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Structure of Enteric Neurons

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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).