

A computer simulation of recurrent, excitatory networks of sensory neurons of the gut in guinea-pig

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Received 2 March 2000; received in revised form 15 April 2000; accepted 1 May 2000

Abstract

Intrinsic sensory neurons of the intestine are connected together to form a recurrent network. They interact by slow excitatory post synaptic potentials (EPSPs), which have a complex dependence on the pattern of input. These networks are unstable and unable to give graded responses to sensory input without some form of inhibition, but inhibitory synaptic potentials are rare in this system. Intrinsic sensory neurons have a characteristic after-hyperpolarization (AHP), but this is depressed during slow EPSPs. To test whether AHPs can provide the inhibition necessary for stability, AHPs, slow EPSPs and their interactions were included in a computer simulation of realistic sensory neuron networks. Residual AHPs as small as 1% of control were found to lead to stable networks capable of giving graded responses. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Computer simulation; Neural networks; Recurrent excitation; Enteric nervous system; Inhibition; Sensory neurons

The myenteric plexus of the enteric nervous system provides the primary neural control for gastrointestinal motility [6]. This is a ganglionated network that runs within the intestinal wall and has several functional classes of neurons including intrinsic sensory neurons. The sensory neurons respond to changes in the chemical content of the lumen and to stretch or tension [7]. They make connections with almost every other neuron type in the myenteric plexus, including other sensory neurons [11]. The predominant projections are circumferential, and reciprocal, with a slight oral and anal spread [3], thus forming a recurrent network. They transmit to other sensory neurons by generating slow excitatory post-synaptic potentials (EPSPs) in the target neurons [11], which depolarize the neuron by closing potassium channels. The change in membrane conductance due to slow EPSPs induced by internodal strand stimulation is non-linear in both the frequency and the number of stimuli [12]. More stimuli lead to faster rise times and longer time courses, but do not depolarize the membrane beyond a maximum of 10–15 mV. Inhibitory post-synaptic potentials (IPSPs) are rare or absent. Sensory neurons also show a prominent after-hyperpolarization (AHP) lasting from 2 to

20 s following an action potential (AP) [7]. This is caused by calcium entering the cell during the AP, leading to the opening of calcium activated potassium channels. The slow EPSP closes the same channels so it significantly reduces or abolishes the AHP [7].

Recurrent neural networks with excitatory feedback, and no inhibition, are only stable for small stimuli [5]. Any stimulus or spontaneous activity, larger than some threshold, will amplify until the network is firing at its maximum rate and it will not return to a low firing state. Even if some other mechanism, with a longer time course, came into play to return the network to the basal state, such a network cannot give a graded response to stimuli above the threshold. In some cases, intestinal reflexes give all-or-nothing responses [4], but most intestinal reflexes are graded and, in those that are not, electrical responses recorded in the circular muscle are graded with stimulus strength [4]. Thus, the sensory neuron circuits must also be producing a graded response.

The aim of this study was to examine the role that AHPs may play in network transduction of sensory stimuli, despite being diminished during slow EPSPs. This was done by computer simulation of networks of sensory neurons with anatomically realistic numbers of neurons and patterns of connections. Synaptic currents were realistically modelled and a novel model of the interaction between slow EPSPs and the AHPs was included. Input was modelled as

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described below and output was measured as the neuron firing rate averaged across the network.

Computer experiments were performed as follows. Networks were constructed and solved using the 'Plexus' network simulator [2,13] to which was added a model of the interaction between slow EPSPs and the AHPs. The slow EPSP equations are a set of three coupled differential equations modelling the creation of a diffusible second messenger, which disassociates the catalytic subunit from protein kinase A, which in turn phosphorylates calcium activated potassium channels [1]. A single AHP, in the absence of a slow EPSP, is modelled by a stereotyped time course describing the number of calcium bound channels. The number of channels for multiple AHPs sums linearly. The proportion of channels open depends on four cases. When nothing is bound, the conductance is assigned a low basal value. Thus, if no AHP or slow EPSP are present the basal conductance contributes to the resting membrane properties. When channels are only phosphorylated or only calcium is bound they are closed or open, respectively. When channels are phosphorylated and calcium is bound, the channels have a residual conductance. This was systematically varied during the study. The proportion of channels in each state is determined from the slow EPSP and AHP models. A maximal slow EPSP, by definition, results in all channels being phosphorylated. At its peak, a single AHP is associated with 25% of channels binding calcium, allowing summation of AHPs for multiple APs.

