppl 1) 143-147
De Laet M-H, Dassonville M, Steyaert H, Vanderveken E, Lotstra F, Vierendeels G, Przedborski S, Vaudry H, Vanderhaeghen J-J (1989) Decrease of vasoactive intestinal peptide, methionine-enkephalin, substance P and increase of neuropeptide Y immunoreactive nerve fibres in aganglionic colon of Hirschsprung's disease. Neurochem Int 14:135–141

De Souza RR, de Carvalho CAF, Watanabe I (1988) Identification of collagen, elastic, elaunin and oxytalan fibres in ganglia of the myenteric plexus of the human oesophagus. Anat Embryol (Berl) 179:97–102

Dhatt N, Buchan AMJ (1994) Colocalization of neuropeptides with Calbindin D_{28k} and NADPH diaphorase in the enteric nerve plexuses of normal human ileum. Gastroenterology 197:680–690

Dogiel AS (1895a) Zur Frage über die Ganglien der Darmgeflechte bei den Säugetieren. Anat Anz 10:517–528

Dogiel AS (1895b) Zur Frage über den feineren Bau des sympathischen Nervensystems bei den Säugethieren. Arch Mikrosk Anat 46:305–344

Dogiel AS (1896) Zwei Arten sympathischer Nervenzellen. Anat Anz 11:679–687

Dogiel AS (1899) Ueber den Bau der Ganglien in den Geflechten des Darmes und der Gallenblase des Menschen und der Säugethiere. Arch Anat Physiol Anat Abt (Leipzig) 130-158

Domoto T, Bishop AE, Oki M, Polak JM (1990) An in vitro study of the projections of enteric vasoactive intestinal polypeptide-immunoreactive neurons in the human colon. Gastroenterology 98:819–827

Drasch O (1881) Beiträge zur Kenntnis des feineren Baues des Dünndarms, insbesondere über die Nerven desselben. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften in Wien. Abtheil III, 82:168–198

Eaker EY (1997) Neurofilament and intermediate filament immunoreactivity in human intestinal myenteric neurons. Dig Dis Sci 42:1926–1932

Eaker EY, Sallustio JE (1994) The distribution of novel intermediate filament proteins defines subpopulations of myenteric neurons in rat intestine. Gastroenterology 107:666–674

Ekblad E, Bauer AJ (2004) Role of vasoactive intestinal peptide and inflammatory mediators in enteric neuronal plasticity. Neurogastroenterol Motil 16, 123-128

Euler T, Denk W (2001) Dendritic processing. Curr Opin Neurobiol 11:415–422

Fehér E, Vajda J (1972) Cell types in the nerve plexus of the small intestine. Acta Morphol Acad Sci Hung 20:13–25

Ferri G-L, Botti PL, Vezzadini P, Biliotti G, Bloom SR, Polak JM (1982) Peptide-containing innervation of the human intestinal mucosa. Histochemistry 76:413–420

Fiala JC (2005) Reconstruct: a free editor for serial section microscopy. J Microsc 218:52–61

Firth M, Prather CM (2002) Gastrointestinal motility problems in the elderly patient. Gastroenterology 122:1688–1700

Foxx-Orenstein AE, Kuemmerle JF, Grider JR (1996) Distinct 5-HT receptors mediate the peristaltic reflex induced by mucosal stimuli in human and guinea pig intestine. Gastroenterology 111:1281–1290

Furness JB (2006) The enteric nervous system. Blackwell, Oxford

Furness JB, Costa M (1980) Types of nerves in the enteric nervous system. Neuroscience 5:1–20

Furness JB, Costa M (1987) The enteric nervous system. Churchill Livingstone, Edinburgh

Furness JB, Costa M, Eckenstein F (1983a) Neurons localized with antibodies against choline acetyltransferase in the enteric nervous system. Neurosci Lett 40:105–109

Furness JB, Costa M, Emson PC, Hakanson R, Moghimzadeh E, Sundler F, Taylor IL, Chance RE (1983b) Distribution, pathways and reactions to drug treatment of nerves with neuropeptide Y- and pancreatic polypeptide-like immunoreactivity in the guinea-pig digestive tract. Cell Tissue Res 234:71–92

Furness JB, Costa M, Gibbins IL, Llewellyn-Smith IJ, Oliver JR (1985) Neurochemically similar myenteric and submucous neurons directly traced to the mucosa of the small intestine. Cell Tissue Res, 241:155–163

Furness JB, Bornstein JC, Trussell DC (1988) Shapes of nerve cells in the myenteric plexus of the guinea-pig small intestine revealed by the intracellular injection of dye. Cell Tissue Res 254:561–571

Furness JB, Trussell DC, Pompolo S, Bornstein JC, Maley BE, Storm-Mathisen J (1989) Shapes and projections of neurons with immunoreactivity for gamma-aminobutyric acid in the guinea-pig small intestine. Cell Tissue Res 256:293–301

Furness JB, Lloyd KCK, Sternini C, Walsh JH (1990a) Projections of substance P, vasoactive intestinal peptide and tyrosine hydroxylase immunoreactive nerve fibres in the canine intestine, with special reference to the innervation of the circular muscle. Arch Histol Cytol 53:129–140

Furness JB, Trussell DC, Pompolo S, Bornstein JC, Smith TK (1990b) Calbindin neurons of the guinea-pig small intestine: quantitative analysis of their numbers and projections. Cell Tissue Res 260:261–272

Furness JB, Lloyd KCK, Sternini C, Walsh JH (1991) Evidence that myenteric neurons of the gastric corpus project to both the mucosa and the external muscle: myectomy operations on the canine stomach. Cell Tissue Res 266:475–481

Furness JB, Clerc N, Gola M, Kunze WAA, Fletcher EL (2000a) Identification of component neurons and organisation of enteric nerve circuits. In: Krammer H-J, Singer MV (eds) Neurogastroenterology from the basics to the clinics. Kluwer, Dordrecht, pp 137–150

Furness JB, Clerc N, Lomax AEG, Bornstein JC, Kunze WAA (2000b) Shapes and projections of tertiary plexus neurons of the guinea-pig small intestine. Cell Tissue Res 300:383–387

Furness JB, Alex G, Clark MJ, Lal VV (2003) Morphologies and projections of defined classes of neurons in the submucosa of the guinea-pig small intestine. Anat Rec 272A:475–483

Furness JB, Jones C, Nurgali K, Clerc N (2004a) Intrinsic primary afferent neurons and nerve circuits within the intestine. Progr Neurobiol 72:143–164

Furness JB, Robbins HL, Xiao J, Stebbing MJ, Nurgali K (2004b) Projections and chemistry of Dogiel type II neurons in the mouse colon. Cell Tissue Res 317:1–12

