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Architecture of enteric neural circuits involved in intestinal motility

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Abstract. – This short review describes the conceptual development in the search for the enteric neural circuits with the initial identifications of the classes of enteric neurons on the bases of their morphology, neurochemistry, biophysical properties, projections and connectivity. The discovery of the presence of multiple neurochemicals in the same nerve cells in specific combinations led to the concept of "chemical coding" and of "plurichemical transmission". The proposal that enteric reflexes are largely responsible for the propulsion of contents led to investigations of polarised reflex pathways and how these may be activated to generate the coordinated propulsive behaviour of the intestine. The research over the past decades attempted to integrate information of chemical neuroanatomy with functional studies, with the development of methods combining anatomical, functional and pharmacological techniques. This multidisciplinary strategy led to a full accounting of all functional classes of enteric neurons in the guinea-pig, and advanced wiring diagrams of the enteric neural circuits have been proposed. In parallel, investigations of the actual behaviour of the intestine during physiological motor activity have advanced with the development of spatio-temporal analysis from video recordings. The relation between neural pathways, their activities and the generation of patterns of motor activity remain largely unexplained. The enteric neural circuits appear not set in rigid programs but respond to different physico-chemical contents in an adaptable way (neuromechanical hypothesis). The generation of the complex repertoire of motor patterns results from the interplay of myogenic and neuromechanical mechanisms with spontaneous generation of migratory motor activity by enteric circuits.

Key Words:

Enteric neurons, Neural transmission, Motor patterns, Spatio-temporal maps

Introduction

Intestinal motility is a field that has a high relevance for interpreting health of individuals and is the subject of a large number of publications over more than 100 years. Yet it remains an ill defined collection of descriptions barely relevant to what actually happens in a living higher organism. The reason is relatively simple. The digestive tube is made of a muscular wall that contracts and relaxes in complex fashion, resulting eventually in the effective progress of contents from the oral to the anal end. The first obstacle to explain this progress is that its movements are mostly hidden from direct observation. Thus the simple description of the actual movements is per se a task not to be underestimated. The simple measurement of how long it takes for some ingested content to reach a particular point has been named "transit studies", whereby some labelled material is introduced and the time taken to traverse a given length of intestine measured. Of course this objective measurement at a single time point tells little of the way in which the progress is achieved by intestinal movements.

Opening the abdominal cavity to reveal movements was used in the very early times, and soon researchers realized that such descriptions were quite un-physiological. Attempts to open unobtrusive windows for viewing movements directly were made in the last century with limited success, due to a second major obstacle. The loops of intestine vary in position and form a knot hard to unravel visually even when in full view.

Much of the description of intestinal movements was thus left to observations made by interfering with the intestine in order to "visualise" its movements. The most powerful method was the application of X rays by W.B. Cannon at the turn of the 19th century. By giving an inert radio

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opaque meal he described for the first time some of the most common patterns of motor activity, particularly of the stomach with its pumping action. Indirect measures of movements by studying intraluminal pressure become feasible with the development of the Kymograph by Ludwig, first used to detect small rhythmic changes in intraluminal pressure by two French investigators[1]. In the following few decades much of the terminology was introduced to describe the rather complex and often ill-defined motions of the intestinal tube. Peristalsis and segmentation were the main descriptors of movements, with attributes of propelling content aborally and mixing contents, respectively. This terminology survives to date mostly because it is hard to argue against them; contents can usually only go either forward, remain localized, or go short distances back and forth. With a few examples of antiperistalsis (or retroperistalsis), all possible movements were covered by what appears a foolproof and comprehensive terminology[2]. Significant progress was made by the early recognition that in segments of the digestive tube isolated from animals, complex movements occur similar to those surmised in intact animals. The descriptive terms *peristalsis* and *segmentation* translated well in such isolated preparations of intestine in a classic paper recently translated from the original in 1917[3]. This apparent simplicity of intestinal movements led investigators in the 20th century to begin to search for the actual mechanisms underlying such patterns of motor activity.

The discovery by German neuroanatomists in the second half of the 19th century of a rich network of neurons, embedded within the intestinal wall (Meissner and Auerbach), opened a parallel history of neuroscience of the digestive system. The assumption that this neural tissue was responsible for much of the coordination of intestinal movements (neurogenic hypothesis) inspired much of last 140 years research in the neuroanatomy of the enteric nervous system (ENS).

The systematic description in the first half of the 1900s of spontaneous quasi-rhythmic motor activity of the intestine, even when nerves were absent or paralysed, led to a competing interpretation of intestinal movements as a unique characteristic of intestinal smooth muscle endowed with a spontaneous oscillatory mechanism (myogenic hypothesis). This controversy remains alive even today, despite the inadequacy of either hypothesis to explain all motor patterns.

The discovery that cells, originally described by Ramon y Cajal, and now known as interstitial cells of Cajal (ICCs), act as pacemakers for intestinal muscle has enabled recent reviewers of intestinal motility to integrate both neurogenic and myogenic mechanisms to explain the otherwise unintelligible complexity of movements of the digestive tract.

A further significant obstacle to an adequate description of intestinal motor patterns is their variation along the digestive tract. The filling and emptying of the stomach is very different from the propulsion of a solid bolus in the rectum. Also the physico-chemical nature of contents, influenced by the herbivorous, omnivorous or carnivorous diets, affects both the structure and movements of the digestive tube. Thus, a generalisation across species is very problematic.

In this short article we will critically review the advances made in the study of the physiological and anatomical bases of intestinal motility. The process of developing a multidisciplinary approach, despite the large variety of the methodological approaches from different disciplines, will be discussed. We will address some of the existing controversies and point to the misunderstandings that often underlie these, in the hope that this may help new investigators to navigate across the deceptively simple behaviour of the digestive tract.

Architecture of Muscular Apparatus

The tissues that form the gastro-intestinal muscular apparatus include the multiple layers of smooth muscle cells linked to form a functional syncytium that includes also nets of ICCs. These keep the muscle in a state of oscillating excitability as described in a number of excellent reviews by Sanders and Ward[4,5]. The spontaneous depolarisation of ICCs, which form nets of interacting oscillators, generate spatiotemporal patterns of excitation, which are then transmitted to the adjacent smooth muscle cells. The oscillations of the membrane potential of the muscle, originally called “slow waves”, can generate contraction when muscle contractile mechanisms are activated[4,5]. The resulting spatio-temporal patterns of contraction correspond to the “myogenic” motor activity. The mechanical consequences vary depending on the conditions and regions of the digestive tract. In the stomach, slow waves are highly spatio-temporally organised, starting in the corpus and propagating to the pylorus. This results in “antral peristalsis”, a robust pattern of

motor activity responsible for the steady pumping of content across the pylorus into the duodenum. In the small intestine, slow waves generally propagate aborally for varying distances. Sometimes they reach contractile threshold and thus generate rather irregular motor activity often described as "segmentation" or "mixing movements". In the large intestine the role of the slow waves in motor activity is even less clear due to the variability between species and with varied diet.

