Longitudinal and circumferential spike patches in the canine small intestine in vivo

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INTESTINAL MOTILITY IS INITIATED by slow waves and by action potentials (spikes) that may or may not occur in the wake of the slow wave (6, 18, 19, 23). Several studies have investigated the temporal relationship between slow waves and spikes and between spikes and motility (2, 3, 5, 7, 16, 25, 26). Not much attention, however, has been given to the spatial pattern of spike propagation (6, 17) or to possible regional variations along the small intestine. Recently, it was shown that spikes propagate for a limited extent in time and space before terminating spontaneously, thereby activating a relatively small area termed a spike “patch” (11). These patches were demonstrated in isolated tissues in vitro, in a single species, the cat, and in one part of the intestine, the duodenum. The question therefore arises as to whether spike patches also occur in another species, in the whole organism, and in other parts of the small intestine.

We are now presenting an approach enabling us to record in vivo electrical signals from 240 extracellular sites simultaneously from the serosal surface of the intact canine small intestine using an open-abdomen anesthetized animal model. With a custom-designed 240-electrode array, we sampled the electrical activities in a 2 × 5-cm area in different parts of the small intestine, ranging from the duodenum to the distal parts of the ileum. In all recordings, slow waves were visible that were often followed by one or more spikes. Analysis of the propagation of these spikes revealed the presence of spike patches at all levels in the small intestine. Two types of spikes were found; the first type, previously described in the cat duodenum (11, 12), propagated in the longitudinal direction, originated anywhere around the intestinal tube, and occurred predominantly in the duodenum. The second type, which occurred much more frequently in the jejunum and the ileum, originated in most cases along the antimesenteric line and propagated in the circumferential direction. Both types of spikes conducted over limited space and time before terminating spontaneously.

METHODS

This study required the use of 12 female beagles (12.2 ± 2.1 kg) that had been fasted the day before the experiments. Anesthesia was induced with scopolamine (0.015 mg/kg), lofentanil (0.070 mg/kg), and succinylcholine (1 mg/kg) and maintained throughout the duration of the experiments (3–4 h) with etomidate (1.5 mg·kg⁻¹·h⁻¹) and fentanyl (0.025 mg·kg⁻¹·h⁻¹; see Ref. 27). The animals were ventilated through a tracheal tube, and the left femoral artery was
cannulated to record systemic blood pressure. The average systolic and diastolic pressures in all experiments were 156.8 ± 23.4 and 90.8 ± 15.0 mmHg, respectively. There was a small but not significant drop in the average blood pressure during the course of the experiments from 118 ± 15 to 105 ± 10 mmHg over the 3- to 4-h experiments, accompanied by a reduction in heart rate during the same time period (83.5 ± 20.2 to 59.9 ± 8.5 beats/min).

After a median laparotomy, the abdominal walls were carefully retracted, and a loop of the small intestine was identified (Fig. 1A). A wet cotton pad was positioned under the loop, and a 240-electrode assembly was gradually lowered on the serosal surface until physical contact was made between the tissue and bottom surface of the assembly. In eight dogs, as shown in Fig. 1, the electrodes covered one-half of the intestinal circumference, from the mesenteric to the antimesenteric borders. In a second series of four dogs, the area around the antimesenteric border was recorded.

In contrast to previous mapping studies (13, 11), the electrode tips were flush with the bottom surface of the assembly (Fig. 1B) so that there were no protruding sharp edges, avoiding the risk of damage to the underlying tissue. Care was also taken not to put too much pressure on the intestinal tube. After the electrode assembly was positioned (Fig. 1, C and D), a temperature probe was located alongside the intestine, and the whole region was packed with moist cotton pads to prevent drying of the tissue (Fig. 1E). A heat lamp kept the experimental area at body temperature during subsequent control and recording periods (36.4 ± 0.6°C). After recordings from one area had been performed, another part of the intestine was chosen, and the procedure was repeated. The order in which the areas were chosen was changed in every experiment. Figure 1G shows the location of the recording sites along the small intestine in all dogs.

