Spontaneous Action Potential from Threadlike Structures on the Surface of Abdominal Organs

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The action potential is classified as a long-lasting limit cycle (repetitive pattern) of the electrical potential difference over a biological membrane resulted from the nonlinear transport of ions across the membrane. A threadlike structure with nodes in the abdominal cavity closely resembled the primo vascular system (Bonghan system) which was proposed to be the anatomical reality of acupuncture meridians. The threadlike structure consisted of thread parts and nodes.

The membrane potential of cells, from the thread parts and nods, was measured to investigate the electrophysiological characteristics of the cells and the existence of excitable cells. To this end, intracellular recording technique was applied to measure action potentials. We examined dozens of thread part and node samples of rats in order to measure spontaneous action potentials, and interestingly, we succeeded in three cases; two cases for thread parts and one case for a node. Although the three cases of action potentials were different from one another in the characteristics of action potentials and resting potentials, the full width at half maximum (FWHM) of action potentials was tens of milliseconds in common. Their FWHMs were significantly different from that of a neuron, a skeletal muscle cell and a cardiac muscle cell. This result suggests that these action potentials were measured from smooth muscle cells, secreting cells, or immune cells.

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I. INTRODUCTION

In the early 1960's, Bong-Han Kim claimed to have discovered a novel circulatory system which is a body-wide web of vascular threadlike structures in an animal and in which a new fluid, named the primo fluid (Bonghan liquid) [1]. This system is more primordial than the vascular and the lymphatic systems in the developmental sense and possibly in the evolutionary sense. The primo fluid keeps an animal healthy by regenerating damaged tissues and healing wounds. It is a transparent fluid with microcells that do cell therapy like totipotent stem cells in modern terminology. He proposed that the primo vascular system (PVS, Bonghan system) was an anatomical structure corresponding to the acupuncture points and meridians. Bong-Han Kim did not, however, disclose his methods of observing the system, so no one could confirm his claims, despite intensive attempts in China, Japan, and Russia, except for one Japanese anatomist, Fujiwara [2].

Starting from 2002, researchers at Seoul National University (SNU) have developed methods employing modern technologies to rediscover most of the PVS in various parts of an animal's body [3] and to investigate the morphological aspects of the primo microcells [4]. Especially, an effective staining method with Trypan blue, which stains the PVS preferentially to other tissues like blood vessels, lymph vessels, nerves, and membranes, was found [5]. This method led to a surprising discovery of the PVS on surfaces of tumor tissues [6], which was a hitherto unknown metastasis route of cancer [7].

The anatomical gross features [8] and the histological characteristics [9] of the PVS are distinctively different from those of blood vessels, lymphatic vessels, nerves, and fibrin strings. However, there have been only a few reports on the functional aspects of the PVS [3]. Especially, an electrophysiological study of the PVS was begun only recently, and preliminary results for the action potentials (APs) of primo nodes (Bonghan corpuscles) were presented [10].

An important aim of an electrophysiological study of the PVS is to investigate its function. The expected one is a new circulatory function. The supporting evidence for circulation in primo vessels (Bonghan ducts) was obtained by observing the flow of a staining dye, Alcian blue, which was injected into a primo node [17]. The presence of channels in a primo vessel [18] and the existence of endothelial cells in the wall of the channels [9] provided support for the flow of a fluid in the PVS. However, the means by which the fluid is being moved along the vessel has not yet been determined. We assumed that the circulation of primo fluid is brought about by peristaltic contraction of smooth muscle. In order to identify this assumption, we tried finding them by histological analyses such as H&E staining and IHC method but failed. One thing that can be considered as the main cause of the failure is the possibility of the existence of 'smooth muscle-like cells' instead of 'smooth muscle cells' as Bong-Han Kim described in his paper.

Therefore it became necessary to study spontaneous and stimulated APs of the PVS. In the present study, we found threadlike structures on the surface of abdominal organs which resembled the primo vascular system. We measured the spontaneous APs of the cells in the threadlike structures. The characteristics of the action potentials were analyzed and compared with various kinds of excitable cells.

II. MATERIALS AND METHODS

1. Sample preparation

Sprague-Dowley rats (male, 6-week old, weighing 200-250g) were obtained from Hanlym Animal Lab for this study. The animals were housed in a temperature-controlled environment (constant temperature of 23℃) with a 60% relative humidity under a 12-hour light/darkness cycle. The procedures involving the animals and their care fully complied with the institutional guidelines of Seoul National University and with current international laws and policies (Guide for the Care and Use

of Laboratory Animals, National Academy Press, 1996).

The threadlike structures can be found by using a stereomicroscope (Olympus, SZX12). The morphological characteristics of the threadlike structures follow (Fig. 1). First, they were freely moving threadlike structures connecting nodes. Second, their colors were milky white, but sometimes the nodes were reddish. Third, the diameter of thread parts were 10~50 μm.