Gabella G (1972) Fine structure of the myenteric plexus in the guinea-pig ileum. J Anat 111:69–97

Gabella G (1982) On the ultrastructure of the enteric nerve ganglia. In: Polak JM, Bloom SR, Wright NA, Daly MJ (eds) Basic science in Gastroenterology: Structure of the gut. Glaxo, Ware, pp 193–203

Gábriel R, Pásztor I, Dénes V, Wilhelm M (1997) Some neurohistochemical properties of nerve elements in myenteric plexus of rabbit ileum: similarities and dissimilarities to the rodent pattern. Cell Tissue Res 292:283–291

Ganns D, Schrödl F, Neuhuber W, Brehmer A (2006) Investigation of general and cytoskeletal markers to estimate numbers and proportions of neurons in the human intestine. Histol Histopathol 21:41–51

Geboes K, Collins S (1998) Structural abnormalities of the nervous system in Crohn's disease and ulcerative colitis. Neurogastroenterol Motil 10:189–202

Gershon MD (1997) Genes and lineages in the formation of the enteric nervous system. Curr Opinion Neurobiol 7:101–109

Gershon MD, Bursztajn S (1978) Properties of the enteric nervous system: limitation of access of intravascular macromolecules to the myenteric plexus and muscularis externa. J Comp Neurol 180:467–488

Gershon MD, Erde SM (1981) The nervous system of the gut. Gastroenterology 80:1571–1594

Gershon MD, Rothman TP (1991) Enteric glia. Glia 4:195–204

Gershon MD, Kirchgessner AL, Wade PR (1994) Functional anatomy of the enteric nervous system. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Raven Press, New York, pp 381–422

Giaroni C, De Ponti F, Cosentino M, Lecchini S, Frigo G (1999) Plasticity in the enteric nervous system. Gastroenterology 117:1438–1458

Goniaew K (1875) Die Nerven des Nahrungsschlauches. Arch Mikrosk Anat 11:479–496

Goyal RK, Hirano I (1996) The enteric nervous system. N Engl J Med 334:1106–1115

Greving R (1951a) Histologische Studien am Plexus myentericus des Magens. 1. Der Plexus nyentericus und seine Zelltypen. Dtsch Z Nervenheilk 165:622–643

Greving R (1951b) Histologische Studien am Plexus myentericus des Magens. 2. Das Problem der Ganglienzellfortsätze. Z Anat Entwicklungsgesch 115:541–554

Gunn M (1959) Cell types in the myenteric plexus of the cat. J Comp Neurol 111:83–99

Gunn M (1968) Histological and histochemical observations on the myenteric and submucous plexuses of mammals. J Anat 102:223–239

Häusser M, Mel B (2003) Dendrites: bug or feature? Curr Opin Neurobiol 13:372–383

Hager H, Tafuri WL (1959) Elektronenoptische Untersuchungen über die Feinstruktur des Plexus myentericus (Auerbach) im Colon des Meerschweinchens (Cava cobaya). Arch Psychiat Nervenkr 199:437–471

Hahn M, Bishop J (2001) Expression pattern of Drosophila ret suggests a common ancestral origin between the metamorphosis precursors in insect endoderm and the vertebrate enteric neurons. Proc Natl Acad Sci USA 98:1053–1058

Hanani M, Reichenbach A (1994) Morphology of horseradish peroxidase (HRP)-injected glial cells in the myenteric plexus of the guinea-pig. Cell Tissue Res 278:153–160

Harting K (1931) Über die feinere Innervation der extrahepatischen Gallenwege. I. Über die mikroskopische Innervation der Gallenblase. Z Zellforsch 12:518–543

Hendriks R, Bornstein JC, Furness JB (1990) An electrophysiological study of the projections of putative sensory neurons within the myenteric plexus of the guinea pig ileum. Neurosci Lett 110:286–290

Henle J (1871) Handbuch der systematischen Anatomie des Menschen. 3. Bd, 2. Abt.: Nervenlehre. Vieweg, Braunschweig

Hens J, Schrödl F, Brehmer A, Adriaensen D, Neuhuber W, Scheuermann DW, Schemann M, Timmermans J-P (2000) Mucosal projections of enteric neurons in the porcine small intestine. J Comp Neurol 421:429–436

Hens J, Vanderwinden J-M, De Laet M-H, Scheuermann DW, Timmermans J-P (2001) Morphological and neurochemical identification of enteric neurones with mucosal projections in the human small intestine. J Neurochem 76:464–471

Hens J, Gajda M, Scheuermann DW, Adriaensen D, Timmermans J-P (2002) The longitudinal smooth muscle layer of the pig small intestine is innervated by both myenteric and submucous neurons. Histochem Cell Biol 117:481–492

Hill CJ (1927) A contribution to our knowledge of the enteric plexuses. Philos Trans R Soc London, Ser B 215:355–387

Holzer P, Schicho R, Holzer-Petsche U, Lippe IT (2001) The gut as a neurological organ. Wien Klin Wochenschr 113:647–660

Honig MG (1993) DiI labelling. Neurosci Prot 93-050-16-01-20

Honjin R, Izumi S, Ôsugi H (1959) The distribution and morphology of argentophile and argentophobe nerve cells in the myenteric plexus of the digestive tube of the mouse: a quantitative study. J Comp Neurol 111:291–319

Hoyle CHV, Burnstock G (1989a) Neuronal populations in the submucous plexus of the human colon. J Anat 166:7–22

Hoyle CHV, Burnstock G (1989b) Galanin-like immunoreactivity in enteric neurons of the human colon. J Anat 166:23–33

Huizinga JD, Golden CM, Zhu Y, White EJ (2004) Ion channels in interstitial cells of Cajal as targets for neurotransmitter action. Neurogastroenterol Motil 16 (Suppl 1):106–111

Iljina WJ, Lawrentjew BJ (1932) Zur Lehre von der Cytoarchitektonik des peripherischen autonomen Nervensystems. III. Ganglien des Rektums und ihre Beziehungen zu dem sakralen Parasympathikus. Z Mikrosk Anat Forsch 30:530–542

Irwin DA (1931) The anatomy of Auerbach's plexus. Am J Anat 49:141–166

Ito T (1936) Zytologische Untersuchungen über die intramuralen Ganglienzellen des Verdauungstraktes. Über die Ganglienzellen der menschlichen Wurmfortsätze, mit besonderer Berücksichtigung auf Golgiapparat, Mitochondrien, Nisslsubstanz und Pigmentgranula. Okajimas Fol Anat Jpn 14:621–663

