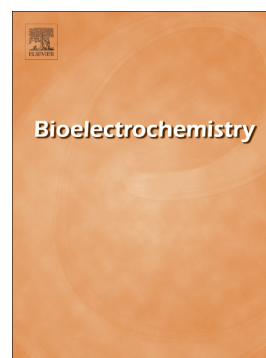


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The origin of hyperpolarization based on the directional conduction of action potential using a model nerve cell system

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ABSTRACT

In nerve cells, changes in local membrane potentials are generated and propagated along a nerve axon mainly by the function of K^+ and Na^+ channels. Generally, concurrent monitoring of multi-points on an axon is performed based on the voltage-clamp method. As the respective membrane potentials have been evaluated by considering the relations between the applied potential, the local current, and conductance, experimental values are not directly evaluated. We directly measured the actual membrane potentials and local currents of the respective cells using a nerve-model system comprising liquid-membrane cells. It was then proven that the action potential spreads along the axon toward the axon terminal due to the function of both the channel-type receptors in the synapse and voltage-gated Na^+ channels on the axon, and that hyperpolarization cannot be caused by only the operation of the delayed- K^+ and the voltage-gated Na^+ channels.

Keywords: Directional nerve conduction, Action potential, Liquid-membrane cell, Ion transport, Membrane potential, Hyperpolarization

1. Introduction

In nerve cells, the action potential is generated and propagated mainly by the operation of K^+ and Na^+ channels [1,2]. The normal membrane potential of a nerve cell is called the resting potential, and it is determined by the ratio of the K^+ concentration inside to that outside. When an external stimulus promotes the release of neurotransmitters, channel-type receptors at the synapse open by binding to them. Since Na^+ mainly flows into the nerve cell, the membrane potential of the surrounding area starts to change from the resting potential to the action potential determined by the ratio of the Na^+ concentration inside to that outside. The change in the membrane potential then spreads nearby. The membrane potential at the axon then exceeds the threshold, and voltage-gated Na^+ channels on the axon surface open with short open-lifetimes (about 1 ms). The membrane potential subsequently returns to the resting potential within about 1 ms due to both closing of the voltage-gated Na^+ channels and opening of the delayed- K^+ channels. It appears that the part demonstrating the action potential runs along the axon toward the synaptic terminal. Hyperpolarization is frequently observed at this time.

Concurrent monitoring of the membrane potentials at the multi-points on a certain axon was performed based on the voltage-clamp method as usual [1-3]. However, the actual membrane potentials were not directly measured at the respective points as they were estimated considering the constant membrane potential, the potential differences converted from both the local current flows and the conductivities. On the other hand, many papers have been published on nerve conduction using voltage-sensitive dyes [4,5], but hyperpolarization has not been clearly observed due to the low time-resolution of charge-coupled device cameras. Therefore, the details on hyperpolarization have not been revealed yet. On the other hand, various neural models including the oscillator and the cable theory have been proposed [6-9]. Further complicated simulations on propagation of the action potential have been also conducted by considering various channel functions [10,11]. Several

research groups referred to generation and propagation of the action potential caused by the electromagnetic field [12-15].

The author's group determined the propagation of the change in membrane potential using liquid-membrane (LM) cells as a model of the nerve system [16-23]. Ueya et al. initially built a U-shaped LM cell to investigate how to propagate the change in the membrane potential by adding the transfer ion to one aqueous phase [16]. Kushida et al. constructed LM-cell systems to mimic the resting and action potentials and investigated the conduction mechanism by connecting multiple LM-cells through a switch [17-19]. Additionally, Takano et al. developed LM-cell systems possessing the functions of K^+ and Na^+ channels to mimic actual nerve cells [20]. They also improved the cells to mimic voltage-gated Na^+ channels using relay switches within the electric circuit [21]. Kaji, et al. then studied the generation and propagation of the action potential by an external electric stimulus using the LM-cell system [22].

In the present study, a model system mimicking the nerve cell was built by connecting several LM cells possessing the functions of the always-open K^+ channel, the delayed- K^+ channel, the channel-type receptor (Na^+ channel) in the synapse, and the voltage-gated Na^+ channel on the axon. The changes in the membrane potential and membrane current for all the LM cells were recorded, and the directional conduction of the action potential was actually determined. The effect of the delayed- K^+ channel on the action potential propagation and the origin of hyperpolarization were also assessed.

2. Experimental

2.1. Chemicals

Sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB) were synthesized and purified in a similar manner to that described in a previous study [24]. The potassium (KTFPB) and tetrabutylammonium (TBATFPB) salts of TFPB⁻ were obtained analogously to a previous study [24]. All other chemicals were of analytical reagent grade and were used without further purification.

2.2. Electrochemical cells

An LM cell consisted of two aqueous phases (W1 and W2) and a nitrobenzene phase (NB), as illustrated in Fig. SI-1. The NB phase was impregnated in a T100A025A polytetrafluoroethylene membrane filter (pore size: 1 μm , thickness: 75 μm , Advantec Tokyo, Ltd.), and the filter was employed as a structural material to stabilize the NB phase. W1 and W2 were saturated with NB to prevent NB outflow from the filter.

Fig. SI-1

2.3. Apparatus

Potentiometric measurements were performed by use of electrometers (HJE-100, Hokuto Denko Co., Japan). The local circulating currents were measured by ammeters (HM-103, Hokuto Denko Co., Japan). All data were recorded using an A/D converter model GL7000 (Graphtec. Co., Japan) and a personal computer. Switching of the electric circuit of the model nerve cell system was performed with a rotary switch (MR-3-4-Z, NIDEC Copal Electronics, Inc., Japan). A reed relay 291-9669 (RS Components, UK) was employed as a relay switch to mimic the open condition of the voltage-gated Na⁺ channels, and an LMC555CINS Timer (Texas Instruments Inc., USA) was used as a timer to mimic both the open life of the voltage-gated Na⁺ channels and the delay time for opening the delayed-K⁺ channel. In the potential sending site corresponding to the

synapse, the RP (Resting Potential) cell and the AP (Action Potential) cell mimicked K^+ channels and channel type receptors (Na^+ channels) in the synapse, respectively. On the other hand, the Rec cells and the V-ap cells in the potential receiving site performed functions of K^+ channels and the voltage-gated Na^+ channels, respectively, on the nodes of Ranvier. When the membrane potential (E_{W2-W1}) of the Rec cell exceeded the threshold as the standard (0 mV), the V-ap cell was connected to the electric circuit for a given time (0.011 s) with the relay switch and the timer. The ionic compositions of the RP cell, AP cell, Rec cells 1-3, and V-ap cells 1-3 are given by cell configurations (1), (2), (3), and (4), respectively.

(W1)	(LM)	(W2)	(1)
1×10^{-3} M KCl	1×10^{-3} M KTFPB	2×10^{-2} M KCl	
4.5×10^{-2} M $MgCl_2$	1×10^{-2} M TBATFPB	4×10^{-2} M $MgCl_2$	
T1-1, B1-1		T1-2, B1-2	
(W1)	(LM)	(W2)	(2)
2×10^{-2} M NaCl	4×10^{-3} M NaTFPB	1×10^{-3} M NaCl	
4×10^{-2} M $MgCl_2$	1×10^{-2} M TBATFPB	4.5×10^{-2} M $MgCl_2$	
	2×10^{-2} M DB18C6		
T2-1, B2-1		T2-2, B2-2	
(W1)	(LM)	(W2)	(3)
1×10^{-3} M KCl	1×10^{-4} M KTFPB	2×10^{-2} M KCl	
4.5×10^{-2} M $MgCl_2$	1×10^{-2} M TBATFPB	4×10^{-2} M $MgCl_2$	
T3-1, B3-1		T3-2, B3-2	
T5-1, B5-1		T5-2, B5-2	
T7-1, B7-1		T7-2, B7-2	

(W1)	(LM)	(W2)	(4)
2×10^{-2} M NaCl	1×10^{-4} M NaTFPB	1×10^{-3} M NaCl	
4×10^{-2} M MgCl_2	1×10^{-2} M TBATFPB	4.5×10^{-2} M MgCl_2	
	2×10^{-2} M DB18C6		
T4-1, B4-1		T4-2, B4-2	
T6-1, B6-1		T6-2, B6-2	
T8-1, B8-1		T8-2, B8-2	

In each cell, aqueous phase 1 (W1), aqueous phase 2 (W2), and the organic phase (LM) mimicked the extracellular solution, intracellular solution, and cell membrane, respectively. All the W1 and W2 phases of the eight cells contained the same Cl^- concentration of (0.1 M: $\text{M} = \text{mol dm}^{-3}$). The Ag|AgCl electrodes (T1-1, T1-2, T2-1, T2-2, T3-1, T3-2, T4-1, T4-2, T5-1, T5-2, T6-1, T6-2, T7-1, T7-2, T8-1, and T8-2) were immersed in both W1 and W2 of the respective cells to electrically connect with each other through the electric circuit. The $E_{\text{W2-W1}}$ values of all cells were measured with Ag|AgCl electrodes (B1-1, B1-2, B2-1, B2-2, B3-1, B3-2, B4-1, B4-2, B5-1, B5-2, B6-1, B6-2, B7-1, B7-2, B8-1, and B8-2) inserted in both W1 and W2 of the respective cells. Here the potentials of the Ag|AgCl electrodes in the W1 side were treated as a standard.

