Spatial Analysis of Slowly Oscillating Electric Activity in the Gut of Mice Using Low Impedance Arrayed Microelectrodes

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Abstract

Smooth and elaborate gut motility is based on cellular cooperation, including smooth muscle, enteric neurons and special interstitial cells acting as pacemaker cells. Therefore, spatial characterization of electric activity in tissues containing these electric excitable cells is required for a precise understanding of gut motility. Furthermore, tools to evaluate spatial electric activity in a small area would be useful for the investigation of model animals. We thus employed a microelectrode array (MEA) system to simultaneously measure a set of 8 x 8 field potentials in a square area of ~1 mm². The size of each recording electrode was 50 x 50 μm², however the surface area was increased by fixing platinum black particles. The impedance of microelectrode was sufficiently low to apply a high-pass filter of 0.1 Hz. Mapping of spectral power, and auto-correlation and cross-correlation parameters characterized the spatial properties of spontaneous electric activity in the ileum of wild-type (WT) and W/Wv mice, the latter serving as a model of impaired network of pacemaking interstitial cells. Namely, electric activities measured varied in both size and cooperativity in W/Wv mice, despite the small area. In the ileum of WT mice, procedures suppressing the excitability of smooth muscle and neurons altered the propagation of spontaneous electric activity, but had little change in the period of oscillations. In conclusion, MEA with low impedance electrodes enables to measure slowly oscillating electric activity, and is useful to evaluate both histological and functional changes in the spatio-temporal property of gut electric activity.

Introduction

Cellular electrical cooperation produces smooth and elaborate motions of various biological systems. In the gut, it is well known that a network of intrinsic neurones simultaneously induce ascending contraction and descending relaxation of smooth muscle, leading to peristaltic movements [1,2]. Also, basal slow electric oscillations occur in most sections of the gastrointestinal tract [3,4]. Relatively recent studies have revealed that special interstitial cells, referred to as interstitial cells of Cajal (ICC) act as pacemaker cells for the basal electric activity [5–9]. These cells are likely to contribute to spatial organization of gut excitability through their network of long processes. In agreement with this notion, there is a growing body of evidence that gut motility disorders, such as diabetic gastroparesis and inflammatory bowel diseases (IBD) among other diseases, contain alterations of the network-forming pacemaker cells as well as neurons and smooth muscle cells [10–14]. Thus, investigation into the spatial property of electrical activity, including in pacemaker cells, benefits a more precise understanding of gut motility and medical therapy. In addition, interstitial cells mimicking ICC are distributed over the body, for instance in urinary tracts, lymph ducts and small vessels, and are now considered to play a crucial role in generating spontaneous electric activity.

Using an 8 x 8 microelectrode array (MEA), we previously compared spontaneous basal electrical activity of the ileum between wild-type (WT) and W/Wv mice. In the latter, it is well known that the number of ICC is reduced thereby their pacemaker and network functions are impaired due to a loss-of-function mutation of c-Kit receptor gene [5,7,15]. A power spectrum integrating the whole recording area could distinguish these preparations [16] in the presence of nifedipine and tetrodotoxin (TTX), which suppress the electrical activity of neurones and smooth muscle, respectively. Also, potential mapping videos qualitatively suggested the uncoordinated spontaneous electric activity in the ileum of W/Wv mice. However it was preliminary to display the coordinated actions between basal slow electric oscillations over the whole recording area. In this study, we thus analyzed the MEA field potential recordings by using auto-correlation and cross-correlation parameters as well as spectral power. Examples show that mapping analyses could well characterize spatial properties of gut spontaneous electric activity based on both functional and histological alterations. The ICC network appears to play a crucial role in coordinating gut electric activity with a delay of several seconds per millimetre, and requires...
the support of other cellular components to enhance the coupling. Also, we carefully explain the requirements of MEA systems for the measurement of slowly oscillating electric potentials in a small area, in order to address recent controversies on the frequency of gut spontaneous electric activity [17–21].

Materials and Methods

Animals and Preparations

Animals were treated ethically, in accordance with the guidelines for proper conduct of animal experiments in Science Council Japan. All procedures were approved by the Animal Care and Use Committee in Nagoya University Graduate School of Medicine (Permission #23337). C57BL/6J (WT) and W/W mice (~8 weeks after birth) were sacrificed by cervical dislocation after deeply anaesthetising with diethyl ether. The ileum was quickly excised, and cut along the mesentery. The whole-muscle layer (~5 mm × 20 mm) containing the myenteric plexus was isolated using fine forceps. The mice used were purchased from Chubu Kagaku Shizai Co., Ltd. (Nagoya, Japan).