After the positioning of the electrode assemblies at a location, a 10-min stabilization period was allowed, followed by 5 min of

Fig. 1. Overview of the experimental field. A: opened canine abdomen with an intestinal loop positioned on a wet cotton pad without any fixation. B: view on the 240 electrode array with the electrode tips, arranged in a 24 × 10 array, flush with the bottom of the assembly. Interelectrode distance 2 mm, total dimensions 46 × 18 mm. C and D: controlled lowering of the electrode assembly on the intestinal loop until electrode tips made contact with the serosal surface. Dashed white line is drawn to indicate the antimesenteric border. E: covering of the experimental area with additional wet cotton pads. A heat lamp (not shown) keeps the area at body temperature, as monitored by a thermistor (cable visible in bottom left). F: diagram of the intestinal loop with the 24 × 10 electrode array superimposed. G: histogram showing the frequency of the recording sites along the (normalized) length of small intestine in 12 dogs.
recording. Electrical recordings were performed unipolarly with a subcutaneous needle in the back right leg as the indifferent pole. All electrodes were connected through shielded wires to 240 AC preamplifiers where the signals were amplified (×4,000), filtered (2–400 Hz), digitized (1-kHz sampling rate), and stored on the hard disk of a laptop. In each dog, this recording procedure was repeated at four other sites along the length of the small intestine. The location of each recording site was marked with a loose ligature looped around the intestinal tube. At the end of the experiment, the animals were killed, the small intestines were removed in toto, and the total length and the distance of the recording sites from the pylorus were measured. The average length of the canine small intestine in 12 animals was 290 ± 48 cm (range 222–366 cm).

Analysis was performed by choosing at random a 16-s window from the 1st, 3rd, and 5th min of each 5-min recording. From each 16-s window, the propagation of one slow wave and all spikes after that slow wave were analyzed. The signals were filtered digitally (20-point moving average) and displayed on-screen in sets of 20–24 electrograms at a time (Fig. 2A). The local activation time of a slow wave was identified by the moment of maximum negative slope (4, 13) and marked with a cursor. The local activation times of the spikes were marked by the steepest slope of the signal, which was usually negative (11). All local activation times are related to a common reference time, determined by the timing of the first detected slow wave in the mapped area. After time marking all slow waves and spikes, their activation times were plotted in maps (B–E).

**Fig. 2.** Reconstructing the pattern of propagation of slow waves and spikes (canine jejunum). A: plot of 23 electrograms recorded from a single line of electrodes (oral to aboral orientation) with the individual locations shown in B–E. A slow wave is displayed, propagating aborally, followed by spikes. The spikes occurred in several clusters, as indicated by ovals. The timing of both the slow waves and the spikes was determined by the most rapid negative slope in each signal. These times, in milliseconds (relative to the occurrence of the first slow wave in channel 1), are plotted in activation maps in B–E. B: all individual slow-wave activation times, measured during this particular cycle, are plotted in a grid representing the original electrode array. The background color at each electrode site is determined by the local activation time in time steps of 200 ms. Electrodes located outside of the intestinal loop did not record any signal, and these sites were left empty. C: final activation map of the slow wave, with the actual activation times left out for the sake of clarity. Isochrones were drawn in time steps of 200 ms, and additional details such as an arrow are added to demonstrate the major propagation pathway. D and E: procedure for a single spike with an isochrone time step of 25 ms. In this case, the spike originated from an area located in the top right region, close to the antimesenteric border, and propagated predominantly in the circumferential direction. The spike did not propagate >6–8 mm in the longitudinal direction. In this recording, the contact of the electrode assembly was not optimal at the aboral end of the intestinal segment, as indicated by a lack of slow waves in electrograms 21–23. Therefore, this part of the map was not analyzed.
times were displayed in the format of a grid of the original recording array (24 × 10; Fig. 2B). For slow waves, isochrones were drawn manually around areas activated in steps of 50–1,000 ms (Fig. 2, B and C) and for spikes in steps of 10–100 ms (Fig. 2, D and E).