Samples on the organ surface satisfying above criteria were taken carefully and moved to a prepared Krebs solution (composition, mM: NaCl, 115.48; KCl, 4.61; $MgSO_4$, 1.16; $CaCl_2$, 2.50; $NaHCO_3$, 21.90; NaH_2PO_4, 1.14; glucose, 10.10) gassed with carbogen (95% O_2, 5% CO_2). The PVS sample was fixed on sylgard by using L-shaped pins, as shown in Fig. 2, and it was placed in a chamber (volume, 0.4 ml), which was mounted on a stage of a inverted microscope for measurement. The sample was perfused with warmed (36℃) and carboxygenated Krebs solution at a constant flow rate (2 ml/min) and left in this condition for at least 1 hour to equilibrate before microelectrode recording. Sometimes 50uM acetylcholine was added to the solution for 5minutes during measurement.

2. Experimental setup

The electrode was made by pulling a quartz tube (Sutter Instruments, QF100-70-7.5) with the puller (Sutter Instruments, P-2000). Individual cells in a primo vessel were impaled with the micropipette electrode filled with 3M KCl (tip resistance, 30-60 MΩ). A short piece of Ag/AgCl wire was inserted into it. Ag/AgCl pellet wrapped in cotton which was moistened with 3M KCl was used for a reference electrode. The signal from the electrode was amplified in the headstage, digitized in the digital multimeter (HP, 3458A) at a sampling rate of 1 kHz. The sampled data was sent to a computer through a GPIB cable and saved by LabVIEW 8.5 (National Instruments) software.

The pulse generator (Keithley 236) generates a square pulse with a constant voltage and it is

converted into a constant current in the headstage. A constant current pulse of 1nA was supplied to the microelectrode and it was used for measuring resistance of the microelectrode.

For the intracellular recording, the electrode can be moved by a motorized manipulator (Sutter Instruments, MP-225), where speed and resolution of movement are easily selected. First, the electrode was moved as close to the sample as possible at a fast moving mode; then, it was inserted into the sample at a precise moving mode. The microscopic view from the CCD camera (Infinity 1, Lumenera) is displayed on the LCD monitor.

Krebs solution was heated right before it enters the chamber. The temperature of the solution kept constant at a set value by a feedback control system. The electric power of the heater was supplied by DC battery in other to prevent external power noise interference.

The most components of the setup were kept in a Faraday cage to minimize electrical noise and placed on an anti-vibration table to reduce mechanical noise (Fig. 3).

3. System calibration

The cell membrane potential of a colonic circular smooth muscle cell from a rat was measured in order to test our equipment. The average cell membrane potential was -45 mV, and the spontaneous APs (amplitude: 40 mV) with slow waves (amplitude: 6 mV, time period: 33 s) was observed (Fig. 4). The measurement data were in good agreement with typical values for a smooth muscle.

III. RESULTS

We measured spontaneous APs of cells in the thread parts and the respectively; we succeed two times in the thread parts and once in a node during the attempt of dozens of times. Several uncertain cases were excluded; they were measured by extracellular recording technique or resting potential was changed abruptly. In clear three cases, regularly oscillating APs were observed but the characteristics

of them were all different.

In the first case of a thread part (TP1), the resting potential was -22mV while the average amplitude of the APs was 20mV. The frequency of APs was 3.9min^{-1}. Stimulation by acetylcholine (50uM in Krebs solution) changed the frequency to a lower value (2.3min^{-1}). After the sample had been washed with Krebs solution, the frequency was slightly higher (2.7min^{-1}) (fig.5)

The resting potential was -90mV in the second case of the thread part (TP2) while the average amplitude of the APs was 80mV. Spontaneous APs with time interval of 2 seconds were generated regularly. There are small peaks between APs; these are estimated to occur from adjacent cells. Response of acetylcholine could not be observed in this case (fig.6)

In the case of the node, the resting potential was about -60mV although it is unstable. The average amplitude of APs was regular 40mV, whereas the time intervals of the APs were irregularly variable from 0.44 to 1.6 seconds. Generation of APs was suddenly disappeared at one moment but it was reappeared for a while after applying of acetylcholine (fig.7)

Some characteristics of APs were analyzed for several time sections as summarized in Table 1~3. Number of peaks, resting potential, average and standard deviation of time interval, amplitude and full width at half maximum (FWHM) in each time section were calculated by Origin 8.5.