Electrical Recordings

To measure ileal electric activity, an array of 8×8 planar microelectrodes with an inter-electrode distance of 150 μm was used (Alpha MED Scientific, Ibaraki, Japan). Ileal musculatures were fixed in a recording chamber with this MEA, with the longitudinal muscle layer placed directly on microelectrodes under the strings of a slice anchor (SDH series, Harvard Apparatus Japan). Platinum black particles are fixed on a small square (~5 mm × 20 mm) containing the myenteric plexus was isolated using fine forceps. The mice used were purchased from Chubu Kagaku Shizai Co., Ltd. (Nagoya, Japan).

Each recording electrode is a ~50 μm square, made by platinum black nanoparticles [22], thereby the surface area is increased by ~200-folds, being equivalent to 0.5 mm² (Fig. 1C–D; see also Fig. S1). Platinum black particles are fixed on a small square (~50 μm × 50 μm) area of an indium tin oxide (ITO) plate (50 and 500 μm thick, respectively). To insulate the connecting lines (extended ITO plate), the upper surface of the recording chamber was coated with polyimide, leaving the square areas of microelectrodes.

The impedance of the recording microelectrode (ZME) is given as a function of signal frequency (f):

$$Z_{ME} = \sqrt{\left( \frac{1}{2\pi f \times C_{ME}} \right)^2 + (R_{ME})^2}.$$  

The capacitance (CME) and resistance (RME) of each microelectrode measured by an LCR meter (ZM2353, NF Corp., Yokohama, Japan) were 0.052 μF and 15 kΩ, respectively. The impedance of the recording electrode at 0.1 Hz was therefore small enough (~31 MΩ) to follow oscillating potentials, compared to the input impedance of the multi-channel amplifier (100 MΩ at 0.1 Hz).

The efficacy of electric signal transmission (Tf) is estimated from the input resistance of the amplifier (Rin) and the impedance of the electrode at a certain frequency:

$$\frac{R_{in}}{\sqrt{(R_{in})^2 + (Z_{ME})^2}}.$$  

Thus, Tf was estimated to be ~95% at 0.1 Hz: 100 MΩ/√{100 MΩ² + 31 MΩ²} (Fig. 2). The low resistance (15 kΩ) of electrodes are advantageous for reducing the thermal noise (Nf):

$$N_T = \sqrt{4kT \times R_{ME} \times f},$$

where k and T are the Boltzmann constant and absolute temperature, respectively. The calculated Nf was ~1.6 μV at the low-pass frequency (10 kHz) of the amplifier. Also, in order to reduce the interface tension, recording electrodes were immersed in 0.1% polyethylenimine overnight prior to the first use, and kept in distilled water.

When the noise level of one recording channel was twice larger than that of other channels, the arrayed data were judged to be inappropriate for 2D pseudo-colour images, described below. Air bubbles on microelectrodes were frequently the cause of large noise levels. When removals of platinum black nanoparticles from microelectrodes (i.e. high resistance and impedance) were the cause, the recording chamber was replaced with new one.

A set of 8×8 field potentials (Fig. 1E–F) were recorded in a personal computer via a computer-controlled, multi-channel AC amplifier with low-pass filtering at 10 kHz, and 14 bit A/D converters with a sampling rate of 20 kHz. The dynamic range of A/D conversion applied was usually ±1 mV. A high-pass filter of 0.1 Hz was applied to stabilize the baseline drift of the microelectrode potential. It is known that such low cut-off frequency of high-pass filtering is necessary to follow slow electric activity in the gut [23,24].

MEA measurements were started after perfusing the recording chamber with a normal extracellular solution for at least 30 min. In some experiments, nifedipine (1 μM) and TTX (250 nM) were added to suppress smooth muscle and neuronal electric activity [16,25] and predominantly measure ICC electric activity. Nifedipine also suppressed mechanical activity by blocking L-type Ca²⁺ channels, a major pathway for Ca²⁺ influx in visceral smooth muscle [14].

Solutions and Drugs

The “normal” extracellular solution, a modified Krebs solution, had the following composition (in mM): NaCl, 125; KCl, 5.9; MgCl₂ 1.2; CaCl₂ 2.4; glucose 11; Tris-HEPES, 11.8 (pH 7.4). Nifedipine and TTX were purchased from Sigma-Aldrich (St Louis, MO, USA).

