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SPIKE PROPAGATION IN THE SMOOTH MUSCLE OF THE GUINEA-PIG TAENIA COLI

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SUMMARY

1. Spike activity was produced by external field stimulation of the guinea-pig taenia coli. Spikes were evoked by depolarization of the muscle membrane. Though the activity was usually also observed during hyperpolarization, this was shown to be conducted activity from the depolarized region of the tissue. Spike conduction was blocked when hyperpolarization exceeded 10 mV.

2. The shape of the conducted spike was influenced by membrane polarization. Sometimes a notch appeared on the spike and sometimes the spike was split into two by hyperpolarization. This is probably due to the fact that functional bundles form a network and that the branches between bundles are more susceptible to the membrane polarization.

3. There was a critical spike amplitude for normal propagation. Therefore, different spike amplitudes were observed near the stimulating electrode, but only spikes of nearly full size amplitude were recorded far from the stimulating electrode, i.e. at a distance of more than 5 mm.

4. When repetitive stimulation was applied, the spike amplitude decreased with increasing frequency of stimulation. No steady level was reached, however, but the spike amplitude fluctuated at about 0·3 c/s.

INTRODUCTION

The smooth muscle, both of the taenia coli and of the vas deferens of the guinea-pig, has cable properties (Tomita, 1966*a*, 1967). Therefore, it is reasonable to assume that spike conduction in smooth muscle is due to a local circuit current as in nerve or skeletal muscle. In fact, it has been shown that the early time course of the conducted spike in the taenia coli fits the prediction based on the local circuit current in a cable-like fibre (Tomita, 1966*b*). However, the spike conduction in smooth muscle is complicated because it is composed of functional bundles which branch to form a network.

The aim of the present investigation was to study the properties of the

conducted spike in the taenia which was kept quiescent in a hypertonic solution. Hypertonicity has two advantages: first, the reduction of mechanical disturbance and, secondly, the absence of interference by spontaneous electrical activity (Holman, 1957; Tomita, 1966*a*).

METHODS

The preparation (Bülbring, 1954) and the Krebs solution (Kuriyama, 1963) were the same as previously described. Most of the experiments were done in hypertonic solution which was made by adding 10 g sucrose to 100 ml. Krebs solution. The hypertonic solution was always introduced after the tissue was mounted in the organ-bath and equilibrated for at least 10 min in normal Krebs solution. To eliminate the possible nervous contribution to the muscle activity tetrodotoxin (10^{-6} g/ml., Sankyo Co.) was sometimes added to the solution, but the results were essentially the same whether it was used or not.

The electrical recording and stimulating arrangements were similar to those previously described (Tomita, 1966*a*). The organ-bath was divided by an insulating partition into two chambers. One contained the stimulating electrodes which were 10 mm apart. The tissue was pulled through a small hole of the insulating partition so that 5–8 mm of the tissue was in the stimulating chamber and 10 mm in the other chamber, from which the intracellular records were taken, unless otherwise stated.

Sometimes, another pair of stimulating electrodes composed of two rings of platinum wire, 3 mm apart, was placed at one end of the tissue in the recording chamber for triggering the spike.

RESULTS

High-frequency responses during hyperpolarization. The muscle was stimulated with large external electrodes and the response was recorded intracellularly as shown in the diagram of Fig. 1. When the muscle membrane was hyperpolarized by a long current pulse (more than 1 sec), small spike-like potentials were often observed. These have been interpreted as responses due to the spike activity produced near the cathode, because their frequency pattern was often similar to that of the spikes produced by depolarizing current of the same intensity (Tomita, 1966*a*). However, it was sometimes found that hyperpolarizing currents produced small repetitive potential changes at a higher frequency than that of the spikes produced by depolarizing current of equal intensity, as shown in Fig. 1. In order to find the mechanism by which these high-frequency responses during hyperpolarization were produced experiments were carried out, in which the electrical activities near each stimulating electrode were compared. After the responses to polarizing currents were observed near the partition (*a* and *c* in Fig. 2), the micro-electrode was inserted near the end of the tissue in the stimulating chamber (*b* and *d* in Fig. 2), and the same polarizing currents were again applied to the tissue. As seen in Fig. 2, the frequency pattern of the response to hyperpolarization at (*a*) is similar to that of the depolarization at (*b*), while that of the depolarization at (*c*) is similar to that of the hyperpolarization at (*d*). Therefore, the frequency

