Mathematical modelling of enteric neural motor patterns

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Summary

1. The enteric nervous system modulates intestinal behaviours such as motor patterns and secretion. While much is known about different types of neurons and simple reflexes in the intestine, it remains unclear how complex behaviours are generated. Mathematical modelling is an important tool for assisting the understanding of how the neurons and reflexes can be pieced together to generate intestinal behaviours.

2. Models have identified a functional role for slow excitatory post synaptic potentials (EPSPs) by distinguishing between fast and slow EPSPs in the ascending excitation reflex. These models also discovered coordinated firing of similarly located neurons as emergent properties of feedforward networks of interneurons in the intestine.

3. A model of the recurrent network of intrinsic sensory neurons identified important control mechanisms to prevent uncontrolled firing due to positive feedback, and that the interaction between these control mechanisms and slow EPSPs is necessary for the networks to encode ongoing sensory stimuli. This model also showed that such networks may mediate migrating motor complexes.

4. A network model of VIP neurons in the submucosal plexus found this relatively sparse recurrent network could produce uncontrolled firing under conditions that appear to be related to cholera toxin induced hypersecretion.

5. Abstract modelling of the intestinal fed-state motor patterns has identified how stationary contractions can arise from a polarized network. These models have also helped predict and explain pharmacological evidence for two rhythm generators and the requirement of feedback from contractions in the circular muscle.

Introduction

The enteric nervous system (ENS) is contained entirely within the walls of the gastrointestinal tract. While there is communication between the ENS and central nervous system, the gastrointestinal tract can perform many functions without external input.[1] Thus the ENS must contain complete neural circuits that control gastrointestinal functions.

In addition to containing functionally complete neural circuits, the ENS is readily accessible, so much is known about the chemistry, morphology and connectivity of each type of neuron, especially in the guinea-pig small intestine. In most cases the neurons have also been physiologically characterized. Furthermore, the output of the ENS (such as muscle contractions and mucosal secretion) can be readily observed and measured when compared to functional output from other parts of the nervous system. This has allowed enteric reflexes, such as ascending excitation, descending excitation and descending inhibition, to be well characterized. However, it is not obvious how such reflexes come together to form complex motor patterns, such as peristalsis, segmentation and migrating motor complexes.

Mathematical and computational modelling are excellent tools for piecing together experimental details to link the properties of single neurons to the behaviour of the whole system. In doing so they help to clarify how the system works, how things can change to produce a pathological state, and what other pieces of information may be missing and hence are required to produce a global behaviour of the system. Here, we review the role computational modelling has played in understanding the function of the ENS over the last 15 years.

Types of neurons in the ENS

The vast majority of neuronal cell bodies in the ENS are found in the myenteric plexus and the submucosal plexus. The myenteric plexus provides most neural input to both the circular and longitudinal muscle layers.[1] It is believed to regulate the motor activity of the gastrointestinal tract and modulate secretion and blood flow (directly and *via* the submucosal plexus). The submucosal plexus provides most neural input to the mucosa and regulates secretion and blood flow.[1]

The ENS contains up to 18 different populations of neurons depending on the classification used.[2] These neurons have the anatomical structure and electrophysiological properties to form reflex circuits. Rather than analysing the potential role of every possible population of neurons in the ENS, the functional roles of broad groups of neurons will be discussed here. The focus will be on the information processing and roles played within reflex circuits. The broad groups of neurons discussed are intrinsic sensory neurons (ISNs), ascending interneurons, descending interneurons, excitatory motor neurons to the circular muscle, inhibitory motor neurons to the circular muscle, and secretomotor neurons immunoreactive for vasoactive intestinal peptide (VIP neurons). The neurotransmitters and receptors involved in synaptic transmission within, and between, populations of neurons have been reviewed elsewhere,[3] so we focus on the dynamics of transmission, rather than the pharmacology.

Since much of the knowledge of the ENS comes from the guinea-pig small intestine, this review concentrates on this species and region unless otherwise stated. The mathematical models described in this review are also based on data from guinea-pigs for the same reason. In recent times, more electrophysiological data have become available from the mouse ENS and so far these mathematical models are also a good representation of this species because the main electrophysiological parameters are comparable to those in the guinea-pig. Currently there is virtually no data on the electrophysiological properties of human myenteric neurons, but when such data become available the important parameters in these models can be set to represent human physiology.

