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Movement based artifacts may contaminate extracellular electrical recordings from GI muscles

O. BAYGUINOV, G. W. HENNIG, and K. M. SANDERS

Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV 89557, USA

Abstract

Background—Electrical slow waves drive peristaltic contractions in the stomach and facilitate gastric emptying. In gastroparesis and other disorders associated with altered gastric emptying, motility defects have been related to altered slow wave frequency and disordered propagation. Experimental and clinical measurements of slow waves are made with extracellular or abdominal surface recording.

Methods—We tested the consequences of muscle contractions and movement on biopotentials recorded from murine gastric muscles with array electrodes and pairs of silver electrodes.

Key Results—Propagating biopotentials were readily recorded from gastric sheets composed of the entire murine stomach. The biopotentials were completely blocked by nifedipine ($2 \mu\text{mol L}^{-1}$) that blocked contractile movements and peristaltic contractions. Wortmannin, an inhibitor of myosin light chain kinase, also blocked contractions and biopotentials. Stimulation of muscles with carbachol increased the frequency of biopotentials in control conditions but failed to elicit

Address for Correspondence: K. M. Sanders, Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV 89557, USA. Tel: +(775) 784 6908; fax: +(775) 784 6903; ksanders@medicine.nevada.edu.

CONFLICT OF INTEREST

There are no conflicts of interest to disclose.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Block of spontaneous contractions by nifedipine. (A) Shows spontaneous contractions of an antral muscle strip cut parallel to the circular muscle layer. (B) Shows substantial reduction in contractile amplitude caused by addition of nifedipine ($1 \mu\text{mol L}^{-1}$) added to the perfusion solution. (C) Shows effects of cumulative addition of nifedipine ($2 \mu\text{mol L}^{-1}$).

Figure S2. Extracellular records displayed using different bandpass windows. (Aa) Shows a record of monopolar electrical recording with a passband between 0.3 and 100 Hz. (Ab) Shows the same trace using a passband of 0.3–3 Hz (postacquisition filtering). (Ac) Shows the same data as in Aa with a passband of 3–100 Hz. (B) Shows the same filtering characteristics after addition of nifedipine ($2 \mu\text{mol L}^{-1}$). (Ba) Shows a recording with a passband between 0.3 and 100 Hz. (Bb) Shows the same trace using a passband of 0.3–3 Hz (postacquisition filtering). (Bc) Shows the same data as in Ba with a passband of 3–100 Hz.

Movie S1. Displacement of surface markers during the antral peristaltic waves (speed = $6.7 \times$ normal speed). Bottom of the movie frame is at the level of the esophagus (two halves of the esophagus are the white regions at the bottom left and bottom right), and the top of the movie frame is at the level of the pylorus. The greater curvature runs vertically midway through the frame (middle row of surface markers). The markers closest to the esophagus are separated by ~ 2 mm circumferentially. The instantaneous velocity and direction of each surface marker is denoted by a yellow arrow above the marker, and the degree of circumferential contraction between pairs of markers is denoted by a red line [red = closest (-0.05 mm per 1 mm separation), white = furthest apart ($+0.05$ mm per 1 mm separation)]. Similarly, longitudinal separation between pairs of markers is denoted by green lines (green = closest, white = furthest apart). Note the varied relationship between circumferential contraction and longitudinal movement during the progression of an antral peristaltic wave.

Movie S2. This movie was taken in the presence of nifedipine ($2 \mu\text{mol L}^{-1}$). Muscle preparation and all dimensions and scalings are the same as in Movie S1. Note the decrease in the amplitude of movements.

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biopotentials with nifedipine or wortmannin present. Intracellular recording with microelectrodes showed that authentic gastric slow waves occur at a faster frequency typically than biopotentials recorded with extracellular electrodes, and electrical slow waves recorded with intracellular electrodes were unaffected by suppression of movement. Electrical transients, equal in amplitude to biopotentials recorded with extracellular electrodes, were induced by movements produced by small transient stretches (<1 mm) of paralyzed or formalin fixed gastric sheets.

Conclusions & Inferences—These data demonstrate significant movement artifacts in extracellular recordings of biopotentials from murine gastric muscles and suggest that movement suppression should be an obligatory control when monitoring electrical activity and characterizing propagation and coordination of electrical events with extracellular recording techniques.

Keywords

electrical slow waves; GI motility; smooth muscle

INTRODUCTION

Since the first recordings of electrical activity were made from the stomach with extracellular electrodes,¹ many techniques have been developed to study the electrical behavior of smooth muscles. Techniques such as pressure or suction electrodes^{2–4} and sucrose gap^{5–7} are limited in use today, but intracellular microelectrodes,^{8,9} metal extracellular electrodes^{10–12} and electrogastrogram (EGG)^{13–17} are used widely. Intracellular recording with microelectrodes provides measurements of resting membrane potentials and high fidelity recordings of common electrical behaviors of gastrointestinal (GI) smooth muscles such as slow waves and action potentials.^{18–20} Simultaneous intracellular recording and contractions can describe electrical–mechanical coupling in well-coupled smooth muscles.²⁰

Extracellular metal electrodes and surface EGG electrodes are used to monitor electrical events *in vivo* or in organ segments where intracellular recording would be very difficult with current technology. Measurements of membrane potential and amplitudes of transmembrane electrical events cannot be obtained from extracellular recording,⁴ but it is widely thought that the frequency of electrical events can be recorded with fidelity. Based on this belief, EGG is used clinically to evaluate electrical rhythmicity in patients. Extracellular recording has also been used to establish classical parameters of normal motility, such as to pinpoint the dominant pacemaker in the stomach: ²¹ slow waves originate from the mid- or upper corpus, propagate aborally with increasing velocity and amplitude, and do not spread to the fundus.²¹

The electrical activity recorded from the stomach with extracellular electrodes can be in the form of triphasic potentials or complexes with multiple potential oscillations. The basic triphasic events were attributed to slow waves and the oscillatory transients to Ca²⁺ action potentials superimposed upon slow waves. Extracellular electrical signals require filtering to remove competing bioelectrical signals, ambient electrical noise and enhance the signal-to-noise ratio. Using multichannel amplifiers and computerized analysis, arrays of extracellular electrodes are being used to map the spread of electrical activity in gastrointestinal

organs.^{11,12,22–26} O’Grady and coworkers,²² proposed that large extracellular electrode arrays provide an improved method for monitoring the electrical activity of the human stomach and the effects of gastric pacing on slow wave activity. This group has confirmed and modeled the classical concepts about the site of origin of pacemaker activity, direction and speed of slow wave propagation, and organization of gastric electrical behavior.²²

