Of Slow Waves and Spike Patches

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In the small intestines, the major task of the slow wave is to induce mechanical movements in the intestinal wall by generating local calcium spikes. High resolution electrical mapping reveals fundamental differences in propagation between slow waves and calcium spikes. These differences suggest that slow waves and spikes are propagated by different mechanisms through different cell networks.

In the small intestine, the propagating slow wave is thought to act as a pacemaker signal that induces the muscle layers to contract. Recent work has shown that slow waves are probably conducted in the organ not by the smooth muscle cells themselves but by a network of interstitial cells of Cajal (ICC; see Refs. 6 and 13). Smooth muscle cells appear to act chiefly as recipients of the slow wave, which they in turn transform into patterns of contractions. Smooth muscle spikes, initiated in response to the slow wave, appear to propagate through the smooth muscle layer and are often the first step in the excitation coupling mechanism leading to contraction. Understanding the spatial and temporal propagation patterns of these two electrical waves, the slow wave and the spike, should therefore help to clarify not only the mechanisms involved in their generation and propagation but also the processes that underlie the different types of small intestine movement.

Temporal relationship between slow waves and spikes

Many studies, in different ways, have shown the close temporal relationship between slow waves, spikes, and contractions (5, 12). Intracellular recordings from strips of the intestinal wall have consistently shown the presence of a slow wave, on top of which one or more spikes may or may not occur (4). Whether or not spikes are initiated seems to depend on the magnitude of depolarization generated in smooth muscle cells by the slow wave. Recently, Huizinga and colleagues (9) have shown in isolated cells that slow waves and spikes occur in two different cell types; slow waves could only be demonstrated in ICC, whereas spikes were only recorded in isolated smooth muscle cells. Furthermore, isolated smooth muscle cells did not generate spikes spontaneously; these only occurred when the membrane potential was depolarized above a well defined threshold (9). In intact tissue, this suprathreshold depolarization is presumably provided by the slow wave.

Spatial pattern of slow wave propagation

Most of the studies described above used only one or at most a few electrodes to record the electrical activity in segments of the intestines. This electrical activity, however, propagates throughout the organ, and it is the pattern of propagation that may also determine the pattern of contraction in the small intestine. Recently, by using methodologies developed for reconstructing the spatial pattern of electrical propagation in cardiac muscle, it has become possible to visualize the conduction of electrical activity over the surface of smooth muscle organs (8). The technique requires simultaneous recordings from a large number of extracellular electrodes (high resolution electrical mapping). The timing of the waveforms as they reach every electrode in the recording array provides the data required for reconstructing the propagation of the waveforms.

Figure 1 demonstrates high resolution mapping of the spontaneous electrical activity from the feline duodenum, isolated and superfused in a tissue bath, using an array of 240 extracellular recording electrodes (8). Figure 1A displays, at low speed, one of the 240 electrograms recorded from the serosal surface of the duodenum and shows the regular rhythm of slow waves, sometimes followed by spikes. Figure 1C presents at faster speed a set of 24 electrograms selected from the period indicated within the box in Fig. 1A. The 24 signals were recorded from sites located along the longitudinal axis of the organ, as shown in Fig. 1B. Two types of waveforms are visible in Fig. 1C; the first signal, a slow wave, consists of a negative-moving deflection that slowly returned to baseline. In several but not all leads, this slow wave was followed by a second type of waveform, a spike, which displays a much more rapid bidirectional or triphasic deflection.

Figures 1A, C show that the timing of the slow wave recorded at each electrode was marked at the moment of the most rapid negative deflection. All of these times, relative to the time of the first detected slow wave (that on electrode 6), were then plotted on a grid representing the original recording sites, as shown in Fig. 1D. To visualize the spread of propagation, lines (isochrones) were drawn around areas that were activated in successive time steps. In the case of the slow wave, isochrones were drawn every 250 ms. The slow wave map in Fig. 1E shows the gradual spread of the waveform through the tissue by using isochrones separated by different-colored bands. The sites of the recording electrodes are shown by the black dots, which also provide an indication of the resolution used in the reconstruction of this propagation. If the quality of the recording at an electrode was too poor or if no slow wave was detected, then the electrode site was left blank (indicated by empty circles). Slow wave maps reconstructed in this way visualize the...
spread of the most rapidly depolarizing leading edge of the slow wave (2).