Intrinsic sensory neurons (ISNs)

ISNs in the ENS detect and respond to contents in the lumen. They are sometimes called intrinsic primary afferent neurons, intrinsic sensory neurons, or AH/Dogiel type II neurons. These neurons are characterized electrophysiologically as AH because they display a prominent after-hyperpolarizing potential (AHP) after an action potential.[4] They have Dogiel type II morphology which is characterized by smooth cell bodies and multiple long processes.[4] ISNs have multiple long processes with at least one projection to the mucosa,[5] which allows them to respond to the contents of the lumen, while the other projections allow these cells to activate other neurons. ISNs are activated by chemical stimulation of the mucosa and/or mechanical deformation of the mucosa.[6-8] In addition to mucosal stimulation, ISNs also respond to stretch and tension in the circular muscle.[9,10]

ISNs may activate all other types of neurons in the myenteric plexus because they provide varicose terminals around all cell bodies in their own and adjacent ganglia in the myenteric plexus.[11,12] Indeed, slow excitatory post-synaptic potential (EPSP) transmission from ISNs to other ISNs and to interneurons has been directly demonstrated,[13] while indirect evidence suggests fast EPSPs are also evoked in most interneurons.[14]

One synapse type that appears to largely affect the functionality of the ISNs is the connection to other ISNs. Synaptic connections between ISNs have been demonstrated by electron microscopy[12] and electrophysiologically.[13] Given their largely circumferential projections,[11] this produces a feedback loop within the population of ISNs. Synaptic transmission from ISN to ISN is *via* slow EPSPs.[13] In conjunction with the reciprocal synaptic connections, this produces a positive feedback loop. That is, if one neuron is activated, it will activate other neurons in the network, which will in turn activate the initial neuron. This will continue until all neurons reach their highest activity level and remain there even if the initial stimulus is removed. Obviously this does not occur under normal physiological conditions, indicating other mechanisms must be in place to control the activity levels, such as AHPs, inhibitory postsynaptic potentials (IPSPs), alterations in transmitter release or other conductances.

Ascending interneurons

Ascending interneurons project orally through the myenteric plexus. It was originally thought that ascending interneurons receive synaptic input only from other ascending interneurons and ISNs,[11,15,16] but recently it has been shown they also receive synaptic input from descending interneurons.[17] Also, ascending interneurons have recently been shown to be mechanosensitive in the guinea-pig small intestine[18] and distal colon.[19] That is, these neurons are activated by stretching the circular muscle without synaptic input from the ISNs. This means the ascending interneurons can also act as ISNs and initiate reflexes, but the functional role of this direct activation is currently unknown.

The synaptic inputs from ISNs to ascending interneurons appear to be mediated by fast EPSPs and slow EPSPs,[20,21] but slow EPSPs have not always been observed in ascending neurons.[15]

Anatomical studies indicate that ascending interneurons do not have synaptic outputs until 3 mm oral to the cell body, but further from the cell body they provide numerous synaptic connections for the remaining length of the projection.[15] Immunohistochemical labelling suggests these neurons provide output to most other neurons, including some descending interneurons,[15,17] while electrophysiological studies confirm they excite other ascending interneurons and excitatory and inhibitory motor neurons.[22,23] Transmission between ascending interneurons appears to be *via* fast EPSPs only,[20] while transmission from ascending interneurons to excitatory motor neurons may be *via* fast and slow EPSPs.[20,21]

The oral projections in conjunction with synaptic connections to other ascending neurons mean this population of neurons forms a feedforward network. That is, when ISNs activate ascending interneurons at one location, this activity will pass on, provided the stimulus is strong enough, to all other ascending interneurons oral to the stimulus location. Since the ascending interneurons also form connections with excitatory motor neurons, this feedforward network is the most likely mechanism behind contractions observed oral to a stimulus. This reflex is usually referred to as ascending excitation.[24]