After many years of motility research, there is general agreement among clinicians and investigators about gastric slow wave frequencies in humans²⁷ and many experimental animals. However, when slow waves are measured with intracellular and extracellular recording techniques, clear differences in slow wave frequency have been reported. For example, slow waves at 5.2 cycles per minute (CPM) were recorded from the intact canine stomach with extracellular recording *in vivo*²⁰ and at 3.7 CPM in muscles of the gastric corpus greater curvature (near region of dominant pacemaker) with intracellular recording *in vitro*.⁸ Slow waves were recorded at 2.83 CPM with extracellular electrodes from intact stomachs of humans *in vivo*²² and at 6.17 CPM from muscles of the gastric corpus (near pacemaker region) with intracellular techniques *in vitro*.⁸ Slow waves were recorded in the intact mouse stomach *in vivo* at 2.9 CPM with extracellular electrodes²⁸ and at 6.3 CPM in the pacemaker region of the corpus with intracellular recording.²⁹ No suitable explanation exists for the differences in frequency recorded with intracellular and extracellular techniques. However, slow wave frequency (i.e. electrical rhythmicity at 3 CPM) is used as a clinical standard of normal activity in patients²⁷ and patients outside this norm are considered to have syndromes such as bradygastria, tachygastria, or mixed dysrhythmias. Thus, clarification of true slow wave frequency and an explanation of why differences are observed with different recording methods is an important goal to pursue.

We tested whether muscle movements impact electrical signals recorded with extracellular electrodes. We used a standardized gastric sheet preparation consisting of the entire corpus and antrum of murine stomach.²⁹ We monitored extracellular and intracellular activity in muscles before and after inhibiting isotonic contractions (as verified by high resolution video recording and measurements of contractions) downstream of the membrane ionic events responsible for electrical slow waves. Our data demonstrate that electrical transients recorded from murine gastric muscles by extracellular metal electrodes result predominantly from mechanical activity.

METHODS

Tissue preparation

BALB/C mice (60–90 days old) of either sex were anesthetized with carbon dioxide and killed by cervical dislocation and decapitation. The animals were maintained, and all protocols performed, in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The experimental protocols were approved by the Institutional Animal Use and Care Committee at the University of Nevada, Reno.

Stomachs were excised and opened along the lesser curvature and contents were removed by rinsing with Krebs–Ringer bicarbonate buffer (KRB; see ‘Solutions and drugs’ below). The gastric sheets were pinned to the base of a Sylgard (Dow Corning, Midland, MI, USA)-

coated dish and the mucosa and submucosa were dissected away from the muscularis. The chamber was perfused with oxygenated KRB maintained at 37.5 ± 0.5 °C throughout all experiments.

Extracellular recordings

Bipolar or monopolar extracellular recordings were made using an array of 32 silver electrodes (250 μ m diameter) arranged in a 4×8 matrix (2 mm distance between positive and negative terminals in the case of bipolar recording). Sixteen of the 32 electrodes were wired in reference to a common ground electrode (Ag/AgCl electrode pellet in bath; EP2; World Precision Instruments, Sarasota, FL, USA) in the case of monopolar recording. The recording electrodes were imbedded in a Sylgard-layered recording chamber, forming a rectangular array (8×16 mm), and connected with shielded wires to a 16-channel extracellular amplifier (Model 3500; A-M System Inc., Carlsborg, WA, USA). The recordings were filtered with 2-pole Bessel bandpass filters set to collect data between 3 and 100 Hz.

The gastric sheets were pinned with stainless steel pins (0.1 mm) along the pyloric and fundic margins to the Sylgard surrounding the electrode array. A small degree of longitudinal stretch was applied to the sheet to keep it against the array. After securing the gastric sheet into the recording chamber, it was allowed to equilibrate for 90–120 min before commencement of recordings. In most of experiments the gastric sheets were oriented such that the circular muscle faced the electrodes, but in some experiments muscles were pinned with the serosal surface facing the electrodes, and the recordings were identical in waveform.

For comparison of techniques, recordings were also made with silver wire electrodes (~3 cm long; 250 μ m in diameter). The electrodes were insulated, save for the tips. Silver wire electrodes, either coated with silver chloride or not, generated similar signals. The chlorided electrodes generated larger amplitude signals, but the signal to noise relationship was similar, so there did not appear to be a technical advantage to chlorided tips. We also tested commercial tungsten electrodes (1 μ m tip; 1.0 M Ω resistance, 75–100 μ m tip separation; World Precision Instruments). As with chlorided silver electrodes, the amplitude of events was larger than with silver wires, but the signal-to-noise ratio was similar. Recordings with a single electrode pair were amplified with a single channel amplifier (ISO-80; World Precision Instruments) and filtered with a 2-pole Butterworth bandpass filters set to collect data between 5 and 100 Hz. Extracellular electrical transients (biopotentials) were amplified at 1 K, digitized at 200 Hz, and recorded *via* a computerized data acquisition and analysis system (MP 100; BIOPAC Systems Inc., Santa Barbara, CA, USA).

Intracellular recordings

For microelectrode recordings circular muscle cells were impaled with glass microelectrodes having resistances of 50–90 M Ω . Transmembrane potentials were recorded with a high-input impedance electrometer (Duo 773; World Precision Instruments). Data were digitized at 200 Hz and recorded on computerized data acquisition and analysis system (MP 100, BIOPAC Systems Inc.).

Recordings of contractile activity

Muscle strips (5×10 mm) were cut parallel to the circular muscle fibers, mounted in organ bath under a resting tension of 5 mN, and allowed to equilibrate for 90–120 min. The baths were continuously perfused with oxygenated KRB maintained at 37.5 ± 0.5 °C throughout all experiments. Tension was monitored *via* isometric force transducers (TSD 125C, BIOPAC Systems Inc.). Contractile signals were digitized at 200 Hz and recorded on computerized data acquisition and analysis system (MP 100, BIOPAC Systems Inc.).