Ito T, Nagahiro K (1937) Zytologische Untersuchungen über die intramuralen Ganglienzellen des Verdauungstraktes. Über die Ganglienzellen der Darmwand der Ratte, mit besonderer Berücksichtigung auf die Sekretkörnchen ähnlichen Granula in den intramuralen Ganglienzellen. Okajimas Fol Anat Jpn 15:609–634

Iwanow IF (1930) Die sympathische Innervation des Verdauungstraktes einiger Vogelarten. Z Mikrosk Anat Forsch 22:469–492

Iwanowa TS (1958) Über den Bau der Nervenzellen vom Typus II nach Dogiel. Z Mikrosk Anat Forsch 63:523–528

Jabonero V (1951) Études sur le système neurovégétatif périphérique. III. Innervation de l'estomac humain. Acta Anat 11:490–532

Jabonero V (1958) Mikroskopische Studien über die Innervation des Verdauungstraktes. I. Ösophagus. Acta Neuroveg 17:308–353

Jacobs JM (1977) Penetration of systemically injected horseradish peroxidase into ganglia and nerves of the autonomic nervous system. J Neurocytol 6:607–618

Jessen KR (2004) Glial cells. Int J Biochem Cell Biol 36:1861–1867

Johanson JF, Carney JA, Go VLW, Koch TR (1991) Segmental distribution of colonic neuropeptides in Hirschsprung's disease. Regul Pept 36:59–69

Johnson SE (1925) Experimental degeneration of the extrinsic nerves of the small intestine in relation to the structure of the myenteric plexus. J Comp Neurol 38:299–314

Jones EG (1984) History of cortical cytology. In: Peters A, Jones EG (eds) Cerebral cortex. Vol. 1: Cellular components of the cerebral cortex. Plenum Press, New York, London, pp 1–32

Kapadia SE, Kapadia CR (1986) Ultrastructure and localization of substance P and met-enkephalin immunoreactivity in the human fetal gastric antrum. Cell Tissue Res 243:289–297

Karaosmanoglu T, Aygun B, Wade PR, Gershon MD (1996) Regional differences in the number of neurons in the myenteric plexus of guinea pig small intestine and colon: an evaluation of markers used to count neurons. Anat Rec 244:470–480

Katayama Y, Lees GM, Pearson GT (1986) Electrophysiology and morphology of vasoactive-intestinal-peptide-immunoreactive neurones of the guinea-pig ileum. J Physiol (Lond) 378:1–11

Keast JR, Furness JB, Costa M (1985) Distribution of certain peptide-containing nerve fibres and endocrine cells in the gastrointestinal mucosa in five mammalian species. J Comp Neurol 236:403–422

Keast JR, Furness JB, Costa M (1987) Distribution of peptide-containing neurons and endocrine cells in the rabbit gastrointestinal tract, with particular reference to the mucosa. Cell Tissue Res 248:565–577

Keilhoff G, Fansa H, Wolf G (2002) Neuronal nitric oxide synthase is the dominant nitric oxide supplier for the survival of dorsal root ganglia after peripheral nerve axotomy. J Chem Neuroanat 24:181–187

Kellow JE, Borody TJPhillips SF, Tucker RL, Haddad AC (1986) Human interdigestive motility: variations in patterns from esophagus to colon. Gastroenterology 91:386–395

Keppel Hesselink JM (1996) Evolution of concepts and definitions of Parkinson's disease since 1817. J Hist Neurosci 5:200–207

Keränen U, Vanhatalo S, Kiviluoto T, Kivilaakso E, Soinila S (1995) Co-localization of NADPH diaphorase reactivity and vasoactive intestinal polypeptide in human colon. J Auton Nerv Syst 54:177–183

Kirsche W (1984) Ramón y Cajals Lebenswerk im Wandel der Wertung. Ein Beitrag aus Anlaß der 50. Wiederkehr seines Todestages. Z Mikrosk Anat Forsch 98:641–658

Kiss F (1933) Senile und experimentelle Veränderungen an den Zellen der peripherischen Ganglien. Vorläufige Mitteilung. Beitr Path Anat 92:127–131

Kobayashi S, Suzuki M, Uchida T, Yanaihara N (1984) Enkephalin neurons in the guinea pig duodenum: a light and electron microscopic immunocytochemical study using an antiserum to methionine-enkephalin-Arg^6-Gly^7-Leu^8. Biomed Res 5:489–506

Kobayashi S, Suzuki M, Yanaihara N (1985) Enkephalin neurons in the guinea pig proximal colon: an immunocytochemical study using an antiserum to methionine-enkephalin-Arg^6-Gly^7-Leu^8. Arch Histol Jpn 48:27–44

Koelliker A (1896) Handbuch der Gewebelehre des Menschen. Engelmann, Leipzig

Kolossow NG, Milochin AA (1963) Die afferente Innervation der Ganglien des vegetativen Nervensystems. Z Mikrosk Anat Forsch 70:426–464

Kolossow NG, Sabussow GH (1932) Zur Frage der Innervation des menschlichen Magen-Darmkanals. Z Mikrosk Anat Forsch 29:541–560

Komuro T, Baluk P, Burnstock G (1982) An ultrastructural study of neurons and non-neuronal cells in the myenteric plexus of the rabbit colon. Neuroscience 7:1797–1806

Kopin IJ (1993) Parkinson's disease: past, presence, and future. Neuropsychopharmacology 9:1–12

Krammer H-J, Kühnel W (1992) Immunohistochemistry for intermediate filaments in the enteric nervous system of the porcine small intestine. Ann Anat 174:275–278

Krammer H-J, Karahan ST, Mayer B, Zhang M, Kühnel W (1993a) Distribution of nitric oxide synthase-immunoreactive neurons in the submucosal plexus of the porcine small intestine. Ann Anat 175:225–230

Krammer H-J, Karahan ST, Rumpel E, Klinger M, Kühnel W (1993b) Immunohistochemical visualization of the enteric nervous system using antibodies against protein gene product (PGP) 9.5. Ann Anat 175:321–325

Krantis A, Nichols K, Staines W (1998) Neurochemical characterization and distribution of enteric GABAergic neurons and nerve fibres in the human colon. J Auton Nerv Syst 68:33–42

Krishnamurthy S, Schuffler MD, Rohrmann CA, Pope CE (1985) Severe idiopathic constipation is associated with a distinctive abnormality of the colonic myenteric plexus. Gastroenterology 88:26–34

Kuntz A (1913) On the innervation of the digestive tube. J Comp Neurol 23:173–192

Kuntz A (1923) On the occurrence of reflex arcs in the myenteric and submucous plexuses. Anat Rec 24:193–210

Langley JN (1900) The sympathetic and other related systems of nerves. In: Schäfer EA (ed) Text-Book of Physiology. Pentland, Edinburgh, pp 616–696