Descending interneurons

Descending interneurons project in the anal direction in the myenteric plexus. Usually this population of neurons in guinea-pig ileum is broken up into three populations according to their immunoreactivity for vasoactive intestinal peptide (VIP), 5-hydroxytryptamine (5-HT) or somatostatin. Descending interneurons receive input from ISNs, other descending interneurons and ascending interneurons.[17,22,25,26] This synaptic transmission appears to be mediated by both fast and slow EPSPs.[27,28] Synaptic outputs can be identified as little as 100 μm from the cell body[29] and are formed with other descending interneurons, ascending interneurons, inhibitory motor neurons, excitatory motor neurons, and ISNs.[17,29-32]

Like the ascending interneurons, descending

interneurons have outputs to other descending interneurons, producing a feedforward network.[17,29] Transmission in this pathway appears to be mediated in part by slow EPSPs,[27] indicating that slow EPSPs are not present just to increase the excitability of neurons, but can conduct reflexes on their own.[28] Descending interneurons also transmit to inhibitory motor neurons *via* fast EPSPs.[25] The feedforward network formed by the descending interneurons and output to the inhibitory motor neurons is the most likely mechanism for the observed relaxations anal to a stimulus. This reflex is usually referred to as descending inhibition.[24]

In addition to the simple feedforward network, the descending interneurons have a more complex functional role because of the synaptic connections formed with ISNs, excitatory motor neurons and ascending interneurons. Similar to ascending interneurons, the functional role of descending interneurons has been further complicated in recent years by the finding that these neurons may also act as mechanosensitive neurons.[18,19] Therefore, these neurons may be an important adjunct to the AH/Dogiel type II class of ISN.

It appears that a subpopulation of descending interneurons immunoreactive for vasoactive intestinal peptide (VIP) and ascending interneurons form a recurrent network.[17] These two populations have reciprocal connections and are likely to transmit to each other *via* fast and/or slow EPSP (see above and below), which will produce positive feedback. The potential effects of such a recurrent network are difficult to predict because these neurons are also in feedforward networks heading in different directions, both feedforward networks receive input and provide feedback to the network of ISNs, and, ascending interneurons do not have synaptic outputs in the first 3 mm of their projection which could create regions or bands of activity. The potential effects of this recurrent network formed by ascending and descending interneurons are an untapped area of research that may provide insight into unknown mechanisms underlying complex motor patterns.

Descending interneurons also appear to be involved in transmission to excitatory motor neurons.[33,34] Given that the descending interneurons form a feedforward network, this provides a possible mechanism to produce contractions anal to a stimulus (referred to as descending excitation) and to produce contractions that propagate in the anal direction. However, as descending interneurons have strong connections with inhibitory motor neurons, there may be something more complex occurring because these two outputs from descending interneurons oppose each other. While it appears that 5-HT containing descending interneurons are involved in transmission to excitatory motor neurons[30,31] and descending interneurons immunoreactive for either somatostatin or VIP are involved in transmission to inhibitory motor neurons,[35] there must be mechanisms to ensure that all subpopulations of descending interneurons are not activated simultaneously.

Excitatory motor neurons

Excitatory motor neurons receive input from other neurons and transfer this information to the circular muscle, where they can elicit contractions. The excitatory motor neurons receive synaptic input from ISNs,[14] ascending interneurons[15,17] and a subpopulation of descending interneurons.[17] Both fast and slow EPSPs have been recorded in excitatory motor neurons.[14] One source of the fast and slow EPSPs is the ascending interneurons,[20,36] but as acetylcholine and tachykinins are involved in transmission to excitatory motor neurons,[20,36] there are probably also monosynaptic connections from ISNs to excitatory motor neurons involving fast and slow EPSPs. The fast EPSPs probably mediate rapid reflex responses in the ascending reflex pathway.[20,21,23,36,37] However, blocking fast EPSP transmission onto excitatory motor neurons depresses, but does not abolish, contractions in the circular muscle,[36] indicating a role for slow EPSPs.