Optical recordings of movement

Stomachs were opened along the lesser curvature from the pylorus to the apex of the fundus and were pinned out as a flat sheet (mucosa side topmost) in a Sylgard-lined organ bath used for video imaging. The mucosa and submucosa were removed by sharp dissection and small opaque surface markers were placed on the circular muscle of the antrum in a 3×3 or 5×3 mm arrays.²⁹ Relative positions of the surface markers and edges of the gastric sheet were recorded at 7.5 frames per second (fps) using a HD camera (DMK 41AF02; Imaging Source, Charlotte, NC, USA) onto a computer (iMac; Apple, Cupertino, CA, USA). Edge tracking and particle tracking routines were used to determine diameter changes and displacement of surface markers, respectively. The positions of surface markers were tracked using a least-difference algorithm throughout each movie. Trajectories were plotted with circumferential movements plotted in the *Y*-axis, and longitudinal movements in the *X*-axis. To determine the amplitude of displacement, the average *X* and *Y* position was calculated and the distance from each marker to the *XY* average was determined. Spatio-temporal maps of diameter were constructed and line traces of the displacement of surface markers from their average position plotted.

Solutions and drugs

The KRB used in this study contained: 120 mmol L⁻¹ NaCl, 5.9 mmol L⁻¹ KCl, 2.5 mmol L⁻¹ CaCl₂, 1.2 mmol L⁻¹ MgCl₂, 15.5 mmol L⁻¹ NaHCO₃, 1.2 mmol L⁻¹ NaH₂PO₄, and 11.5 mmol L⁻¹ dextrose. This solution had a final pH of 7.3–7.4 after equilibration with 97% O₂–3% CO₂. Nifedipine, carbachol, and wortmannin were obtained from Sigma (MO, USA). Nifedipine was dissolved in 95% ethanol, and wortmannin was dissolved in dimethyl sulphoxide (DMSO) to make 10 mmol L⁻¹ stock solutions. These were aliquoted and stored at –20 °C until the day of use. Carbachol was dissolved in deionized water at 10 mmol L⁻¹ and kept at 4 °C. Final concentrations of drugs were made by diluting stock solutions in KRB. Nifedipine and wortmannin are light sensitive, so these drugs were used in a darkened laboratory.

Statistical analysis

The frequencies were tabulated as CPM. Data are expressed as means $\pm$ SEM, and differences between data sets were determined using Student's *t*-tests ($\alpha = 0.05$). Statistical analyses were made using Sigma Stat 2.03 (Aspire Software International, Ashburn, VA, USA).

RESULTS

Biopotentials were recorded from the murine stomach with extracellular array electrodes at 4.7 ± 0.42 CPM ($n = 10$; Fig. 1A). Biopotentials typically consisted of oscillatory or ‘spike-like’ activity. The electrical activity was similar to the activity recorded with extracellular electrodes from visceral organs of various species.²⁰ Biopotential complexes propagated from the proximal end of the gastric sheet toward the pylorus at 2.5 ± 0.1 mm s⁻¹ ($n = 10$; Fig. 1A). Recordings were also made, for comparison, with 250 μ m wires placed within gastric sheets to optimize and maintain contact with muscle fibers during movement. Recordings of similar form and frequency as with the array electrodes were obtained with these electrodes (Fig. 1B).

Slow waves cause Ca²⁺ influx and excitation–contraction coupling in gastric muscles due to voltage- dependent activation of Ca²⁺ channels in smooth muscle cells.^{30,31} Ca²⁺ entry can be blocked by dihydropyridines in mice, reducing contractile activity to low levels without substantially reducing the amplitudes of slow waves (which are due to Ca²⁺-activated Cl⁻ currents; see Ref. 8). We tested the effects of nifedipine on biopotentials recorded by extracellular electrodes to determine whether muscle movements contribute to these signals. Nifedipine (1 μ mol L⁻¹) reduced biopotential amplitude and eliminated ‘spike-like’ oscillations without affecting biopotential frequency (Fig. 2A,B). This concentration of nifedipine, however, did not block all movement and small contractions were still apparent by eye, and this was verified by standard organ bath contractile recordings (Figure S1). Thus, we increased the dose of nifedipine to 2 μ mol L⁻¹, and this abolished movement and biopotentials recorded with bipolar and monopolar extracellular electrodes (Fig. 2C; $n = 5$). We also made recordings from stomachs of five mice with bipolar and a monopolar configuration in which single electrodes within the arrays were referenced against a bath ground. The monopolar recordings had similar characteristics as the bipolar array recordings and the signals recorded by either technique were blocked by 2 μ mol L⁻¹ nifedipine (Fig. 3).

Previous studies have used different filtering characteristics to record or display biopotentials obtained *via* array electrodes. For example, studies by Lammers and coworkers have routinely used a bandpass filter in the range of 2–400 Hz (e.g. Ref. 11), but more recent studies by O’Grady and coworkers have filtered their recordings with low pass filtering with a cut off of 2 Hz.²² We performed an additional five experiments in which monopolar recordings were acquired with a bandpass of 0.3–100 Hz. Data were then postacquisition filtered within the range of 0.3–3 Hz or from 3 to 100 Hz. Periodic signals similar to the recordings in Fig. 2 were observed with both filtering paradigms, however, different signal characteristics were emphasized. All periodic signals were blocked by nifedipine (2 μ mol L⁻¹). An example of one of these experiments is shown in Figure S2.

In most of our experiments, extracellular and intracellular recordings were performed on separate tissues in separate recording chambers. The intracellular experiments were performed to verify that the movement suppression techniques did not block slow waves. However, we also did experiments in which extracellular and intracellular recordings were performed on the same tissue under the same conditions to avoid possible differences in electrical activity that might have been introduced by different treatments of the muscles. In

these experiments intracellular impalements were very difficult before movement suppression due to the loose pinning of the gastric sheets and significant contractile activity of the muscles that rapidly dislodged microelectrodes. However, contractions of gastric sheets were irregular or weak in three experiments, and cell impalements could be maintained for several minutes during extracellular recording (Fig. 4A–D). In five additional experiments we verified that when biopotentials recorded with extracellular electrodes were blocked by nifedipine, slow waves persisted, as recorded by intracellular microelectrodes in the same muscles (Fig. 4E–G).