2.4. Electrochemical Measurements

As illustrated in schematic 1(A), the cell system mimics one part of the nerve cell. The parts corresponding to the synapse and the nodes of Ranvier of the axon are labeled the sending and receiving sites, respectively. Here, it is assumed that three nodes of Ranvier (receiving sites (I)-(III)) are present on the model axon. As shown in schematic 1(B), eight LM cells were connected in parallel. In the sending site corresponding to the synapse, the RP and AP cells were set to imitate

Schematic 1

always-open K^+ channels and channel-type receptors (Na^+ channels), respectively. The cell configurations of the RP and AP cells are given by cell configurations (1) and (2), respectively. The change in E_{W2-W1} from the resting to the action potential at the synapse was mimicked by switching the electrical connection with receiving sites (I)-(III) from the RP to the AP cell. In the receiving site, the Rec cells (Rec1, Rec2, and Rec3) corresponding to the always-open K^+ channels were always connected to the electric circuit. The cell configurations of the Rec cells are given by cell configuration (3). Similarly, the V-ap cells (V-ap1, V-ap2, and V-ap3) correspond to the voltage-gated Na^+ channels in respective receiving sites (I)-(III). The cell configurations of the V-ap cells are given by cell configuration (4). In the same receiving site, the adjacent V-ap cell was connected to the electric circuit for 0.011 ms if E_{W2-W1} of the RP cell exceeded 0 mV, using the relay switch and timer. Here, 0 mV is the threshold for opening the voltage-gated Na^+ channels. The currents flowing across the respective LM cells corresponded to those induced by ion transport across the respective channels at each node of Ranvier. As the resistivity of the inner solution between two adjacent nodes of Ranvier exceeds $100 \Omega \text{ cm}^{-1}$ in the myelinated nerve [23], the resistances were assumed to be 100Ω when the inner diameter of the axon was $1 \mu\text{m}$ and the length 1 cm. The resistors (100Ω) were then set between the respective receiving sites in the present experiment. For the sake of conciseness, it was hypothesized that the extracellular resistance is negligible. The current (i_{W2-W1}) flowing through each cell was measured by the ammeter connected to each cell. Here, it was defined that the direction of the current flow due to cation transfer from W2 (outside) to W1 (inside) is positive. Furthermore, the effect of the delayed- K^+ channels was investigated.

A D-RP cell imitating the delayed- K^+ channel was connected to the electric circuit, as shown in schematic 1(C). The ionic composition of the D-RP cell is given by cell configuration (3). The D-RP cell was connected via the relay switch to the electric circuit when the E_{W2-W1} value of the Rec1 cell

was below 40 mV after breaking the connection of V-ap1 and V-ap2 cells with the electric circuit.

3. Results and discussion

3.1. Directional propagation of the action potential due to the channel-type receptors on the synapse and the voltage-gated Na^+ channels on the axon

Fig. 1(A) shows the change in the E_{W2-W1} value of each cell. The enlarged figure indicates the change after switching. The membrane current of each cell is represented in Fig. 1(B), and an enlarged figure is also shown. The membrane potentials of the RP cell and Rec cells 1-3 were approximately -75 mV (-76.0, -76.0, -75.8, and -75.8 mV, respectively) before switching. The membrane potentials of the AP cell and V-ap cells 1-3 were approximately +70 mV (+83.9, +67.9, +71.0, and +70.9 mV, respectively) before switching, and these were determined by the ratio of the Na^+ concentration in W1 to that in W2. When the AP cell was connected to the electric circuit and the RP cell was cut off with the switch (at $t = 0.000$ s), circulating currents were locally generated by local ion transfers, as illustrated in schematic 2. The change in E_{W2-W1} was then larger for a cell that was closer to the synapse (the sending site). When the E_{W2-W1} value of the Rec1 cell exceeded 0 mV ($t = 0.044$ s), the V-ap1 cell was connected to the electric circuit with the relay switch. This accelerated the propagation of the change in E_{W2-W1} in the neighboring site. Thus, the E_{W2-W1} values of all cells mutually changed with the occurrence of circulating currents. When the E_{W2-W1} values of the Rec2 and Rec3 cells exceeded 0 mV ($t = 0.048$ and 0.052 s, respectively), the V-ap2 and V-ap3 cells were connected to the electric circuit with the relay switch. When the RP cell was reconnected to the electric circuit and the AP cell was cut off by the switch (at $t = 5.000$ s), oppositely circulating currents were immediately generated, and the membrane potential of each Rec1-3 cell was gradually

Fig. 1

Schematic 2

Fig.

restored to its initial resting potential. Therefore, no hyperpolarization was observed in the present model system. To analyze the propagation behavior of the action potential, profiles of the membrane potential were plotted and are shown in Fig. 2. The author's group has proposed a conduction mechanism for the action potential of each part. In the conventional theory [15,16], the effect of the channel-type receptors (Na^+ channels) in the synapse has not been evaluated, though conduction of the action potential in the axon has been reasonably explained. When the resting potential was determined by the ratio of the K^+ concentration inside the cell to that outside, hyperpolarization was not normally observed. Because the open time of the channel-type receptors in the synapse is several ms, and that of the voltage-gated Na^+ channels on the axon is about 1 ms, it is believed that the channel-type receptors in the synapse and the voltage-gated Na^+ channels on the axon correspond to the AP and V-ap cells, respectively. The open times of the AP cell and the V-ap cells were selected at 5 s and 0.011 s, respectively. The channel-type receptors and voltage-gated Na^+ channels would then serve as the main and additional electric power sources, respectively, to propagate the action potential toward the synapse terminus. Thus, not only the influx of Na^+ at the synapse but also that at the activated part on the axon causes the change in the electric field along the axon. The author's group mentioned the propagation mechanism due to both the change in the electric field caused by the local circulating current and the keep of the electroneutrality in each phase [16-23]. Although the local circulating current due to various ionic channels partly generates the magnetic field, it is thought that the change in the electric field mainly works on the change in the membrane potential around the activated area.

3.2. Effect of delayed- K^+ channels on the propagation of the action potential and the origin of hyperpolarization

The effect of K^+ channels that open after closing the voltage-gated Na^+ channels (delayed- K^+ channels) was investigated. In actual nerve conduction, some K^+ channels are always open, while in signal propagation, other K^+ channels gradually open when the voltage-gated Na^+ channels are almost closed [1,2,27]. Considering the K^+ channels that gradually open when the voltage-gated Na^+ are almost closed, we performed an experiment. The changes in E_{W2-W1} and i_{W2-W1} for each cell are represented in Fig. 3(A) and (B), respectively. As shown in schematic