IV. DISCUSSION

Despite anatomical existence of the PVS, its physiological function has not yet been clear. One of possible functions is the circulation of primo fluid through the PVS not blood or lymph vessel; there are several supporting evidences [9, 17, 18]. Another possible function is secreting hormones in response to external mechanical stimulus such as acupuncture. To clarify this subject, electrophysiological study is expected to provide what kind of cell types are existed in the PVS. Indeed, in the current study, the spontaneous APs were measured from cells of the threadlike structure which

closely resembled the PVS and the APs were compared with known excitable cells.

Generally, the resting potential of a neuron is about -70mV. A cardiac muscle cell and a skeletal muscle cell are about -80mV and -90mV respectively. In the case of a smooth muscle cell, the resting potential is about -50mV. Furthermore, only neurons, various kinds of muscle cells, epithelial cells of glands, immune cells and fertilized eggs are known to excitable cells generating APs.

The patterns of bursts in the threadlike structures we studied showed three distinctive patterns. The resting potential of TP1 was -20mV. This value is considerably different with excitable cells of other type. Although the resting potential in TP2 is similar to that of a skeletal muscle, the FWHM is quite longer than that of a skeletal muscle; FWHM in TP2 was 19.5ms whereas that of a skeletal muscle is below 1ms.

FWHM of measured APs from the PVS was ranged from 20 to 78ms. This is significantly different with a neuron, a skeletal muscle cell, and a cardiac muscle cell; the FWHM of an AP of a neuron and a skeletal muscle cell is below 1ms and that of a cardiac muscle cell is over 200ms. Therefore we can exclude these kinds of cells from measured data. The rest were smooth muscle cell, secreting cell, or immune cell. In the previous study for the PVS, it was revealed that the PVS produces catecholamine as a novel endocrine tissue and also it has many immune cells like lymphocytes.

Based on the facts that have been discussed so far, we concluded that the measured APs are from smooth muscle cell, secreting cell or immune cell. This result does not seem surprising but it is significant in the sense that the threadlike structures have excitable cells and they produce spontaneous APs. From this we may assume as follows.

APs are produced by opening and closing of voltage sensitive ion channels. Ligand gated ion channels and mechanically gated ion channels are also involved in producing of them. In particular, mechanically gated ion channels play a prominent role in sensory neurons such as Meissner's corpuscle.

If the node of threadlike structure is relevant to an acupuncture point, the cell of the threadlike structures must have mechanically gated ion channels on its membrane in order to response for a

mechanical stimulus of acupuncture. It would be of great interest to investigate this further and it may reveal the mechanism of acupuncture treatment.

To this end, we need to separate cells from the threadlike structures with collagenase for the patch clamp study. Another reason for cell isolation is the low success rate of our measurements, i.e., only three cases out of dozens of samples. The cells exhibiting APs lie not on the surface of tissue, but deep inside, the threadlike structures. Due to the fragility of the thin and weak tips of microelectrode, reaching cells lying deep inside tissue without breaking is demanding.

Further research on the AP to elucidate the electrophysiological properties, such as the presence of Cajal cells [20], the receptor types, and the channel characteristics, by using stimulating chemicals [21] is worth pursuing in the future.

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REREFENCES

[1] B.H. Kim, J. Acad. Med. Sci. DPR Korea **90**, 1-41 (1963).

[2] S. Fujiwara and S. B. Yu, Igaku no Ayumi **60**, 567-77 (1967) [In Japanese].

[3] K. S. Soh, J. Acupunct. Meridian Stud. **2**, 93-106 (2009).

[4]. K. Y. Baik, V. Ogay, S. C. Jeoung, and K. S. Soh,. J. Acupunct. Meridian Stud. **2**, 124-9 (2009).

[5] B. C. Lee, K. S. Kim and K. S. Soh, J. Acupunct. Meridian Stud. **2**, 66-70 (2009).

[6] J. S. Yoo, H. B. Kim, V. Ogay, B. C. Lee, S. Ahn and K. S. Soh, J. Acupunct. Meridian Stud. **2**, 113-23 (2009).

[7] J. S. Yoo., *In vivo Tracking of Cancer Cells in Bonghan Channels using Multispectral Fluorescence Imaging System*. (Ph.D. Thesis, Seoul National University, 2009).

[8] H. S. Shin, H. Johng, B. C. Lee, S. Cho, K. Y. Baik, J. S. Yoo, et al., Anat. Rec. B New Anatomist **284**, 35-40 (2005).

[9] V. Ogay, K. H. Bae, K. W. Kim and K. S. Soh, J. Acupunct. Meridian Stud. **2**, 107-17 (2009).

[10] S. H. Park, B. C. Lee, C. J. Choi, K. S. Soh, J. H. Choi, S. Y. Lee, and P. D. Ryu, J. Kor. Phys. Soc. **55**, 688-93 (2009).