CCh ($1 \mu\text{mol L}^{-1}$) increased the frequency of biopotentials under control conditions to 9.2 ± 0.38 CPM ($n = 5$) and induced small, irregular fluctuations during periods between biopotential complexes (Fig. 5Aa). After nifedipine ($2 \mu\text{mol L}^{-1}$), CCh caused a few irregular fluctuations, but nothing typical of electrical slowwaves was elicited by muscarinic agonists (Fig. 5Ab).^{8,29,32}

The effects of movement suppression were also tested with intracellular recording. Cells were impaled and slow waves, 37 ± 0.2 mV in amplitude, 5.7 ± 0.05 s in duration, occurred at 8.25 ± 0.75 CPM ($n = 5$; Fig. 5Ba). These parameters are consistent with previous electrophysiological studies of murine stomach.^{29,32} The frequency of slow waves recorded with intracellular electrodes was significantly greater ($P < 0.001$) than the biopotential complexes recorded with extracellular electrodes (values above). Nifedipine ($2 \mu\text{mol L}^{-1}$) did not significantly affect the electrical activity of gastric muscles (Fig. 5Bb). Slow waves persisted at 37 ± 0.2 mV amplitude, 5.6 ± 0.03 s duration and 8.3 ± 0.5 CPM. These parameters were not significantly different (each $P > 0.1$) from slow waves recorded from the same cells prior to nifedipine. CCh causes depolarization and increases the frequency of murine gastric slow waves.³² In the presence of nifedipine, CCh caused depolarization (24 ± 0.4 mV) and increased the frequency of slow waves (to 9.6 ± 0.5 CPM, $n = 5$; $P = 0.028$; Fig. 5Bb). Thus, movement suppression by nifedipine did not interfere with the electrical response to CCh.

Although nifedipine does not block gastric slow waves,^{30,32,33} we also tested wortmannin, an irreversible inhibitor of myosin light-chain kinase,³⁴ as a second means of movement suppression and shown previously not interfere with electrical activity.³⁵ Biopotentials were recorded under control conditions, and CCh ($1 \mu\text{mol L}^{-1}$) increased the frequency to 9.6 ± 0.24 CPM ($n = 5$) and caused small, irregular fluctuations between biopotential complexes (Fig. 6Aa). Wortmannin ($10 \mu\text{mol L}^{-1}$ for 30 min and then wortmannin was removed) blocked contractile movements and completely blocked biopotentials recorded with extracellular electrodes (Fig. 6Ab). No electrical signals were evoked by CCh after treatment with wortmannin (Fig. 4B).

Intracellular experiments were also performed to test the effects of wortmannin on gastric slow waves. Slow waves averaging 37 ± 0.2 mV in amplitude and 5.4 ± 0.1 s in duration occurred at 8.25 ± 0.75 CPM ($n = 5$) in control conditions (Fig. 4Ba). Wortmannin had no effect on electrical activity (Fig. 6Bb); slow waves persisted at 37 ± 0.2 mV in amplitude, 5.6 ± 0.1 s in duration at a frequency of 8.6 ± 0.3 CPM ($n = 5$; $P > 0.1$ for each parameter). Movement suppression by wortmannin did not alter electrical responses to CCh. After

wortmannin, CCh depolarized cells (22.5 ± 0.6 mV) and increased slow wave frequency to 10.2 ± 0.2 CPM ($n = 5$; $P = 0.001$; Fig. 6Bb).

Gastric movements and the stabilizing effects of nifedipine were characterized with high-resolution video imaging (Fig. 7A). Spontaneous contractions, corresponding in frequency and spatial characteristics to contractions of intact stomachs²⁹ were recorded in gastric sheet preparations at 5.04 ± 0.7 CPM (Fig. 7B,D; $n = 4$; Movie S1). Contractions developed in the proximal end of gastric sheets and spread to the pyloric terminus as shown by spatio-temporal maps (Fig. 7C). Nifedipine ($2 \mu\text{mol L}^{-1}$) blocked movements as shown in spatio-temporal maps and by measurements of displacement of tissue markers (Fig. 7C,D). Circular and longitudinal movements were more accurately characterized by tracking positions of small opaque markers placed on the surface of the gastric sheets. The average displacement amplitude of markers in control conditions was $142 \pm 14 \mu\text{m}$, which was significantly reduced to $23 \pm 10 \mu\text{m}$ after the addition of nifedipine ($2 \mu\text{mol L}^{-1}$; $n = 6$; $P < 0.01$, Fig. 8C,D; Movie S2). Frequency was difficult to determine with nifedipine due to the small amplitude of contractions.

We also tested whether passive muscle movements can induce biopotentials of the shape and magnitude recorded during active contractions. In these experiments a small stainless steel hook connected to a micromanipulator was attached to the pyloric end of gastric sheets. Extracellular recordings were made with silver electrodes (Fig. 9A), and then active contractions and biopotentials were blocked by nifedipine (Fig. 9B). After paralysis, tiny (<1 mm) passive stretches were applied that were approximately equivalent in magnitude to the movements observed by eye during gastric contractions. These stretches were applied to the distal end of the stomach, several mm from the site where electrodes were placed. Thus, the actual movements at the site of recording were likely to have been less than at the site of the applied stretches. Passive stretching of gastric sheets resulted in electrical transients that were approximately equivalent in magnitude and shape to the transients accompanying active contractions (Fig. 9C; $n = 5$). Similar tests were performed on formalin-fixed gastric sheets, and stretching initiated voltage transients similar to the events observed in pharmacologically paralyzed muscles (not shown).

DISCUSSION

Electrical activity in visceral smooth muscles is important and desirable to monitor because it is a major determinant of excitation–contraction coupling and patterning of digestive movements. Depolarization of smooth muscle cells increases open probability of voltage-dependent Ca^{2+} channels, facilitating Ca^{2+} entry and contraction.³⁶ In organs and patients it is far easier and safer to use extracellular recording. There are obstacles to extracellular recording from visceral muscles, however: (i) membrane potential changes during slow waves and Ca^{2+} action potentials are smaller in amplitude than in nerve, skeletal muscle, or cardiac action potentials; (ii) dV/dt of membrane potential changes in GI muscles is at least two orders of magnitude less than in nerves, skeletal and cardiac muscles; (iii) there are larger amplitude, larger dV/dt signals occurring in cells surrounding pacemaker cells in visceral smooth muscles that must be de-emphasized by filtering; (iv) a small percentage of cells ($<10\%$) within the *tunica muscularis* [i.e. interstitial cells of Cajal (ICC)] generate slow

waves; slow waves are conducted passively to smooth muscle cells;^{37,38} and (v) visceral smooth muscles contract. Recording of biopotentials is further complicated by distance between the bioelectric source (ICC) and the recording electrodes. The present study shows that movement also contaminates extracellular recording, making slow waves difficult to resolve. Suppression of movement, downstream of electrical pacemaker activity, completely blocked resolution of biopotentials in gastric sheets. The obvious conclusion from our observations is that biopotentials, recorded by extracellular electrodes, may be contaminated by movement artifacts.