Fig. 3

1(C), the D-RP cell was connected to receiving site (I) in parallel via the relay switch. Profiles of the change in E_{W2-W1} in the model containing the D-RP cell are shown in Fig. 4. When the D-RP cell was connected with the circuit at 0.122 s, the E_{W2-W1} value started to shift in the positive direction and the current flowed. After the connection, a part where the E_{W2-W1} value was more negative than that observed in the absence of the D-RP cell appeared in each Rec cell (cf. Fig. 2). The local E_{W2-W1} values became more negative as the amount of K^+ transported from W2 to W1 increased. In a whole nerve cell, the increase in the amount of K^+ transported from W2 to W1 inhibited the transfer of Na^+ from W1 to W2. Therefore, the delayed- K^+ channels appear to inhibit further propagation of the action potential in comparison with the case where the delayed- K^+ channels are absent. If the channel-type receptors at the synapse do not function, nerve conduction may be weakened by the delayed- K^+ channels. That is, it is important that the channel-type receptors at the synapse remain open for as long as possible during nerve conduction. If the membrane potential is more positive than the threshold of the opening of the voltage-gated Na^+ channels, the action potential can be propagated. However, the rapid return of the membrane potential from the action potential to the resting potential induces rapid reopening of the voltage-gated Na^+ channels. In this model cell system, hyperpolarization did not occur. If the resting potential is determined by not only the transport of K^+ but also that of other ions including Na^+ , HPO_4^- , and Cl^- , the initial resting potential is a mixed potential and is more positive than the ideal resting potential generated by the

Fig. 4

transport of K^+ only [23]. Therefore, hyperpolarization appears to occur under actual nerve conduction. On the other hand, it is possible that the inflow of K^+ (and the outflow of Na^+) due to the functions of the Na^+/K^+ -ATPase enzyme causes the negative shift of the resting potential to generate hyperpolarization. In such a case, the amounts of the inflow of K^+ by the Na^+/K^+ -ATPase enzyme is larger than that of the outflow of K^+ by K^+ channels. However, it is unclear how to conjugate the function of the Na^+/K^+ -ATPase enzyme with the nerve conduction. Dyachok, et al. and So, et al. reported that clear hyperpolarization is generated by the membrane electroporation or the function of specific channels [28,29]. On the other hand, we successfully explained the appearance of hyperpolarization by considering the relation between the membrane potential caused by the fluxes of multiple ions and the function of the delayed- K^+ channels.

4. Conclusion

We built a nerve model system mimicking the function of channels in nerve cells. Based on the results, nerve conduction can be explained as follows. The local circulating currents are generated by expression of the action potential at the synapse, and the membrane potential in the axon changes locally from the resting potential to the action potential. Channel-type receptors (Na^+ channels) at the synapse serve as the main electric power source to propagate the action potential. When the membrane potential of the node of Ranvier exceeded the threshold, it changes, and the action potential is further propagated along the axon. Namely, the voltage-gated Na^+ channels at the axon function as additional electric power sources to propagate the action potential toward the synapse terminus. When the resting potential is determined by the ratio of the K^+ concentration inside the cell to that outside, hyperpolarization is not observed. When the resting potential is a mixed potential generated by not only K^+ transport but also that of other ions, hyperpolarization appears to be

generated by delayed- K^+ channels.

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References

- [1] B. Hille, *Ion Channels of Excitable Membranes*, 3 rd. ed., Sinauer Associate, Inc., Sunderland, U.S.A., 2001.
- [2] B. Sackmann, E. Neher, *Single-Channel Recordings*, 2 nd. ed., Kluwer Academic-Plenum Publishers, New York, U.S.A., 1995.
- [3] R.H. Adrian, W.K. Chandler, A.L. Hodgkin, Voltage Clamp Experiments in Striated Muscle Fibres, *J. Physiol.* 208 (1970) 607-644. doi:10.1007/978-1-4612-2596-6014.
- [4] M. Zochowski, M. Wachowiak, C.X. Falk, L.B. Cohen, Y.W. Lam, S. Antic, D. Zecevic, Imaging Membrane Potential with Voltage-Sensitive Dyes, *Biol. Bull.* 198 (2000) 1-21. doi:10.2307/1542798.
- [5] S. Chemla, F. Chavane, Voltage-sensitive dye imaging-Technique review and models, *J. Physiol.* 104 (2010) 40-50. doi:10.1016/j.jphysparis.2009.11.009.
- [6] R. Fitzhugh, Impulses and physiological states in theoretical models of nerve membrane, *Biophys. J.* 1 (1961) 4445-466.
- [7] N.H. Sabah, K.N. Leibovic, Subthreshold oscillatory responses of the Hodgkin-Huxley cable model for the squid giant axon, *Biophys. J.* 9 (1969) 1206-1222.
- [8] J.L. Johnson, M.L. Podgett, PCNN models and applications, *IEEE Trans. Neural Networks*, 10 (1999) 480-498.
- [9] A.N. Burkitt, A review of the integrate-and-fire neuron model: I. Homogeneous synaptic input, *Biol. Cybern.* 95 (2006) 1-19. doi:10.1007/s00422-006-0068-6.
- [10] S.-N. Wu, Y.-C. Lo, B.-S. Chen, E.C. So, L.-T. Chen, Contribution of blocked potassium current conductance and increased conductance of persistent sodium current to the afterdischarge in myelinated neuron, *Muscle & Nerve* (2012) 297-299. doi:10.1002/mus.23395.
- [11] A.G. Dimitrov, N.A. Dimitrova, A possible link of oxaliplatin-induced neuropathy with potassium channel deficit, *Muscle & Nerve* (2012) 403-411. doi:10.1002/mus.22311.
- [12] B.J. Roth, P.J. Bassar, A model of the stimulation of a nerve fiber by electromagnetic induction, *IEEE Trans. Biomed. Eng.* 37 (1990) 588-597.
- [13] J.A. Brown, J.A. Tuszynski, Dipole interactions in axonal microtubules as a mechanism of signal propagation, *Phys. Rev. E* 56 (1997) 5834-5840.
- [14] J. Ma, G. Zhang, T. Hayat, G. Ren, Model electrical activity of neuron under electric field, *Nonlinear Dyn* 11 (2018). doi:org/10.1007/s11071-018-4646-7.
- [15] F. Wu, J. Ma, G. Zhang, A new neuron model under electromagnetic field, *Appl. Math. Comp.* 347 (2019) 590-599. doi:10.1016/j.amc.2018.10.087.
- [16] N. Ueya, O. Shirai, Y. Kushida, S. Tsujimura, K. Kano, Transmission mechanism of the change in membrane potential by use of organic liquid membrane system, *J. Electroanal. Chem.* 673 (2012) 8-12. doi:10.1016/j.jelechem.2012.03.012.

- [17] Y. Kushida, O. Shirai, Y. Takano, Y. Kitazumi, K. Kano, Influence of the circulating current on the propagation of the change in membrane potential, *Electroanalysis* 26 (2014) 1858-1865. doi:10.1002/elan.201400195.
- [18] Y. Kushida, O. Shirai, Y. Kitazumi, K. Kano, Influence of Charging Current and Potential Drop on the propagation of the change in membrane potential, *Anal. Sci.* 31 (2015) 677-683. doi:org/10.1002/elan.201400195.
- [19] Y. Kushida, O. Shirai, Y. Kitazumi, K. Kano, Propagation of the Change in Membrane Potential Owing to the Circulating Current within a Membrane System in Analogy with Neurotransmission, *Bull. Chem. Soc. Jpn.* 87 (2014) 110-112. doi:10.1246-bcsj.20130240.
- [20] Y. Takano, O. Shirai, Y. Kitazumi, K. Kano, Propagation of the change in the membrane potential using a biocell-model, *Phys. Chem. Chem. Phys.* 18 (2016) 12689-12695. doi:10.1039/c5cp07446k.
- [21] Y. Takano, O. Shirai, Y. Kitazumi, K. Kano, Proposal of a new mechanism for the directional propagation of the action potential using a mimicking system, *Phys. Chem. Chem. Phys.* 19 (2017) 5310-5317. doi:10.1039/c6cp07603c.
- [22] M. Kaji, O. Shirai, Y. Kitazumi, K. Kano, Generating change in membrane potential by external electric stimulation and propagating the change by using nerve model cell systems, *Electrochim. Acta* 282 (2018) 89-96. doi:10.1016/j.electacta.2018.06.023.
- [23] O. Shirai, Y. Kitazumi, K. Kano, Electrochemical Interpretation of Propagation of the Change in the Membrane Potential Using the Goldman-Hodgkin-Katz Equation, *Electroanalysis* 29 (2017) 1-10. doi:10.1002/elan.201700368.
- [24] Y. Yoshida, M. Matsui, O. Shirai, K. Maeda, S. Kihara, Evaluation of distribution ratio in ion pair extraction using fundamental thermodynamic quantities, *Anal. Chim. Acta* 373 (1998) 213-225. doi:10.1016/S0003-2670(98)00367-5.
- [25] R.C. Thomas, The Effect of Carbon Dioxide on the Intracellular pH and Buffering Power of Snail Neurons, *J. Physiol.* 255 (1976) 715-735. doi:10.1113/jphysiol.1976.sp011305.
- [26] I. Tasaki, G. Matsumoto, On the Cable Theory of Nerve Conduction, *Bull. Math. Biol.* 64 (2002) 1069-1082. doi:10.1006/bulm.2002.0310.
- [27] F.L. Burton, S.M. Cobbe, Dispersion of ventricular repolarization and refractory period, *Cardiovasc Res.* 50 (2001) 10-23. doi:10.1016/S0008-6363(01)00197-3.
- [28] O. Dyachok, P. Zhabyeyev, T.F. McDonald, *J. Membrane Biol.* 238 (2010) 69-80. doi:10.1007/s00232-010-9320-z.
- [29] E.-C. So, K.-L. Tsai, F.-T. Wu, M.-C. Hsu, K.-C. Wu, S.-N. Wu, Identification of minuscule inward currents as precursors to membrane electroporation-induced currents: Real-time prediction of pore appearance, *Cell. Physiol. Biochem.* 32 (2013) 402-416. doi:10.1159/000354447.