[11] S. J. Lee, B. C. Lee, C. H. Nam, W. C. Lee, S. U. Jhang, H. S. Park, et al., J. Acupunct. Meridian Stud. **1**, 97-109 (2008).

[12] K. G. Bau and F. der Haut, Dtsch Zschr Akup **15,** 1-31 (1966) [In German].

[13] W. S. Kroger, Am. J. Psychiatry **130**, 855−60 (1973).

[14] E. Meng, J. Young, et al., Neurourology and urodynamics **27**, 79 (2008).

[15] D. Budai, Acta Biol. Szeged. **48**, 13-17 (2004).

[16] S. Ward, S. Harney, et al., J. Physiol. **505**, 241-258 (1997).

[17] B. Sung, M. S. Kim, B. C. Lee, J. S. Yoo, S. H. Lee, Y. J. Kim, et al. Naturwissenschaften **95**, 117-24 (2008).

[18] B. C. Lee, J. S. Yoo, V. Ogay, K. W. Kim, H. Dobberstein, K. S. Soh, et al., Microsc. Res. Tech. **70**, 34-43 (2007).

[19] E. Kandel, Sci. Am. **241**, 61-70 (1979).

[20] L. Thomson, T. L. Robinson, et al., Nat. Med. **4**, 848-851 (1998).

[21] S. H. Woo, H. L. Byung, K. I. Kwon, and C. O. Lee, Arch. Pharm. Res. **28**, 930-935 (2005).

Table 1. Characteristics of action potential for TP1

Time section (s)	200–360	680–1180	1300–1780
Number of peaks	7	19	20
Resting potential (mV)	-21.6	-21.9	-27.9
Interval (s)	15.2 ± 1.8	25.9 ± 3.0	22.2 ± 2.4
Amplitude (mV)	20.7 ± 0.8	16.0 ± 1.1	18.7 ± 1.6
Half-width (ms)	28.0 ± 4.2	43.0 ± 6.0	74.9 ± 15.4

Table 2. Characteristics of action potential for TP2

Time section (s)	0–30
Number of peaks	14
Resting potential (mV)	-88.7
Interval (s)	2.1 ± 0.1
Amplitude (mV)	79.8 ± 0.3
Half-width (ms)	19.5 ± 0.8

Table 3. Characteristics of action potential for the node of a threadlike structure

Time section (s)	0–30	80–105	130–150	330–350
Number of peaks	67	15	26	14
Resting potential (mV)	-56.6	-55.3	-57.4	-52.8
Interval (s)	0.44 ± 0.034	1.6 ± 1.0	0.73 ± 0.14	1.26 ± 0.17
Amplitude (mV)	40.5 ± 0.7	39.2 ± 1.7	42.3 ± 0.54	39.1 ± 0.89
Half-width (ms)	60.1 ± 5.7	64.4 ± 16.9	78.3 ± 14.0	64.2 ± 7.2

Figures

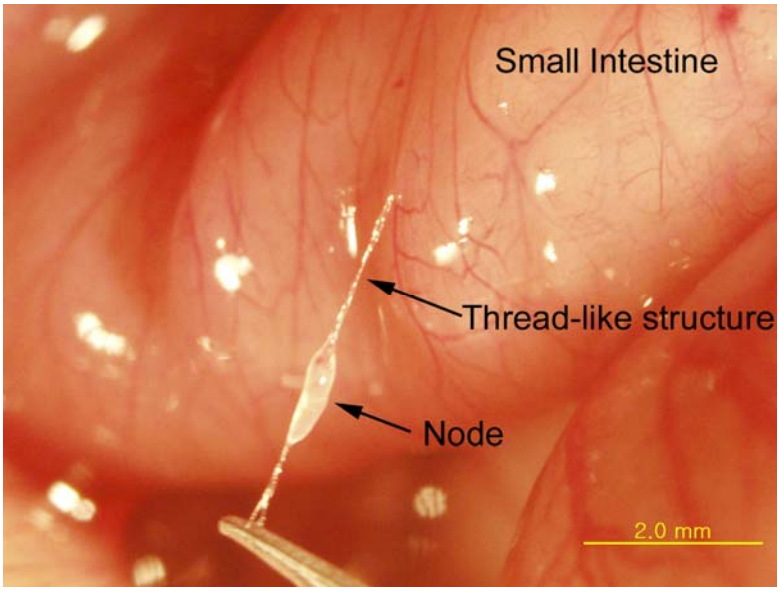

Fig. 1. Primo vessel on the surface of the small intestine of a rat.