The mechanism of slow wave generation and propagation, and conduction of these events into smooth muscle cells may be a major obstacle to extracellular recording. Slow waves are generated in ICC by activation of large amplitude, inward Ca^{2+} -activated Cl^- currents.^{9,39} Unlike other excitable cells, which have reciprocal voltage-dependent outward currents to enhance the velocity of repolarization, participation of a voltage-dependent outward current in slow wave repolarization has never been demonstrated in ICC.^{39,40} Termination of slow waves is accomplished by deactivation of the Ca^{2+} -activated Cl^- current.³⁹ In heart, skeletal muscles, and neurons activation of outward currents at the trailing edge of the active zone (i.e. region of membrane with net inward current) results in localized currents of relatively large current density. In the syncytium formed by ICC and smooth muscle cells, much of the inward current flowing into ICC during slow waves is shunted to smooth muscle cells *via* electrical coupling.³⁸ This current depolarizes smooth muscle cells. These physical features of the circuit model of GI muscles would tend to dissipate field potentials in both time and space. A final confounding feature of recording slow waves with extracellular electrodes is the distance between ICC and electrodes. The magnitude of field potentials falls exponentially with distance in a volume conductor; thus the amplitude is 0 within 15 radii of the active fibers.⁴¹ The ICC network in mice is a loosely knit, planar structure, and cells are only a few microns in diameter. Thus, electrodes touching the serosal surface would record signals attenuated by the thickness of the longitudinal muscle layer. The longitudinal muscle layer in the murine stomach is about 20 μm (S.M. Ward and Y. Bayguinov, unpublished results), so even with tight contact between electrodes and the serosal surface, field potentials would be attenuated significantly.

The very slow voltage transients, typically identified as slow waves in extracellular recordings,²² seem inconsistent with the kinetics of slow wave depolarization and propagation in networks of ICC. Consider that the upstroke phase of ICC slow waves, which is the primary event resolved in extracellular recordings because it is the fastest component, occurs in only about 50–75 ms (Fig. 10A). Volume recordings from excitable cells approximate 2nd derivatives of transmembrane potential changes.² Fig. 10B,C shows 1st and 2nd derivatives of ICC slow waves recorded previously,⁴² and it should be noted that the major component of the 2nd derivatives are biphasic transients that occur within the time course of the upstroke depolarization (i.e. 50–75 ms). Extracellular recordings from GI muscles would not be able to monitor electrical activity from a single cell, but rather would sample more integrated membrane potential changes from a population of ICC beneath the electrode. We have previously monitored propagation of excitability in ICC using Ca^{2+} sensitive dyes.⁴³ These studies showed that upstrokes of Ca^{2+} transients are about three

times longer than slow wave upstroke potentials (Fig. 10A & C),⁴³ and integrated Ca^{2+} transients in ICC within a 400 μm field of view (FOV, which is larger than the tips of silver electrodes in our arrays) display a rise time of about 350 ms (Fig. 10D). Using the observation that electrical depolarization is 3.3 times faster than the rise of Ca^{2+} transients in a single cell suggests that the depolarization wave in an ICC network (here in a 400 μm FOV) occurs within about 100 ms. Thus, authentic slow wave upstroke transients recorded with extracellular electrodes should be of approximately this duration, but published extracellular recordings considered to represent slow waves are far longer in time course (e.g. Ref. 22). Our data suggest that the slow biopotential transients, displayed in many studies utilizing extracellular recording, may be the result of muscle movements.

While the current study raises questions about the suitability of extracellular recording for analysis of slow waves, biopotentials may be a useful assay of contractile activity. One could relate the mechanical activity to the electrical events if there is one-to-one coupling between slow waves and contractions. However, (i) if coupling between slow waves and contractions is asymmetrical as often happens in GI muscles and as we have shown in murine stomach (slow wave frequency was ~8 CPM, but biopotential (contractile) frequency was about 5 CPM), (ii) if the coupling ratio between slow waves and contractions cannot be monitored, as in non-invasive recording, (iii) if the coupling ratio changes dynamically during recording sessions, or (iv) if two regions of muscle are electrically uncoupled but mechanically coupled (i.e. slow waves do not propagate between regions, but contraction in one region causes movement in adjacent regions), then conclusions about electrical activity from extracellular recording would be questionable. Our data do not necessarily discredit extracellular recording of GI electrical activity in previous studies, however, the high degree of mechanical artifact present in our *in vitro* recordings, where positions of electrodes were optimized, muscles were secure as flat sheets, and noise from other sources of electrical activity and movement were minimized, suggest that assessment of movement artifacts should be a control when extracellular recording is used to study electrical behavior. Control of movement is difficult or impossible *in vivo*, but testing of electrode arrangements, filtering characteristics, and amplifier sensitivity on *in vitro* sheets from a given region of the GI tract could be performed.

Classical descriptions of GI organs, such as the basic electrical rhythms of the stomach (3 CPM in humans; 4–5 CPM in dogs), were deduced from extracellular recordings. We observed a significant mismatch, however, in frequencies recorded with extracellular and intracellular recording techniques. Intracellular microelectrodes recorded 1.75 $\times$ faster slow wave frequency than extracellular recording. The probable explanation for this discrepancy is less than one-to-one coupling between slow waves and contractions. It should also be noted that the 3 CPM in human stomach is much slower than slow wave frequency recorded by intracellular electrodes.⁸ Thus, it is possible that the ‘normal’ slow wave frequency in the human stomach has been underestimated by extracellular recording, if movement artifacts also contaminate these recordings.