Although the pacemaker-muscle syncytium is capable of generating complex motor patterns it is clear that these would be insufficient to generate all of the filling and emptying patterns needed for digestive functions. The essential role of the nervous system in providing a more responsive means to control aboral progress of digesta is now beyond reasonable doubt. The enteric neural circuits, interacting with the muscular apparatus, are capable of producing specific adaptable motor patterns responsible for appropriate progression of contents along the gut.

The Enteric Nervous System

The ENS comprises all of the neurons embedded within the digestive tract and their extrinsic connections with sympathetic, parasympathetic and sensory ganglia. Over the past 25 years significant advances have been made on the details of the circuits that operate in the ENS. At the end of the 1980s a book dedicated to the ENS was written for the first time[6]. By the mid nineties a definitive account of all the major classes of enteric neurons, at least in the small intestine of the guinea-pig, was published[63]. Another update of the book with the same title appeared recently[7], following several review articles on the subject[8-10].

The major guiding principle of these studies has been the assumption that the enteric neurons are organised into different functional classes with characteristic morphological, chemical and biophysical identities, and that understanding the organization of the enteric circuits will clarify their function and their role in controlling intestinal motor functions. This reductive approach is based on the reasonable assumption that understanding the parts helps understand the whole. The difficulty of this task was well expressed in the pioneer work of Bayliss and Starling[11]. Only few laboratories since the 1970s have addressed this challenge in a systematic way.

In addition to motor functions, the ENS is involved in the control of mucosal transport, in control of local blood supply and in modulating cellular defence mechanisms. The circuits underlying the different functions work in concert. For instance, neurally mediated changes of motility are usually associated with changes in secretion and blood flow. Although extensive analysis of enteric neural circuits is well advanced, how they integrate enteric motor, circulatory and mucosal transport behaviours is less well understood. These aspects will not be reviewed here.

The cell bodies of enteric neurons are mainly located in ganglia of the myenteric (Auerbach) plexus and in ganglia of the submucous (Meissner's) plexus. They form neural nets continuous from the oesophagus to the anal canal. Since their discovery many descriptions of these plexuses have been published but were unable to decipher the neural pathways and circuits hidden within this distributed network of ganglia. The axons and dendrites of the enteric neurons run between ganglia and from these to the muscle layers and to other intestinal tissues including blood vessels, glands and epithelium. The methods developed by Golgi and Ramon y Cajal, based on silver impregnation, gave a picture of a homogeneous complexity, which was impossible to unravel. The origin of nerve fibres running in any single internodal strand, joining two ganglia, could not be ascertained and the contribution of the enteric and extrinsic neurons to these nets remained elusive for more than 100 years. The original methods used to visualise enteric neurons, just as in the central nervous system, were based on non-selective stains, which at their best gave an idea of the shape of the cell bodies. Dogiel at the end of the 19th century used methylene blue to reveal the shape of enteric neurons and described two main classes, one with many short stubby dendrites and one axon (Type I) and another with several long processes emerging from a smooth cell body (Type II). This morphological classification, with some minor additions, remains generally valid, but did not enable functional correlates to be proposed on a testable basis. The suspicion that Dogiel's morphological classes corresponded to distinct functional classes of neurons grew with evidence that reflex motor activity persisted in isolated segments of intestine. The work of Magnus in the early 1900s[12] and the seminal paper by Trendelenburg in 1917[3] demonstrated that isolated segments of small experimental animals showed coordinated motor activity triggered by mechanical stimuli, simulating what happens normally in living animals.

Propulsion, described as peristalsis, could then be attributed to enteric reflexes (myenteric reflex or peristaltic reflex). The history of the identification of motor and sensory enteric neurons had begun then.

However, it was only after the 1970s that identification of functional classes of enteric neurons could be addressed directly. Key advances in the past 30 years involved the development of specific staining techniques based on the distinct chemistries of nerve cells (histochemistry), the development of techniques to reveal the "projections" of enteric neurons, and finally the combination of these with intracellular electrophysiological recordings of identified enteric neurons.

Why the Guinea-Pig Intestine?

The major classes of neurons have been mainly identified in the widely used guinea-pig small intestine. The choice of this model was justified on the basis of its being the segment of intestine most studied since the initial physiological work of Trendelenburg[3] and the morphological work of Dogiel[13]. A boost for the use of the guinea-pig small intestine came with the simple but influential work by Paton[14] and Ambache et al[15] who, using pharmacological agents, demonstrated that excitatory motor nerves could be activated by passing electrical pulses across the entire wall (transmural or field stimulation). This elicited fast contractions of the longitudinal muscle, which were blocked by atropine, a muscarinic receptor antagonist. Acetylcholine was then, correctly, deemed to be the major excitatory transmitter of the motor neurons. The deceptive simplicity of such preparations ensured its wide use in most pharmacological studies of drug effects on the nervous system. Interestingly the distal 10 cm of the small intestine (ileum) was mostly avoided. This advice was given on the bases of little known work of Munro[16] who observed unexpected contractions using adrenergic agonists. Since then this has hardly been reinvestigated. Less clear is the limitation of most studies to the ileum. There is not a well-defined morphological boundary with the jejunum. Indeed there is a linear increase in the thickness of the longitudinal muscle from the duodenum to the ileocaecal junction[17]. The literature on the pharmacology of the "cholinergic twitch of the guinea-pig ileum" has grown extensively. The significance of the wealth of pharmacological receptors on the cholinergic motor neurons projecting to the longitudinal muscle of the guinea-pig small intestine remains a biological mystery. Nevertheless, a large number of papers has been dedicated to testing agonists and antagonists on this preparation. It was in this very preparation that evidence for non-cholinergic excitatory transmission first led to the discovery that a peptide (a tachykinin, possibly substance P) was a co-transmitter with acetylcholine[18].