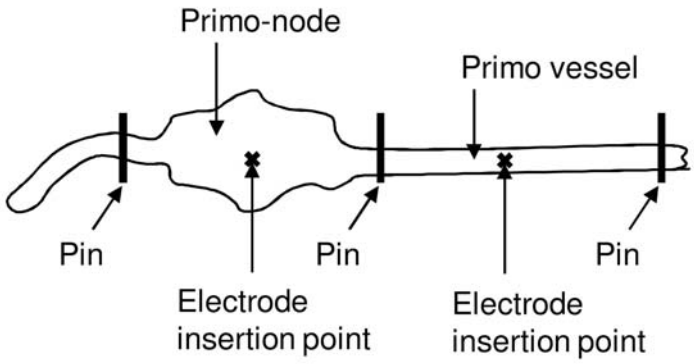

Fig. 2. Primo vessel fixed with pins on sylgard in the sample chamber.

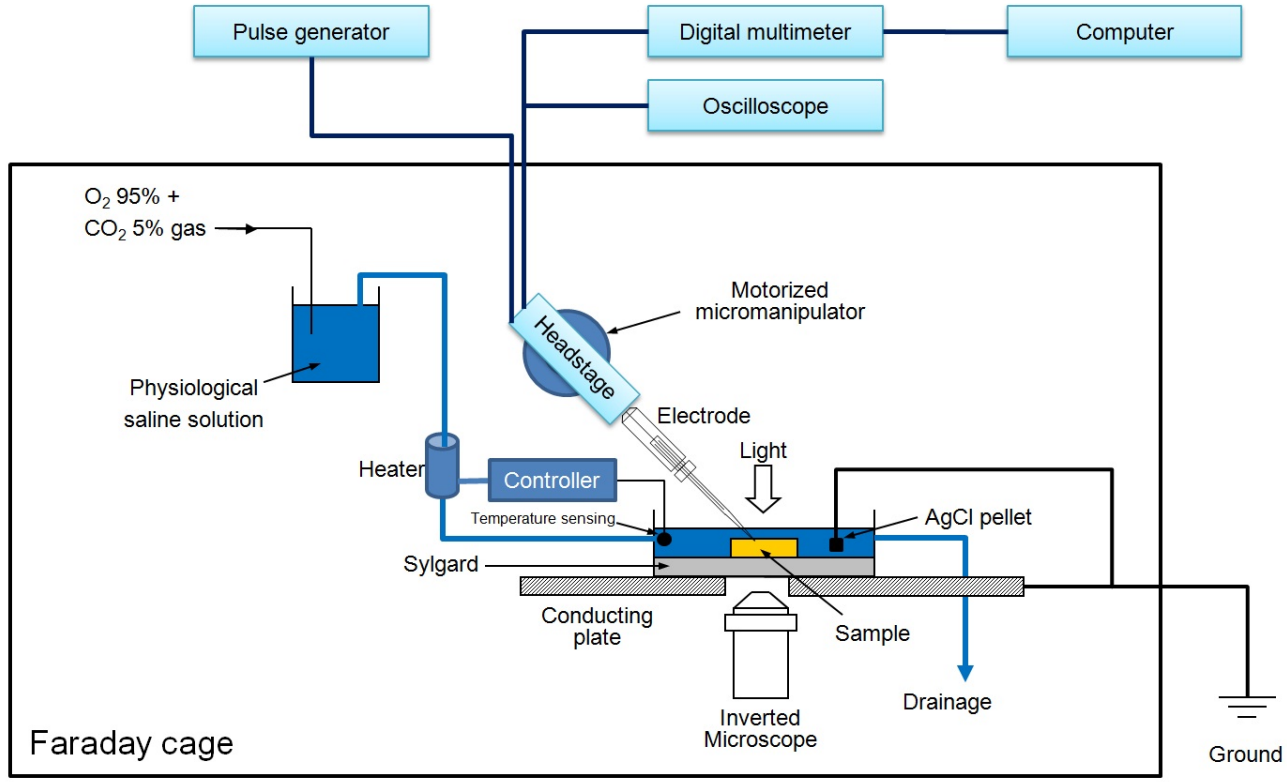

Fig. 3. Diagram of the experimental setup.