Excitatory motor neurons have a single long axon that projects orally through the myenteric plexus before travelling down into the circular muscle. The vast majority of axons project orally through the myenteric plexus for short distances of 0 - 2 mm, but some project up to 8 mm through the myenteric plexus from the cell body.[38] These axons do not give rise to branches or varicosities within the myenteric plexus,[39] thus they do not have synaptic outputs to other myenteric neurons. Once the axon enters the circular muscle, many branches and varicosities are observed.[39] Accordingly, the excitatory motor neurons have a sole purpose of relaying information from other myenteric neurons to the circular muscle. However, the oral projection of excitatory motor neurons can be expected to be important in ascending excitation in response to a local stimulus.

Inhibitory motor neurons

Inhibitory motor neurons receive input from other neurons and relay this information to the circular muscle where they can cause active relaxations often leading to dilations. The inhibitory motor neurons receive synaptic inputs from ISNs and descending interneurons.[11,17,40] Similar to the excitatory motor neurons, the inhibitory motor neurons display both fast and slow EPSPs.[11,27,28,41] Fast EPSP transmission appears to predominate here, but transmission along the chain of descending interneurons may be mediated by slow EPSPs.[28] This is consistent with the long latency of the inhibitory junction potentials observed in circular muscle evoked by distension and mucosal stimulations.[20,25,35] However, fast EPSPs could be involved in a rapid response through direct connections from ISNs to inhibitory motor neurons,[20,25] providing a method for muscle relaxation/dilation to precede an anally propagating contraction.

The long single axon of the inhibitory motor neurons projects anally through the myenteric plexus for 0.5-25 mm[39,42] before entering the circular muscle. While projecting through the myenteric plexus, these axons do not give rise to any branches or varicosities,[39] so they do not

form synaptic connections with other myenteric neurons. Once the projection enters the circular muscle, many branches and varicosities are observed.[39]

Secretomotor neurons

The ENS is capable of modulating chloride secretion in the gastrointestinal tract[43] and neurons in the submucous plexus appear to be the final common output in neurally mediated secretion because they provide the majority of projections to the mucosa and are required for neurally evoked secretion.[43] Almost half the neurons in the submucous plexus are immunoreactive for vasoactive intestinal peptide (VIP neurons) and these are the most likely candidate for the final step in neurogenic hypersecretion.[43] These neurons receive synaptic inputs from ISNs and interneurons in both the submucous plexus and myenteric plexus. In addition to these synaptic inputs, the VIP neurons can occasionally provide varicose collaterals in submucosal ganglia and it has been electrophysiologically shown they can elicit slow EPSPs in other VIP neurons.[44] Thus, the VIP neurons form a recurrent network, but this recurrent network is much weaker (in terms of the synaptic connections) when compared to recurrent networks of ISNs in the myenteric plexus or submucous plexus.

Modelling

Early modelling

Mathematical modelling of the ENS and surrounds as a method to explain intestinal behaviour first appeared in the 1990s through work done by Miftkahov and colleagues.[45-47] They aimed to build models using as much biophysical detail as possible. The complexity of these models meant they were solved numerically and only small regions of the parameter space could be examined with the resources available at the time. The authors concentrated mainly on synaptic transmission from transmitter release to currents generated in the postsynaptic membrane. Unfortunately, because of the limited biophysical data available at that time, the insight provided by the models and comparison with experiments was limited. This work is also marred by some physiological errors; for example, EPSPs were calculated to have an absolute value of 87mV (well above the observed driving potential of -10 to +10 mV) and IPSPs were calculated to hyperpolarize membrane potential to 70mV below resting membrane potential. Such physiological errors combined with the small regions of the parameter spaces examined led to suggestions such as slow waves as the result of a feedback loop between mechanosensitive neurons, motor neurons and motor contraction[45] despite the evidence that they are generated by the interstitial cells of Cajal.[48-50]

Leaky integrate-and-fire models

Another approach to modelling ENS function is to use leaky integrate-and-fire models for neurons. Leaky integrate-and-fire models do not explicitly model action potentials, instead once a threshold is reached an instantaneous event occurs. Therefore, these models do not require large amounts of biophysical detail, such as information regarding the voltage-dependence of ion channels. Also, the numerical methods required to solve the equations are simpler, which allows larger parameter spaces to be explored. Therefore, leaky integrate-and-fire models are widely used in the central nervous system (particularly when simulating 1000s of neurons) and were required to simulate small regions of intestine with the computer resources available during the late 1990s and early 2000s.