Another preparation from the guinea-pig gut, the "taenia coli", consists of the narrow bundles of longitudinal muscle running along the guinea-pig caecum. This preparation revealed an inhibitory response to electrical transmural stimulation[19]. The advent of pharmacological antagonists to sympathetic noradrenergic transmission led to these nerve-mediated relaxations being attributed to an unknown set of non-sympathetic inhibitory nerves[20]. Paradoxically, the existence of non-sympathetic intrinsic inhibitory neurons had been surmised already by Langley in his monograph of 1921[21]. Langley identified the enteric nervous system as the third division of the autonomic nervous system, containing neurons capable of excitation and inhibition of intestinal muscle. This suggested that enteric neurons could not be regarded as simply postganglionic parasympathetic neurons even though some enteric motor neurons received inputs from the parasympathetic preganglionic nerves. The relative autonomy of the ENS was not described by Langley in full, probably because of his untimely death. It was not until the late 1960s that intrinsic inhibitory neurons were rediscovered and called non adrenergic-non cholinergic (NANC)[20,22].

The use of the guinea-pig small intestine to study more complex motor functions in vitro started with Trendelenburg in 1917[3]. More detailed studies with improved recording methods and extensive pharmacological analysis continued in the 1950s and 1960s and still flourish into the present[23-29].

Another important reason for the widespread adoption of the guinea-pig intestine for studies of the structure and function of the ENS was its suitability for fine dissection. Replacing histological sections with "whole mount" preparations of intestinal layers containing intact enteric plexuses enabled the study of intact enteric neurons with their full morphology and biophysical properties retained[31]. In recent years similar whole mount approaches have been extended to other species (mouse, rat) and humans[7,8]. The guinea-pig small intestine became the preparation in which many of the most significant advances in

intestinal neuroscience and motor behaviour occurred. The functional demonstration of enteric excitatory and inhibitory motor neurons in the guinea-pig intestine opened the possibility of identifying them anatomically within the maze of the enteric plexuses.

However, the extent to which the principles of organization and function of enteric circuits in the guinea-pig small intestine apply to other non-herbivore species and to other regions of the gut is an open question.

Development of Histochemistry to Study Enteric Neuronal Populations

The development of histochemical techniques to visualise chemical substances in cells and the realisation that this may help in distinguishing different groups of enteric neurons played a critical role in the strategy for a modern neuroscience of the intestine.

One of the first histochemical techniques developed in the 1950s was the visualisation of the acetylcholinesterases. This enzyme is involved in the hydrolysis of acetylcholine, and had already proved important in the termination of neuromuscular somatic transmission to striated muscle. However cholinesterases were abundant in nervous and non-nervous tissues, with functions yet unknown, but probably unrelated to the hydrolysis of the transmitter acetylcholine. Despite mounting evidence that this histochemical technique did not accurately demonstrate the localisation of cholinergic nerves, it took several decades for it to be abandoned, mostly because of its wide use by histopathologists.

The demonstration of the actual distribution of cholinergic nerves had to wait until the early 1990s when antibodies to the enzyme choline acetyltransferase (ChAT) enabled the visualisation and accounting of the populations that contained it[32].

A selective histochemical technique was developed in the early 1960s by Swedish investigators to visualise directly some of the monoamines such as noradrenaline, dopamine, adrenaline and 5-hydroxytryptamine (5-HT), now known as the Falk and Hillarp technique. This method was based on a reaction between these amines and vaporised hot aldehydes to produce fluorescent compounds. The initial mapping of the amine-containing neuronal groups in the brain ushered in a histochemical and pharmacological revolution in brain sciences. The identification of the dopamine containing neurons in the substantia nigra and the parallel development of drugs such as L-DOPA for Parkinson Disease quickly followed.

In the mid 1960s, one of us (M.C.) was fortunate to apply this technique for the first time to "whole mount" preparations of guinea-pig intestine, providing colour pictures of the astonishingly beautiful networks of sympathetic nerves terminals[33]. These histochemical techniques with some subsequent improvements using aqueous aldehyde solutions (glyoxylic acid and FAGLU) were then superseded in the 1970s by new methods based on immunology[31].

The crucial step that opened a new era in visualisation of substances in enteric neurons was the development of immunohistochemistry, based on the visualisation of antibodies raised against different neuronal substances[31]. Antibodies to the enzymes for the synthesis of catecholamines and indolamines confirmed and extended the findings obtained with aldehyde induced fluorescence histochemistry. The suspicion that 5-HT is a transmitter in the ENS found support with the demonstration of enteric neurons showing immunoreactivity for this indolamine[34]. Its exact role as a transmitter however remains unclear despite significant interest from the pharmaceutical industry. Much of the potential interest in 5-HT as mediator of slow excitatory transmission in myenteric ganglia was proven unjustified following experiments in which the 5-HT containing nerve fibres in the myenteric ganglia were lesioned and degenerated, without affecting the slow transmission in myenteric ganglia[35]. This evidence should have led to reassessment of the importance of 5-HT in the enteric circuits. However, strong advocacy for a major role delayed a more appropriate appreciation of its minor role as an enteric neurotransmitter. Some pharmaceutical research on 5-HT payed substantial price for this misdirected enthusiasm. Most of the effect of endogenous 5-HT are probably better explained by its role as a mediator released by the non-neural enterochromaffin cells.

The discovery that small peptides were present in enteric neurons[36] ushered in several descriptions of populations of enteric neurons containing specific neuropeptides. The application of immunohistochemistry to whole mount preparations of the guinea-pig intestine[31] enabled a very fertile period of investigating the distribution of a variety of peptides including substance P, opioid peptides, vasoactive intestinal polypeptide (VIP) and somatostatin, to name just a few. From these

studies, the previously hidden highly organized nature of the enteric circuits began to emerge. Somatostatin nerve cells in the myenteric ganglia give rise to processes that terminate only in enteric ganglia, thus indicating that these are interneurons and could not be motor neurons. The situation for other peptides was not as simple. Substance P immunoreactivity was present in a vast number of nerve fibres in most potential target tissues including myenteric and submucous ganglia, blood vessels, all muscle layers and mucosa. Similar findings applied to VIP and opioid peptides[37,38]. A major problem associated for such descriptive approaches was that the terminations of identified enteric neurons could not be readily established.

The proliferation of immunohistochemical descriptions of peptides in histological sections of gut increased the problem, with many papers making easy but unsubstantiated statements about the importance of neuropeptides in intestinal functions.