Analyses and Statistics

The procedures for analysing 8×8 MEA data are summarized in Fig. 3. Arrayed data of field potential recordings were thinned by a 1000-fold time domain, thereby the sampling interval was increased to 50 ms. This sampling frequency was enough to follow ICC pacemaker activity. Digital band-pass filter, power spectrum and cross-correlation analyses were performed using commercial add-in software (Kyowa Electronic Instruments, Tokyo, Japan).

1) In power spectrum analysis, Fourier transformation with a Hanning window was applied to each field potential recording (1024 points for ~32 s) of the 8×8 array data. The spectral power
in the frequency range between 9.4 to 27.0 cpm (Pw9.4–27.0) was plotted as a 2D pseudo-colour image (Power map) (Fig. 4E and F), using MATLAB software. 2) The auto-correlation function was derived from each field potential recording (1024 points) of 8×8 array data, after digital band-pass filtering of 0.05–0.5 Hz. The shift and amplitude of the peak adjacent to the centre peak were plotted as 2D pseudo-colour images: period map (Fig. 5B and E) and auto-correlation map, respectively (Fig. 5C and F). 3) The cross-correlation function was derived from each field potential recording (1024 points) of 8×8 array data using Ch28 (or Ch29) as the base channel, after digital band-pass filtering of 0.05–0.5 Hz. The shift (time-lags) and amplitude of the peak of cross-correlation functions (curves) were plotted as 2D pseudo-colour images: phase-shift map (Fig. 6B, E and H) and cross-correlation map, respectively (Fig. 6C, F and I). In the ileum of in W/W’ mice, no digital band-pass filter was applied to 8×8 array data, because oscillating spontaneous electric activity was so small in this tissue that cross-correlation functions reflected the base-line drift after digital band-pass filtering. Also, it is noted that in auto-correlation analysis and cross-correlation analysis, the band-pass filter of 0.05–0.5 Hz was appropriate to mainly detect a basal oscillation frequency of ICC pacemaker activity, but reduced spike-like transient components in field potential traces.

Numerical data are expressed as means±S.D. Significant differences were evaluate by paired and unpaired t-tests.

Results

Microelectrode Array Recording of Ileal Pacemaker Activity

Isolated ileal musculatures were mounted in a recording chamber with an 8×8 MEA on the bottom (Fig. 1A), and field potentials were simultaneously recorded through a multi-channel AC amplifier. Microelectrodes were aligned with a distance between them of 150 μm, reflecting ileal pacemaker activity of a ~1 mm² area (B). Due to the increased surface area of the electrodes made by fixing platinum black particles (Fig. 1C–D), a high-pass filter of 0.1 Hz could be applied in the AC amplifier in order to follow slowly oscillating electric activity. The transmission of the electric signal was ~95% at 0.1 Hz (see Materials and methods). The panels in Fig. 1E and F show example recordings of 8×8 field potentials from isolated musculatures of WT and W/W’ mice, respectively (n = 8 vs 5), under superfusion with a ‘normal’ extracellular solution. Field potentials were smaller in the latter, agreeing well with previous reports on the impairment of the ICC (i.e. pacemaker cells) network [5,15]. During prolonged recordings for more than several minutes, slow potentials were continuously observed in WT mice.

Spectral Power in WT and W/W’ Mice

First, spectral analysis was performed to compare these preparations. The panel in Fig. 4A shows the power spectrum transformed from a field potential recording at 28Ch for ~52 s:
1024 points) in the same preparation of WT mouse shown in Fig. 1E. Based on the spectral power between 9.4 and 27.0 cpm \((P_{9.4-27.0})\) covering the ileal pacemaker frequency under normal conditions, the pseudo-colour map in Fig. 4B was constructed by normalizing with \(P_{9.4-27.0}\) at 28Ch, in order to indicate the distribution of the magnitude of spontaneous electric activity.

Figure 2. Simplified electric circuit diagram, and simulation of the frequency-dependence of circuit parameters. A) Three operational amplifiers (OPA-1, 2 and 3) are sequentially connected in each recording channel, and electric signals are recorded through an A/D converter (ADC). The input resistance \(R_{IN}\) of OPA-1 is set to be 100 M\(\Omega\) by connecting a resistor to the reference (ground). B–C) The impedance of microelectrode \(Z_{\text{ME}}\) and the efficiency of signal transmission in OPA-1 are plotted against the frequency. Continuous and dotted lines represent microelectrodes with and without platinum black nanoparticles (+P and –P), respectively. The capacitance and resistance of microelectrodes \(C_{\text{ME}}\) and \(R_{\text{ME}}\) with platinum black were measured to be 0.052 \(\mu\)F and 15 k\(\Omega\), respectively. \(C_{\text{ME}}\) without platinum black was assumed to be smaller by 200-folds.