The role of ascending excitation

A model of ISNs and orally directed interneurons was used to investigate the role of slow and fast excitatory transmission from ISNs to these interneurons.[51] This model showed when transmission from ISNs to ascending interneurons was *via* fast EPSPs, the latencies and durations of the simulated responses were too brief to match the electrophysiologically recorded responses. When transmission from ISNs was *via* slow EPSPs, the latencies were very similar to those recorded physiologically, and the durations matched the recorded responses when the firing of ascending interneurons was limited to the beginning of a slow EPSP. Thus, this model showed the functional transmission from ISNs to orally directed interneurons is generated by slow excitatory transmission for ascending excitation and not fast excitatory transmission. This illustrates how modelling can provide functional information that is unattainable from traditional physiological experiments.

This model of ascending excitation was extended to include excitatory motor neurons and the circular muscle to investigate the role of ascending excitation in peristalsis.[52] This model was anatomically realistic in the number of neurons simulated and the patterns of connections between neurons. A leaky integrate-and-fire model was used for the neurons, whereas the circular muscle was described by voltage-dependent currents and these currents could flow through the muscle syncytium. ISNs were activated in a manner consistent with a brief mechanical stimulus. Transmission between ISNs and first-order interneurons was by slow EPSPs. Interneurons then transmitted to higher order interneurons by fast EPSPs. As the activity propagated along the pathway, random firing became progressively more synchronized between neurons, until the network as a whole was firing in a coordinated manner. This was a robust phenomenon in this feedforward network and the emergence of this property could only be identified by mathematical modelling. The smooth muscle model indicated that bursting input to the muscle may increase the likelihood of muscle cells firing action potentials when compared with uniform input. In addition, the syncytium model explains how the predicted muscle excitation might be related to current experimental observations such as the changes in circular muscle activity when stimulating the mucosa.[53,54]

A model of slow EPSP transmission and recurrent networks

Investigating the ascending reflex showed the importance of slow EPSPs in transmission from ISNs to interneurons.[52] Slow EPSPs are also present in many other synaptic connections in the ENS (see introduction). Therefore, a better mathematical description of the slow EPSPs was developed.[55]

This description involved breaking down the second-messenger cascade that mediates slow EPSPs[56] into three stages. The first stage describes the activation of receptors, G-proteins and the production of cyclic adenosine monophosphate. The second stage corresponds to the release of active protein kinase A catalytic subunit. Finally, the third stage represents simple phosphorylation of potassium channels, thereby closing them. Each stage had a decay based on first-order, mass-action kinetics. These equations were found to fit the experimental data from myenteric neurons of the guinea-pig ileum better than variations still based on describing the second-messenger cascade. The values of the parameters within these equations were determined by fitting to electrophysiological data. The final output of the model produced a slow EPSP with physiologically realistic rise time, amplitude and duration. Also, the model produced a nonlinear stimulus-response relationship, an important feature of slow EPSPs, which resulted from the underlying kinetics of the signalling cascade.

This slow EPSP model was then incorporated into a large-scale computer simulation of ISNs.[57] As stated above the ISNs are interconnected *via* slow EPSPs, hereby forming a recurrent network. Such networks are unstable and unable to give a graded response to sensory input without some form of inhibition. It seemed likely that this was due to their characteristic AHPs. However, the AHP is suppressed during slow EPSPs.[4] Therefore, AHPs, slow EPSPs and the interactions between the two were included in computer simulations of networks ISNs to test whether AHPs provide enough inhibition to allow this recurrent network to give a graded response to ongoing stimuli as would be expected with nutrients in the intestinal lumen. These simulations showed the AHPs can readily stabilize a network of ISNs allowing the network to encode the magnitude of a fluctuating sensory stimulus. Even when the slow EPSPs suppressed the AHP to as little as 1% of control, the network of ISNs was still able to give graded responses. This highlighted the importance of the AHP in ISNs which help lead other investigations into a potential role in disease states such as irritable bowel syndrome[58] and inflammation of the colon.[59,60] Further investigations into this ISN recurrent network also revealed that inhibitory cotransmission could allow this network to encode biological inputs in a useful manner.[61]