Despite the initial promise of discovering the roles of enteric neuropeptides, a number of problems emerged. For example, peptides come in "families" with related sequences, but often with dissimilar biological actions on different receptors. A full analytical study requires innumerable combinations and permutations of experiments just to establish which molecular forms are actually present in the enteric neurons. It was well known that imunohistochemistry by itself is unable to establish the chemical identity of peptides. Proper terminology was needed, and is still needed when referring to tissues labelled with antibodies. Labelling with an antibody raised against chemical X needs appropriate specificity (e.g., absorption with the antigen) tests to prove that the labelling is due to "X-like immunoreactivity". Further highly technical analytical methods of purification, separation and synthesis are necessary to unravel the sheer complexity of peptide families. Few laboratories developed the necessary combination of methods for a systematic investigation of the increasing variety of peptides discovered.

The process of identifying the molecular forms of enteric peptides is far from being complete and raises the question of how much analytical work is justified, and how it affects the interpretation of histochemical and pharmacological results. The excessive focus often on a single peptide or subfamily of peptides exposed much of the published work to the legitimate criticism of ignoring the bigger context.

Projections of Enteric Neurons

The unravelling of enteric circuits needed methods to establish the origin of nerve fibres in different targets within the gut wall. This refers to a related problem: finding the sources of enteric nerve endings and establishing the fields of innervation of particular neurons. Answers to these complementary problems would give an idea of the "projections" of enteric neurons. The methods developed for such studies in the 1980s were based on classic lesioning techniques of axons, with subsequent degeneration and disappearance of the corresponding nerve endings. In addition, accumulation of immunoreactive material by axonal transport indicated the direction of the projections. The direction of axons running along the intestine gave rise to the idea of oral or aboral polarity of enteric projections. However, a more accurate and powerful technique to establish enteric projections was the development of retrograde tracing techniques suitable for the enteric microcircuitries. This required the application of retrograde tracers in very discrete locations within the intestinal wall. This could be achieved only in isolated preparations kept for several days alive to enable the retrograde transport to take place. The use of organ cultures for this purpose was introduced in the early nineties[39,40]. Much of the detailed wiring diagrams of enteric circuits available to date can be attributed to such studies.

Identification of Enteric Motor Neurons

The existence of excitatory and inhibitory motor neurons was well established on functional bases by the 1970s. However, which of the enteric neurons in the enteric plexuses are motor neurons to the different muscle layers remained uncertain.

The simple experiment of removing the myenteric plexus in vivo in the guinea-pig small intestine answered this problem. Following "myectomy" all nerve fibres disappeared from the circular muscle, demonstrating that they originated in or traversed myenteric ganglia[41]. In small experimental animals the motor neurons to this muscle layer involved in propulsion are entirely localised in the myenteric ganglia. In larger species similar lesion studies showed that some motor neurons are also located in submucous ganglia[42].

The question of whether only two classes of axons are present in the muscle and correspond unequivocally to the excitatory and inhibitory neurons was addressed by using two good im-

munohistochemical markers for nerve fibres in the circular muscle. Substance P, or a related tachykinin, had already been proposed as a co-transmitter of excitatory motor neurons. VIP was at the time a strong candidate for being an important transmitter of enteric inhibitory neurons[43]. Both substance P and VIP are present in nerve fibres within the circular muscle. Using immunocytochemistry each of the markers were found to account for about half of the total nerve fibres in the circular muscle. To establish beyond reasonable doubt that there are no other classes of fibres, "occlusion" experiments were used in which antibodies to both VIP and substance P were simultaneously applied: every axon in the circular muscle was labelled. This excluded the possibility that some nerve fibres may contain neither VIP nor substance P[44]. The full extent of the origin and projections of enteric motor neurons to the circular muscle was achieved in 1991 by using the retrograde labelling technique in vitro. Excitatory motor neurons were identified on the accepted evidence that they used acetylcholine for transmission which was detected by immunoreactivity for the enzyme ChAT. The immunohistochemical distribution of this enzyme confirmed the major role of acetylcholine as excitatory transmitter of enteric motor neurons[32]. However, transmural stimulation elicited nerve-mediated contractions that were resistant to acetylcholine receptor antagonists. Initial evidence that the tachykinin substance P was probably responsible was supported by the anatomical finding that tachykinins are colocalised with ChAT in enteric neurons projecting to the muscle. The idea of multiple transmission mechanisms, now widely accepted for most neurons, was anatomically grounded by these observations.

The identification of the enteric inhibitory motor neurons to the circular muscle was also completed in the early nineties by using retrograde tracing combined with immunohistochemistry for the enzyme nitric oxide synthase (NOS)[45] (and see below). Since their functional discovery in the taenia coli[19], their function as part of the polarised set of reflex pathways involved in propulsive motor activity had been proposed[46]. The search for the unknown inhibitory transmitter was swayed by the powerful proposal that adenosine triphosphate (ATP) was the best candidate[47]. Despite the relative weakness of the evidence, the hypothesis of purinergic transmission gave rise to a fruitful field of research in other biological processes[48]. The subsequent discovery that VIP extracted from intestinal tissue had a relaxing action on intestinal muscle[43,49] and its localisation in nerve endings in the muscle layers[50] led to an equally strong proposal that this peptide was the unknown inhibitory transmitter[51]. The resolution of this apparently insoluble problem began to emerge when it was demonstrated that inhibitory neurons may utilise more than one mechanism to relax the muscle. Transmural stimulation of inhibitory motor neurons in different parts of the guinea-pig intestine was affected differentially by drugs that antagonised neurally mediated "NANC" inhibitory transmission[52]. The later discovery of NO as mediator of vasodilatation[53] led to the discovery that it also acts as inhibitory transmitter in the intestine[54]. The localisation of the synthetic enzyme, NOS, in myenteric neurons projecting to the muscle[45], and its subcellular localisation in myenteric neurons[55], confirmed that nitric oxide (NO) is one of the inhibitory transmitters of the enteric inhibitory motor neurons. The idea of multiple transmission was vindicated, opening more comprehensive and less-adversarial explanations for the unknown inhibitory transmission in the gut. The evidence that there is probably only one class of such inhibitory neurons that contain and utilise a combination of VIP, NO and ATP is now convincing. Because of these coexisting mechanisms, the task has evolved to establish the relative importance of each of the transmitter mechanisms in different parts of the gut, in different species and in different physiological or pathological conditions. Very few of the original laboratories that proposed one or the other compound as inhibitory transmitter were equipped to address this complex issue.