The example in Fig. 1E shows that the first activity \( (t = 0) \) was detected in the upper part of the preparation and that the slow wave propagated from this site in all directions. After 1.75 s, a second slow wave originated in the lower right corner of the mapped area. Both slow waves then propagated toward each other and collided at \( t \sim 2 \) s. In the feline duodenum, such pairs of pacemaker areas are regularly seen. In addition, areas of pacemaking are not fixed and commonly shift from one site to another (8).

Spatial pattern of slow wave and spike propagation

Figure 1 also displays the pattern of propagation of a single spike. The procedure for measuring and plotting the activation times of spikes is essentially the same as that for slow waves. In Fig. 1C, spike activation times were marked by thin short lines. Figure 1F shows the activation time map for this spike. Because of the fast spike conduction velocity, isochrones were drawn at intervals of 50 ms. On comparing the maps of the propagating slow wave and spike (Fig. 1, E and F), three fundamental differences between these two electrical waves are immediately visible.

In the first place, the location at which the spike originated (Fig. 1F, spike origin at \( t = 0 \) ms) is different from that at which the two slow waves originated (Fig. 1E, slow wave origins at \( t = 0 \) and \( t = 1,750 \) ms). The site of origin of slow waves is presumably dictated by the behavior of one or a group of ICC (13), the dynamic mechanisms of which are unknown. The mechanisms responsible for determining the origin of the spike are also not known, but their spatial relationship to slow waves (discussed below) implies a causal relationship.

In the second place, there is a marked difference in the spatial pattern of slow wave and spike propagation. The isochrones plotted around the site of initiation of the spike are shaped in the form of concentric ellipses, with their long axes parallel to the longitudinal axes of the intestine, indicating that the speed of propagation of the spikes is faster in the longitudinal than in the transverse direction (anisotropic conduction). The slow wave, however, does not propagate anisotropically. This is clear in Fig. 1E, where, in the upper part of the map, the isochrones are arranged in concentric circles, indicating that conduction velocity is similar in the longitudinal and in the circumferential directions (isotropic conduction). This difference in the pattern of propagation supports the concept that slow waves propagate via an isotropic network of ICC, whereas spikes propagate predominantly within the longitudinal layer of muscle cells, which are coupled in an electrophysiological anisotropic network (15).

A third crucial difference between spike and slow wave propagation is that the conduction of the spike stops abruptly after traveling only a short distance and appears to be self limiting. By contrast, the slow wave propagates throughout the entire area of tissue monitored by the recording array. As displayed in Fig. 1F, the spike spontaneously stopped propagating in both the longitudinal and the circumferential directions and, in so doing, activated only a very limited area of the tissue, called a “patch” (7). In fact, in this example, the majority of the recorded area was not activated by a spike at all. This self-limiting propagation stands in sharp contrast to the propagation of the slow wave, which, once initiated, does not stop propagating until it is either blocked by collision with another slow wave (Fig. 1E) or until it reaches the borders of the preparation.

Spatial distribution of multiple spike patches

Following a slow wave, spikes often occur in groups or bursts. Figure 2 displays such an event. In the set of 24 electrograms displayed at top, 7 clusters of spike discharges can be identified following the first slow wave. Figure 2, bottom left, displays the pattern of propagation of the slow wave. At bottom middle, the propagation of three of the seven spike patches is shown. Each spike propagated over small, self-limited areas before terminating spontaneously. In the case of patch 4, two origins are visible and two spike wavefronts propagated toward each other before colliding \( \sim 300 \) ms later. This is an example of two patches occurring at approximately the same time and in each other’s near neighborhood.