Housing, care, type of anesthesia, and experimental procedures were approved by the institutional ethics committee. All pooled data are given as averages and SDs. Significance was tested with Student’s t-test.

RESULTS

Two types of spike patches were found in the canine small intestine (Fig. 3). The first type, the longitudinal spike patch, resembled that described earlier in the isolated cat duodenum in vitro (11) and showed predominant propagation in the longitudinal direction. A second type, not described before, showed mostly propagation from the antimesenteric border in the circumferential direction and toward the mesenteric border of the intestinal tube. Both types of spikes stopped propagating spontaneously in all directions after spreading over a relatively small area, termed a patch (11).

Spike patches occurred throughout the length of the small intestines but showed regional variations in type and frequencies. Figures 4–7 display representative samples of the recorded electrograms (A) and the reconstructed activation maps of slow waves (B) and of

![Diagram of Slow Wave Propagation](image)

![Diagram of Longitudinal Spike Patch](image)

![Diagram of Circular Spike Patch](image)

![Diagram of Conduction Velocities](image)

Fig. 3. Origin, size, and electrograms of longitudinal and circumferential spikes. A: propagation of a slow wave along a canine duodenum. This was followed by several spike patches of which two examples are shown. B: map on left shows a longitudinal spike originating from a location approximately halfway between the mesenteric and the antimesenteric border, and propagating in the aboral direction for >100 ms before terminating spontaneously. In map on right, a patch was initiated along the antimesenteric border and propagated predominantly in the circular direction before stopping spontaneously, after ~30 ms. The central isochrone bar relates to the longitudinal patch in steps of 50 ms and to the circumferential patch in steps of 10 ms. In C, a and b, the 2 patches are repeated, and electrograms, recorded along the dominant propagation pathway (nos. 1 to 10 along the longitudinal direction for the longitudinal spike and letters a to h along the circular direction for the circumferential spike), are displayed on right. From the time latency and the covered distance, conduction velocities were calculated, showing that circumferential spike conduction is faster than longitudinal spikes.
longitudinal or circumferential spike patches (C and E) from the duodenum, the jejunum, the proximal, and the distal ileum, respectively.

At all sites, slow waves conducted as uniform homogeneous broad waves of excitation. Most of the slow waves propagated in the aboral direction, although sometimes oral propagation did occur, as shown in Fig. 4. In addition, the speed of slow wave propagation declined from the duodenum toward the distal ileum (1, 15). Therefore, in the duodenum, it took the slow wave ~0.5 s to propagate along a distance of 5 cm (Fig. 4B), whereas in the distal ileum, the slow wave needed >6 s to cover the same distance (Fig. 7B).

At all locations throughout the small intestine, spikes occurred after the slow wave in varying numbers and at various sites. The pattern of propagation of 945 individual spikes (Table 1) was analyzed, and representative samples, obtained at different levels in the small intestine, are presented in Figs. 4C–7C. In all cases, spikes propagated in limited areas and stopped conducting abruptly and spontaneously, thereby activating a circumscribed area (11). In addi-
there were significant variations in the direction of spike propagation and the shape of spike patches along the small intestine. In the duodenum (Fig. 3C), spikes propagated predominantly in the longitudinal direction, either in the oral or in the aboral direction, before stopping spontaneously. Hence these patches tended to be longer in the longitudinal direction and narrow in the circumferential direction. The reverse was true in the jejunum and ileum, where spikes propagated predominantly in the circumferential direction, and the patches were wider in the circular and smaller in the longitudinal direction (Figs. 5C, 6C, and 7C). In the vast majority of cases (>90%), circumferential spikes propagated from the antimesenteric toward the mesenteric border.

Table 1 details the dimensions of all 945 analyzed spike patches. Both types of spikes could occur anywhere along the small intestine, but the majority of longitudinal spikes was located in the duodenum, whereas the majority of circumferential spikes was...
found in jejunum and ileum. In addition, circumferential patches are not the same as longitudinal patches, which have been rotated by 90 degrees. For example, in the duodenum, the distance in propagation of both spikes was similar (10.7 mm in the longitudinal direction for longitudinal spikes and 11.5 mm in the circular direction for circumferential spikes), but the width of the longitudinal spike patches was significantly narrower (3.1 mm) than that of the circumferential spike patches (11.0 mm; \( P < 0.001 \)).