The success in the identification of the excitatory and inhibitory classes of motor neurons supplying the circular muscle was completed with the identification of the enteric neurons that supplied the longitudinal muscle in the guinea-pig small intestine. The calcium binding protein, calretinin, turned out to be a valuable marker for these motor neurons, which also contain ChAT and substance P. The projections of these neurons are non-polarised and relatively short, supplying the longitudinal muscle around the myenteric ganglia of origin[56]. The apparent absence of nerve fibres within the longitudinal muscle of the guinea-pig small intestine made it hard to explain the existence of the "cholinergic twitch" in this preparation. Detailed ultrastructural analysis re-

vealed that the "tertiary" component of the myenteric plexus provided a suitable substrate[57]. More surprising was the finding that longitudinal muscle motor neurons represented a quarter of all myenteric neurons[56] despite this layer playing only a minor role in the propulsive behaviour of the small intestine (see below).

The identification of enteric motor neurons was not without its mistakes. For example, in early studies we described nerve cell bodies (with Dogiel type III morphology) with VIP-like immunoreactivity in myenteric ganglia and proposed them to be the origin of the nerve endings in the circular muscle and hence enteric inhibitory motor neurons. It turned out from subsequent experiments that these cells were in fact secretomotor neurons projecting to the mucosa. The mistake was due to the very low constitutive immunoreactivity of VIP in the nerve cell bodies of the real inhibitory neurons. Better antibodies, increased immunohistochemical sensitivity and the use of substances that increased peptide content in the cell bodies by blocking axonal transport (e.g., colchicine) eventually enabled the proper nerve cell bodies of the inhibitory motor neurons to be correctly identified.

Accounting for Enteric Populations

The simple idea to find out how many classes of enteric neurons exist emerged naturally and implied the ability to find ways to first reliably identify and classify groups of enteric neurons, and then to establish what proportion of the total each population represented. This would eventually account for all classes of enteric neurons, and it would be possible to begin attributing to each specific functions. Classification of natural phenomena in the natural sciences represent a good beginning to go beyond personal observations. It was clear in the 1970s that enteric neurons could be classified according to several, very different parameters. Firstly, they can be classified according to shape, a process that started with Dogiel at the end of the 1800s using methylene blue as a stain. The early distinction based on the morphology of the cell body and its processes between Dogiel type I and type II neurons found relevance in the 1990s with the demonstration that these shapes correlated quite well with differences in the biophysical properties of enteric neurons (see below). In the early 1900s the differential affinity for silver impregnation distinguished "argyrophilic" and "argyrophobic" enteric neurons. Interestingly, this distinction corresponded to Dogiel type I and type II neurons, respectively, better than many investigators expected. This staining difference turned out to be due to the differential presence of neurofilament proteins in Dogiel type I and type II neurons. While silver impregnation was deemed to be a universal stain for neurons, already in the early 1900s Bielchowsky and Brodmann showed that only a small proportion of cortical neurons were labelled with silver impregnation techniques. An affinity for silver appears to be a property of some specific neurofilament proteins; not all neurons contain these particular proteins[58]. In the enteric nervous system neurofilament immunoreactivity distinguishes well between Dogiel type I and type II neurons[59].

Another important feature in the accounting of enteric neurons is provided by their biophysical properties. Pioneering work in the 1970s[60,61] recorded electrical activity with intracellular electrodes in myenteric neurons distinguishing two main types of neurons, type 1 with fast synaptic input and repetitive firing (called then S neurons) and type 2 with only slow synaptic inputs and unable to fire repetitively (hence called subsequently AH neurons for after-hyperpolarising). Subsequent work demonstrated that there is an excellent correlation between the two main Dogiel type neurons classified by shape and the two main electrophysiological classes[8]. Dogiel type I neurons are in general S neurons and Dogiel type II neurons are usually AH neurons. This identity enabled structure and function to be correlated on firm bases in enteric neuroscience.

The best evidence that there are more than two classes of enteric neurons came from immunohistochemistry. It became apparent already in the early 1980s that the number of histochemical markers was growing in excess of realistic subpopulations of enteric neurons. Almost every new peptide antibody labelled a subset of enteric neurons. In order to establish the proportion of neurons labelled by a particular marker, a universal label was needed. Early calculations of the total number of neurons had been performed using a simple histochemical reaction based on visualisation of ubiquitous respiratory enzymes (e.g., NADH)[62]. This technique was incompatible with multiple immunohistochemical labelling necessary to study other markers. An antibody that labelled all guinea-pig enteric neurons was discovered by chance, yet the nature of the actual antigen remains unknown. Using this marker and a large number of combinations and permutations of other markers, a first se-

rious attempt to account for all major subpopulations of myenteric neurons succeeded[63]. Subsequent calculations fundamentally confirmed the results of this study with only minor corrections of the relative proportions.

One major consequence of this successful process of accounting was the possibility to extend the classification scheme to other species and parts of the intestine and that in such preparations any new substance could be relatively easily be localised in one or more known subpopulations.

Chemical Coding of Enteric Neurons

With the initial detailed studies of the immunohistochemistry of enteric subpopulations in the 1980s it became clear that certain markers were associated with specific morphological populations and that combinations of markers appeared to better identify such populations than single labelling. This gave rise to the concept of "chemical coding" that proposed that every class of enteric neuron with specific shapes and combinations of chemical markers was probably associated with a specific function. Likewise, a single functional class of enteric neuron was expected to share similar shapes and combinations of markers[38]. The search for similarities or differences in the chemical code was associated with expected functional similarities or differences. While the use of "chemical coding" is now widespread and has been successfully applied to other parts of the nervous system, the molecular, developmental and evolutionary bases for the associations between chemical markers, shapes, biophysical properties and functions remain to be ascertained.