A model of migrating motor complexes

Migrating motor complexes (MMCs) are cyclic motor patterns with several phases observed in the fasted state. Since Phase III has slow propagation speeds of 4-17 cm/min in the guinea-pig,[62] fast EPSPs and interneurons cannot account for this motor pattern. Accordingly, the recurrent network of ISNs was used to explore whether slow EPSP transmission between ISNs underlies MMCs.[63] In addition to the features of the previous studies of this recurrent network, this version had an activity-dependent synaptic depression to represent physiological mechanisms such as receptor internalization/desensitization or presynaptic inhibition. The basis of the model was that the recurrent connections would drive high firing rate, which would activate the adjacent regions of the network in the anal direction because of the anal bias in projections of ISNs,[11] and then the activity-dependent inhibition would switch off the initial activity. Computer simulations of this model were able to relate properties of neuron to properties of the MMC cycle. Phase III migration speed was governed by neuron excitability, MMC cycle length was related to the rate of recovery of synaptic efficacy, and phase III duration was determined by the duration of slow EPSPs in ISNs. By relating the properties of single neurons to the properties of the MMCs, the model made experimental predictions that can be tested using standard techniques. Therefore, this model produced the first plausible mechanism to explain the generation and propagation of MMCs.

A model of pathogenic secretion

Since modelling studies and experimental studies indicate a crucial role for recurrent networks of ISNs in producing complex motor patterns, it naturally led to the question of the importance of other recurrent networks in the ENS. One such network is formed by secretomotor neurons immunoreactive for VIP in the submucous plexus. This recurrent network of VIP neurons is also interconnected *via* slow EPSPs, but differs from the myenteric ISNs in that there are far fewer synaptic connections and they do not display an AHP to control firing. Simulations showed that this recurrent network was unlikely to be bistable under normal physiological conditions, but instead activity decayed with a similar time course to the decay in activity observed in secretion across the mucosa.[64] However, if slow EPSPs were larger than under normal physiological conditions, then the network of VIP neurons became bistable, responding to any input with maximal and sustained firing. These results predict that enterotoxins such as cholera toxin could cause hypersecretion by switching the VIP network to a bistable state. It has subsequently been shown cholera toxin dramatically increases the excitability of VIP neurons,[65] which would switch the VIP network to a bistable state. Such a switch can account for some findings about cholera-induced hypersecretion, such as a VIP antagonist prevents this hypersecretion when applied before cholera toxin but not blocking it when cholera has already elicited a response.[66] Further, when interactions between the recurrent VIP neuron network and a recurrent network of ISNs located within the submucosal plexus were modelled, the simulations predicted that VIP neurons would maintain a low level of spontaneous firing at rest, which would produce a basal level of secretion. This is currently being

tested.

Abstract modelling

Abstract models involve describing complex systems with only a small number of variables, which drastically reduces the simulation time, allowing many different scenarios to be investigated. When investigating the mechanisms to control firing in the recurrent network of ISNs in the myenteric plexus, a Wilson and Cowan[67] type model had been used to perform a preliminary analysis.[61] This abstract model produced the same results as the anatomically realistic and electrophysiologically realistic for synaptic potentials model described above. In addition to accurately reproducing these results, this abstract model had previously reproduced experimental observations in the central nervous system.[68] Thus, it provided a good way to investigate general mechanisms because a large number of parameters could be quickly explored while still been able to produce physiologically realistic results.

Segmentation is the motor pattern observed in the fed-state. It is a complex pattern that could not be readily observed *via* traditional techniques. The emergence of video imaging methods enabled development of an *in vitro* approach to directly observe and analyse segmentation. This revealed that the most prominent pattern is stationary contractions that repeat at regular intervals and these are mixed in with short-length propagating contractions and whole-length propagating contractions.[69,70] This immediately raised the question of how can a polarized network produce stationary contractions.