Our data help explain long-standing discrepancies between intracellular and extracellular electrical recordings. Many extracellular studies of gastric muscles concluded that postprandial (Phase 3) contractions only occur when Ca^{2+} action potentials (aka ‘spikes’)

are superimposed upon electrical slow waves. This idea formed the basis for the terminology used by many investigators: electrical control activity (i.e. slow waves without spikes) and electrical response activity (i.e. slow waves with superimposed spikes),^{44,45} which can still be found in textbooks and reviews of gastric electrophysiology.²⁷ In some studies intraluminal pressure was recorded simultaneously with extracellular electrical recording, and significant pressure events (i.e. peristaltic contractions) were always associated with biopotentials with superimposed spikes. Thus, it seemed that gastric peristaltic waves occur only when slow waves exceed the action potential threshold. In contrast, intracellular recording showed that action potentials only occur in muscles of the most terminal antrum and pylorus,^{8,46} and contractions of gastric muscles occur in the absence of action potentials.⁴⁷ Even powerful contractions elicited by excitatory agonists failed to evoke action potentials in corpus and antrum.⁴⁷ Simultaneous measurement of intracellular electrical activity, intracellular Ca^{2+} transients and contractions demonstrated that slow waves accomplish excitation–contraction coupling and increasing the amplitude and duration of slow waves enhances the force of contraction,³⁰ as Szurszewski and colleagues proposed.⁴⁷ Explanations for discrepancies between intracellular and extracellular recording have never been provided. Evidence that oscillations in membrane potential superimposed upon slow waves in classic studies were *bona fide* action potentials was based on the finding that inhibitory substances blocked these events, and when the spikes were blocked, contractions were also blocked. Our experiments recapitulate these classic findings. Gastric biopotentials recorded with extracellular electrodes consisted of spikes superimposed upon the underlying basal electrical rhythm. However, our data demonstrate that the spiky oscillations in membrane potential were not action potentials, but are likely to be movement artifacts as: (i) there are no action potentials superimposed upon slow waves in the murine gastric antrum in control or during muscarinic stimulation;³² and (ii) paralyzing movement blocked spikes but did not affect electrical activity recorded with intracellular microelectrodes. Our data suggest that classic extracellular recordings may have been misinterpreted: contractions are not dependent upon the occurrence of action potentials, but instead, the ‘spikes’ appear to be caused by contractile movements.

Electrode array recordings are used widely to study electrical propagation in GI and non-GI organs.^{11,12,22–25,48} From descriptions of recording conditions in these studies, tissues and organ segments could move relative to electrode arrays during recording. In fact in some cases, large changes in electrode position relative to muscle segments occurred during recording.⁴⁹ As we showed, even small movements, hard to visualize by eye, induced electrical deflections. Movement is not easily predictable, and even adjacent regions can have quite different movement trajectories (Fig. 8). Thus, tiny localized contractions could be a serious contaminant in electrode array recordings, and differences in movement trajectories might explain the variability in waveforms of recorded events, even in adjacent electrodes (e.g. fig. 13 in Ref. 50). Suppression of movement *in vivo* may be very difficult to accomplish and verify, but movement suppression *in vitro* is possible and rarely tested. No previous studies appear to have tested whether effects of movement suppression were due to block of slow waves or block of contractions without effects on slow waves. Nifedipine was used to reduce movements in guinea pig small intestine,¹² and as we observed, $1 \mu\text{mol L}^{-1}$ nifedipine reduced electrical signals, but did not block all activity.¹² Higher concentrations

of nifedipine were apparently not tested, and no comment was made about whether residual movements persisted with $1 \mu\text{mol L}^{-1}$ nifedipine. In our studies, further stabilization of movement blocked biopotentials. In another study, murine small intestinal segments were treated with verapamil ($10 \mu\text{m}$), and this was reported to block movements as monitored in video images.⁴⁹ In three of four intestinal segments, however, slow wave signals were also blocked by verapamil, suggesting either: (i) In 75% of the muscles studied, slow waves recorded were actually movement artifacts; (ii) In 100% of recordings slow waves were movement artifacts but verapamil failed to block all movement in one of four muscles tested, or (iii) verapamil blocked or reduced slow waves so the events could not be resolved by the array electrodes. Verapamil is an L-type Ca^{2+} channel blocker, so like nifedipine, this compound may not block slow waves in the small intestine.⁵¹ Persistence of slow waves after verapamil was not checked in their study.⁴⁹

Array electrodes have also been used to characterize propagation of electrical activity and conclusions have been made about the velocity of slow wave propagation, coupling of slow waves to action potentials, sites of slow wave origin, and spread of slow waves along the length of the small intestine.^{11,12,22–25,48} Smooth muscles are viscoelastic in nature and muscle bundles are mechanically interconnected to form tissues and organs. E–C coupling in one region of muscle results in active stress development and muscle shortening (movement) by the cells reaching threshold. Movement is transmitted to adjacent regions by passive stretch whether electrical events propagate or not. As we have shown, the dynamics of tissue movement in regions stretched by active contraction in adjoining areas of muscle are complicated; application of strain or relaxation of strain in one region may not produce constant length changes in adjoining regions of tissue. Thus, contamination of electrical recordings by movement might lead to misconceptions about the characteristics of electrical propagation. Without excluding movement from electrical recordings, one cannot assume that sequential electrical deflections in extracellular signals are due only to electrical propagation.

In summary, contractions are a significant contaminant in extracellular recordings from murine gastric smooth muscles. Controls for movement should accompany studies using extracellular recording to evaluate electrophysiological behavior of visceral muscles. Movement suppression drugs (e.g. with dihydropyridines) may not be equally effective in all tissues and species; thus techniques to suppress movement should be tested to establish effective doses.* Biopotential frequencies assessed by extracellular recording may incorrectly monitor true slow wave frequency, and such artifacts could be more serious in tissues and organs with poorly coordinated electrical and mechanical activities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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***Note on stabilization of movement:** As this paper suggests, an important control to determine the effects of movements on movement on extracellular recording is to make recordings before and after application of agents to block contraction without significantly affecting electrical slow waves. The contractile responses of GI smooth muscles are initiated by Ca^{2+} entry. However, removal of extracellular Ca^{2+} to stabilize movement would not be useful, because this also blocks slow waves in many muscles. GI smooth muscles typically utilize $\text{Ca}_v1.2$ channels for excitation–contraction coupling (i.e. L-type Ca^{2+} channels are a major pathway for Ca^{2+} entry during excitation–contraction coupling), but L-type Ca^{2+} channels are not a fundamental conductance in generating or propagating slow waves in many species. So blocking these channels is a potential avenue for suppressing movement without blocking slow waves (and this was the rationale for using this compound in the present study). However, there are other Ca^{2+} channels expressed in smooth muscle cells and ICC (e.g. $\text{Ca}_v1.3$ and Ca_v3 family channels) and expression is likely to vary with species. Thus, the effectiveness of Ca^{2+} channel blocking drugs needs to be tested in movement or contractile studies of each type of muscle. We have found, for example, that $1 \mu\text{mol L}^{-1}$ nifedipine (or nicardipine), which is quite potent in some smooth muscles, does not fully suppress contractions in rodent GI muscles, including the mouse. Thus, higher concentrations of nifedipine are needed to accomplish movement stabilization. We have also tested quite high concentrations of nifedipine in gastric muscles of the monkey *Macaca fascicularis* ($>10 \mu\text{mol L}^{-1}$), and this compound failed to block contractions. Thus, careful evaluation of the technique chosen for movement stabilization is needed. We have also used wortmannin, an inhibitor of myosin light chain kinase (MLCK), as another means to stabilize movement, because previous studies showed this compound had little or no effect on electrical activity.³⁵ Additional compounds, such as ML-7 or blebbistatin, could also be tested.