Consequences of the Chemical Coding for Plurichemical Transmission

The discovery that each neuron may contain a multitude of substances, some with similar actions, some with opposing actions and some with completely different effects, has created conceptual difficulties to view neurons as simple transmitting elements with a straightforward "computational" role[64]. One of the first casualties was the idea of "one neuron one transmitter". This idea has been wrongly attributed to Sir Henry Dale. In the 1930s he proposed that if a transmitter substance was found at one ending, e.g., the central process of a sensory neuron, the same substance was also present in the peripheral processes. He coined the terms "adrenergic" and "cholinergic" to describe the chemical nature of the transmitter substance through which specific neurons did their work (thus "ergic"). The idea that neurons could be described by a single transmitter mechanism became engrained; "chemical coding" dispelled this misunderstanding. If neurons could utilise more than one transmitter, then the terminology with the ending "ergic" should surely be abandoned. The issue is simply one of correct terminology. A neuron uses a "cholinergic", "nitrergic" or "VIPergic" mechanism, but is not unequivocally identified by such terms. A neuron thus is not "cholinergic", it may use a "cholinergic" mechanism, but also a "tachykinergic" one at the same time. This argument should dissuade investigators from using the ending "ergic" after any substance has been visualised histochemically in a neuron. The presence of a substance in a neuron may raise the possibility that it plays a role as a transmitter, but proving that this is the case requires a long and difficult series of studies. The criteria established in the 1950s for a substance to be a transmitter demand evidence of presence, synthesis, release, inactivation, and mimicking. They have often been applied in a rather lawyer-like fashion with advocacy rather than genuine evidence. Certainly mere presence does not entitle a substance to be regarded ipso facto as transmitter.

The second lesson to be learned from "chemical coding" is that the same transmitter or related substances can be present in different functional classes of neurons. The search for a single unique function of a new transmitter is thus conceptually flawed. Yet even in the central nervous system, terms such as the "noradrenaline" or "serotonin" system are still in widespread use. Similarly, the absence of a transmitter substance, demonstrated by immunohistochemistry, may not imply its total absence from the neuron. For example, in some pathological human gut specimens, tachykinin immunoreactivity in motor nerve fibres was apparently absent[65]. Yet the motor neurons were still present containing the other transmitter-related chemical marker, ChAT[66]. Thus, the pathology appeared to relate to only one of the several co-transmitter substances. Simplistic conclusions about the etiology of diseases deduced from the absence of a single neurochemical can easily be avoided by adopting the concept of chemical coding. A significant number of papers show that in Hirschsprung's disease, defined by the absence of all enteric neurons, a particular histochemical marker is absent. Too often the authors of such

papers conclude, in a nonsensical way, that the absent chemical is likely to be involved in the etiology of the disease.

A more difficult problem raised by the multiplicity of transmission mechanisms is the presence of redundant transmission mechanisms and the assessment of their relative importance in transmission. For example, inhibitory transmission in the guinea-pig colon was reported to be resistant to apamin. It was subsequently shown to be mediated by NO. When NO-mediated transmission was blocked by NOS inhibitors, an apamin-dependent transmission was revealed in this preparation. Thus, the absence of an effect of an antagonist is not sufficient to rule out a role for the endogenous substance.

A last, and even more important lesson from the discovery of multiple transmitter substances is the coexistence of substances with apparently opposite actions. Release of acetylcholine and tachykinins from enteric motor neurons result both in excitation of the muscle. The same population of neurons also contain and release opioid peptides, which inhibit excitatory transmission. This suggests perhaps that neurons are not always working at their maximal capacity and that they are under an intrinsic physiological "brake". Indeed this interpretation was proposed as an explanation for the increase in propulsive efficacy of the guinea-pig small intestine after blocking opioid receptors[67]. Such modulatory processes may be the norm rather than the exception, given the diversity of peptides, modulators and receptors in enteric neurons.

Identification of all Functional Classes of Enteric Neurons

As mentioned above, the fullest accounting of functional classes of enteric neurons has been accomplished in the guinea-pig small intestine. The successful identification of the functional classes of enteric excitatory and inhibitory motor neurons to muscle layers on the bases of shape (Dogiel type I), electrophysiology (S neurons), histochemistry, pharmacology of transmission and projections (lesions and retrograde tracing) opened the way for the identification of the other functional classes of enteric neurons.

The existence of enteric interneurons was predicted on the bases of functional studies. In particular nicotinic antagonists interfere with reflex responses (see below), at the level of enteric ganglia, hence the rather inaccurate term "ganglion blocking drugs". The morphological identification of myenteric interneurons had to await the use of retrograde tracing methods[68]. The first application of a retrograde tracer on a single nerve strand joining two adjacent myenteric ganglia (internodal strands) labelled an average of over 800 neurons, 80% of which were located in myenteric ganglia oral to the application site at distances of more that 10 cm, while only 20% were located in myenteric ganglia on the aboral side all within 15 mm. This was the first evidence for a dramatic polarity of enteric interneurons in addition to the polarity of motor neurons. It also showed that most enteric neurons project aborally within the myenteric plexus. Subsequent analysis of such projections with specific immunohistochemical markers showed the presence of a single population of ascending interneurons which contained tachykinins, opioid peptides, the calcium binding protein calretinin and ChAT. The latter finding was consistent with the nicotinic cholinergic transmission of these neurons, which form long ascending excitatory chains running up the gut. There are several populations of long descending interneurons, one with a filamentous shape, containing somatostatin and the enzyme ChAT representing descending cholinergic interneurons. 5-HT is present in another subpopulation of cholinergic interneurons and thus may be minor co-transmitter, with acetylcholine playing the major transmitter role. A third class of descending interneurons contains VIP, NOS, dynorphin, gastrin-releasing peptide and sometimes also ChAT. Synaptic transmission from these descending interneurons in myenteric ganglia remains a fertile area of advancing studies. Acetylcholine, tachykinins, 5-HT, purines and other mediators are all involved in fast or slow synaptic transmission[8].

The search for enteric primary afferent neurons has been an interesting story of ingenuity mixed with preconceptions. The functional evidence that there must be intrinsic primary afferent neurons was based on extensive experiments in which isolated segments of gut showed neurally mediated responses to mechanical and chemical stimuli. The remote possibility that cut nerve terminals of extrinsic afferent neurons were responsible was eliminated by using segments extrinsically denervated by surgery[69]. The claim that in the colon such a mechanism operates[70] remains surprisingly open. The proponents of this idea have not performed the appropriate denervation experiments that would resolve the issue; the burden of proof should belong naturally to the proponent of an hypothesis.