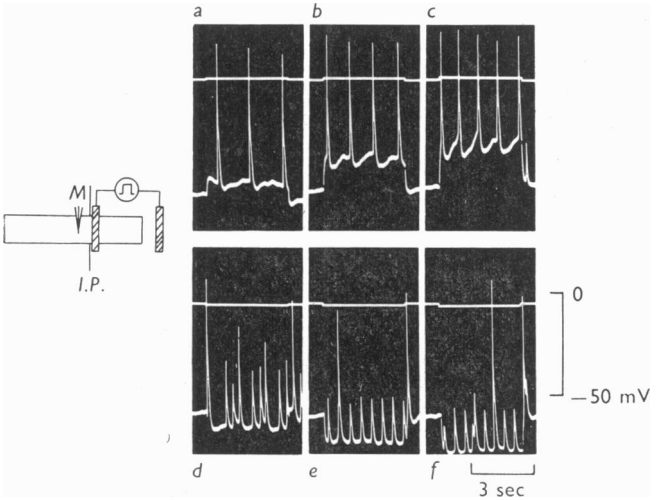

Fig. 1. Guinea-pig taenia coli in hypertonic Krebs solution. Intracellular records (lower trace) taken from the same cell at a distance of 0·3 mm (micro-electrode (*M*)) from the insulating partition (*I.P.*). *a–c*: responses to depolarizing current (intensity increased from *a* to *c*, monitored in upper trace); *d–f*: responses to hyperpolarizing currents of the same intensities.

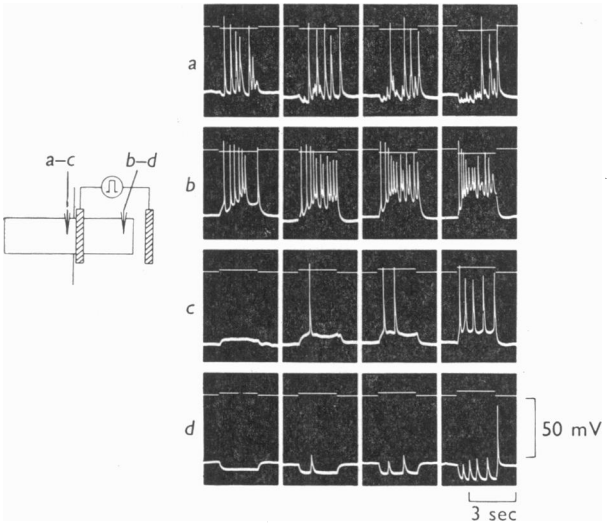

Fig. 2. Intracellular records (lower trace), *a* and *c* from a cell in the recording chamber at a distance of 0·3 mm from the partition, in response to hyperpolarizing (*a*) and depolarizing (*c*) currents (monitored in upper trace), *b* and *d* from a cell in the stimulating chamber at a distance of 7 mm from the partition (the tissue length in the stimulating chamber was 8 mm). Records *b* were in response to the same stimulating currents as in records *a*, and records *d* were in response to the same stimulating currents as in records *c*. In hypertonic Krebs solution containing tetrodotoxin (10^{-6} g/ml.).

pattern of the spike-like activity observed in the hyperpolarized region appears to be determined by the frequency in the depolarized region. This is clearly shown in a preparation as that in Fig. 2 in which the same current intensity produced different frequency patterns in different parts of the tissue. This observation explains why, in the same cell, the spike frequency during hyperpolarization may be higher than that during depolarization, as shown in Fig. 1 and also when the records (*a*) and (*c*) of Fig. 2 are compared.