So, an abstract model was used to investigate general mechanisms by which a polarized network could produce stationary contractions.[71] This computational model simulated the mean neuron firing rate in the feedforward ascending and descending reflex pathways. A stimulus driven pacemaker was located in the afferent pathway or in a feedforward pathway. Output of the feedforward pathways was fed into a simple model to determine the response of the muscle. In the computational model, a local stimulus produced an oral contraction and anal dilation, similar to *in vitro* responses to local distension or mucosal deformation. Introducing outputs from descending interneurons to ascending interneurons produced an anally propagating excitation that followed descending inhibition, thereby mimicking descending excitation and suggesting a role for at least part of the recurrent circuits formed by the interneurons. When the stimulus was distributed, representing a nutrient load, the result was either a sustained small amplitude contraction/relaxation along the length of the simulated segment or globally synchronized oscillations. However, when we introduced local variations in synaptic coupling, stationary contractions occurred around these locations. This predicts that severing the ascending and descending pathways with an acute lesion will induce stationary contractions on either side. This prediction was tested using video imaging of guinea pig jejunum infused with decanoic acid to induce segmentation *in vitro*. An acute lesion *in vitro* significantly increased the number of stationary contractions immediately oral and anal to the lesion, as predicted by the computer model. Therefore, this abstract computer model produced the first plausible mechanism by which stationary contractions observed during segmentation could arise from a polarized network. The results suggest that spatially localized rhythmic contractions arise from a local imbalance between ascending excitatory and descending inhibitory muscle inputs and require a distributed stimulus and a rhythm generator in the afferent pathway.

Following on from the prediction that a rhythm generator was located in the afferent pathway, pharmacological studies were performed to try and uncover the rhythm generator by targeting forms of inhibition potentially involved in producing the oscillations. Pharmacological agents that target the intermediate potassium (IK) current underlying the AHP in ISNs affected the rhythm of contraction episodes (comprising short bursts of stationary and short-length propagating contractions mixed with whole-length propagating contractions) and quiescent periods. The durations of the contraction episodes were increased, but the quiescence periods were unaltered. It is unusual to affect the period of one part of an oscillation without effecting the period of the other part of the oscillation. To understand this phenomenon, the principles of an abstract Wilson and Cowan type model and principles from the slow EPSP model and its interaction with the AHP were combined to produce a simple abstract model that incorporated synaptic potentials and interactions with calcium dependent currents.[72] The model described activity in ISNs, excitatory and inhibitory motor neurons and the muscle. It reproduced alternating activity and quiescence with physiologically accurate durations for both the control response and the pharmacological data on inhibiting the AHP in ISNs. The model predicted that feedback to ISNs from contractions in the circular muscle was required to produce contraction episodes and quiescence. The model also predicted that transmission from ISNs to excitatory motor neurons is mainly *via* fast excitatory synaptic potentials and transmission from ISNs to inhibitory motor neurons is mainly *via* slow EPSPs.

The prediction that feedback to ISNs from contractions in the circular muscle is required to produce contraction episodes and quiescence[72] was tested pharmacologically by blocking activation of $5\text{-HT}_{3/4}$ receptors.[69] This would block responses to mucosal 5-HT release evoked by contractions in the circular muscle[73] feeding back onto the ISNs. It is also likely cholecystokinin (CCK) 1/2 receptors are involved in the nutrient stimulus activating the ISNs, so antagonists for these receptors were also tested.[69] The addition of 5-HT antagonists caused a large reduction in the amount of segmentation observed, agreeing with the computer model prediction, but also indicating there must be a residual effect of 5-HT or other mechanisms providing the feedback. Addition of CCK antagonists decreased the amount of segmentation observed, which was replicated in the model. The combined 5-HT and CCK antagonists did not further reduce the

amount of segmentation observed and this could only be replicated in the model if the nutrient stimulus activated two pathways in the mucosa that converge within the enteric neural circuitry.