The conduction velocity of both types of spikes is also different. In 80 large patches, an estimate of the conduction velocity of the spikes was obtained by measuring the time difference between the initiation and the termination of the spike and the distance between those two sites (as shown in Fig. 3C). This was performed in the longitudinal direction for longitudinal spikes (\( n = 20 \)) and in the circular direction for circumferential spikes (\( n = 60 \)). The conduction velocity of circumferential spikes, 17.1 ± 6.1 cm/s, is significantly higher than that of longitudinal spikes, 6.9 ± 1.7 cm/s (\( P < 0.01 \)).

In several cases, apparent deviations from the usual pattern of propagation of circumferential spikes were
observed, and Fig. 8 presents examples of two types of events. In the first type, described in A, it was sometimes possible to record spike signals propagating in the opposite direction, from the mesenteric toward the antimesenteric border. This always occurred in conjunction with a circumferential spike propagating from the antimesenteric line. Our explanation for this phenomenon is that the circumferential spike, initiated at the antimesenteric border, had also propagated along the reverse, not mapped, part of the intestinal tube. It then crossed the mesenteric border and continued to propagate along the mapped side of the tube. In other words, the spike conducted along the back wall of the intestine for more than half the circumference and “wrapped” itself around to the other side. Such an event occurred 30 times in the ileum but never in the jejunum or the duodenum (Table 1).

The second event is shown in Fig. 8B and shows the initiation of a circumferential spike at a site other than at the antimesenteric border. In this case, the second spike originated approximately halfway between the antimesenteric and the mesenteric border.
In the four dogs that were mapped along the antimesenteric border (see METHODS), signal analysis of the spikes revealed several more striking features, displayed in Fig. 9. As shown in the three isochrone maps from examples recorded in the duodenum, jejunum, and ileum (Fig. 9, A–C), the initial activation, which colored the isochrone red, was not only located in the neighborhood of the antimesenteric border, as expected, but was also relatively large compared with later isochrones. In fact, in the first 10 ms (the width of the first isochrone), no pattern of propagation could be determined. This seems to indicate near-simultaneous activation of the whole area. It is only later, in the following isochrones, that a pattern of conduction becomes clear as the spike propagates away from the antimesenteric border. This last point is also visible in Fig. 9D, where the intestine was mapped along one side of the tube. Electrodes 1, 2, and 3, located close to the antimesenteric border, were activated virtually simultaneously, whereas electrodes 4–7 were activated progressively later in time, as also shown by the electrograms displayed in Fig. 9D, right. In conjunction with this feature, the morphology of the spikes also differed. In the area of initiation, the spike waveform starts with an initial negativity (indicated in electrograms 1–3 in Fig. 9D), whereas an initial positive upstroke appears in electrograms 4–7, in conjunction with propagation. Such an initial negativity was also visible in the electrograms recorded in the examples displayed in Fig. 9, A–C, as shown by the signals displayed on the right side of each corresponding map. In all areas of initial activity, initial negativity was found in all signals. Further away from this area, a positive signal appeared, as shown in Fig. 9, A and C, indicating propagation (23). Such large areas of initial negativity were never seen in longitudinal spike patches.

Aside from the two types of spike patches in the small intestine, another feature was consistently observed, and this relates to the spatiotemporal sequence of spike patches after the slow wave. In the lower intestine, spike patches tended to follow each other regularly in time and space, whereas in the upper part of the duodenum, such an order was often absent. An example of this difference in behavior is shown in Fig. 10. Figure 10, left, shows the situation in the duodenum, whereas Fig. 10, right, displays the situation in the distal ileum, as measured in three dogs. Maps in Fig. 10A display the origin of individual spikes that occurred after a single slow wave. The chronological sequence of the origin of every spike is indicated. The locations of the origin of the first spike and of the last spike are also indicated. The propagation of the slow wave in all instances was uniform and occurred from oral to aboral.