Langley JN (1921) The autonomic nervous system, part 1. Heffer, Cambridge

La Villa I (1898) Estructura de los ganglios intestinales. Revista Trimestral Micrografica III:1–13

Lawrentjew BJ (1929) Experimentell-morphologische Studien über den feineren Bau des autonomen Nervensystems. II. Über den Aufbau der Ganglien der Speiseröhre nebst einigen Bemerkungen über das Vorkommen und die Verteilung zweier Arten von Nervenzellen in dem autonomen Nervensystem. Z Mikrosk Anat Forsch 18:233–262

Lawrentjew BJ (1931) Zur Lehre von der Cytoarchitektonik des peripherischen Nervensystems. I. Die Cytoarchitektonik der Ganglien des Verdauungskanals beim Hunde. Z Mikrosk Anat Forsch 23:527–551

Lees GM, Mackenzie GM, Pearson GT (1992) Complex correlations between the morphology, electrophysiology and peptide immunohistochemistry of guinea-pig enteric neurons. Eur J Morphol 30:123–136

Levitt JB (2001) Function following form. Science 292:232–233

Li ZS, Furness JB (2000) Inputs from intrinsic primary afferent neurons to nitric oxide synthase-immunoreactive neurons in the myenteric plexus of guinea pig ileum. Cell Tissue Res 299:1–8

Li ZS, Young HM, Furness JB (1995) Do vasoactive intestinal peptide (VIP)- and nitric oxide synthase-immunoreactive terminals synapse exclusively with VIP cell bodies in the submucous plexus of the guinea-pig ileum? Cell Tissue Res 281:485–491

Llewellyn-Smith IJ, Costa M, Furness JB, Bornstein JC (1993) Structure of the tertiary component of the myenteric plexus in the guinea-pig small intestine. Cell Tissue Res 272:509–516

Lolova I, Davidoff M, Itzev D, Apostolov A (1986) Histochemical, immunocytochemical and ultrastructural data on the innervation of the smooth muscle of the large intestine in Hirschsprung's disease. Acta Physiol Pharmacol Bulg 12:5–62

Lomax AE, Furness JB (2000) Neurochemical classification of enteric neurons in the guinea-pig distal colon. Cell Tissue Res 302:59–72

Lomax AEG, Sharkey KA, Bertrand PP, Low AM, Bornstein JC, Furness JB (1999) Correlation of morphology, electrophysiology and chemistry of neurons in the myenteric plexus of the guinea-pig distal colon. J Auton Nerv Syst 76:45–61

Lomax AE, Fernández E, Sharkey KA (2005) Plasticity of the enteric nervous system during intestinal inflammation. Neurogastroenterol Motil 17:4–15

Luebke JI, Rosene DL (2003) Aging alters dendritic morphology, input resistance, and inhibitory signaling in dentate granule cells of the rhesus monkey. J Comp Neurol 460:573–584

Mann PT, Southwell BR, Ding YQ, Shigemoto R, Mizuno N, Furness JB (1997) Localisation of neurokinin 3 (NK3) receptor immunoreactivity in the rat gastrointestinal tract. Cell Tissue Res 289:1–9

Mann PT, Furness JB, Southwell BR (1999) Choline acetyltransferase immunoreactivity of putative intrinsic primary afferent neurons in the rat ileum. Cell Tissue Res 297:241–248

Mannl A, Pospischil A, Dahme E (1986) Der Plexus submucosus (Meissner und Schabadasch) im Darm des Schweines. I. Licht- und elektronenmikroskopische Untersuchung der Normalstruktur. J Vet Med A 33:647–659

Marín-Padilla M (1998) Cajal-Retzius cells and the development of the neocortex. TINS 21:64–71

Mawe GM, Collins SM, Shea-Donohue T (2004) Changes in enteric neural circuitry and smooth muscle in the inflamed and infected gut. Neurogastroenterol Motil 16 (Suppl 1):133–136

Meissner G (1857) Über die Nerven der Darmwand. Z Ration Med N F 8:364–366

Messenger JB, Bornstein JC, Furness JB (1994) Electrophysiological and morphological classification of myenteric neurons in the proximal colon of the guinea-pig. Neuroscience 60:227–244

Michail S, Karamanlidis A (1967) Morphologie du plexus myentérique d'Auerbach de l'intestin grêle du chien. Acta Anat 67:424–436

Michel K, Reiche D, Schemann M (2000) Projections and neurochemical coding of motor neurones to the circular and longitudinal muscle of the guinea pig gastric corpus. Pflügers Arch - Eur J Physiol 440:393–408

Miller SM, Reed D, Sarr MG, Farrugia G, Szurszewski JH (2001) Haem oxygenase in enteric nervous system of human stomach and jejunum and co-localization with nitric oxide synthase. Neurogastroenterol Motil 13:121–131

Miyake H, Oda M (1938) Über die klinische Bedeutung des Ileumdivertikels, mit besonderer Berücksichtigung der Pathologie des intramuralen Nervengeflechts. Dtsch Z Chir 251:111–119

Müller E (1892) Zur Kenntnis der Ausbreitung und Endigungsweise der Magen-, Darm- und Pankreas-Nerven. Arch Mikrosk Anat 40:390–409

Müller E (1921) Über das Darmnervensystem. Ups Läkaref Förh 26: XXII. 1-22

Müller LR (1912) Die Darminnervation. Dtsch Arch Klin Med 105:1–43

Munakata K, Tomita R, Kurosu Y (1997) Preliminary immunohistochemical new findings in the myenteric plexus of patients with intestinal neuronal dysplasia type B. Eur J Pediatr Surg 7:21–29

Murat VN (1933) Sur la question de la cytoarchitectonique des ganglions nerveux de l'intestin de l'homme. Trab Lab Invest Biol Univ Madrid 28:387–401

Nada O, Kawana T (1988) Immunohistochemical identification of supportive cell types in the enteric nervous system of the rat colon and rectum. Cell Tissue Res 251:523–529

Nakajima K, Tooyama I, Yasuhara O, Aimi Y, Kimura H (2000) Immunohistochemical demonstration of choline acetyltransferase of a peripheral type (pChAT) in the enteric nervous system of rats. J Chem Neuroanat 18:31–40

Neuhuber WL, Raab M, Berthoud H-R, Wörl J (2006) Innervation of the mammalian esophagus. Adv Anat Embryol Cell Biol 185:1–76

Neunlist M, Aubert P, Toquet C, Oreshkova T, Barouk, J, Lehur PA, Schemann M, Galmiche JP (2003) Changes in chemical coding of myenteric neurones in ulcerative colitis. Gut 52:84–90