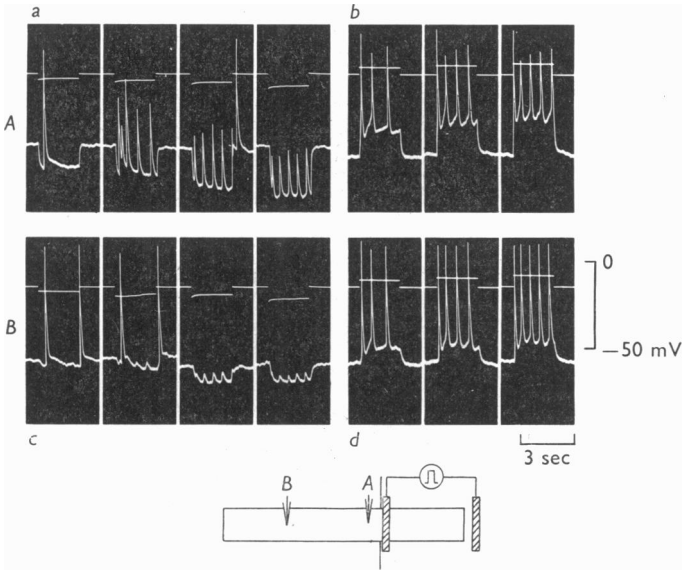

Fig. 3. Intracellular records (lower trace) from two different cells at distances of 0·2 mm (*A*) and 2 mm (*B*) from the partition. *a* and *c*: responses to hyperpolarizing currents, intensity increased stepwise (monitored in upper trace). *b* and *d*: responses to depolarizing current. In hypertonic solution containing tetrodotoxin (10^{-6} g/ml.).

The cause of the high-frequency response near the cut end of the tissue was not investigated in the present experiments. It could be due to a different distribution of the stimulating current in the tissue or to a temperature gradient.

The conclusion that the spike-like responses were due to spike activity at the depolarized region, but not due to the hyperpolarization itself, was also supported by the results shown in Fig. 3. In this experiment, the records were taken from two cells in the recording chamber at about 0·2 mm (*A*) and at about 2 mm (*B*) from the insulating partition. The frequency pattern of the two cells in the two different parts of the tissue was very similar, although the membrane polarization was large in one cell (*A*) and small in the other cell (*B*) far from the partition, because of

the spatial decay. If hyperpolarization itself were responsible for the spike-like activity, a similar frequency pattern should be produced by a similar magnitude of hyperpolarization. It was not; instead it was related to the frequency pattern produced by depolarization.

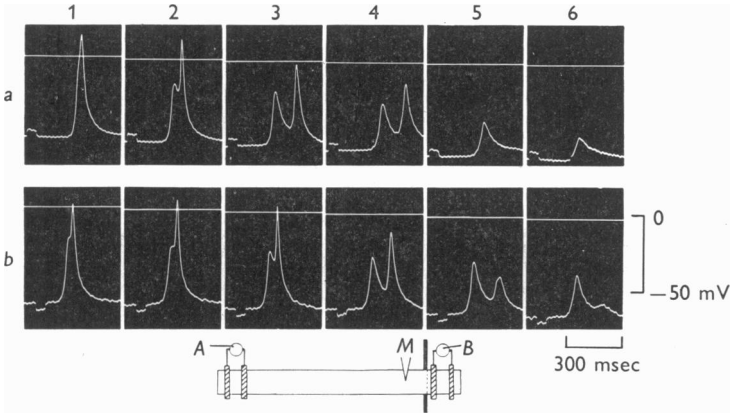

Fig. 4. Effects of external polarization on the conducted spike (upper trace = current monitor, lower trace = intracellular record). The records were taken at about 0·3 mm from the partition. Conditioning polarization (by electrodes *B*) was started 300 msec before the stimulus (by electrodes *A*) used to evoke a response and then was maintained through the period of recording. Distance between the nearest stimulating electrode and the recording site was about 8 mm. The nearest stimulating electrode was the anode in (*a*) and the cathode in (*b*); the intensity was kept constant, slightly above threshold. In both (*a*) and (*b*), the anode of the polarizing electrodes was near the micro-electrode (*M*). In hypertonic solution containing tetrodotoxin (10^{-6} g/ml.).

Effect of polarizing current on spike size. Two further points should be mentioned about the results shown in Fig. 3. One was that in the cell near the partition (*A*) the amplitude of the responses was gradually reduced by increasing hyperpolarization while in the cells far from the partition (*B*) no intermediate amplitude was observed but only two extremes.

The other point was the disproportion of the spatial decay of the electrotonic potential and that of the spike-like potentials. The sharper decay of the spike-like potential may be simply explained by the cable-like properties of the fibres, particularly when the time constant of the fibre membrane is long (80 msec, Tomita, 1966*a*). Polarization of long duration is not affected by a capacity component. In the ephaptic synapse of the crayfish motor nerve (Furshpan & Potter, 1959) or of the leech ganglion (Eckert, 1963), it is found that the spikes are more attenuated than the long electronic potential. Therefore it is possible, also in the smooth muscle, that the resistance and capacity components of junctions between cells might act as some barrier.