In the duodenum (Fig. 10, maps on left), successive spikes originated often far away from each other, and the distances between successive origins were therefore often large. This is also shown in Fig. 10B, top left, wherein the distances are plotted sequentially for all three cases (mean distance 17.8 ± 10.9 mm). The situation is very different in the distal ileum, as shown in Fig. 10, maps on right. There, the origins of successive spikes follow each other closely, and the distances between origins were usually quite small, as is also visible in Fig. 10B, top right (mean distance 3.5 ± 2.8 mm; P < 0.001). If a large step occurred between successive spike origins, then it usually took place in the circumferential direction and rarely in the longitudinal direction. Moreover, in the ileum, nearly all spikes originated along the antimesenteric border, whereas this was not the case in the duodenum.

This spatial difference in spike origin behavior between the proximal and the distal small intestines does, however, not take place in the temporal dimension. In Fig. 10B, bottom, the timing of each spike was plotted in relation to the time of the leading edge of the slow wave at that site. As indicated by the histograms, spikes occurred in a relatively broad time period ranging from 0 to 3 s after the passage of the slow wave (1.47 ± 0.43 and 1.42 ± 0.73 s for duodenum and distal ileum, respectively; P = not significant).

**DISCUSSION**

In previous studies (11, 14), the demonstration of spike patches was limited to the feline duodenum in vitro. This study now extends these findings to support
the concept that spike patches also occur in vivo, in another species, and that they occur at all levels along the small intestine.

In addition, this study reveals the existence of two types of spikes. The first type, longitudinal spikes, has been shown previously in the isolated feline duodenum (11), and current results are in agreement with previous descriptions as follows: 1) these spikes propagate predominantly in the longitudinal direction, 2) spike propagation stops spontaneously in all directions, thereby activating a limited area, termed a patch, 3) several patches may occur after a slow wave (Fig. 4A), and 4) within a patch, spikes may propagate in the oral or in the aboral direction (Fig. 4, A and C).

The second type of spikes, labeled circumferential spikes based on their dominant direction of propagation, has not been described before. The following properties characterize circumferential spikes and differentiate them from longitudinal spikes: 1) spike propagation occurs in the circumferential direction, 2) spike conduction velocity in the circumferential direction is faster than longitudinal spike velocity in the longitudinal direction, 3) the large majority of circumferential spikes are initiated along the an-
timesenteric border (Figs. 3 and 5–10), whereas longitudinal spikes may be initiated anywhere along the intestinal tube’s circumference (Figs. 3, 4, and 10), and 4) at its site of initiation, the waveform of the circumferential spikes shows prominent initial negativity over relatively large areas (Fig. 9) in contrast to longitudinal spikes, where such areas were never seen (Figs. 3 and 4).

The topographical distributions of the two spike patches are also different. Longitudinal spike patches occur mainly in the duodenum, whereas circumferential patches occur much more frequently in the jejunum and the ileum (Table 1) so that a countergradient in the probability of both types is present along the length of the small intestine. Furthermore, circumferential spike patches are not simply longitudinal patches turned 90 degrees around. Circumferential patches are much wider in the longitudinal dimension than longitudinal patches are in the circular direction. In the duodenum, for example, the circumferential spike patches are 11.5 mm long in the longitudinal direction, whereas the longitudinal spike patches are much more narrow and measure only 3.1 mm in the circular direction (Table 1).
The pattern of spike propagation within the two types of spike patches is also different. Circumferential spikes are predominantly initiated along the antimesenteric border; therefore, conduction may occur along both sides of the intestinal wall toward the mesenteric border, effectively straddling the intestine to varying degrees. In several cases, conduction along one side of the “saddle” may even reach the opposite mesenteric border and continue its propagation along the other side, as shown by the occurrence of several spike “wraps” (Fig. 8A). Such a pattern of propagation could not occur with longitudinal spikes.