Nichols K, Staines W, Wu J-Y, Krantis A (1995) Immunopositive GABAergic neural sites display nitric oxide synthase-related NADPH diaphorase activity in the human colon. J Auton Nerv Syst 50:253–262

Nurgali K, Stebbing MJ, Furness JB (2004) Correlation of electrophysiological and morphological characteristics of enteric neurons in the mouse colon. J Comp Neurol 468:112–124

Okamura C (1934) Über die Darstellung des Nervenapparates in der Magen-Darmwand mittels der Vergoldungsmethode. Z Mikrosk Anat Forsch 35:218–253

Oshima Dr (1929) Über die Innervation des Darmes. Z Anat Entwicklungsgesch 90:725–767

Ottaviani G (1940) Histologisch-anatomische Untersuchungen über die Innervation des Mastdarmes (Intestinum terminale-Rectum). Z Mikrosk Anat Forsch 47:151–182

Patzelt V (1936) Die Nerven der Darmwand. In: von Möllendorf W (ed) Handbuch der Mikroskopischen Anatomie des Menschen. Verdauungsapparat, Atemapparat (V/3). Springer, Berlin, pp 313–328

Peaire AE, Krantis A, Staines WA (1997) Distribution of the NPY receptor subtype Y1 within human colon: evidence for NPY targeting a subpopulation of nitrergic neurons. J Auton Nerv Syst 67:168–175

Pearson GT (1994) Structural organization and neuropeptide distributions in the equine enteric nervous system: an immunohistochemical study using whole-mount preparations from the small intestine. Cell Tissue Res 276:523–534

Peters A, Palay SL, Webster H deF (1991) The fine structure of the nervous system. Oxford University Press, New York

Pfannkuche H, Reiche D, Sann H, Schemann M (1998) Different subpopulations of cholinergic and nitrergic myenteric neurones project to mucosa and circular muscle of the guinea-pig gastric fundus. Cell Tissue Res 292:463–475

Phillips RJ, Kieffer JK, Powley TL (2003) Aging of the myenteric plexus: neuronal loss is specific to cholinergic neurons. Auton Neurosci 106:69–83

Phillips JR, Hargrave SL, Rhodes BS, Zopf DA, Powley TL (2004) Quantification of neurons in the myenteric plexus: an evaluation of putative pan-neuronal markers. J Neurosci Meth 133:99–107

Pieper S, Halle M, Kikinis R (2004) 3D Slicer. Proceedings of IEEE International Symposium on Biomedical Imaging: From Nano to Macro 2004. SPL Technical Report 448, 632-635

Pimont S, Des Varannes SB, Le Neel JC, Aubert P, Galmiche JP, Neunlist M (2003) Neurochemical coding of myenteric neurones in the human gastric fundus. Neurogastroenterol Motil 15:655–662

Pompolo S, Furness JB (1988) Ultrastructure and synaptic relationships of calbindin-reactive, Dogiel type II neurons, in myenteric ganglia of guinea-pig small intestine. J Neurocytol 17:771–782

Pompolo S, Furness JB (1990) Ultrastructure and synaptology of neurons immunoreactive for gamma-aminobutyric acid in the myenteric plexus of the guinea pig small intestine. J Neurocytol 19:539–549

Pompolo S, Furness JB (1995) Sources of inputs to longitudinal muscle motor neurons and ascending interneurons in the guinea-pig small intestine. Cell Tissue Res 280:549–560

Pompolo S, Furness JB (1998) Quantitative analysis of inputs to somatostatin-immunoreactive descending interneurons in the myenteric plexus of the guinea-pig small intestine. Cell Tissue Res 294:219–226

Portbury AL, Pompolo S, Furness JB, Stebbing MJ, Kunze WAA, Bornstein JC, Hughes S (1995) Cholinergic, somatostatin-immunoreactive interneurons in the guinea pig intestine: morphology, ultrastructure, connections and projections. J Anat 187:303–321

Portbury AL, Grkovic I, Young HM, Furness JB (2001) Relationship between postsynaptic NK1 receptor distribution and nerve terminals innervating myenteric neurons in the guinea-pig ileum. Anat Rec 263:248–254

Porter AJ, Wattchow DA, Brookes SJH, Schemann M, Costa M (1996) Choline acetyltransferase immunoreactivity in the human small and large intestine. Gastroenterology 111:401–408

Porter AJ, Wattchow DA, Brookes SJH, Costa M (1997) The neurochemical coding and projections of circular muscle motor neurons in the human colon. Gastroenterology 113:1916–1923

Porter AJ, Wattchow DA, Hunter A, Costa M (1998) Abnormalities of nerve fibres in the circular muscle of patients with slow transit constipation. Int J Colorectal Dis 13:208–216

Porter AJ, Wattchow DA, Brookes SJH, Costa M (1999) Projections of nitric oxide synthase and vasoactive intestinal polypeptide-reactive submucosal neurons in the human colon. J Gastroenterol Hepatol 14:1180–1187

Porter AJ, Wattchow DA, Brookes SJH, Costa M (2002) Cholinergic and nitrergic interneurones in the myenteric plexus of the human colon. Gut 51:70–75

Purves D, Lichtmann JW (1985) Geometrical differences among homologous neurons in mammals. Science 228:298–302

Purves D, Rubin E, Snider WD, Lichtmann J (1986) Relation of animal size to convergence, divergence, and neuronal number in peripheral sympathetic pathways. J Neurosci 6:158–163