Figure captions and schematic captions

Schematic 1. (A) Overview of a neuron and a model system, (B) The LM model system used to explain the mechanism of the action potential propagation, (C) The LM model system containing D-RP cell.

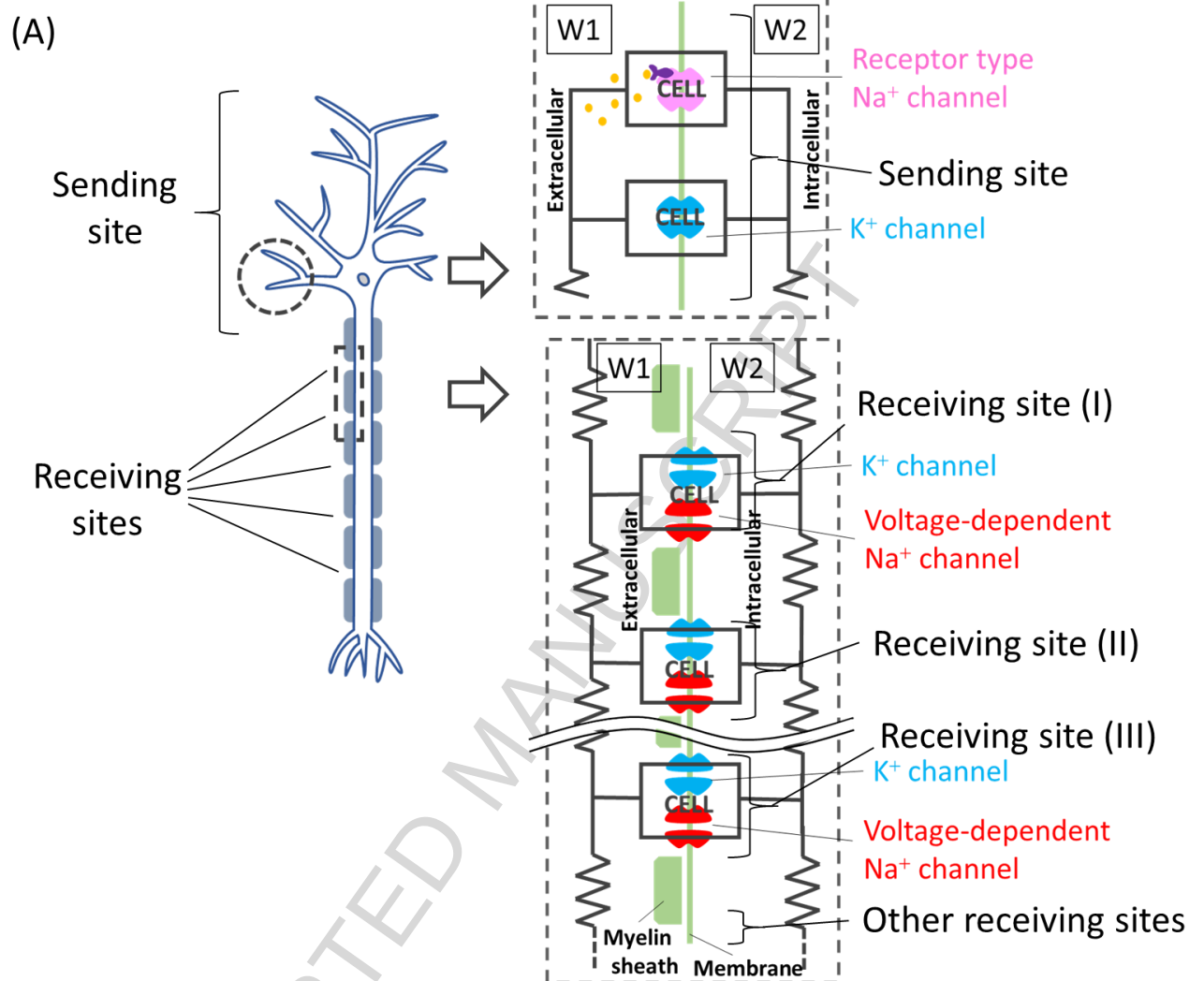
Schematic 2. The cell system as an electric circuit. (A) Initial condition of the cell system as an electric circuit. (B) The electric circuit when switching between the RP and AP cells.

Fig.1. (A) Time course of the membrane potential (E_{w2-w1}) and an enlarged figure and (B) that of the membrane current (i_{w2-w1}) and an enlarged figure.

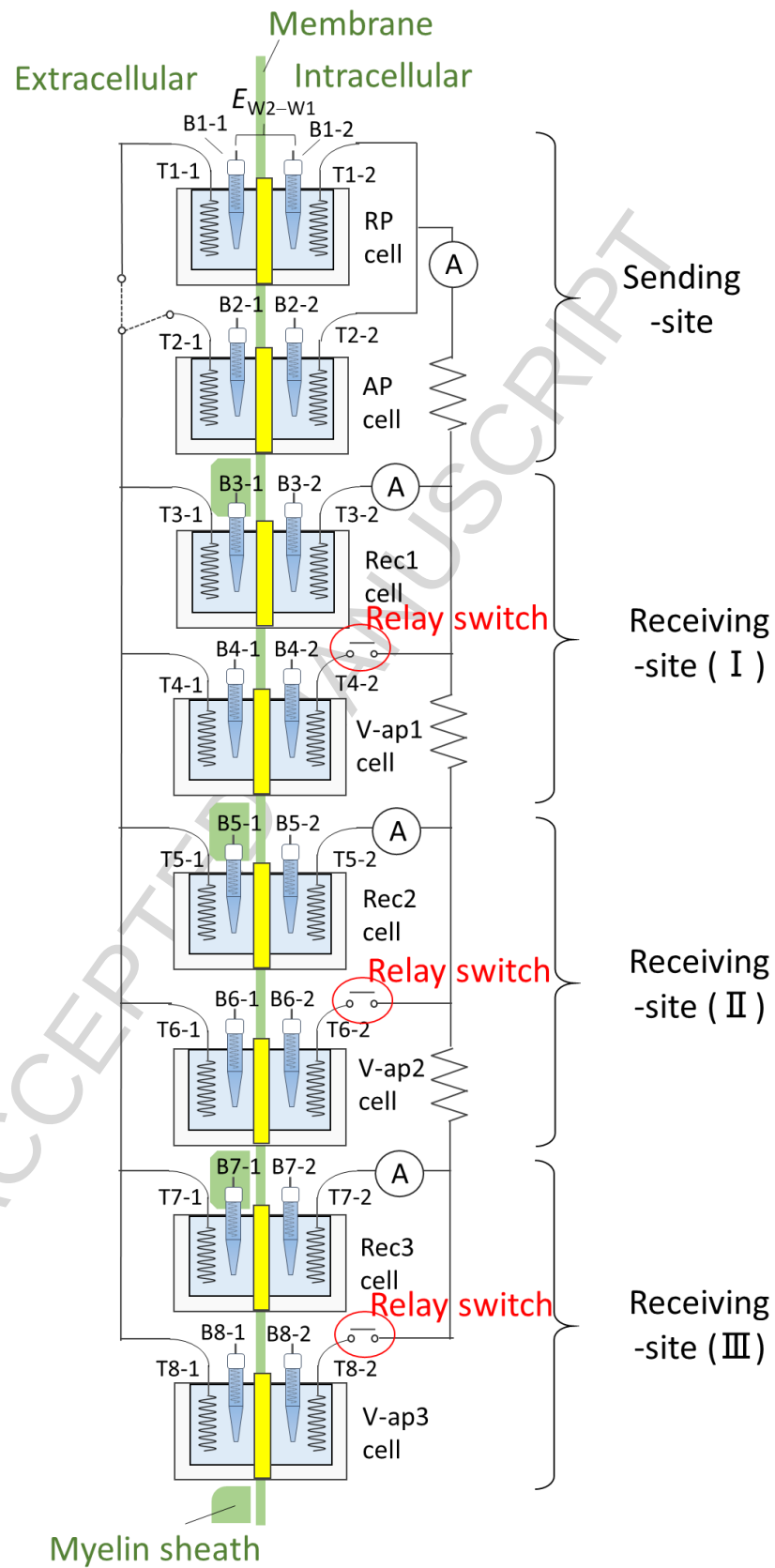
Fig. 2. Profiles of the membrane potential at each time in the model nerve sell system.