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Figure 3. Procedures used in MEA analyses. 8×8 array data of field potentials were normally thinned by a 1000-fold time domain, thereby the sampling interval was increased to 50 ms. 1) In power spectrum analysis, Fourier transformation was applied to each field potential recording (1024 points for ~52 s), and the spectral power in the frequency between 9.4 to 27.0 cpm \((P_{9.4-27.0})\) covering the ileal pacemaker frequency under normal conditions, the pseudo-colour map in Fig. 4B was constructed by normalizing with \(P_{9.4-27.0}\) at 28Ch, in order to indicate the distribution of the magnitude of spontaneous electric activity.

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Similarly, the power spectrum (Fig. 4C) and pseudo-colour map (Fig. 4D) were constructed for the ileal musculature preparation of the W/Wv mouse shown in Fig. 1F. The comparison between these preparations indicated that in addition to a large difference of the total spectral power over the recording area, the magnitude of electric activity gradually changed in WT mice (Fig. 4C), while it was rather randomly distributed in W/Wv mice (Fig. 4D), agreeing well with the notion that the ICC network plays an important role in coordinating the local electric activity to produce smooth contraction [16].

Smooth muscle and enteric neurones are major components of electrically excitable cells other than ICC. Nifedipine and TTX suppress these cells by blocking voltage-gated L-type Ca\(^{2+}\) channels and Na\(^{+}\) channels, respectively [16,25], thus these drugs can be used to examine how much electric current is generated by ICC. After the application of nifedipine (1 \(\mu\)M) and TTX (250 nM), the sum of Pw\(_{9.4-27.0}\) in all 64 channels was largely reduced in ileal musculature preparations of WT mice (from 0.11±0.02 to 0.0062±0.0052 mV\(^2\), P<0.05, paired t-test, n = 8) (Fig. 4E). On the other hand, Pw\(_{9.4-27.0}\) was small in ileal musculatures of W/Wv mice even in normal solution. Application of these drugs again reduced Pw\(_{9.4-27.0}\) in these preparations (from 0.00083±0.00098 to 0.00033±0.00025 mV\(^2\), P=0.20, paired t-test, n = 5) (Fig. 4F), but the ratio of the remaining power was larger in W/Wv mice (5.5% vs 40.3%). The results suggest that ICC facilitated the electric activity of other excitable cells in ileal musculatures.

### Auto-correlation Analysis

Next, auto-correlation analysis was performed to compare these preparations in terms of the frequency of spontaneous electric activity. Panels in Fig. 5A–C show an example of auto-correlation analysis obtained from the ileal musculature of WT mice in normal solution: the array data in Fig. 1E (and Fig. 4B) was used. An auto-correlation function (curve in A) was derived from a field potential recording at Ch 28 (centre channel). Likewise, auto-correlation functions were derived from all MEA channels. The shift toward the adjacent peak (x-axis) was used for mapping the period of electric oscillation in each channel (period map: Fig. 5B), while the amplitude of the adjacent peak (y-axis) was used as an index of similarity of electric oscillations in a time-domain (auto-correlation map: Fig. 5C). The same procedures of auto-correlation analysis were applied to the array data in Fig. 1F (and Fig. 4D) to draw Fig. 4D–F: an auto-correlation function derived from a field potential data at Ch28, period and auto-correlation maps, respectively. The period and auto-correlation maps differed significantly between WT and W/Wv mice.

The periods of oscillations estimated over the recording area were distributed within a small range (4.35–4.5 s in 64 channels: Fig. 5B) in WT mice, but varied largely in W/Wv mice (4.8–8.55 s, in 35 channels: Fig. 5E; Peaks were not detected in 29 out of 64 channels in the frequency range of 9.4–27.0 cpm). Correspondingly, the period map was monotonous in colour in WT mice, but was bumpy in W/Wv mice. The average of the period was 4.2±0.6 s (n = 8) in WT, and 6.1±1.9 s (n = 5) in W/Wv mice. The standard deviation of the mean of the period in 64 channels over the recording area was 0.099±0.059 (n = 8) in WT, and 0.38±0.17 (n = 5) in W/Wv mice, indicating that the frequency estimated were spatially much more varied in W/Wv mice than in WT mice (P=0.02, unpaired t-test). These results reinforced that pacemaker cells are impaired in ileal musculature preparations in W/Wv mice.