Ramón y Cajal S (1893) Sur les ganglions et plexus nerveux de l'intestin. C R Soc Biol 5:217–223
Ramón y Cajal S (1911) Histologie du système nerveux de l'homme et des vertébrés. Maloine, Paris
Rasquin-Weber A, Hyman PE, Cucchiara S, Fleisher DR, Hyams JS, Milla PJ, Staiano A (1999) Childhood functional gastrointestinal disorders. Gut 45 (Suppl II): II60-II68
Reiche D, Schemann M (1998) Ascending choline acetyltransferase and descending nitric oxide synthase immunoreactive neurones of the myenteric plexus project to the mucosa of the guinea-pig gastric corpus. Neurosci Lett 241:61–64
Reiser KA (1932) Der Nervenapparat im Processus vermiformis nebst einigen Bemerkungen über seine Veränderungen bei chronischer Appendicitis. Z Zellforsch 15:761–799
Rieder W (1935) Mikroskopische Untersuchungen des intramuralen Plexus bei chirurgischen Erkrankungen des Magens. Dtsch Z Chir 244:471–490
Romer AS (1972) The vertebrate as a dual animal - somatic and visceral. Evol Biol 6:121–156
Rossi O (1929) Contributo alla conoscenza degli apparati nervosi intramurali dell' intestino tenue. Arch Ital Anat Embriol 26:632–644
Rühl A (2005) Glial cells in the gut. Neurogastroenterol Motil 17:777–790
Sandgren K, Lin Z, Svenningsen AF, Ekblad E (2003) Vasoactive intestinal peptide and nitric oxide promote survival of adult rat myenteric neurons in culture. J Neurosci Res 72:595–602
Sang Q, Young HM (1996) Chemical coding of neurons in the myenteric plexus and external muscle of the small and large intestine of the mouse. Cell Tissue Res 284:39–53
Sang Q, Young HM (1998) The identification and chemical coding of cholinergic neurons in the small and large intestine of the mouse. Anat Rec 251:185–199
Sang Q, Williamson S, Young HM (1997) Projections of chemically identified myenteric neurons of the small and large intestine of the mouse. J Anat 190:209–222
Santer RM (1994) Survival of the population of NADPH-diaphorase stained myenteric neurons in the small intestine of aged rats. J Auton Nerv Syst 49:115–121
Santer RM, Baker DM (1988) Enteric neuron numbers and sizes in Auerbach's plexus in the small and large intestine of adult and aged rats. J Auton Nerv Syst 25:59–67
Saur D, Paehge H, Schusdziarra V, Allescher HD (2000) Distinct expression of splice variants of neuronal nitric oxide synthase in the human gastrointestinal tract. Gastroenterology 118:849–858
Schabadasch A (1930) Intramurale Nervengeflechte des Darmrohrs. Z Zellforsch Mikrosk Anat 10:320–385
Scharf J-H (1958) Sensible Ganglien (Ergänzung zu Band IV/1). In: von Möllendorf W, Bargmann W (eds) Handbuch der Mikroskopischen Anatomie des Menschen. Nervensystem (Dritter Teil). Springer, Berlin, 485 pp
Schemann M (1992) Characteristics of myenteric neurons in the gastric corpus. In: Holle GE, Wood JD (eds) Advances in the innervation of the gastrointestinal tract. Excerpta Medica, Amsterdam, pp 147–156
Schemann M, Neunlist M (2004) The human enteric nervous system. Neurogastroenterol Motil 16 (Suppl 1):55–59
Schemann M, Sann H, Schaaf C, Mäder M (1993) Identification of cholinergic neurons in enteric nervous system by antibodies against choline acetyltransferase. Am J Physiol 265: G1005-G1009
Schemann M, Schaaf C, Mäder M (1995) Neurochemical coding of enteric neurons in the guinea-pig stomach. J Comp Neurol 353:161–178
Schemann M, Reiche D, Michel K (2001) Enteric pathways in the stomach. Anat Rec 262:47–57

Scheuermann DW, Stach W (1983) External adrenergic innervation of the three neuron types of Dogiel in the Plexus myentericus and the Plexus submucosus externus of the porcine small intestine. Histochemistry 77:303–311

Scheuermann DW, Stach W, De Groodt-Lasseel MHA, Timmermans J-P (1987) Calcitonin gene-related peptide in morphologically well-defined type II neurons of the enteric nervous system in the porcine small intestine. Acta Anat 129:325–328

Scheuermann DW, Stach W, Timmermans J-P, Adriaensen D, De Groodt-Lasseel MHA (1989) Neuron-specific enolase and S-100 protein immunohistochemistry for defining the structure and topographical relationship of the different enteric nerve plexuses in the small intestine of the pig. Cell Tissue Res 256:65–75

Scheuermann DW, Stach W, Timmermans J-P (1991) Functional morphology of the enteric nervous system. Verh Anat Ges 85:75–85

Schnell SA, Staines WA, Wessendorf MW (1999) Reduction of lipofuscin-like autofluorescence in fluorescently labeled tissue. J Histochem Cytochem 47:719–730

Schneider J, Jehle EC, Starlinger MJ, Neunlist M, Michel K, Hoppe S, Schemann M (2001) Neurotransmitter coding of enteric neurones in the submucous plexus is changed in non-inflamed rectum of patients with Crohn's disease. Neurogastroenterol Motil 13:255–264

Schofield GC (1962) Experimental studies on the myenteric plexus in mammals. J Comp Neurol 119:159–185

Schultzberg M, Hökfelt T, Nilsson G, Terenius L, Rehfeld JF, Brown M, Elde R, Goldstein M, Said S (1980) Distribution of peptide- and catecholamine-containing neurons in the gastrointestinal tract of rat and guinea-pig: immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurotensin and dopamin β-hydroxylase. neuroscience 5:689–744

Schuffler MD, Jonak Z (1982) Chronic idiopathic intestinal pseudoobstruction caused by a degenerative disorder of the myenteric plexus: the use of Smith's method to define the neuropathology. Gastroenterology 82:476–486

Schuffler MD, Bird TD, Sumi SM, Cook A (1978) A familial neuronal disease presenting as intestinal pseudoobstruction. Gastroenterology 75:889–898

Schuffler MD, Leon SH, Krishnamurthy S (1985) Intestinal pseudoobstruction caused by a new form of visceral neuropathy: palliation by radical small bowel resection. Gastroenterology 89:1152–1156

Shimizu H, Koizumi O, Fujisawa T (2004) Three digestive movements in Hydra regulated by the diffuse nerve net in the body column. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 190:623–630

Singaram C, Ashraf W, Gaumnitz EA, Torbey C, Sengupta A, Pfeiffer R (1995) Dopaminergic defect of enteric nervous system in Parkinson's disease patients with chronic constipation. Lancet 346:861–864

Sjolund K, Fasth S, Ekman R, Hulten L, Jiborn H, Nordgren S, Sundler F (1997) Neuropeptides in idiopathic chronic constipation (slow transit constipation). Neurogastroenterol Motil 9:143–150

Smith B (1972) The neuropathology of the alimentary tract. Edward Arnold, London

Sokolowa ML (1931) Zur Lehre von der Cytoarchitektonik des peripherischen autonomen Nervensystems. II. Die Architektur der intramuralen Ganglien des Verdauungstrakts des Rindes. Z Mikrosk Anat Forsch 23:552–570

Song Z-M, Brookes SJH, Costa M (1992) Projections and pathways of submucous neurons to the mucosa of the guinea-pig small intestine. Cell Tissue Res 269:87–98

Song Z-M, Brookes SJH, Llewellyn-Smith IJ, Costa M (1995) Ultrastructural studies of the myenteric plexus and smooth muscle in organotypic cultures of the guinea-pig small intestine. Cell Tissue Res 280:627–637

Song Z-M, Brookes SJH, Neild TO, Costa M (1997a) Immunohistochemical and electrophysiological characterization of submucous neurons from the guinea-pig small intestine in organ culture. J Auton Nerv Syst 63:161–171