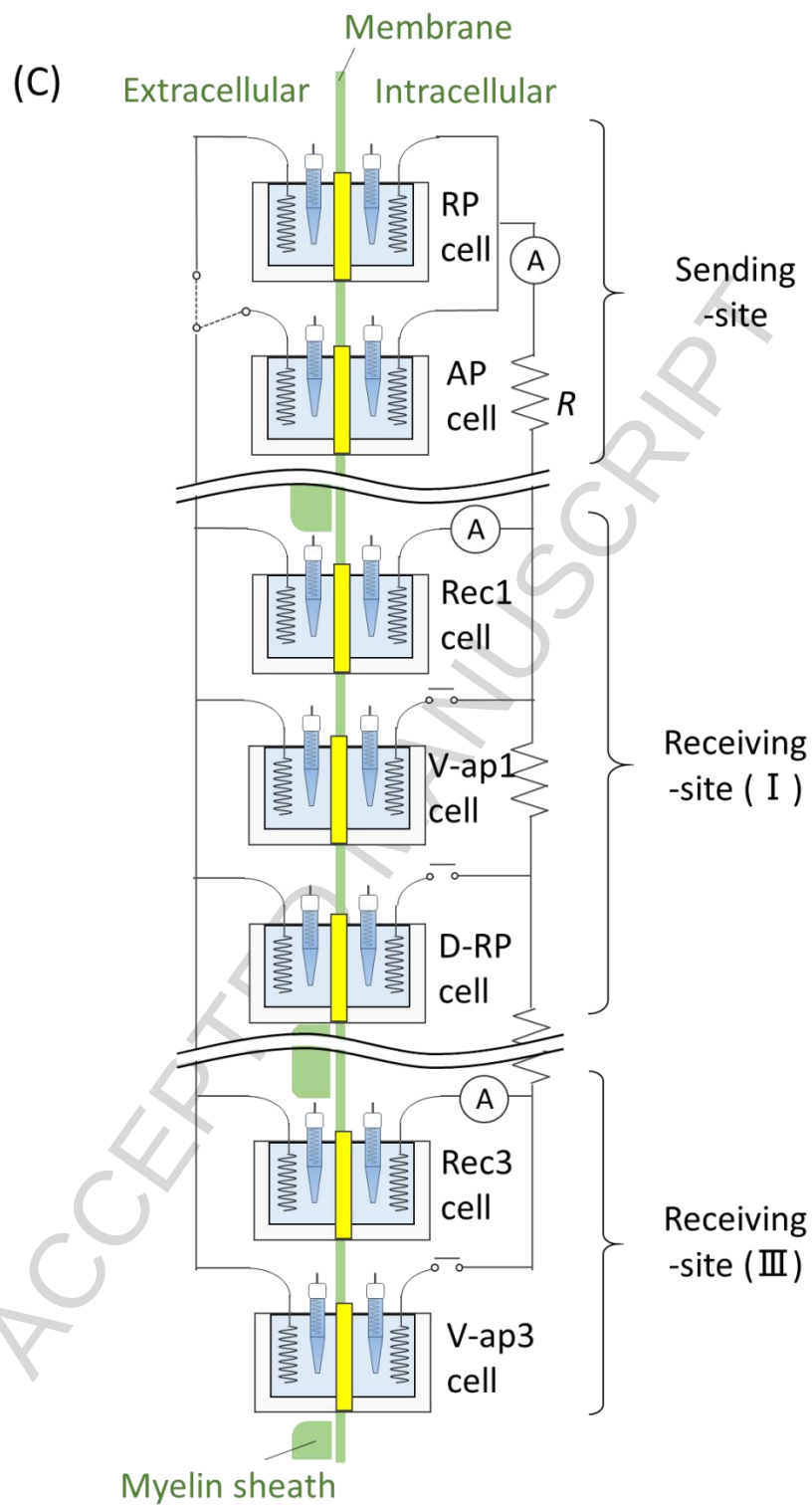
Fig. 3. (A) Time course of the membrane potential (E_{w2-w1}) and an enlarged figure and (B) that of the membrane current (i_{w2-w1}) and an enlarged figure.

Fig. 4. Profiles of the membrane potential at each time in the model nerve cell system considering delayed- K^+ channel.

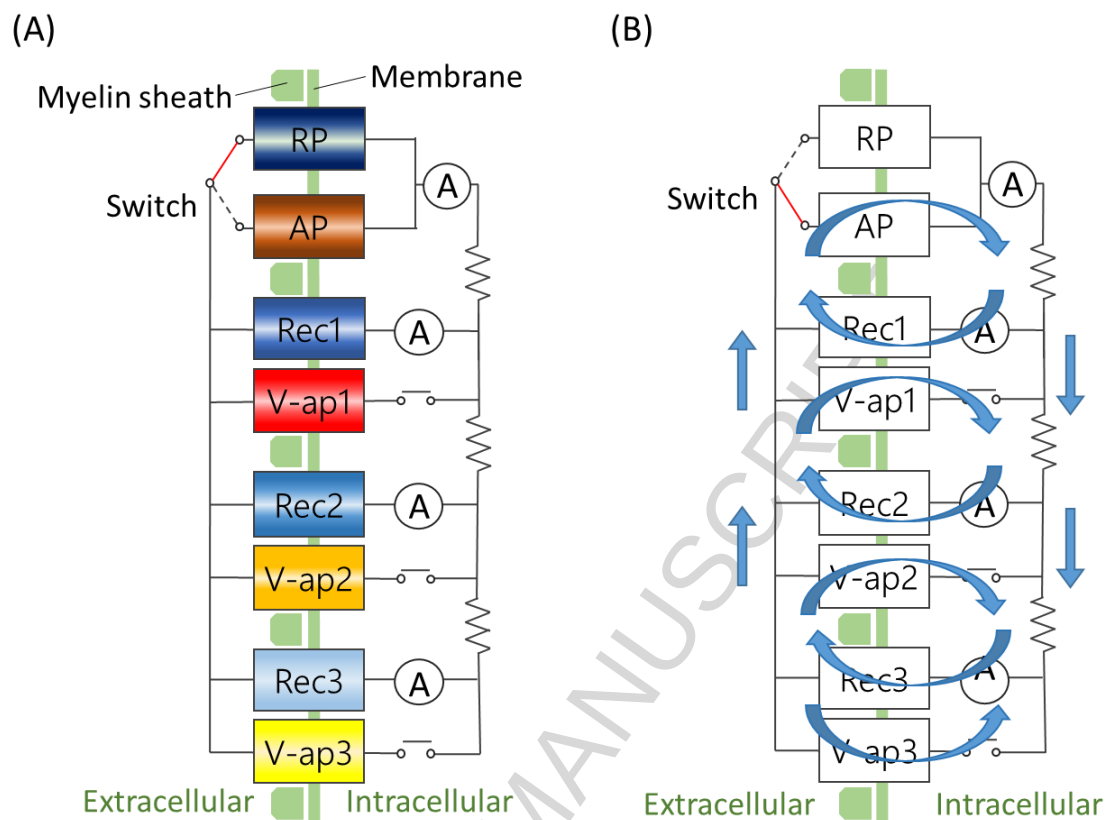


(B)





Schematic 1. M. Kaji, et al.