Effect of polarizing current on spike shape. When the membrane was hyperpolarized, the amplitude of the conducted spike was usually simply decreased, as observed previously (Tomita, 1966*a*). However, sometimes, a notch appeared. In the experiment shown in Fig. 4, a set of stimulating electrodes was placed at each end of the tissue and an insulating partition separated one end with one pair of electrodes (*B*) from the remaining part of the tissue from which records were taken, as shown in the inset of Fig. 4. The electrodes (*A*) were used for triggering the conducted spike and the electrodes (*B*) were used for application of membrane polarization. When the membrane was kept slightly hyperpolarized, a notch appeared on the

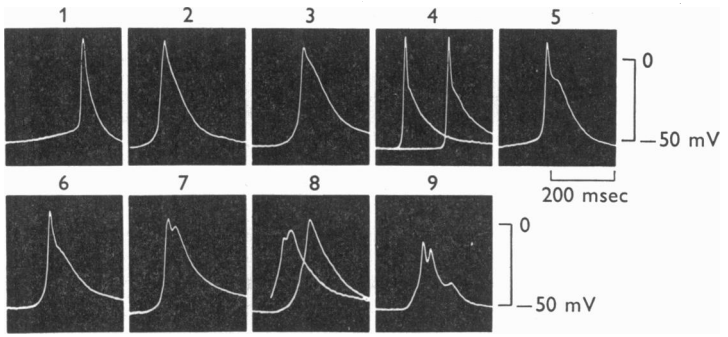

Fig. 5. Examples of various spontaneous spikes recorded intracellularly in normal Krebs solution. 1–3 = cell *A*, 4–5 = cell *B*, 6–9 = cell *C*.

rising phase (*a* 2 in Fig. 4) and the spike was split into two small spike-like potentials during stronger hyperpolarization (*a* 3, 4). The latency of the first potential remained more or less constant, but that of the second potential increased with increasing hyperpolarization and, during strong hyperpolarization, the second potential disappeared and only the first potential was observed (*a* 5, 6).

Occasionally, a notch was observed without conditioning hyperpolarization, as seen in Fig. 4, *b* 1. Then the notch became always clear when the membrane was hyperpolarized (*b* 2, 3). In fact, in Fig. 4*b*, the notch was the peak of the first potential.

Such a splitting up of the conducted spike during hyperpolarization must be another factor in increasing the frequency of the responses during hyperpolarization as shown in Fig. 1, although the main factor appears to be the activity itself at the depolarized region of the tissue.

Notch formation on the spike potential or change of the spike shape are known to appear frequently during spontaneous electrical activity observed in normal Krebs solution (Bülbring, 1957; Holman, 1958). Figure 5 shows

examples of various spike configurations recorded from three different cells in the same tissue (1–3 = cell *A*; 4–5 = cell *B*; 6–9 = cell *C*).

Decremental conduction. When the stimulus duration was short, the spike was often produced in a graded manner, while a long stimulus produced the spike in an all-or-none manner, provided that the record was taken near the partition, as previously shown (Tomita, 1966*a*). Records in column *A* of Fig. 6 show such examples observed at a distance of 0·3 mm from the partition. When a short stimulus (2 msec in *A* 1) was applied, the spike amplitude varied very much at near threshold current intensity even if the stimulus was kept constant. However, in records taken far from