Song Z-M, Brookes SJH, Ramsay GA, Costa M (1997b) Characterization of myenteric interneurons with somatostatin immunoreactivity in the guinea-pig small intestine. Neuroscience 80:907–923

Stach W (1972) Der Plexus entericus extremus des Dickdarmes und seine Beziehungen zu den interstitiellen Zellen (Cajal). Z Mikrosk Anat Forsch 85:245–272

Stach W (1977a) Die differenzierte Gefäßversorgung der Dogielschen Zelltypen und die bevorzugte Vaskularisation der Typ I-Zellen in den Ganglien des Plexus submucosus externus (Schabadasch) des Schweins. Z Mikrosk Anat Forsch 91:421–429

Stach W (1977b) Der Plexus submucosus externus (Schabadasch) im Dünndarm des Schweins. I. Form, Struktur und Verbindungen der Ganglien und Nervenzellen. Z Mikrosk Anat Forsch 91:737–755

Stach W (1979) In Memoriam Arnold Leonowitsch Schabadasch. J Hirnforsch 20:453–454

Stach W (1980) Zur neuronalen Organisation des Plexus myentericus (Auerbach) im Schweinedünndarm. I. Typ I-Neurone. Z Mikrosk Anat Forsch 94:833–849

Stach W (1981) Zur neuronalen Organisation des Plexus myentericus (Auerbach) im Schweinedünndarm. II. Typ II-Neurone. Z Mikrosk Anat Forsch 95:161–182

Stach W (1982a) Zur neuronalen Organisation des Plexus myentericus (Auerbach) im Schweinedünndarm. III. Typ III-Neurone. Z Mikrosk Anat Forsch 96:497–516

Stach W (1982b) Zur neuronalen Organisation des Plexus myentericus (Auerbach) im Schweinedünndarm. IV. Typ IV-Neurone. Z Mikrosk Anat Forsch 96:972–994

Stach W (1983) Über morphologisch definierte vertikale Verbindungen innerhalb des Darmwandnervensystems im Schweinedünndarm. Verh Anat Ges 77:577–578

Stach W (1985) Zur neuronalen Organisation des Plexus myentericus (Auerbach) im Schweinedünndarm. V. Typ V-Neurone. Z Mikrosk Anat Forsch 99:562–582

Stach W (1989) A revised morphological classification of neurons in the enteric nervous system. In: Singer MV, Goebell H (eds) Nerves and the gastrointestinal tract. Kluwer, Lancaster, pp 29–45

Stach W, Krammer H-J, Brehmer A (2000) Structural organization of enteric nerve cells in large mammals including man. In: Krammer H-J, Singer MV (eds) Neurogastroenterology from the basics to the clinics. Kluwer, Dordrecht, pp 3–20

Steele PA, Brookes SJH, Costa M (1991) Immunohistochemical identification of cholinergic neurons in the myenteric plexus of guinea-pig small intestine. Neuroscience 45:227–239

Stöhr P (1931) Mikroskopische Studien zur Innervation des Magen-Darmkanales. Z Zellforsch 12:66–154

Stöhr P (1952) V. Zusammenfassende Ergebnisse über die mikroskopische Innervation des Magen-Darmkanals. Ergeb Anat Entwicklungsgesch 34:250–401

Strausfeld NJ (1976) Atlas of an insect brain. Springer, Berlin

Sundler F, Ekblad E, Hakanson R (1993) Localization and colocalization of gastrointestinal peptides. In: Brown DR (ed) Gastrointestinal regulatory peptides. Springer, Berlin, pp 1–28

Sutherland SD (1967) The neurons of the gall bladder and gut. J Anat 101:701–709

Temesrékási D (1955) Die Synaptologie der Dünndarmgeflechte. Acta Morph Acad Sci Hung 5:53–69

Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Müller-Lissner SA (1999) Functional bowel disorders and functional abdominal pain. Gut 45 (Suppl II): II43-47

Thomsen L, Pearson GT, Hviid Larsen E, Skadhauge E (1997) Electrophysiological properties of neurones in the internal and external submucous plexuses of newborn pig small intestine. J Physiol (Lond) 498:773–785

Thuneberg L (1982) Interstitial cells of cajal: intestinal pacemaker cells? Adv Anat Embryol Cell Biol 71:1–130

Tiegs OW (1927) The structure of the neurone junctions in sympathetic ganglia, and in the ganglia of Auerbach's plexus. Aust J Exp Biol Med Sci 4:79–98

Timmermans J-P, Scheuermann DW, Stach W, Adriaensen D, De Groodt-Lasseel MHA, Polak JM (1989) Neuromedin U-immunoreactivity in the nervous system of the small intestine of the pig and its coexistence with substance P and CGRP. Cell Tissue Res 258:331–337

Timmermans J-P, Scheuermann DW, Stach W, Adriaensen D, De Groodt-Lasseel MHA (1990) Distinct distribution of CGRP-, enkephalin-, galanin-, neuromedin U-, neuropeptide Y-, somatostatin-, substance P-, VIP- and serotonin-containing neurons in the two submucosal ganglionic neural networks of the porcine small intestine. Cell Tissue Res 260:367–379

Timmermans J-P, Scheuermann DW, Barbiers M, Adriaensen D, Stach W, van Hee R, De Groodt-Lasseel MHA (1992) Calcitonin gene-related peptide-like immunoreactivity in the human small intestine. Acta Anat 143:48–53

Timmermans J-P, Barbiers M, Scheuermann DW, Stach W, Adriaensen D, De Groodt-Lasseel MHA (1993) Occurrence, distribution and neurochemical features of small intestinal neurons projecting to the cranial mesenteric ganglion in the pig. Cell Tissue Res 272:49–58

Timmermans J-P, Barbiers M, Scheuermann DW, Stach W, Adriaensen D, Mayer B, DeGroodt-Lasseel MHA (1994) Distribution pattern, neurochemical features and projections of nitrergic neurons in the pig small intestine. Ann Anat 176:515–525

Timmermans J-P, Adriaensen D, Cornelissen W, Scheuermann DW (1997) Structural organization and neuropeptide distribution in the mammalian enteric nervous system, with special attention to those components involved in mucosal reflexes. Comp Biochem Physiol 118A:331–340

Timmermans J-P, Hens J, Adriaensen D (2001) Outer submucous plexus: an intrinsic nerve network involved in both secretory and motility processes in the intestine of mammals and humans. Anat Rec 262:71–78

Toole L, Belai A, Burnstock G (1998) A neurochemical characterisation of the golden hamster myenteric plexus. Cell Tissue Res 291:385–394