In relation to the preceding slow wave, the sequence of both types of spike patches is also very different. With the longitudinal spike patches, these may occur anywhere along the intestine’s circumference and within a relatively wide excitable area following the leading edge of the slow wave. Because of this, sequential spike patches may jump from one location to another, as shown in Fig. 10. In contrast, in the distal ileum, most of the spike origins were located close to the antimesenteric region, and the distances between successive origins were much shorter than in the duodenum. In B, top, the distances between successive spike origins were plotted for the duodenum and for the distal ileum. In the duodenum, the distance graph shows large variations between successive spike origins, whereas in the distal ileum this was reduced to much lower values. In B, bottom, histograms of the timing of each spike origin in relation to the timing of the local slow wave are plotted. Spikes originated both in the duodenum and in the distal ileum during a period of \( \frac{1}{101} \) s after the slow wave.
narrow ring of excitability propagates much more slowly in the distal ileum, offering more time for spike initiation, thereby counterbalancing the smaller area available for initiation.

The fact that circumferential spikes originate along the antimesenteric border may have consequences for the motility in the jejunum and the ileum, similar to the situation with peristaltic contraction (21, 22). Because these origins are clustered along the antimesenteric line, and as the zone of excitability propagates as a ring down the intestinal tube, propagation of individual spikes may occur predominantly in the circular direction. As discussed previously, the spike may propagate in two wavelets from its point of origin, activating simultaneously both sides of the tubes for varying distances and “straddling” both sides of the intestinal tube. In addition, because the zone of excitability is relatively narrow, especially in the ileum, these saddle patches must follow each other very closely behind the front of the slow wave (Fig. 7D). All this could impose a type of “ripple” or squeezing activity on the tube and possibly its contents. The fact that most of these contractions occur at the opposite side of the tube from the anchoring effects of the mesenteric attachments may also be helpful in this regard.

In which muscle layers do these two types of spikes propagate? In the isolated guinea pig ileum, Stevens et al. (24) showed that Ca2+ waves, which are induced by spikes, could occur in both layers. In the current study, longitudinal spikes seem to propagate in the longitudinal muscle layer. This is indicated by spike propagation occurring predominantly in the longitudinal direction. Also, the longitudinal spike velocity (6.9 ± 1.7 cm/s) is comparable with that measured in the cat (7.7 ± 4.5 cm/s), and its anisotropic pattern of propagation is very similar to that in the duodenum of the cat (14). Similar arguments seem to indicate that circumferential spikes would propagate in the circular muscle layer. The predominant direction of propagation is in the circular direction, whereas oral or aboral propagation of circumferential spikes in the longitudinal direction, from its origin along the antimesenteric border, was never seen. The velocity of propagation of circumferential spikes is faster than that of longitudinal spikes, the reasons for which are not clear. It could be because of the larger mass of available circular tissue, because of a possible longer length of the individual circular muscle cells, because of better connections between cells, or because of other physical or electrical properties of the muscle layer.

The limitations of this study must be quite clear. Although the small intestines were studied in situ, the animals were anesthetized, and the intestines were empty. Some published data show that the intestinal spike activity (10, 20) or phasic activity (8, 9) could be enhanced in dogs exposed to opioids, such as morphine or loperamide. Use of opioid anesthesia could have therefore increased the incidence of spike activity during the course of the experiment. However, preliminary observations in awake dogs implanted with electrode arrays indicate similar patterns of spike activity, as reported here in anesthetized dogs (28). Furthermore, spike patches similar to those reported here have been shown to occur in tissues in vitro (11). Nevertheless, some modulating effect of the used opioids cannot be completely ruled out.

Nevertheless, within these limitations, the results are clear. Spikes and spike patches occur throughout the canine small intestine. Significant topographical variations in morphology and origin of spikes were demonstrated based on the presence of two types of spikes, possibly propagating separately in each of the two muscle layers. These facts seem to suggest that the function of spikes in the generation of patterns of motility in various parts of the small intestine are different, which may induce different patterns of motility and underlie different functions along the length of this organ.

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DISCLOSURES

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