Schematic 2. M. Kaji, et al.

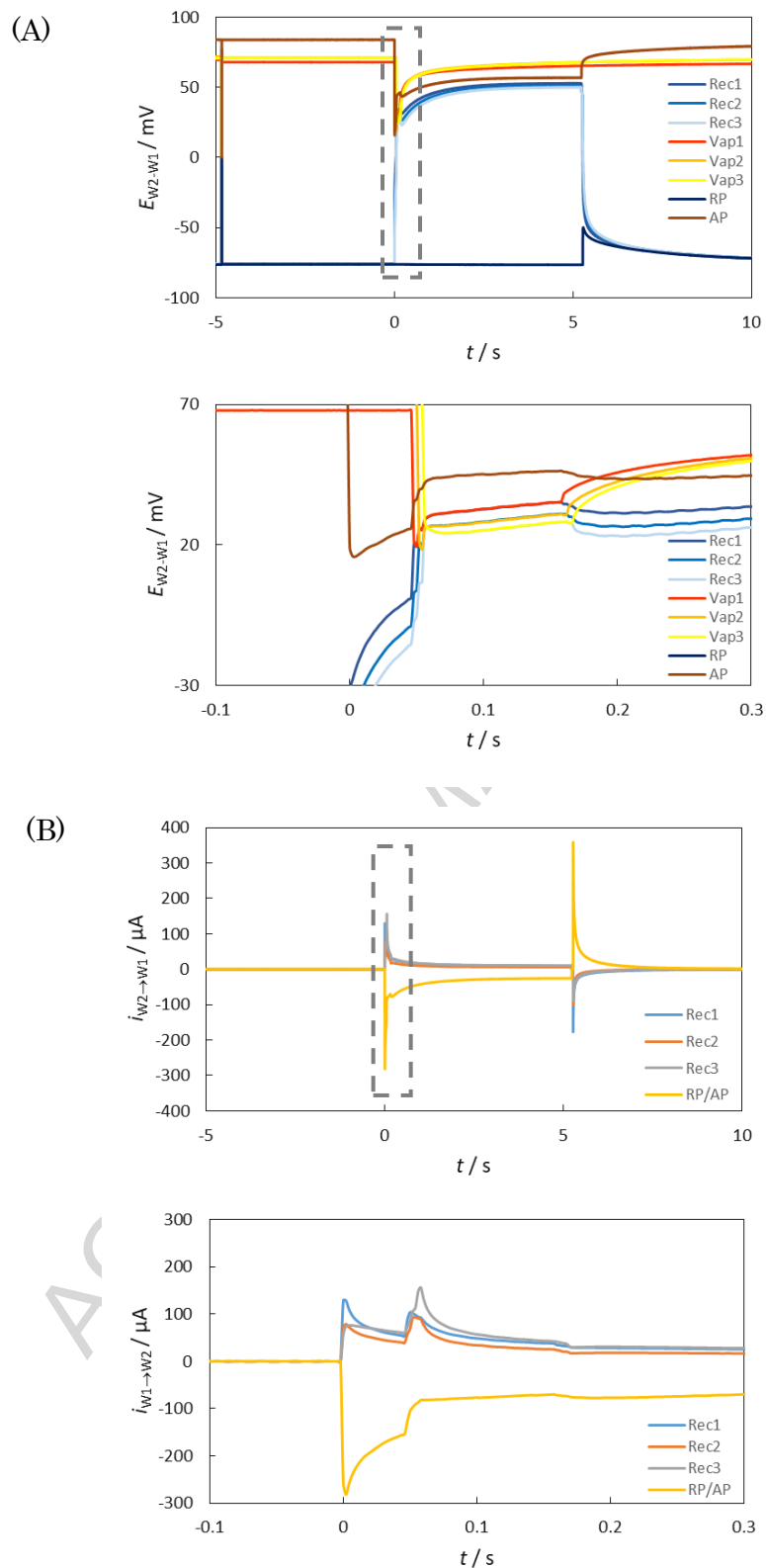
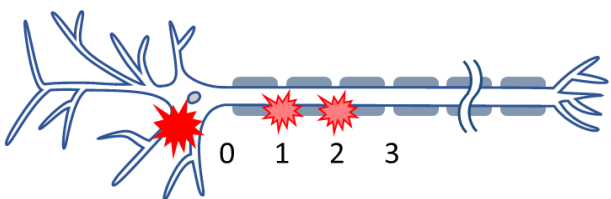
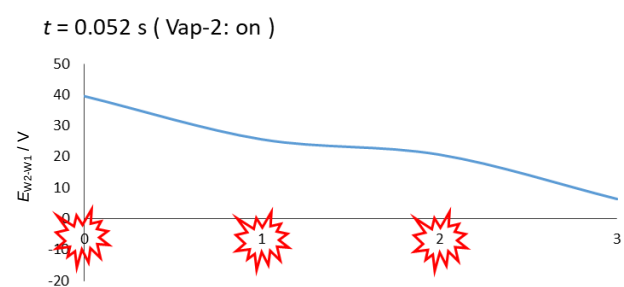
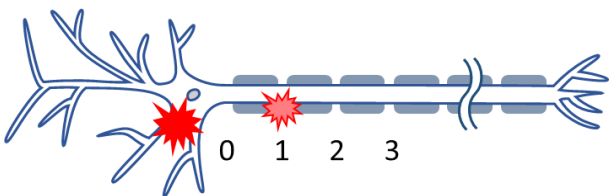
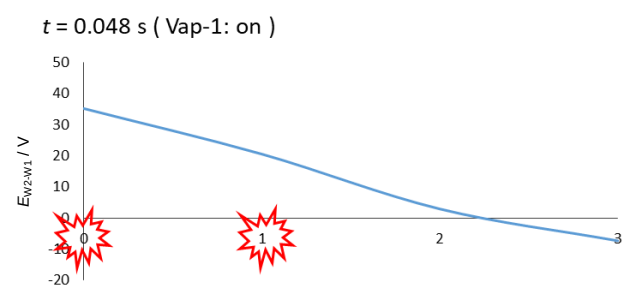
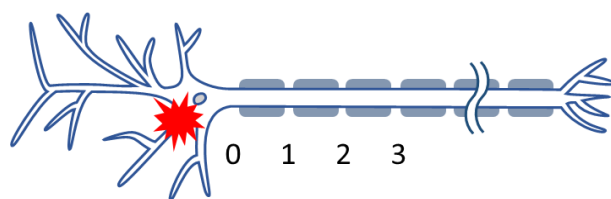
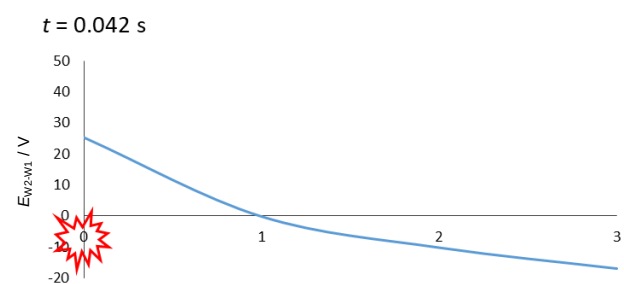