The discovery of the identity of the motor and interneurons in myenteric ganglia left the other major subpopulation, the Dogiel type II AH neurons, as natural candidates for being the enteric intrinsic primary afferent neurons (sometimes called IPANs). Earlier investigators had suspected that the Dogiel type II neurons could well play this role. Their lack of fast synaptic inputs precluded them as candidates for inter- or motor neurons. Some rather heated and not always clear-minded arguments flared between different laboratories, which espoused contradictory views often based on discrepant identification of neuronal properties or shapes[71,72]. The evidence that Dogiel type II, AH myenteric neurons respond directly to mechanical deformation of the gut proved beyond reasonable doubt that these neurons are IPANs. The role of tachykinins in the slow synaptic transmission that IPANs receive has also been established[8]. The role of acetylcholine, also synthesised by these neurons, which contain immunoreactivity for ChAT, is less clear. Most of these IPANs project to other IPANs and to other classes of myenteric neurons, all within close distances. A class of these Dogiel type II neurons has axons that project aborally for significant distances[73]. A large proportion of these IPANs contain the calcium binding calbindin[74]. The morphological identity of the non-calbindin IPANs has been revealed by antibodies to the neuronal marker NeuN which in the gut selectively marks all Dogiel type II neurons. Using this marker we recalculated the proportion of Dogiel type II IPANs which comprise 38% of all myenteric neurons, a figure significantly larger than previous estimates.

The issue of whether enteric IPANs are the only enteric neurons to act as primary afferent neurons is still debated and the evidence that some myenteric S neurons can be directly activated during distension appears to extend the classes of primary afferent neurons in the enteric nervous system[75]. The important issue is that in the ENS there are sufficient neurons capable to respond to chemical and physical stimuli to explain the adaptive properties of the neurally-controlled behaviour.

Other minor classes of myenteric neurons comprise a population of intestinofugal neurons projecting to prevertebral ganglia and two secretomotor neuron classes (with Dogiel type III morphology) projecting to the mucosa, similar to corresponding classes in the submucous ganglia. In addition to these two secretomotor neuronal classes, one cholinergic and one non-cholinergic, the submucous ganglia in the guinea-pig small intestine also contain some IPANs and a unique class of cholinergic vasomotor neurons. These four classes of submucous neurons represent the complete account of submucous plexus neurons. Table I shows the proportions of functional populations of enteric neurons in the guinea-pig small intestine.

Reflexes and Pathways

The study of reflexes in the nervous system goes back a long way. The most successful story relates naturally to spinal cord reflexes[76] investigated in parallel with those of the intestine. The pioneering work of Bayliss and Starling[11] convinced the scientific community that intrinsic reflex pathways in the gut wall showed a complexity suitable for the complex task of ensuring the controlled progress of intestinal contents during digestion.

With the development of the guinea-pig small intestinal preparation to elicit simple behaviours, such as nerve-mediated propulsion in response to filling, the search for the actual neural pathways underlying this behaviour ensued. The oversimplification of dynamic emptying behaviour of intestinal segments as a simple reflex (peristaltic reflex) did little to help the process. However, in analogy with spinal reflexes, localised mechanical stimuli were applied to the wall of the intestine, and electrical and polarised mechanical responses were recorded orally and aborally to the stimulus[11]. Extensive series of such experiments in the small and large intestine in different laboratories built a view that such pathways are robustly wired and could indeed represent the bases of the dynamic behaviour observed during peristalsis.

Thus, the ascending excitatory and descending inhibitory reflexes originally postulated by Bayliss and Starling[11] were identified in the guinea-pig and the classes of neurons involved in the reflex pathways identified[6,7,9]. This represents a significant body of work in neuroscience. The diagrams, now well quoted in the literature, show the excellent consensus on the importance of such pathways.

The enteric pathways are overlapping and cannot simply be regarded as individual reflex pathways. Ascending excitation is sustained by a chain of ascending excitatory interneurons, which extend the contraction oral to the stimulus for different distances depending on the intensity

Table I. Classes of myenteric neurons in the guinea-pig small intestine.

Function	Dogiel type	Chemical coding	%
Short and long ascending excitatory motor neurons to circular muscle	I	SP/ChAT/±ENK/NFP	10
Excitatory motor neurons to longitudinal muscle	I	SP/ChAT/Calret	25
Short and long descending inhibitory motor neurons to circular muscle	I	VIP/NOS/ATP/DYN/GRP/NFP VIP/NOS/ATP/± ENK ± NPY	12
Ascending interneurons	I	SP/ChAT /ENK/NFP	5
Descending interneurons	I	5-HT/ChAT /NFP	1
Descending interneurons filamentous	III	SOM/ChAT	4
Descending interneurons	I	VIP/NOS/±ChAT/DYN/GRP/NFP	1
Primary afferent neurons	II	NeuN/Calb/SP/ChAT	24
Primary afferent neurons	II	NeuN/SP/ChAT	14
Secretomotor neurons	III	VIP/DYN/GAL	1
Secretomotor neurons	III	NPY/ChAT/SOM/CGRP/CCK	1
Secretomotor neurons	I	VIP/ChAT/ENK/DYN/GRP/CCK	1
Intestinofugal	I	ChAT/GRP/VIP/ENK/±NOS/CCK	1
Total			**100**
Classes of submucous neurons in the guinea-pig small intestine			
Primary afferent neurons	II	NeuN/±Calb/SP/ChAT/	14
Secretomotor/vasodilator neurons	I	Calret/ChAT	12
Secretomotor neurons	III	NPY/ChAT/SOM/CGRP/CCK	32
Secretomotor/vasodilator neurons	III	VIP/DYN/GAL	42
Total			**100**

Accounting of the functional classes of enteric neurons in the guinea-pig small intestine based on their shape, projections, neurochemistry and transmission.

of the stimulus. Similarly, descending inhibition, mediated by short and long motor and interneurons, provides a chain of descending pathways, responsible for the significant area of relaxation during filling and in preparation of the advancing solid or liquid bolus.

The pharmacological analysis still under way is slowly revealing fine details and potential roles of different transmitter substances such as ATP, glutamate, 5-HT, NO and various peptides in the transmission along these enteric pathways[8].

However, the knowledge of the relationship between simple reflex pathways and the actual emptying motor patterns is far from being satisfactory. This is partially due to confusion between reflexes and motor patterns.

Reflexes and Motor Patterns

The conceptual distinction between studying reflexes and studying neurally mediated behaviours is nicely exemplified by studies of spinal cord mechanisms. The understanding of spinal reflex pathways has been the basis of modern neurological science and of the elucidation of the integrative functions of the nervous system[76]. Yet for all the advances made in studying such reflexes, their actual role in normal walking (locomotion) remains uncertain. The measurement of the stretch reflex to test the integrity of central motor pathways is a most important method in neurology. However, the understanding of the neural circuits underlying human locomotion has advanced little on these bases. In lower vertebrates locomotion is the result of ongoing activity of entire spinal neural circuits modulated by both higher centres and sensory inputs. This has modified significantly the view on the roles of reflexes. Reflexes as such rarely operate in a naturally evolving motor behaviour. Reflexes are usually elicited in very artificial experimental settings by applying spatially and temporarily restricted "stimuli" and are measured as spatially and temporarily restricted responses. Studying reflexes enabled neuroscientists to relate time, stimulus intensity and response magnitude to cellular and subcellular mechanisms. However, in a

moving animal neurons in the spinal reflex pathways are certainly activated but they do not function in a sequence of simple reflexes to produce adaptive behaviour.