In the composite spike map (Fig. 2, bottom right) the boundaries of all seven spike patches that followed this slow wave are superimposed. The sizes and shapes of the spike patches are not fixed but vary substantially. On average, spike patches occupy an area of \( \sim 12 \times 6 \) mm, covering only 9% of the area of intestine monitored by the electrode array. Patches are limited to this small area even though the slow wave sweeps over the whole of the area monitored by the electrodes (7). In the example shown in Fig. 2, all seven patches in aggregate cover only \( \sim 43\% \), indicating that no spikes were present in the majority of the monitored area.

Spike patches occur in the wake of the propagating slow wave. They are commonly found \( \sim 1 \) s behind the advancing leading edge of the wave (Fig. 2, top). After this period of spike activity, a period of quiescence follows in which no spikes occur until the next slow wave sweeps over the tissue. This is consistent with observations that spikes are initiated (4) near the peak of slow wave depolarization. Since spikes are believed to be generated by inward calcium currents (11, 14), spike patches are expected to delineate the spatial domain over which this calcium influx occurs. In turn the calcium influx is believed to initiate smooth muscle contraction (14). Because most of the mapped area did not generate spikes (Fig. 2), this large area will presumably not contract. Such quiescent areas may, however, show passive movement since they will be stretched by contractions of neighboring areas that did generate spikes. The occurrence of different patterns of movement in neighboring areas may then form the basis for the weak intestinal wall movements required for mixing the luminal contents (10).

Under resting conditions, the majority of the surface of the small intestine does not show spikes, but this can change on stimulation...
FIGURE 1. High resolution mapping of the propagation of a slow wave and a spike in isolated feline duodenum. A: electrogram from a single recording electrode (no. 9) at low speed, showing regular rhythm of the slow wave, sometimes followed by spikes. B: approximate shape and size of preparation and location of the 240 extracellular electrodes (interelectrode distance, 2 mm). Large dots represent the 24 electrodes (no. 1 = most orad) from which the electrograms in C were recorded. C: a set of 24 electrograms, selected from the time within the box in A. These are displayed at a faster speed and show the slow wave propagating caudally and a spike conducting orally. Time of activation of the slow wave at each electrode is indicated by thick vertical lines, whereas numbers show time (ms) relative to time of earliest-detected slow wave (electrode 6, \( t = 0 \) ms). D: activation times of the slow wave measured at each electrode and plotted in the recording grid. Lines (isochrones) were drawn around areas activated in steps of 250 ms. E: activation times have been left out for the sake of clarity and replaced by isochrone colors. This map shows that the slow wave was initiated in the upper part of the preparation and conducted from that site isotropically. F: activation map of the spike, generated in a similar manner using spike activation times shown next to the thin marker lines in C. Spike isochrones (50-ms intervals) show that the spike was initiated at a different site from the slow wave and propagated anisotropically (preferentially in the longitudinal direction). In contrast to the slow wave, spike propagation was limited to a small circumscribed area called a “patch.”
**FIGURE 2.** Multiple spike patches occur in the wake of a slow wave. **Top:** tracings of 24 electrograms were recorded from electrodes aligned in the longitudinal direction as shown at bottom left. Slow waves propagated from oral to caudal. The first slow wave was followed by 7 separate spikes indicated by 7 ellipses. After a period of quiescence, a second slow wave propagated in the same direction, followed once more by several spikes. **Bottom left:** propagation of the slow wave from oral to caudal (isochrone interval 500 ms). A second slow wave emerged from the caudal end at $t = 2.5$ s. **Bottom middle:** propagation of 3 spikes (nos. 2, 4, and 6), all self-limited within circumscribed areas (isochrones at 100-ms intervals). Locations at which spikes were initiated are indicated by ●. **Bottom right:** composite spike map superimposing the areas of all 7 spike patches (numbered in chronological order of excitation). Variability in size, shape, and degree of overlap of spike patches is evident, whereas sequence of patches follow caudal propagation of the slow wave (reproduced with permission from Ref. 7).
of the organ. Exciting the tissue by adding acetylcholine (ACh, 2 \times 10^{-8} \text{ M}), tetrodotoxin (TTX, 10^{-6} \text{ M}), or tetraethylammonium (TEA, 1 \text{ mM}) to the superfusing fluid increases the number of spikes recorded (7). Figure 3 shows the superposition of all spike patches recorded under the four conditions: in the absence of exogenous drugs (A) and during ACh (B), TTX (C), and TEA (D) superfusion. In the absence of drugs (control), nine patches were seen, exciting 37% of the recording area. Addition of ACh, TTX, or TEA increased the area excited to 49, 66, or 70%, respectively, and the number of patches increased to 13, 16, or 22. Stimulating the tissue with either ACh, TTX, or TEA, however, does not increase the average size of the patch (7). The occurrence of more spike patches, not larger patches, activates a larger proportion of the tissue, leading to more powerful mixing movements and possibly even propulsion.