Determination of parameters for the slow EPSP is described in Bertrand et al. [1]. One of these parameters, the 'synaptic strength', which determines the slow EPSP rise time and duration (but not the maximum possible depolarization), was also varied as part of the study. Resting membrane potential, resistance, AP threshold, AHP mean duration and hyperpolarization were taken from Furness et al. [7]. The AHP durations and peak conductances were randomly generated from distributions with SDs of 25% of the means. This was done to qualitatively mimic physiological variation.

Simulated neurons lacking both AHPs and slow EPSPs fire tonically at 20–25 Hz when depolarized by a current injection. The simulated networks were 15 mm in both longitudinal and circumferential directions. They contained about 9000 neurons in 360 ganglia, corresponding to the density of sensory neurons in the guinea-pig ileum [11]. These networks were large enough to avoid size dependent artifacts. Neurons made 7 ± 3 (mean \pm SD, normal distribution) connections with other neurons uniformly in a rectangular region 5.0 ± 2.3 mm in the circumferential direction and 0.7 ± 0.8 mm in the longitudinal direction. The centre of this region of contact is located 0.3 ± 0.8 mm in the anal direction from the cell body. This pattern mimics that seen in the myenteric plexus of the guinea-pig ileum [3]. Input was introduced into the entire network as proximal process potential (PPP) like events, each being large enough to generate an AP in a resting neuron. Events

were generated randomly as a Poisson process, whose average frequency was varied from 0.1 to 50 Hz. Output is reported as the mean firing rate per neuron, averaged over the entire network.

Source code and documentation for Plexus are available from <http://dirac.physiology.unimelb.edu.au/enl/>.

When stimuli greater than 0.1 Hz were applied to a network with no AHP, the network always made a transition to a state in which all neurons were firing maximally. This is shown in Fig. 1A, in which average neuron firing rate is plotted against time for a variety of input frequencies. Furthermore, when the input ceased, the network did not return to the quiescent state (not illustrated), but remained in the excited state indefinitely. On the other hand, when a residual 10^{-1} AHP was present (Fig. 1B), the network firing rate was significantly less than the same network with no AHP. The firing rate was roughly proportional to the input frequency, and hence was graded with the size of the input. Furthermore, the network returned to the quiescent state when the input ceased (not illustrated). When the AHP, but no slow EPSP, was present the response was only graded up to an input frequency of 5 Hz, for which the corresponding output frequency was 1.2 Hz.

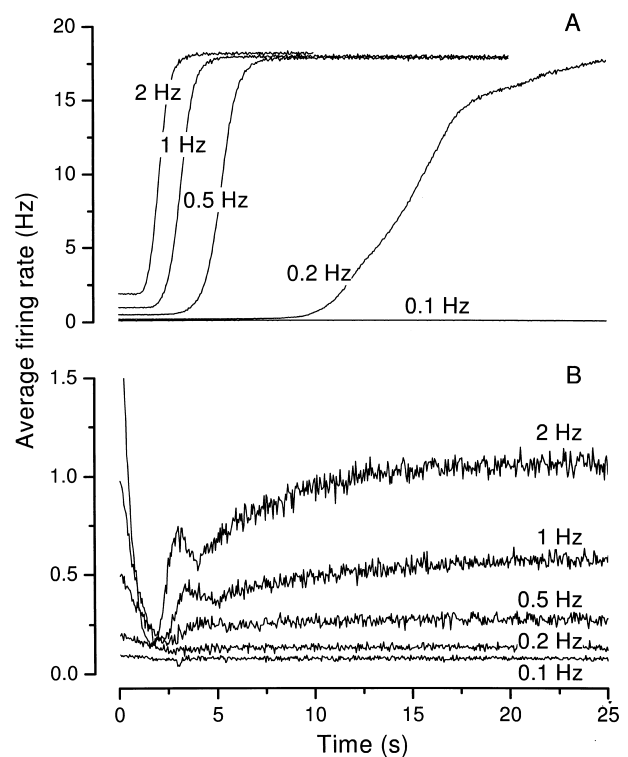


Fig. 1. Action potential firing rate, averaged over the network, is plotted against time, for a variety of input frequencies. (A) No AHP was present in the neurons used to generate these data. All curves, other than for the 0.1 Hz input, eventually reach the maximum firing rate. (B) In the network used here, the neurons had a residual AHP, equal to 10^{-1} of its resting value, during the slow EPSP. The final output firing rate depends on the input firing rate.