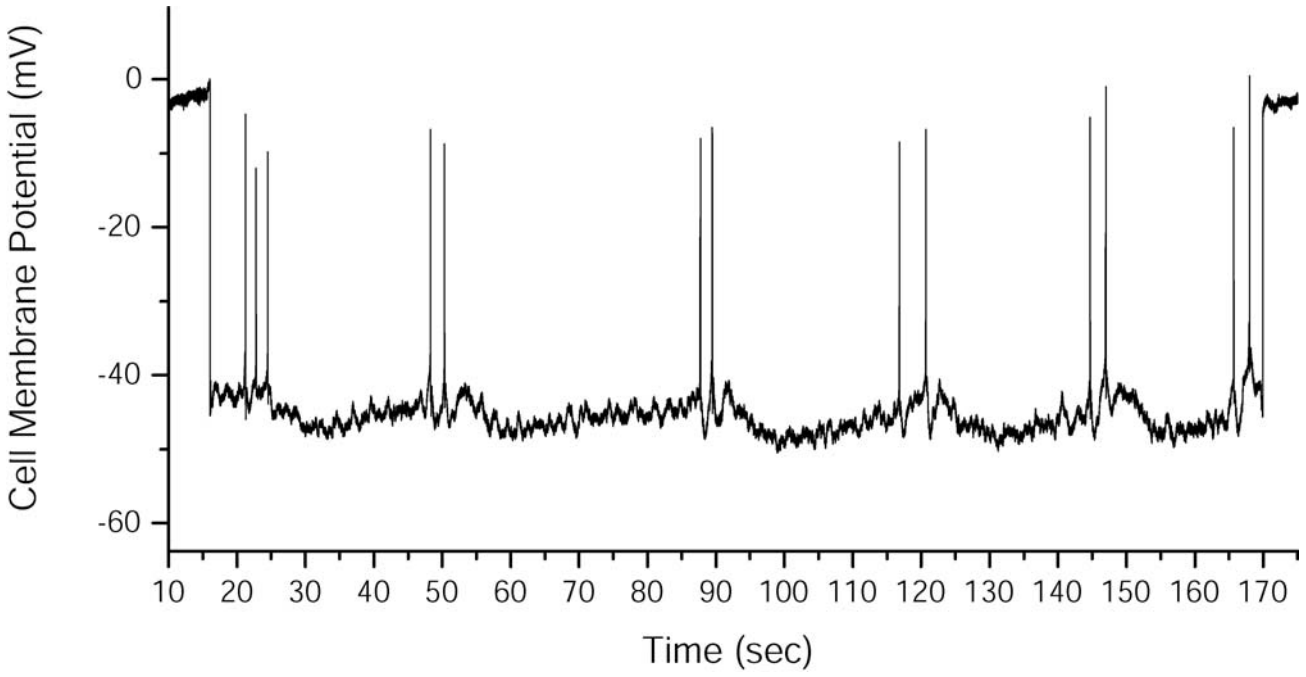

Fig. 4. Spontaneous action potential of a colonic circular smooth muscle cell from a rat.