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**Figure 4. Spectral power analysis.** A–B) Power spectrum transformed from field potentials at Ch 28 and pseudo-colour map constructed from the same array data used in Fig. 1E (the ileum of a WT mouse). C–D) Power spectrum and pseudo-colour map for a W/Wv mouse preparation. The data in Fig. 1F was used. Note the spectral peak corresponding to the oscillation frequency seen only in WT mice. E–F) Bar graphs E and F show the effect of nifedipine (1 \(\mu\)M) and TTX (250 nM) on the spectral power summed in all channels in WT and W/Wv mice, respectively. These drugs were applied to record ICC pacemaker activity by suppressing smooth muscle and enteric neurones. Asterisks represent statistical significance (P<0.05). Note that the Y-axis is expanded in C and F.

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In addition, the amplitudes of the adjacent peak of autocorrelation functions were much smaller in W/W<sup>v</sup> mice (Fig. 5D) than in WT mice (Fig. 5A): 0.41±0.27 (n=8) in WT mice; 0.14±0.02 (n=5) in W/W<sup>v</sup> mice; P=0.01, unpaired t-test. The channels in which the peak of autocorrelation function was below nought in the range of 9.4–27 cpm were not involved in the statistics for the period.

**Cross-correlation Analysis**

In order to assess the synchrony of spontaneous electric activity recorded at MEA channels, cross-correlation analysis was performed (Fig. 6). Panels in Fig. 6A–C show an example of such analysis for spontaneous electric activity in the ileal musculature of WT mice in normal solution. The same array data in Fig. 1E and Fig. 4A was again used. A cross-correlation function (curve in Fig. 6A) was derived from a field potential recording at Ch52 using a centre channel (Ch28) as the reference. Likewise, cross-correlation functions were derived from all MEA channels, and the shift (x-axis) and amplitude (y-axis) of the peak in each cross-correlation function were plotted to draw a phase-shift map (Fig. 6B) and a cross-correlation map (Fig. 6C), respectively.

Panels in Fig. 6D–F show the cross-correlation analysis applied to the MEA data obtained 20 min after application of nifedipine and TTX in the same ileal musculature preparation of WT mice. On the other hand, panels in Fig. 6G–I show the MEA data of W/W<sup>v</sup> mice (normal solution) used in Fig. 1E. Each set of the cross-correlation analysis consists of cross-correlation-function derived from Ch28 and Ch52, phase-shift and cross-correlation maps, respectively. A comparison of the phase-shift and cross-correlation maps between normal solution (Fig. 6B–C) and in the presence of nifedipine and TTX (Fig. 6E–F) indicated that these drugs reduced the propagation velocity and coupling of spontaneous electric activity along the oral-to-anal plane (longitudinal muscle: vertical plane). Similar inhibitory effects on propagation were observed in 5 out of 8 preparations of WT mice: the phase shift at 52Ch was prolonged from 0.23±0.16 s to 0.59±0.49 s (n = 5). The application of drugs, nevertheless, had little effect on the period of spontaneous electric activity estimated by auto-correlation (4.2±0.6 s vs 4.6±0.6 s, P>0.05, paired t-test, n = 8). On the other hand, analyses of W/W<sup>v</sup> mice indicated that spontaneous electric activities were not correlated even with the support of smooth muscle and neurons. Namely, the phase-shifts between electric activities were randomly distributed (Fig. 6H), and the amplitudes of cross-correlation peaks were low, except in the centre channel used as a base channel in cross-correlation analysis (Fig. 6I). These results indicated that the ICC network plays an essential role in propagating electric activity in ileal musculatures.

**Discussion**

To investigate the spatial properties of gut electrical activity, multiple conventional intracellular microelectrodes have previously been applied [26,27]. Also, due to recent progress, optical probes, including fluorescent proteins, could be applicable in future studies [28,29]. However, the former technique largely depends on personal skill and is performed in a limited number of laboratories with specialists. Also, the latter requires voltagesensitive probes with a sufficiently large change in fluorescence, and frequently affects physiological properties of cellular tissues while loading probes. On the other hand, the measurement of MEA is straightforward. After removal of the mucous membrane, musculature preparations are simply mounted on MEA, and MEA measurements on their own never damage preparations. Thus, it is possible to examine the effects of for example, several ionic solutions and chemicals sequentially in the same preparation, and also to compare electric properties of different preparations. In the present study, we carried out MEA measurements in ileal musculatures of WT and W/W<sup>v</sup> mice. In the latter preparations, it is well known that the number of ICC, gut pacemaker cells, is reduced due to a loss-of-function mutation of c-Kit receptor gene [5,7,15]. In agreement with this genetic mutation, spatio-temporal
analyses of the MEA data clearly distinguished these two preparations (Figs. 4–6).