A model of graded contractions induced by local spike patches

On the basis of these results, the spatial interaction between the propagation of slow waves and spikes can now be described. A spike initiated in the area depolarized by the slow wave does not conduct over the whole area depolarized by the slow wave, as shown in Fig. 4B. All of our results indicate that spike conduction is self limited and only propagates over a small area within the zone of slow wave depolarization, as indicated in Fig. 4C. Since contraction is dependent on calcium entering the smooth muscle by the spike, contraction must be limited to an area similar to that of the spike patch. Stronger contractions (induced by ACh, TTX, or TEA) are not induced by increasing the size of the patches but by the initiation of more patches distributed throughout a larger area within the zone of slow wave depolarization (Fig. 4D). This raises the possibility of grading the magnitude of contraction by increasing the number of spike patches in a manner reminiscent of motor unit recruitment in skeletal muscle. What is not yet clear is how the intestines can increase the number of spike patches to induce stronger contractions. One possible mechanism may involve summation of the slow wave and synaptic potentials to increase the area of muscle that is suprathreshold for spike initiation. Alternatively, changes in second messenger levels induced by neurotransmitters may also modify the behavior of the ion channels that generate spikes, thereby contributing to changes in spike patch density.

Mechanisms of spike propagation and spike block

An even more interesting physiological puzzle is the mechanism of spike conduction and especially spike block, a puzzle that shares some similarities with the problem of propagation of regulatory signals responsible for contraction in the

FIGURE 3. Composite maps of all spike patches following a single slow wave during control (A) and during the application of acetylcholine (ACh; B), tetrodotoxin (TTX; C), or tetraethylammonium (TEA; D). Electrogams at top show representative slow waves followed by 1 or more spikes, recorded from a single electrode located at the sites indicated by O. Number beneath each spike in the electrogram indicates spike patch (shown on the map below) that generated it. In composite spike maps, all patches are numbered chronologically. In some of the maps, a few patches have been slightly shifted to reveal underlying patches. In the TEA map, approximate locations of patches 3, 10, 16, and 18 are indicated by lines. Stimulation of the preparation by any of these 3 compounds increased the number of patches from 9 in control to 13, 16, or 22 respectively. In addition, the total area activated by one or more patches increased from 37% during control to 49, 66, or 70%, respectively (reproduced with permission from Ref. 7).
arterial wall presented by Beny (1). In that review, four different types of possible signal propagation were identified: intracellular diffusion of calcium or second messengers, regenerative intracellular diffusion, electrotonic propagation, or action potential-like propagation. In the case of the small intestine, it is important to note that spike propagation in intestine is rapid, especially in the longitudinal direction (8.5 ± 3.3 cm/s) (7). This fact excludes the possibility of spike propagation based on either form of intracellular diffusion mechanism. Such systems propagate much more slowly, typically on the order of 1–100 μm/s. Similarly, conduction of spike potentials cannot take place by electrotonic propagation since spike amplitude is not attenuated as it spreads over the area of the patch. Such decaying potentials have not been found in our recordings of spike patches (Fig. 1C). The only remaining mechanism for spike propagation, compatible with the observed speed of spike conduction, is action potential-like regenerative propagation.