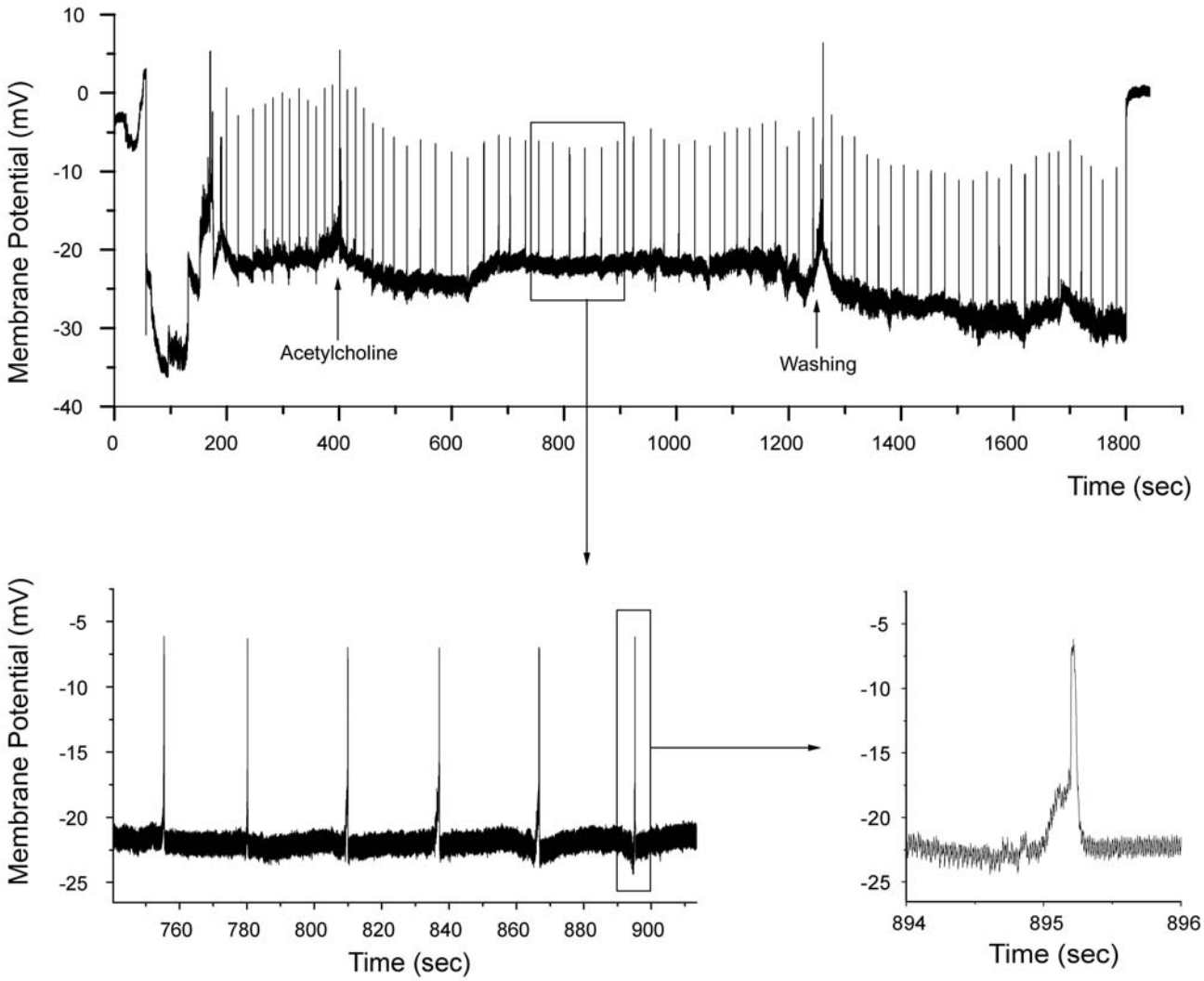

Fig. 5. Spontaneous action potential of TP1

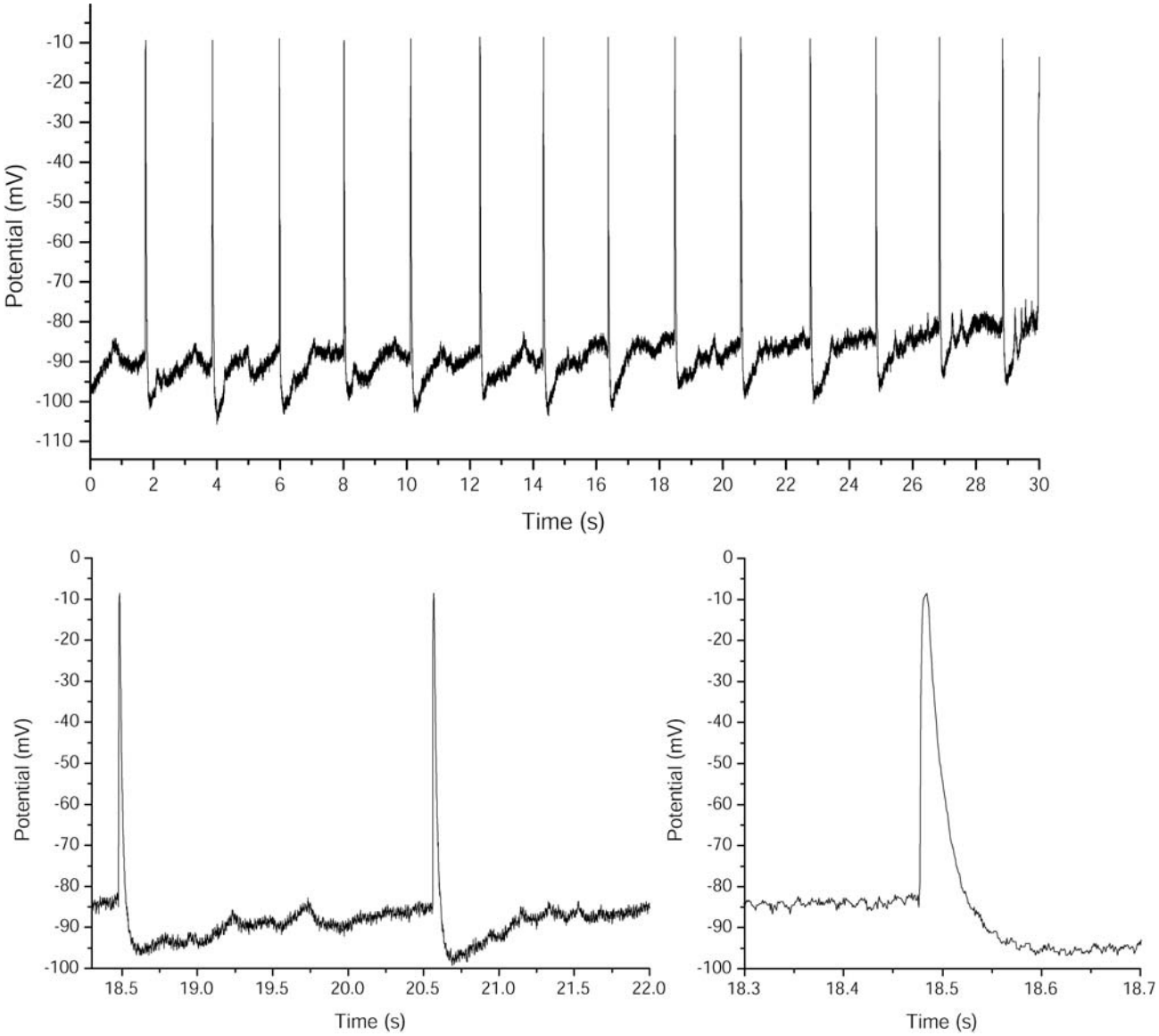