In MEA measurements, nevertheless, several issues that can affect the quality of the study need to be considered. Firstly, if electric activity propagates only in the core of the tissue through intercellular coupling via gap junction channels, and regeneration of cellular electric activity (current) is negligible in the surface of the tissue {see Appendix in [30]}, extracellular electrodes in MEA do not detect changes in field potential. Such spontaneous electric activity may occur in some preparations like detrusor smooth muscle of the urinary bladder [31]. However, pacemaker electric activities are measurable in the stomach and small intestine [16,25], indicating that these potentials reflect local ionic channel current near electrodes at least in these preparations.

Secondly, mechanical activity may affect measurements in normal solution. Such a possibility has been suggested in extracellular recordings of the murine stomach [17]. However, mechanical effects appear to be small in ileal musculature preparations used in the present study, because in contrast to W/Wv mice, random distribution of phase-shift and poor cross-correlation were never observed in WT mice even after the application of nifedipine, where mechanical activity was negligible (Fig. 6D–F). Also, in principle, extracellular recordings detect changes in the field potential ($E_f$) as a product of local membrane current ($I_m$) and the resistance between extracellular electrodes and the reference electrode (referred to as access resistance, $R_a$):

$$E_f = I_m \times R_a.$$

Therefore, it is deduced that oscillating field potentials imply the existence of oscillating membrane currents, and that mechanical activity alone does not induce any oscillations in $E_f$ through changes in $R_a$ if $I_m = 0$.

Thirdly, it is important to apply an appropriate frequency of high-pass filtering. The impedance of the recording microelectrode ($Z_{ME}$) is given as a function of signal frequency ($f$):

$$Z_{ME} = \left\{ \frac{1}{(2\pi f \times C_{ME})^2} + (R_{ME})^2 \right\}^{1/2},$$

where $C_{ME}$ and $R_{ME}$ are the capacitance and resistance of the recording microelectrode, respectively. In recording slow electric oscillations such as in the gut, therefore, $C_{ME}$ is required to increase in order to decrease the impedance, because the former component, capacitive reactance, of the function increases at a low frequency. For this reason, we have employed microelectrodes made of platinum black nano-particles increasing the surface area

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**Figure 6. Cross-correlation analyses.** Panel A shows a cross-correlation function constructed from field potential recordings at Ch28 and Ch52 in an ileal musculature of WT mice (the same preparation shown in Fig. 1E). Panels B and C show the distribution of the phase-shift (x-axis) and the amplitude (y-axis), respectively, of the peak in cross-correlation functions derived from all 64Ch MEA data, in the same ileal preparation shown in A. Ch28 was used as the base channel. Panels D–F show cross-correlation function, phase-shift map and cross-correlation map, respectively, in the presence of nifedipine and TTX in the same preparation shown in A–C. Panels G–I were constructed from MEA data recorded from an ileal musculature of W/Wv mice (the same preparation in Fig. 5D–F).

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permeable channels across the plasma membrane. Among these oscillations in individual ICC, and depolarisations are described as a result of ionic conductances in the membrane. Therefore, it is suggested that the excitability of smooth muscle cells via gap junctions. It is likely that L-type Ca\textsuperscript{2+} channels, a major inward current for depolarization in smooth muscle cells [14], make a contribution to the intercellular coupling between ICC (Fig. S2). For example, when nifedipine suppresses inward current through L-type Ca\textsuperscript{2+} channels in smooth muscle, instead more pacemaker current from ICC is used to charge the plasma membrane in smooth muscle cells, thereby reducing the intercellular coupling between ICC by their own pacemaker current. In addition, if some voltage-sensitive Ca\textsuperscript{2+} channels, e.g. T-type Ca\textsuperscript{2+} channels exist in ICC, albeit a minor conductance [37,44] and/or in a subpopulations of ICC, the intercellular coupling is enhanced to propagate pacemaker potentials [42,45].