Stimulus response curves were obtained for a range of residual AHPs. These are illustrated in Fig. 2, in which output frequency is plotted against input frequency for various residual AHP sizes. When no AHP was present, no graded response was possible, when a 10^{-3} residual AHP was present a graded response was only possible for input frequencies less than 1 Hz, and for frequencies greater than this the network was unstable. However, for a 10^{-1} or 10^{-2} residual AHP a graded response was possible for frequencies up to 50 Hz.

For the values of synaptic strength used to generate the previous results the stable networks always returned to a state of total quiescence after the stimulus ceased. However, when synaptic strength was increased some combinations of residual AHP size and synaptic strength created networks which returned to a state with a level of ongoing activity. Values of the synaptic strength tested were three, five and ten times greater than the value used to generate the previous results. Stable firing rates of 0.3, 0.6 and 1.0 Hz were observed for the residual AHP size and synaptic strength pairs (10^{-1} , 10), (10^{-2} , 5) and (10^{-2} , 10), respectively. In these cases the neurons were firing well below their maximum rates and so were still able to produce graded responses.

These results reveal an important, putative role for the AHP in enteric sensory neurons. Without the AHP, recurrent networks of sensory neurons are unstable, only small stimuli are required to switch the network into the maximal firing state. When a residual AHP is present, however, firing is inhibited in an activity dependent manner leading to stability and graded transduction of input. When no slow EPSP is present, groups of sensory neurons are similarly unable to transduce input because their firing rate is limited. Slow

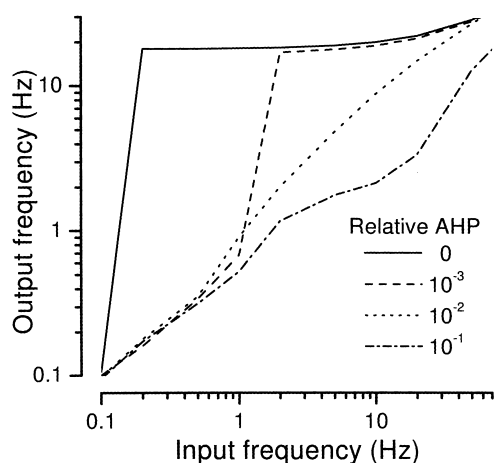


Fig. 2. Neuron action potential firing rate, averaged over the entire network, is plotted against average input rate, for a variety of residual AHP values. Log scales are used for both axes. Networks with no AHP or with a residual AHP of 10^{-3} are unstable for large stimuli, while networks with residual AHPs of 10^{-1} and 10^{-2} are stable for all stimuli.

EPSP excitation and AHP inhibition interact to keep neurons at a high level of excitability while preventing them from firing at high frequency, and hence they are able to convert PPP input to AP output. A theoretical study of the roles of recurrent excitation and inhibition in the visual cortex has shown parallel results [5]. In this system, strong recurrent excitation is present and activity dependent feedback inhibition is provided by IPSPs. The inhibition reduces the gain and increases the range of inputs for which the network is stable, leading to a response that is proportional to the stimulus. The results of our model indicate that this property is not strongly dependent on the precise values of the slow EPSP and AHP parameters, making it a robust observation. Interestingly, in some cases the stable network state does have activity, which can be interpreted as spontaneous firing. Experimental preparations in which the mucosa is intact also display spontaneous activity [10], however, it is unclear whether this is due to sensory drive or is intrinsic to the network. Simulated networks with small amounts of spontaneous activity are still able to transduce stimuli, other than those of very low frequency. This may be related to the observation that, at least for some brief stimuli, sensory neurons fire a burst of action potentials [7]. This would ensure that weak, or highly phasic, stimuli are discernible above spontaneous activity.

The AHP is not the only mechanism for inhibition in enteric sensory neurons for which there is evidence. Firing may be limited by voltage dependent changes in some membrane conductances, such as sodium inactivation. Some neurons display an increased chloride conductance during a slow EPSP [1], and even though it is depolarizing, it can also be inhibitory, either by direct shunting or by enhancing sodium activation [9]. Presynaptic inhibition has also been observed in transmission from sensory neurons [8]. Finally, the possibility that small or infrequent IPSPs can play a role has also not been eliminated. These mechanisms are the subject of further investigation.

This work was supported by a Program Grant (963213) from the National Health and Medical Research Council (Australia). The use of computer facilities was generously provided by the Ormond Supercomputing Facility, a joint venture between the University of Melbourne and the Royal Melbourne Institute of Technology.

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