There is essentially no difficulty in accepting action potential-like propagation of spikes. Many studies have characterized the L-type calcium channels in smooth muscle and demonstrated the dependence of the spike potential on them. What is puzzling is why, after only a short distance of conduction, this mode of propagation always stops spontaneously, thereby activating only a small patch. One possible explanation is the existence of anatomic conduction barriers formed by the pattern of distribution of muscle cell gap junctions, which could delineate “functional” bundles from each other. Our maps of the spike patches do not, however, support such a concept. If anatomic barriers were to play a role, it would be expected that the borders of multiple patches would fall along constant anatomic lines and that patches would always occupy similar areas. In Figs. 2 and 3, however, multiple patches can be seen to overlap each other to varying degrees; patch borders do not seem to align along constant identifiable anatomic lines.

Alternatively, the variability of patches might be accounted for by mechanisms that depend on the time and spatial variability of slow wave depolarization itself. It is expected that this will partly result from variations in the ongoing pattern of synaptic activity over the smooth muscle together with variations in the spatial and temporal amplitude of successive slow waves. The size of membrane currents responsible for generating the spike, including those that flow via L-type calcium channels and voltage- and calcium-dependent potassium channels, are sensitive to such potential changes (11). Moreover, the magnitude of the principle inward current, the calcium current, may be quite small compared with the potassium currents that also overlap it in time (11). Small variations in membrane potential may thus reduce the size of the net

FIGURE 4. Diagrams depicting different possible models of the spatial relationship between slow waves and spike patches. Each diagram shows a segment of intestinal tube with a slow wave propagating caudally. Behind the advancing slow wave front is the depolarized area (grey areas in A, C, and D) following in its wake. B: behavior of a hypothetical spike, initiated at ● in the depolarized area. In this model, the spike propagates to form a patch (red) that extends over the entire area of depolarization. Mapping results are not consistent with this model. C: a spike that is limited to propagate within an area (red) smaller than the depolarized area, which is more consistent with mapping results. D: multiple patches of the kind shown in C can occur at different times and locations within the depolarized area, presumably producing stronger contraction (slightly modified and reproduced with permission from Ref. 9).
inward regenerative current sufficiently to reduce the safety factor and cause propagation to fail, thereby creating patch borders. If this is the case, then it would only be a matter of time before spike conduction stops. In other words, it may be a statistical probability that spike conduction will stop sooner rather than later and small patches should therefore be more likely to occur than large patches. This is indeed found in the isolated feline duodenum (7), where smaller spike patches occurred much more frequently than large ones. However, when slow waves collided or propagated inhomogeneously, large spike patches were found more often, suggesting that other factors may also play a role in determining the size of the spike patch.

Of slow waves and spike patches

High resolution mapping is revealing some of the intricacies involved in the relationship between two distinct electrical waves in the small intestine: the spike and the slow wave. Major differences in the pattern of propagation of these two seem to point to major differences in their mechanisms and in the cell layers in which they originate. On the other hand, the temporal and spatial aspects of spike propagation also reveal the constraints set by the slow wave. This seems to confirm what Daniel and Chapman (3) wrote so many years ago: “A useful and as yet untested hypothesis is that the slow wave is an advancing zone of enhanced excitability which, when further enhanced by local factors, leads to action potentials and contractions.”

Caution, however, must also be expressed in attempting to generalize the present results. This study was performed in a small part of the small intestine and in a single species, and the reported facts are currently limited to this context. More work needs to be done to determine whether spike patches also occur in 1) other parts of the small intestine, 2) other parts of the gut, and 3) in gut of other species and, ultimately, in humans. High resolution electrical mapping may help in part in elucidating some of these issues.

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