In the present study, we characterized movement by high-resolution video imaging. Concluding that movement was stabilized ‘because contractions could not be resolved visually’ is not a rigorous analysis of movement. Small localized contractions, particularly from longitudinal muscle bundles, are capable of inducing movement based artifacts, and these might be missed by visual inspection. If high-resolution video analysis is unavailable, contractile experiments using organ baths should be performed to confirm that a particular drug or solution blocks contraction. Such experiments are usually performed in a manner to measure isometric contraction, but it would probably be better to evaluate isotonic contractions for this question, as muscles are allowed to shorten against a fix load with this technique. For experiments involving extracellular recording from organs *in vivo*, a series of *in vitro* experiments should be performed to check the effects of movement on extracellular recordings from a particular muscle region. Slow waves from different regions have different rates-of-rise, thus it may be possible to resolve slow waves from some tissues or regions of tissues, but not from others. We would recommend the following protocol to verify the effectiveness of treatments on blocking contractions: Muscle strips of the organ and region under investigation should be cut parallel to both the circular and longitudinal muscle layers. The two muscle layers do not necessarily share the same mechanism for contraction, and therefore it cannot be assumed that effective paralysis in one layer is adequate for the adjacent muscle layer. Contraction of either muscle layer may result in movement-based artifacts in extracellular recording. Experiments should be conducted at physiological temperatures for the specific animal under study, and it may also be necessary to inhibit prostaglandin synthesis to restore normal levels of contractile force. After establishing optimal lengths (L_0) for muscles, spontaneous contractions (no applied stimuli) should be recorded. Muscles should then be exposed to at least an ED_{50} concentration of excitatory agonist (e.g. acetylcholine) or to at least 10 Hz (0.3 ms duration pulses, 30 s train) electrical field stimulation (in the presence of drugs to block inhibitory motor input). Spontaneous contractions may be of small amplitude and easily blocked to the level of resolution by lower concentrations of drugs than responses to agonists. In the case of Ca^{2+} channel inhibitors, agonists may recruit additional mechanisms for increasing intracellular Ca^{2+} or by increasing the Ca^{2+} sensitivity of the contractile apparatus. Nerves, hormones and autoids are available *in vivo* and thus movements in intact organs may be more difficult to control than movements of muscle strips *in vitro*. After establishing that a particular concentration of an agent blocks contractions, then electrical activity, both intracellular and extracellular must be recorded. As shown in the present paper, extracellular signals were blocked while intracellular slow waves persisted. This indicates that extracellular signals from murine stomach were movement artifacts. It is important to check intracellular activity, however, because if slow waves are blocked by the treatment used to stabilize movement, then the question of whether extracellular signals are movement-dependent or not will remain unanswered.

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Figure 1.

Biopotentials recorded from gastric sheet with extracellular array electrodes (A; see Methods for details). Events were characterized by oscillatory potentials and propagated from electrode to electrode. (B) Compares recordings of biopotentials with bipolar silver wire electrodes (250 μm) placed on the surface of the gastric sheet.

Figure 2.

Effects of nifedipine on biopotentials recorded with extracellular array electrodes. (A) Shows control recordings of regular complexes of oscillatory biopotentials. After treatment with nifedipine ($1 \mu\text{mol L}^{-1}$; B), the amplitude of the biopotentials decreased and oscillations after the initial deflection were blocked. Movement (contractions) were reduced but not blocked by $1 \mu\text{mol L}^{-1}$ nifedipine. This partial suppression of movement caused the biopotentials to take on the typical waveform associated with extracellular recording (see inset). (C) Shows electrical activity after $2 \mu\text{mol L}^{-1}$ nifedipine which reduced contractile movements (see Figs 7 and 8).

Figure 3.

Comparison of monopolar and bipolar recording. Biopotentials were recorded from single electrodes within the electrode array in reference to a bath ground electrode (Aa) or with two adjacent electrodes within the array in reference to each other (Ba). In both cases the biopotentials had similar frequencies and waveforms, and in both recording configurations, the biopotentials were blocked by nifedipine ($2 \mu\text{mol L}^{-1}$; Ab and Bb).

Figure 4.

Extracellular and intracellular recording in the same muscles. (A and B) Show simultaneous recordings using an extracellular array electrode in monopolar mode and an intracellular microelectrode. Impalements were extremely difficult to maintain under these circumstances because of muscle contractions. In this example contractions were small and the biopotentials had the typical triphasic waveform associated with extracellular recording of slow waves. Recordings were made in different portions of the gastric sheet, so the phasic relationship between biopotentials and slow waves is not of significance. In this stomach, slow waves occurred at the same frequency as the biopotentials. (C and D) Show recordings

from the same muscle after treatment with nifedipine ($2 \mu\text{mol L}^{-1}$). Intracellular recording is from a different impalement than in (B). Note that intracellular recording is lost by nifedipine, but there is no loss of slow waves, as shown by the intracellular recording. (E and F) Show the same effect of nifedipine on biopotentials recorded by extracellular electrodes in another gastric sheet. In this example, and with many other preparations, we were unable to sustain impalements during the extracellular recordings of biopotentials due to muscle contractions. After addition of nifedipine (which suppressed movement) cells were impaled, and these recordings confirmed that slow waves were sustained after loss of biopotentials (G).

Figure 5.