In conclusion, the arrayed microelectrodes with increased surface area successfully measure slowly oscillating electric activity in a small area (~1 mm\textsuperscript{2}) of ileal musculatures, due to the low impedance of microelectrodes (~31 M\textOmega{} at 0.1 Hz). Mapping of spectral power, auto-correlation and cross-correlation parameters further characterized the spatial properties of spontaneous electric activity in the ileum of wild-type (WT) and W/W\textsuperscript{v} mice, in addition to previous studies [16,37]. Also, the electric potentials generated by ICC appear to be coupled via their own network, and enhanced by smooth muscle excitability. We hope that with sufficient biophysical and technical consideration, MEA will be utilized in future studies of slowly oscillating bio-electric potentials in a wide range of tissues and organs including the gut, because ICC-like interstitial cells suspected of generating spontaneous electric activity are distributed all over the body, and can be induced from embryonic stem (ES) cells and induced pluripotent stem (iPS) cells [9,44–50].

Supporting Information

**Figure S1** 3D structure of a recording electrode of a 50 \mu{}m \times 50 \mu{}m square made with platinum black particles. A: A photo of a recording electrode with profiles along with 1A–1B and 2A–2B. The profiles were measured with a confocal laser microscope. B: A pseudo-colour 3D reconstruction. The z-axis is shown expanded. (DOC)

**Figure S2** A hypothetic scheme. The coupling of spontaneous electric activity in an ICC network is supported by the excitability of adjacent cells electrically connected via gap junctions. For example, voltage-gated L-type Ca\textsuperscript{2+} channels generate a major inward current during depolarization. Therefore, when L-type Ca\textsuperscript{2+} channels are suppressed in smooth muscle, more pacemaker current from ICC is required to charge the plasma membrane of smooth muscle cells. As a result, the intercellular coupling between ICC is reduced. (DOC)

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Author Contributions

Conceived and designed the experiments: SN. Performed the experiments: MT SK HBS KS SN. Analyzed the data: MT SK HBS KS SN. Wrote the paper: SN.
References

1. Furness J (2006) The enteric nervous system. 1st ed. Wiley-Blackwell, Inc. Malden, MA pp.:112–28.
2. Wood JD (2006) Integrative function of enteric nervous system. In Physiology of the Gastrointestinal Tract, 4th ed. Vol. 2. (eds Barrett KE, Giyanan FK, Merchant JL, Said HM, Wood JD, Johnson LR), San Diego, USA: Academic Press, 663-684.
3. Tomita T (1991) Electrical activity (spikes and slow waves) in gastrointestinal smooth muscle. In Smooth Muscle: An Assessment of Current Knowledge. Eds. Bulbring E, Bradin AF, Jones AW, Tomita T. Edward Arnold, London. 127–136.
4. Szentagothai JH (1987) Electrical basis for gastrointestinal motility. In Physiology of Gastrointestinal Tract, 2nd edition. ed. Johnson LR. Raven Press, New York. pp:339–422.