These examples show how abstract models can be a powerful tool for investigating general mechanisms. When using abstract models to simulate neural activity, other components of the gastrointestinal tract can readily be added to give a better description of the whole system. Indeed, the abstract model used to predict that feedback to ISNs from contractions in the circular muscle is required to produce contraction episodes and quiescence[72] used a simple model of the circular muscle to produce this feedback. A limitation of this model was the circular muscle could not produce individual contractions, but produced periods of time when contractions were likely to occur due to the relative levels of excitatory and inhibitory input. Since this abstract model was developed, new and improved models of smooth muscle cells[74] and interstitial cells of Cajal[75] have appeared in the literature. Incorporating these models into the physiological feedback loop (described above) should provide new insights into the function of the ENS, the smooth muscle and the gastrointestinal tract as a whole organ. The potential for new insights arising from combining mathematical modelling from different research areas has led to the development of a central repository and framework (http://physiomeproject.org/) to facilitate this process. While current implementations of detailed models of the ENS (such as leaky integrate-and-fire models and conductance based models) would be computationally too exhaustive to be included in such projects, the abstract models discussed here would be ideal.

Current and future direction for modelling in enteric neuroscience

Abstract models are powerful for investigating general mechanisms. However, they need to be closely integrated with wet experiments or more physiologically accurate modelling, otherwise it is not possible know if the abstract values are relevant. Leaky integrate-and-fire models are good for investigating network phenomena and synaptic potentials, but cannot investigate voltage-sensitive currents and their interactions with other currents. Detailed conductance models can be used to investigate the interactions of voltage-sensitive currents and other currents, but these models require a lot of detail and large amounts of computer resources to explore the parameter spaces. Since Miftkahov and colleagues (see above) tried the last type of modelling of the ENS almost 20 years ago, much more data about details of currents in enteric neurons are available and the advance of computer technologies means large parameter spaces can readily be explored when simulating networks of thousands of neurons described by highly detailed conductance base models.

Recently we developed a highly detailed conductance base model of ISNs (Chambers *et al.*, unpublished). This includes voltage-gated sodium and potassium channels, an N-type calcium channel, a big conductance potassium channel, a calcium dependent non-specific cation channel, IK channel, hyperpolarization activated cation (I_h) channels and internal calcium dynamics. We have tested this model against published data and our electrophysiological studies and found the model reproduces the physiological observations of firing in response to multiple current pulses (250pA 10ms duration at 50Hz) or prolonged depolarising current pulses (50-350pA for 500ms), and responses to prolonged hyperpolarizing current pulses. A sensitivity analysis of each of the currents has revealed that IK, I_h, big conductance potassium and calcium dependent non-specific cation currents have the largest influence on the excitability of the ISNs. Furthermore, we have created a network of thousands of ISNs with slow EPSP transmission that can produce propagating activity.

With the development of this highly detailed conductance based model of ISNs, it is now possible to investigate how individual currents and their interactions with other currents can influence the behaviour of ISNs and a network of ISNs. For example, there is evidence that an increased in I_h conductance is involved with inflammation of the colon[59,60] and that cholera-toxin changes the properties of ISNs in the myenteric plexus. This sort of conductance based model can test if such observations are capable of generating the pathology or if other requirements must also be met. Indeed, our single neuron model of ISNs indicates that increasing I_h does increase the excitability of these neurons, but a decrease in IK is also required to produce the increase in duration of action potential firing during a depolarising current injection as observed.[60]

Conclusions

Over the last 15 years, modelling studies in the ENS have helped the understanding of enteric function by relating the behaviour of single neurons to properties of the whole enteric nervous system and its output (such as muscle contractions and secretion). More specifically, models have identified the importance of slow EPSP transmission in the recurrent network of ISNs and some potential control mechanisms. Further investigations of this network model produced the first mechanism of MMC. A similar type of network model of VIP neurons in the submucosal plexus found that this relatively sparse recurrent network could produce uncontrolled firing under certain conditions, which could be related to cholera-induced hypersecretion. Abstract modelling of the intestinal fed-state motor patterns has identified how stationary contractions can arise from a polarized network. These models have also helped predict and explained pharmacological evidence for two rhythm generators and the requirement of feedback from contractions in the circular muscle.

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