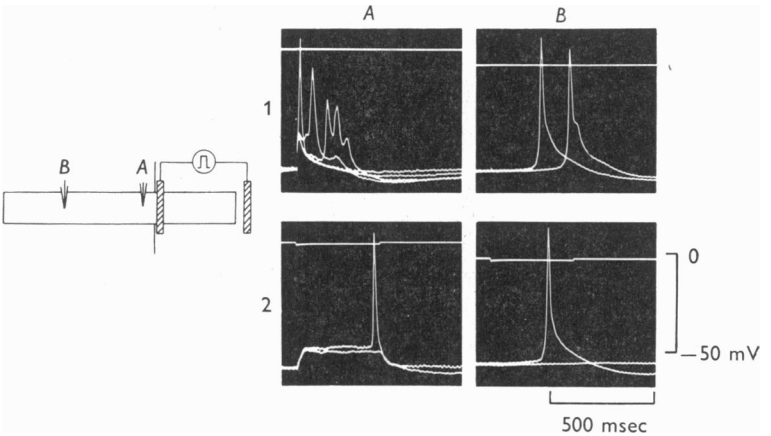

Fig. 6. Intracellular records (lower trace) of the spikes produced by short (2 msec in 1) and long (400 msec in 2) stimuli, and recorded at 0·3 mm (*A*) and at 5 mm (*B*) distance from the partition. Stimulus intensity was near threshold. Hypertonic solution.

the stimulating electrode (5 mm from the partition in column *B*), with the same stimulus parameters, the spike amplitude was more or less constant, but the stimulus often failed to produce the spike.

There seemed to exist a critical amplitude of the spike for normal conduction, although its absolute value might vary in individual bundles. A spike larger than the critical amplitude increased in amplitude to full size during conduction along the tissue. A spike below the critical amplitude decreased in amplitude during conduction and finally became only a passive electrotonic potential (see Fig. 3*B*, *c*).

A similar phenomenon was demonstrated by using repetitive stimulation and recording at two different distances from the partition (Figs. 7 and 8). Records shown in Fig. 7 were recorded from a single cell located 0·3 mm from the partition. When the stimulus frequency was increased from

0·25/sec (interval: 4 sec) to 5/sec (interval: 200 msec), the spike amplitude was progressively reduced because the stimuli were applied during the refractory period. This was more clearly observed with weaker (*a*) than with stronger stimuli (*b*). Moreover, the spike amplitude was not reduced

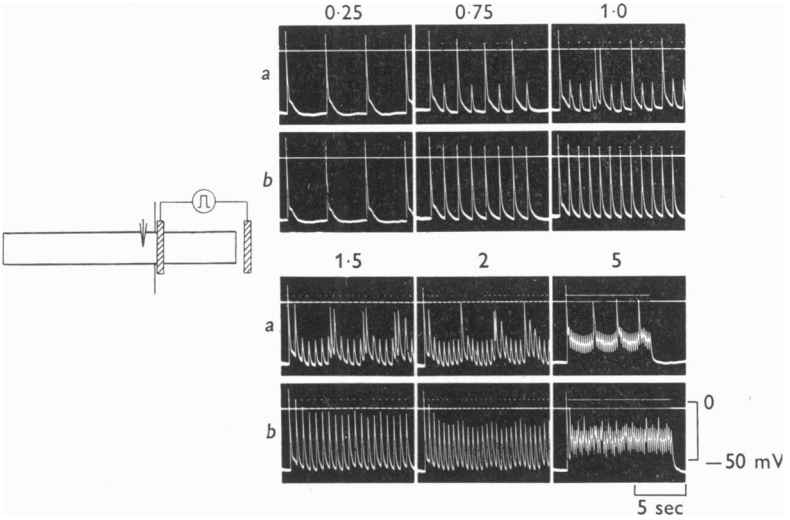

Fig. 7. Responses (lower trace) to repetitive stimulation (100 msec pulses) of increasing frequencies (0·25–5 c/s) recorded intracellularly at 0·3 mm from the partition. (In *b* stimulus intensity was 1·5 times stronger than in *a*.)

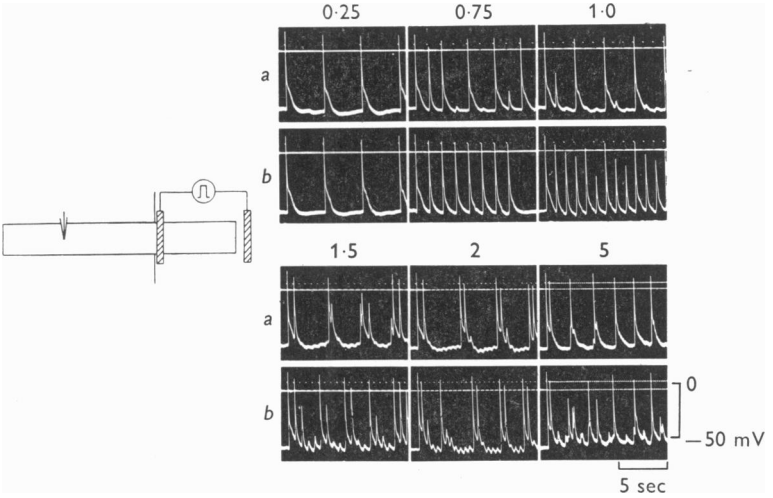

Fig. 8. Recordings similar to Fig. 7, but at a distance of 5·3 mm from the partition. See text for description.

to a constant level but fluctuated rhythmically at about 0·3/sec as seen in Fig. 7.