5. Maeda H, Yamagata A, Nishikawa S, Yoshinaga K, Kobayashi S et al. (2009) Requirement of c-kit for development of intestinal pacemaker system. Development 116: 369–375.
6. Fausone-Pellegrini MS, Thueberg L (1999) Guide to the identification of interstitial cells of Cajal. Microsc Res Tech. 47: 240-266.
7. Sanders KM, Orod T, Koh SD, Touhasi S, Ward SM (1999) Development and plasticity of interstitial cells of Cajal. Neurogastroenterol Motil. 11: 311–338.
8. Rumessen JJ, Vanderwinden J M (2003) Interstitial cells in the musculature of the gastrointestinal tract: Cajal and beyond. Int Rev Cytol. 229: 115–206.
9. Takaki M, Suzuki H, Nakayama S (2010) Recent advances in studies of spontaneous activity in smooth muscle: ubiquitous pacemaker cells. Prog Biophys Mol Biol. 102: 129–135.
10. Camilleri M (2002) Advances in diabetic gastroparesis. Rev Gastroenterol Disord. 2: 47–56.
11. Wang XY, Berezin I, Mikkelsen HB, Der T, Bercik P et al. (2002) Pathology of the gastrointestinal tract: Cajal and beyond. Int Rev Cytol. 229: 115–206.
12. Oka H, Shimono K, Ogawa R, Sugihara H, Taketani M (1999) A new planar magnetoimpedance sensor detection of biomagnetic fields in musculatures with spontaneous electric activity. Biosens Bioelectron 27: 34–39.
13. Brannich NJ, Bradin AF (1996) Electrical properties of smooth muscle in the guinea-pig urinary bladder. J Physiol. 492: 183–198.
14. Yagati AK, Kim SC, Min J, Choi JW (2009) Multi-bit biomemory consisting of coexisting protein variants, azurin. Biosens Bioelectron 24: 1503–1507.
15. Huizinga JD, Ambros K, Der-Silaparut T (1998) Co-operation between neural and myogenic mechanisms in the control of distension-induced peristalsis in the mouse small intestine. J Physiol. 506: 483–496.
16. Akbarali HI, Hawkins EG, Ross GR, Kang M (2010) Ion channel remodeling in interstitial cells of Cajal in the gastrointestinal tract: Cajal and beyond. Int Rev Cytol. 229: 115–206.
17. Bayguinov O, Hennig GW, Sanders KM (2011) Movement based artifacts may occur in extracellular recordings of smooth muscle activity in the human stomach. J Physiol. 589: 6105–6118.
18. Rhee PL, Lee JY, Son HJ, Kim JJ, Rhee JC et al. (2011) Analysis of pacemaker activity of smooth muscle tissue isolated from the guinea-pig stomach antrum. J Physiol. 590: 1299–1300.
19. O’Grady G (2012) Gastrointestinal extracellular electrical recordings: fact or artifact? Neurogastroenterol Motil: 24: 1–6.
20. Imtiaz MS, Katnik CP, Smith DW, van Helden DF (2006) Role of voltage-dependent modulation of store Ca2+ release in synchronization of Ca2+ oscillations. Biophys J. 90: 1–23.
21. Youn JB, Kim N, Han J, Kim E, Joo H et al. (2006) A mathematical model of pacemaker activity recorded from mouse small intestine. Philos Trans A Math Phys Eng Sci. 364: 1135–1154.
22. Fiville RA, Pullan AJ, Sanders KM, Koh SD, Lloyd CM, et al. (2009) Biophysically based mathematical modelling of interstitial cells of Cajal slow wave activity generated from a discrete unitary potential basis. Biophys J. 96: 4834–4852.
23. Means SA, Sneyd J (2010) Spatio-temporal calcium dynamics in pacemaking units of the interstitial cells of Cajal. J Theor Biol. 267: 137–152.
24. Buist ML, Corrias A, Poh YG (2010) A model of slow wave propagation and entrainment along the stomach. Ann Biomed Eng. 38: 3022–3030.
25. van Helden DF, Laver DR, Holdsworth J, Imtiaz MS (2010) Generation and propagation of gastric slow waves. Clin Exp Pharmacol Physiol 37: 516–524.
26. van Helden DF, Laver DR, Holdsworth J, Imtiaz MS (2010) Generation and propagation of gastric slow waves. Clin Exp Pharmacol Physiol 37: 516–524.
27. Hotta A, Kato Y, Suzuki H (2005) The effects of thiol-specific antioxidants on spontaneous activity of smooth muscle tissue isolated from the guinea-pig stomach antrum. J Smooth Muscle Res. 41: 207–220.
28. O’Grady G, Du P, Paskaranandavadivel N, Angeli TR, Lammers WJ et al. (2012) Rapid high-amplitude circumferential slow wave propagation during normal gastric pacemaking and dysrhythmias. Neurogastroenterol Motil. 24: e299–e312.
29. Huizinga JD, Fausone-Pellegrini MS (2005) About the presence of interstitial cells of Cajal outside the musculature of the gastrointestinal tract. J Cell Mol Biol. 306: 468–473.
30. Harhour MI, Pucovsky V, Pustovyan OV, Gortenkov DV, Bolton TB (2005) Interstitial cells in the vasculature. J Cell Mol Med. 9: 232–243.
31. Ishikawa T, Nakayama S, Nakagawa T, Horiguchi K, Misawa H et al. (2004) Characterization of in vitro gut-like organ formed from mouse embryonic stem cells. Am J Physiol Cell Physiol. 286: C1344–1352.
32. Brading AF, McCloskey KD (2005) Mechanisms of disease: specialized interstitial cells of the urinary tract—an assessment of current knowledge. Nat Rev Urol. 2: 546–556.
33. Lang RJ, Tonta MA, Zolkowski BZ, Meelee WF, Wends I et al. (2006) Peyooutereotoxic peristalsis: role of atypical smooth muscle cells and interstitial cells of Cajal-like cells as pacemakers. J Physiol. 576: 695–705.