Tooyama I, Kimura H (2000) A protein encoded by an alternative splice variant of choline acetyltransferase mRNA is localized preferentially in peripheral nerve cells and fibres. J Chem Neuroanat 17:217–226

Trendelenburg P (1917) Physiologische und pharmakologische Versuche über die Dünndarmperistaltik. Arch Exp Pathol Pharmakol 81:55–129

Turner JN, Ancin H, Becker DE, Szarowski DH, Holmes M, O'Connor N, Wang M, Holmes T, Roysam B (1997) Automated image analysis technologies for biological 3D light microscopy. Int J Imag Syst Tech 8:240–254

Turner JN, Shain W, Szarowski DH, Lasek S, Dowell N, Sipple B, Can A, Al-Kofahi K, Roysam B (2000) Three-dimensional light microscopy: Observation of thick objects. J Histotechnol 23:205–217

Vanderwinden J-M, Mailleux P, Schiffmann SN, Vanderhaeghen J-J, De Laet M-H (1992) Nitric oxide synthase activity in infantile hypertrophic pyloric stenosis. N Engl J Med 327:511–515

van Esveld LW (1928) Über die nervösen Elemente in der Darmwand. Z Mikrosk Anat Forsch 15:1–42

Van Ginneken C, Van Meir F, Sommereyns G, Sys S, Weyns A (1998) Nitric oxide synthase expression in enteric neurons during development in the pig duodenum. Anat Embryol 198:399–408

van Pelt J, van Ooyen A, Uylings HBM (2001) The need for integrating neuronal morphology databases and computational environments in exploring neuronal structure and function. Anat Embryol 204:255–265

Vickers JC, Costa M (1992) The neurofilament triplet is present in distinct subpopulations of neurons in the central nervous system of the guinea-pig. Neuroscience 49:73–100

von Boyen GBT, Steinkamp M, Reinshagen M, Schäfer K-H, Adler G, Kirsch J (2004) Proinflammatory cytokines increase glial fibrillary acidic protein expression in enteric glia. Gut 53:222–228

Wade PR (2002) Aging and neural control of the GI tract. I. Age-related changes in the enteric nervous system. Am J Physiol Gastrointest Liver Physiol 283: G489-G495

Wade PR, Cowen T (2004) Neurodegeneration: a key factor in the ageing gut. Neurogastroenterol Motil 16 (Suppl 1):19–23

Wakabayashi K, Takahashi H, Ohama E, Ikuta F (1989) Tyrosine hydroxylase-immunoreactive intrinsic neurons in the Auerbach's and Meissner's plexuses of humans. Neurosci Lett 96:259–263

Waldeyer, W (1891) Über einige neuere Forschungen im Gebiete der Anatomie des Centralnervensystems. Dtsch Med Wochenschr 17:1213–1218; 1244-1246; 1267-1269; 1287-1289; 1331-1332; 1352-1356

Walters JR, Bishop AE, Facer P, Lawson EM, Rogers JH, Polak JM (1993) Calretinin and Calbindin-D_{28k} immunoreactivity in the human gastrointestinal tract. Gastroenterology 104:1381–1389

Wang YF, Mao YK, Xiao Q, Daniel EE, Borkowski KR, McDonald TJ (1997) The distribution of NPY-containing nerves and the catecholamine contents of canine enteric nerve plexuses. Peptides 18:221–234

Ward SM, Sanders KM (2001) Interstitial cells of Cajal: primary targets of enteric motor innervation. Anat Rec 262:125–135

Wattchow DA, Furness JB, Costa M (1988) Distribution and coexistence of peptides in nerve fibres of the external muscle of the human gastrointestinal tract. Gastroenterology 95:32–41

Wattchow DA, Brookes SJH, Costa M (1995) The morphology and projections of retrogradely labeled myenteric neurons in the human intestine. Gastroenterology 109:866–875

Wattchow DA, Porter AJ, Brookes SJH, Costa M (1997) The polarity of neurochemically defined myenteric neurons in the human colon. Gastroenterology 113:497–506

Wedel T, Roblick U, Gleiß J, Schiedeck T, Bruch H-P, Kühnel W, Krammer H-J (1999) Organization of the enteric nervous system in the human colon demonstrated by whole-mount immunohistochemistry with special reference to the submucous plexus. Ann Anat 181:327–337

Wedel T, Spiegler J, Soellner S, Roblick UJ, Schiedeck THK, Bruch H-P, Krammer H-J (2002) Enteric nerves and interstitial cells of Cajal are altered in patients with slow-transit constipation and megacolon. Gastroenterology 123:1459–1467

Wester T, O'Briain DS, Puri P (1999) Notable postnatal alterations in the myenteric plexus of normal human bowel. Gut 44:666–674

Wilson AJ, Furness JB, Costa M (1981a) The fine structure of the submucous plexus of the guinea-pig ileum. I. The ganglia, neurons, Schwann cells and neuropil. J Neurocytol 10:759–784

Wilson AJ, Furness JB, Costa M (1981b) The fine structure of the submucous plexus of the guinea-pig ileum. II. Description and analysis of vesiculated nerve profiles. J Neurocytol 10:785–804

Wong ROL, Ghosh A (2002) Activity-dependent regulation of dendritic growth and patterning. Nat Rev Neurosci 3:803–812

Wood JD, Alpers DH, Andrews PLR (1999) Fundamentals of neurogastroenterology. Gut 45 (Suppl II): II6-II16

Works SJ, Wilson RE, Wellman CL (2004) Age-dependent effect of cholinergic lesion on dendritic morphology in rat frontal cortex. Neurobiol Aging 25:963–974

Wu M, Timmermans J-P, Scheuermann DW (1998) Distributional pattern and targets of GABA-containing neurons in the porcine small and large intestine. Eur J Morphol 36:133–142

Wu M, Van Nassauw L, Kroese ABA, Adriaensen D, Timmermans J-P (2003) Myenteric nitrergic neurons along the rat esophagus: evidence for regional and strain differences in age-related changes. Histochem Cell Biol 119:395–403

Yanagisawa N (1996) Historical review of research on functions of basal ganglia. Eur Neurol 36 (Suppl 1):2–8

Yoshida MM, Schuffler MD, Sumi SM (1988) There are no morphologic abnormalities of the gastric wall or abdominal vagus in patients with diabetic gastroparesis. Gastroenterology 94:907–914

Young HM, Furness JB (1995) Ultrastructural examination of the targets of serotonin-immunoreactive descending interneurons in the guinea-pig small intestine. J Comp Neurol 356:101–114

Young HM, Newgreen D (2001) Enteric neural crest-derived cells: origin, identification, migration, and differentiation. Anat Rec 262:1–15

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