Similarly, enteric reflex pathways underlying localised intestinal reflexes are activated during normal motility including propulsive movements, but are not the result of single reflexes or of sequential activation of reflexes. We propose that that propulsive movements of the intestine, no matter how simple it may appear, should be regarded as a motor pattern, not a reflex.

The main reason for propulsive movements not to be regarded as a reflex is the apparent all-or-none nature of the initiation of the propulsive wave of circular muscle contraction in the guinea-pig small intestine[3]. During slow infusion of liquids into an isolated segment of small intestine, there is activation of neural pathways, including graded activation of longitudinal muscle motor neurons with shortening of the segment. Simultaneous activation of enteric inhibitory pathways to the circular muscle keeps the muscle relaxed (named originally as the "preparatory phase" of peristalsis), until a "threshold" is reached with a sudden dramatic switch from activation of inhibitory pathways to a massive activation of the excitatory pathways (named originally the "emptying phase"). This sudden switch from inhibition to excitation is preserved even in open segments of intestine when slowly stretched[77]. While the inhibition during the preparatory phase affects the full length of the segment, via long descending inhibitory pathways, the contraction starts sharply just aborally to any lesion of the myenteric pathways. This corresponds to the oral end of the commonly used isolated segment. The ring of circular muscle contraction then propagates aborally, acting as a propulsive pump to empty the contents. This sequence of events is clearly a complex one, and some details of its dynamics have been investigated[29,78,79]. The explosive nature of the contraction suggests a highly non-linear process at work. Previous experiments had shown that the initiation of peristalsis is not identical with the activation of the ascending excitatory localised standing reflex[80]. The potential mechanisms behind the non-linear event may include a self-exciting net of the IPANs[81,82], bursting properties of the ascending interneurons and tension-dependent reactivation of the IPANs by the contraction itself. To these processes we should add the movements of the contents that act as ongoing luminal stimuli, as postulated originally by Bayliss and Starling[11].

These processes give the intestine the ability to adapt movements to the circumstances, propelling contents at different rates depending on their physico-chemical composition[83]. Thus enteric circuits for propulsion even in the isolated guinea-pig intestine do not generate rigid motor patterns. They show a remarkable afferent modulation by the contents via what might be called the "neuromechanical hypothesis". Similar processes of motor patterns modulated by sensory inputs operate in the locomotor networks in most vertebrates[84,85].

The almost total quiescence of the guinea-pig segment before the explosive contraction may be due in part to an abnormal state of inhibition possibly due to prostaglandin secretion induced by manipulation of the tissue. The initial claims that slow waves are not normally present in this preparation were proven wrong; slow waves are present both in vivo and in vitro[86,87]. However, the role of myogenic activity in propulsive behaviour may not be easily investigated in the guinea-pig isolated segments.

The complex interplay between these processes makes mechanically induced "peristalsis" in vitro more than a simple reflex. To mistake the study of reflexes with that of motor behaviour does a disservice to the difficult, but important, task of understanding the neural bases of such behaviour.

Despite the significant and long-lasting interest in the peristalsis of the guinea-pig intestine, such dramatic behaviour is unlikely to occur in the intestine of intact animals except during either mechanical obstruction or presence of irritating contents. Full peristalsis elicited in isolated segments of the guinea-pig small intestine may be regarded as a dramatic defence reaction to get rid of contents under pathophysiological conditions, rather than a normal pattern for progression of contents during digestion.

How to go from neural circuits to behaviour is a challenge in all fields of neuroscience. Using information from studies of enteric reflexes and enteric reflex pathways maybe a start but is a long way away from satisfactory explanation of intestinal motor behaviour.

Recording the Motor Behaviour of the Intestine

For digestive function, what counts is the movement of digesta along the digestive tube at a rate suitable for breakdown, absorption and excretion of contents. This is accomplished by mo-

tions of the gut wall. Except in rare situations, the gut mainly mixes the contents or propels them aborally. The relation between wall motion and content propulsion represents a formidable challenge to any hydrodynamic engineer.

While it appears both simple and reproducible, understanding the propulsive behaviour of the isolated intestine requires an accurate description of a rather fast dynamic sequence of mechanical events. Recording a few parameters, such as intraluminal pressure changes, longitudinal muscle tension and ejection of contents was the main strategy for many decades. Kinematic analysis of intestinal movements was restricted to manual analysis of images by few enthusiasts. Several laboratories independently developed methods to record intestinal motions by video imaging producing spatio-temporal maps of movements[29,88,89]. These initial attempts demonstrated new details of the dynamics of muscle movements during peristalsis in the guinea-pig small intestine[29]. In addition, whole new patterns of motor activity were revealed in other preparations for the first time, moving beyond descriptive accounts[90,91]. Such methods are likely to become even more significant when they are extended to experiments in vivo. Then intestinal motility will get the reappraisal of the original descriptions of motor patterns that is so badly needed.

Conclusions

Most patterns of intestinal motor activity are likely to be generated by the interplay of few intrinsic fundamental mechanisms, modulated by extrinsic afferent and efferent neural and humoral influences. These mechanisms include (1) myogenic activity (smooth muscle driven by pacemaker cells generates regular or irregular propagating waves of contractions); (2) neural accommodation (distension-dependent reflex inhibition of circular muscle); (3) neural propulsion of peristalsis (initiated and maintained by intraluminal chemical or mechanical stimuli); (4) migrating motor complexes (spontaneous enteric neural activity apparently independent of luminal contents sweeps the intestine at regular intervals; it may generate segmental contractions when incomplete during the irregular phase).

There can be little doubt that a better knowledge of the fundamental mechanisms and their interplay can lead to an understanding of intestinal motor patterns in health and disease. However, the path to understand the physiological bases of intestinal motility is far from complete. The most appropriate strategy to further advance the field is to carefully evaluate questions and then to use appropriate methods to answer them. This is a formidable challenge for intestinal neuroscience but it will create a solid foundation of intestinal neuroscience for decades.

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