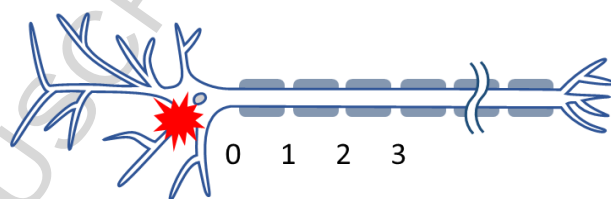
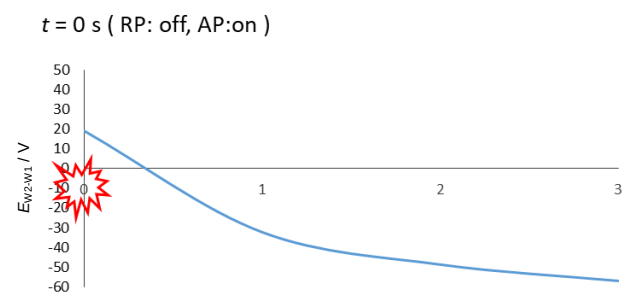
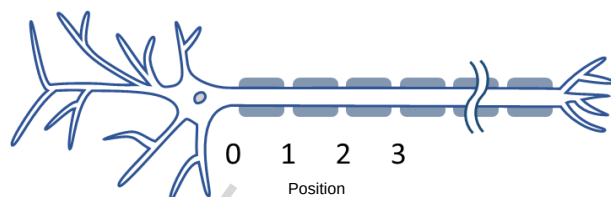
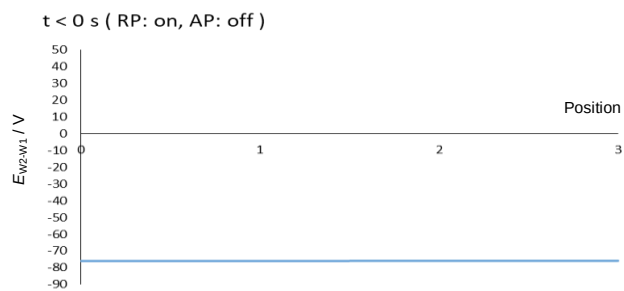
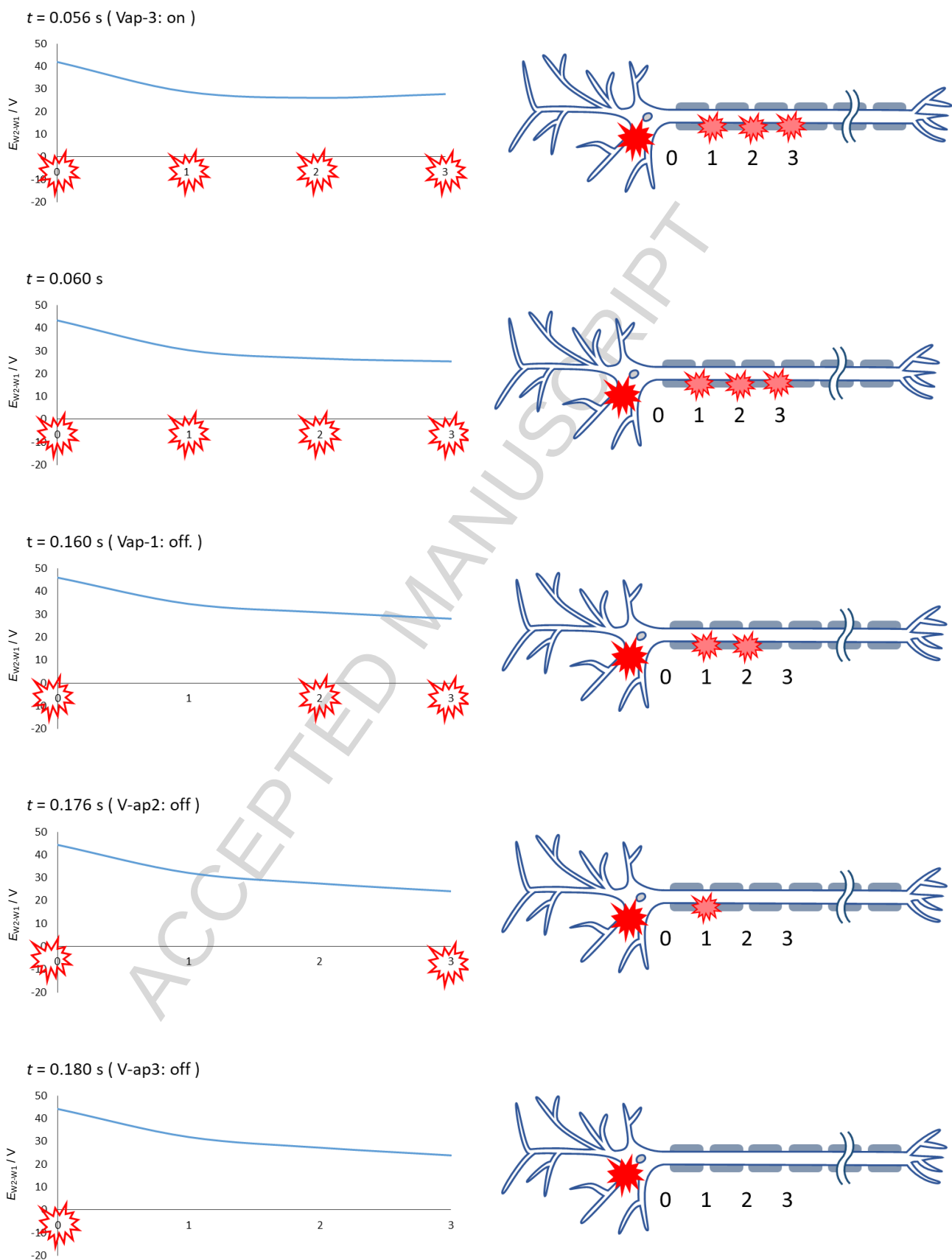
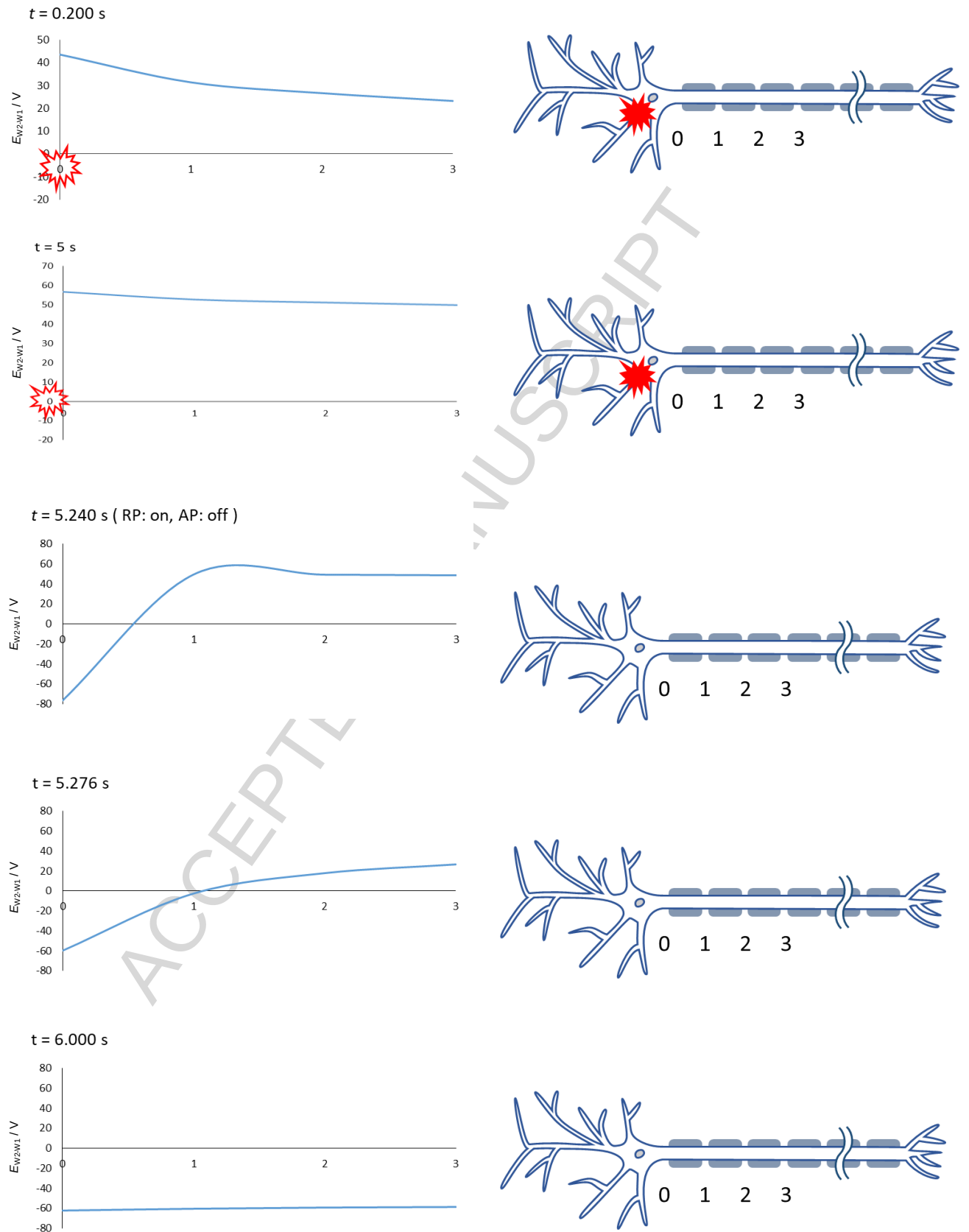


Fig. 1. M. Kaji, et al.