Effects of cholinergic stimulation on biopotentials and electrical slow waves in gastric muscles. (A) Shows recordings *via* extracellular array electrodes. Regular biopotential complexes were noted under control conditions (a). CCh ($1 \mu\text{mol L}^{-1}$) increased the frequency of the biopotentials, and noisy electrical deflections between complexes were often noted at the beginning of responses to CCh. The second trace (b) shows block of the biopotential complexes by nifedipine ($2 \mu\text{mol L}^{-1}$). In the presence of nifedipine, CCh caused only small irregular deflections at the beginning of the response. (B) Compares the electrical activity recorded from gastric sheets with intracellular microelectrodes. In (a) slow wave activity was recorded upon impalement of smooth muscle cells. In (b) nifedipine ($2 \mu\text{mol L}^{-1}$) did not significantly reduce slow waves. Addition of CCh ($1 \mu\text{mol L}^{-1}$), in the presence of nifedipine, caused depolarization and increased slow wave frequency. This is the normal response of gastric muscles to muscarinic stimulation.³²

A Extracellular array electrodes

B Intracellular microelectrode recording

Figure 6.

Effects of wortmannin on biopotentials and electrical slow waves in gastric muscles. (A) Shows biopotentials recorded with extracellular array electrodes. Rhythmic biopotential complexes were noted under control conditions (a). CCh ($1 \mu\text{mol L}^{-1}$) increased the frequency of the biopotentials, and noisy electrical deflections were often noted between complexes at the beginning of responses to CCh. Wortmannin treatment ($10 \mu\text{mol L}^{-1}$; trace b) blocked contractile movements and biopotentials in gastric muscles. In the presence of wortmannin CCh had no effect on electrical activity recorded with extracellular electrodes. (B) Compares effects of wortmannin on electrical activity recorded by an intracellular microelectrode. In (a) normal slow wave activity was recorded in control conditions. Treatment with wortmannin (b) had no effect on slow waves, and CCh ($1 \mu\text{mol L}^{-1}$) caused depolarization and increased slow wave frequency.

Figure 7.

Inhibition of contractile movements by nifedipine. Nifedipine, an inhibitor of voltage-dependent Ca^{2+} channels in gastric muscles, blocked peristaltic contractions in gastric flat sheet preparations. (A) Shows an image of the stomach, opened along the lesser curvature and pinned as a flat-sheet. The two halves of the esophagus are at the top of the image and the duodenum is located at the bottom of the image as labeled. Small inert dots were placed on the surface of the gastric sheet to monitor small changes in position. Movements of the gastric sheet were monitored by video camera. Decreases in gastric diameter (muscle contractions) were calculated as changes in distance between the left and right edges of the gastric sheet and spatio-temporal maps were constructed to display the spread of (peristaltic) contractions. (B) Shows a spatio-temporal map of control activity consisting of regularly occurring, large amplitude contractions that propagated from the corpus to the antrum (white diagonal streaks; arrow depicts direction and rate of propagation). (C) Shows a spatio-temporal map after addition of nifedipine ($2 \mu\text{mol L}^{-1}$). Propagating contractions were not observed after nifedipine. Scale in (C) shows gray-scale mapping of contractions in (B) and

C). (see dotted rectangle in A). (D) Shows traces of movements of a centrally located surface marker (see dotted rectangle in A) during control and after exposure to nifedipine ($2 \mu\text{mol L}^{-1}$). Movement was blocked by nifedipine.

Figure 8.

Analysis of surface marker movements. (A) Shows a flat-sheet preparation of stomach and the trajectories of four surface markers during 4.5 slow wave cycles are overlaid in color. Each surface marker had a unique trajectory, with varying degrees of circumferential movements (Y-axis) and longitudinal movements (X-axis). (B) After the addition of nifedipine (2 $\mu\text{mol L}^{-1}$), movement of surface markers was suppressed significantly. (C) Shows the distance of each marker from its average XY center in control conditions. Note the varied waveforms and phase-relation between traces; even between markers arranged circumferentially to one another (red–blue and green–orange) show varying spatial relations. (D) Shows the reduction in the amplitude of movements after nifedipine (2 $\mu\text{mol L}^{-1}$).

Figure 9.

Movement can induce biopotentials in gastric muscles. (A) Shows biopotentials recorded with extracellular silver electrodes. The biopotentials (B) were blocked by nifedipine ($2 \mu\text{mol L}^{-1}$). (C) Shows induction of electrical transients of equal magnitude to the biopotentials induced by small ($<1 \text{ mm}$) stretches of the gastric sheet from the pyloric end. Stretches of the muscle (1–5 stretches applied as noted in C), resulted in 1: 1 electrical transients picked up by the extracellular electrodes placed in the mid-body region of the gastric sheets.

Figure 10.

Expected kinetics of slow waves in extracellular recordings based on measurements of membrane potential and Ca^{2+} transients in cells of Cajal (ICC) and networks. (A) Shows a transmembrane potential recording of electrical slow waves from an ICC within the ICC-MY network in the murine small intestine. Upstroke velocity exceeds $1 V \cdot s^{-1}$ and the upstroke depolarization is completed in about 50–75 ms. The 1st derivative [dV/dt] (red trace), and 2nd derivative [d^2V/dt^2] (blue trace) are displayed below. The 2nd derivative approximates an extracellular recording in volume from a single cell (Ref. 2). (B) Shows a 400 μm wide field showing the ICC-MY network that was loaded with the Ca^{2+} dye, Fluo 4 (data provided by Dr. Hyun-Tai Lee; Ref. 43). Ca^{2+} transients in ICC within the ICC-MY network during the propagation of a slow wave (Ref. 43) are displayed in the upper three panels, and the progress of a slow wave is noted by the time register in the lower right portion of each panel. The bottom panel (ICC BitMask) was used to calculate the Ca^{2+} -induced fluorescence in the entire ICC-MY network in this field of view (FOV). (C) Shows Ca^{2+} transients calculated from three ICC (ROIs denoted in the bottom panel of B) within the ICC-MY network, and 1st and 2nd derivatives of the Ca^{2+} transient depicted by the black trace are shown in red and blue, respectively. The duration of the upstroke phase of

Ca^{2+} transients associated with slow waves in individual cells is ~250 ms which is 3–4 times slower than the velocity of the electrical upstroke depolarization during slow waves (see A). (D) Shows Ca^{2+} transients calculated from the entire ICC-MY network in the FOV and 1st and 2nd derivatives are shown below in red and blue, respectively. The duration of the upstroke of Ca^{2+} transients calculated from the entire FOV, was ~350 ms. Using the factor (3.33) deduced from (A and C) (difference in rate of rise of the electrical upstroke vs the Ca^{2+} transient associated with slow waves), this suggests that the electrical upstroke of slow waves from ICC within the FOV would have been about 100 ms.