When the records (Fig. 8) were taken from a cell far from the stimulating electrode (5·3 mm from the partition), the responses to repetitive stimulations were quite different from those (Fig. 7) obtained at a short distance. As expected from the results shown in Fig. 6, the small spike could not properly conduct but died out along the tissue and electrotonic potentials, the space constant of which was about 1·6 mm, also decayed. Therefore, in the cell far from the stimulating electrode, the electrical activity showed a tendency to all-or-none behaviour.

The electrical activity was probably also modified by a rhythmic change of the excitability of the membrane. The rhythmic fluctuation appears to have the same basic mechanism as the spontaneous spike activity, and may be related to the observation that all-or-none spikes are most easily set up when external stimulation is applied at a frequency of approximately the same rate as spontaneous discharge in normal Krebs solution (Bülbring Burnstock & Holman, 1958).

DISCUSSION

The experiments which have been described indicate that the action potential of smooth muscle, like that in other tissues, arises by depolarization where current passes outward across the surface membrane. Since the cells are much shorter than the space constant, one has to suppose that there are low resistance paths between cells by which current can pass over many cell lengths without crossing an excitable membrane. All spike-like activity observed during hyperpolarization can be explained as the result of the spikes produced at the depolarized region of the tissue, without assuming a special mechanism. When the effect of membrane polarization is studied, the fact that one end of the tissue is polarized in the opposite direction to the other end may confuse the results.

The spike in the taenia coli probably conducts along the functional bundle, as expected from the cable-like properties (Tomita, 1966*a*, *b*). However, the bundle, unlike a single skeletal muscle fibre, is not a simple cylinder but branches out and joins other bundles forming a mesh. Therefore, it is reasonable to assume that the geometrical factor of branching produces some complications of the spike conduction (Noble, 1966; Tomita, 1967). The local circuit current of a spike which propagates along a bundle must excite a larger area at the branching point. Hence the safety factor would be low and the spike would be more easily blocked than in a simple cylinder-like bundle, especially when the membrane at the branching point is hyperpolarized.

The critical amplitude of the spike which can propagate without decre-

ment cannot be determined exactly because it depends mainly on the geometry of cell arrangement, which is variable. Furthermore, the active (regenerative) component of the spike, which is important for spike conduction, cannot easily be discriminated from the passive (electrotonic) component. However, it may be said that, at a spike amplitude of about half the normal amplitude (30 mV), the conduction becomes probably decremental.

The conduction velocity of the spike may be slightly different in individual bundles and may be affected differently by membrane polarization. When two cells, probably in different bundles, are impaled simultaneously by micro-electrodes, the records show that spontaneous spikes are not necessarily synchronous although they are of the same frequency (Bülbring *et al.* 1958). Therefore, the shape of the spike recorded from the part where different bundles join might be modified depending on the time lag between the arrival of the spikes conducted along the different bundles. This may be an explanation for a notch on the spike.

The mechanism of the spike conduction in smooth muscle is probably the same as in cardiac muscle, the cells being electrically interconnected (Noble, 1966; Weidmann, 1966). The action potential of cardiac muscle is also, under many conditions, not all-or-none. Thus decremental, incremental or unidirectional conduction notches on the rising phase and local block have often been observed in low Na or low Ca solution (Hoffman, 1961; Hoshiko & Sperelakis, 1961). The conduction and configuration of the action potential in such conditions are modified by membrane polarization as in the smooth muscle (Hoshiko & Sperelakis, 1961; Matsuda, Kamiyama & Hoshi, 1967).

The fact that some of the activity near the stimulating electrode is not conducted along the tissue is probably also important when the relation between the electrical activity and the mechanical activity is studied.

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