Fig. 6. Spontaneous action potential of TP2

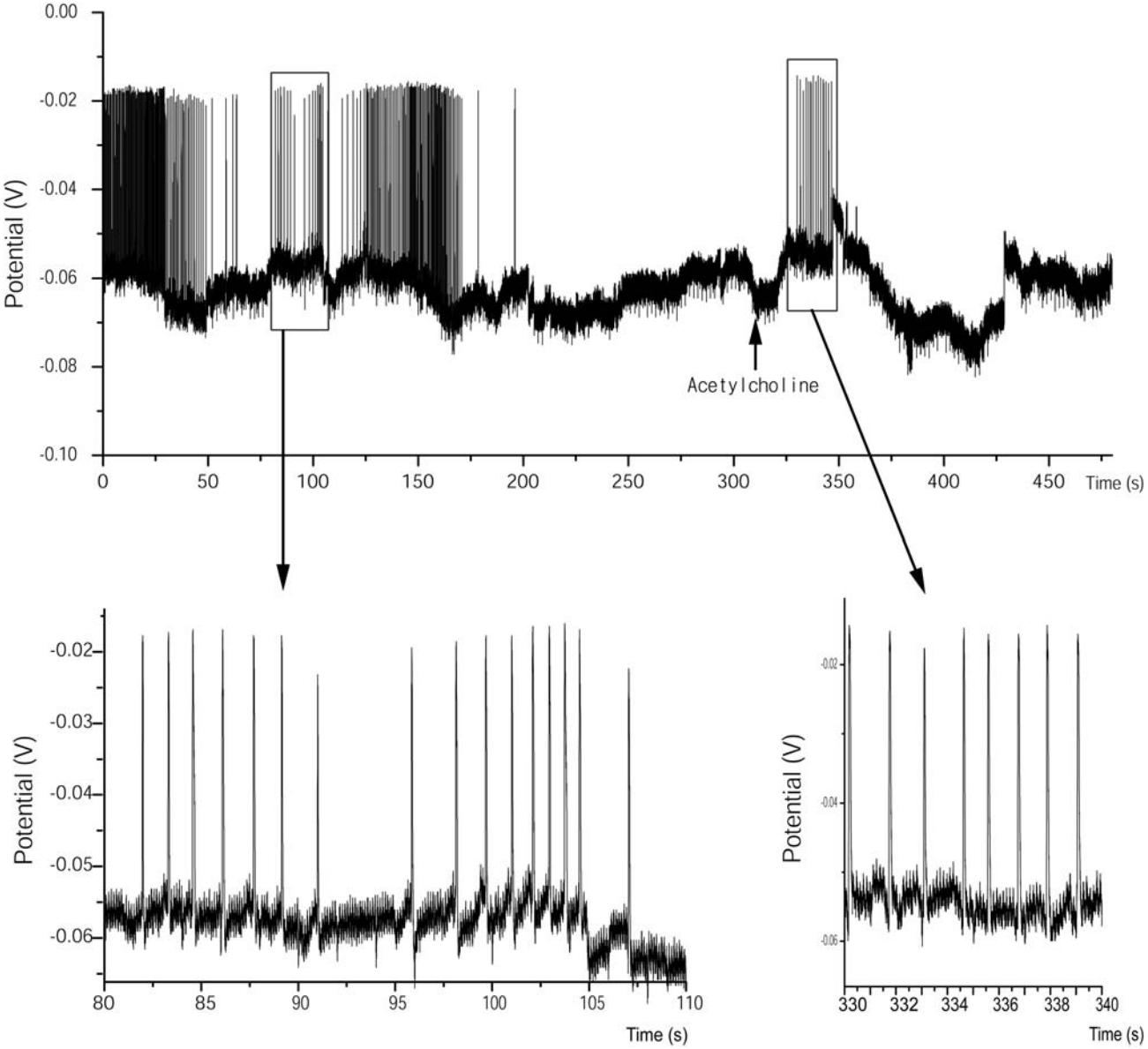

Fig. 7. Spontaneous action potential of the node of a threadlike structure