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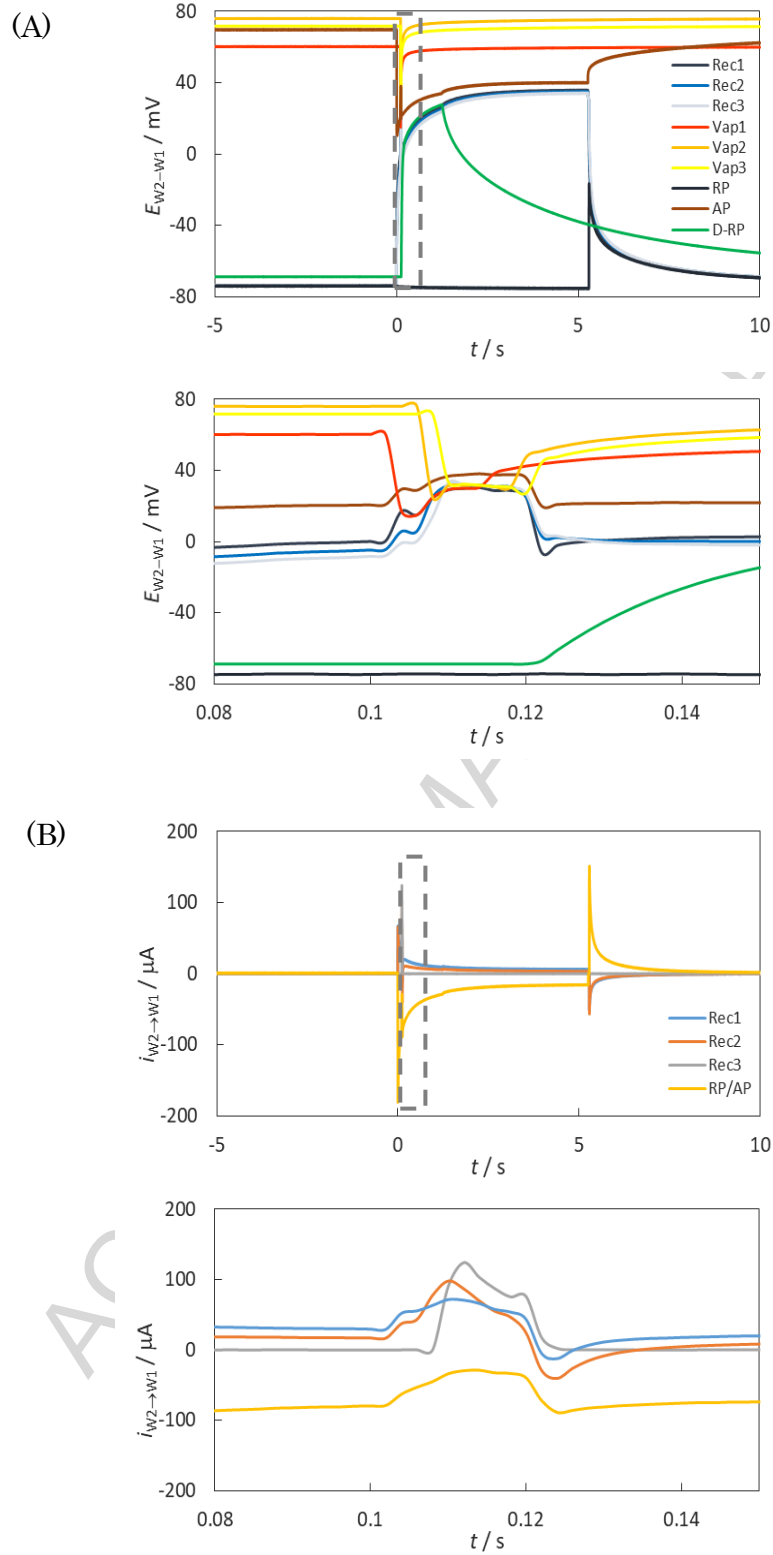
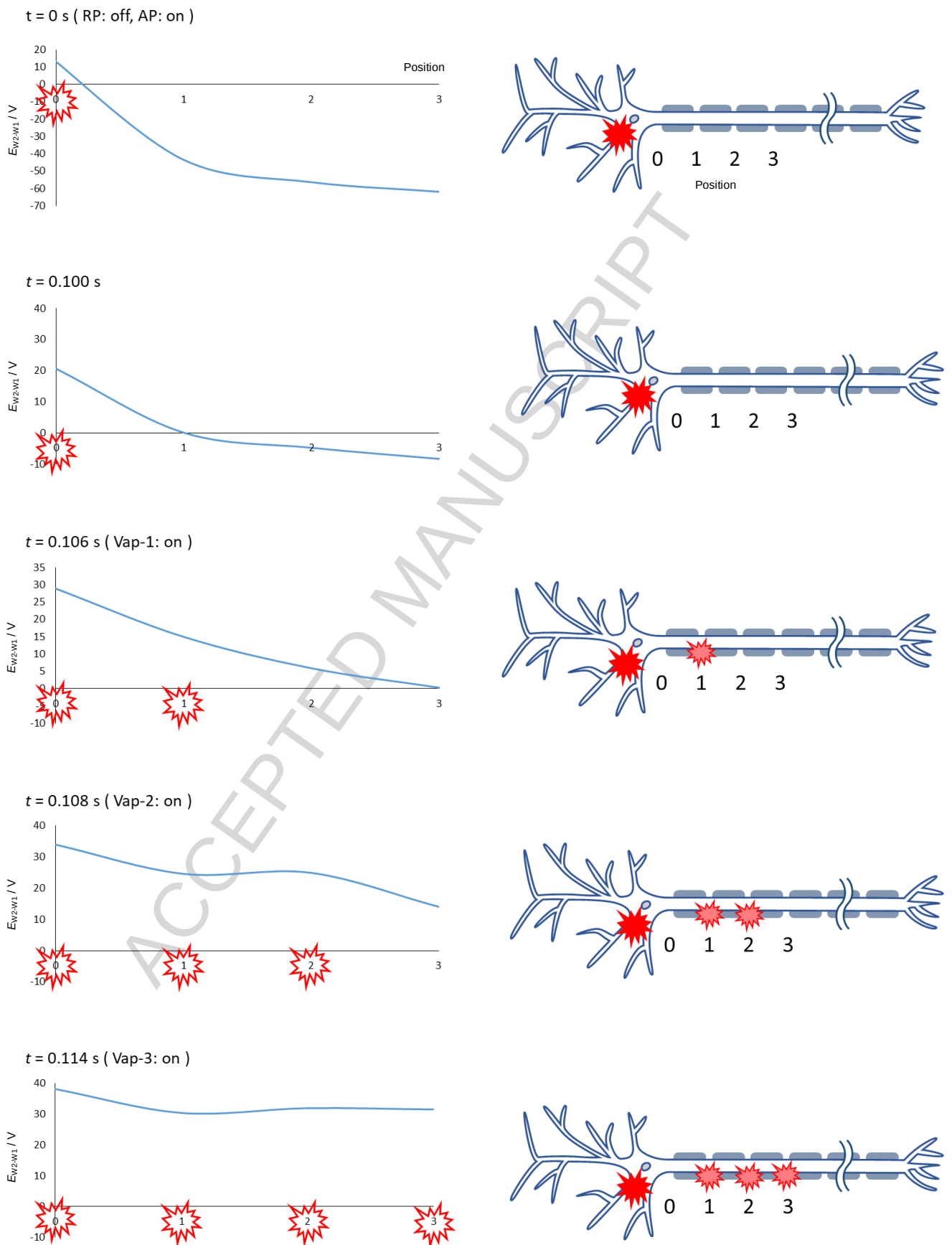


Fig. 3. M. Kaji, et al.



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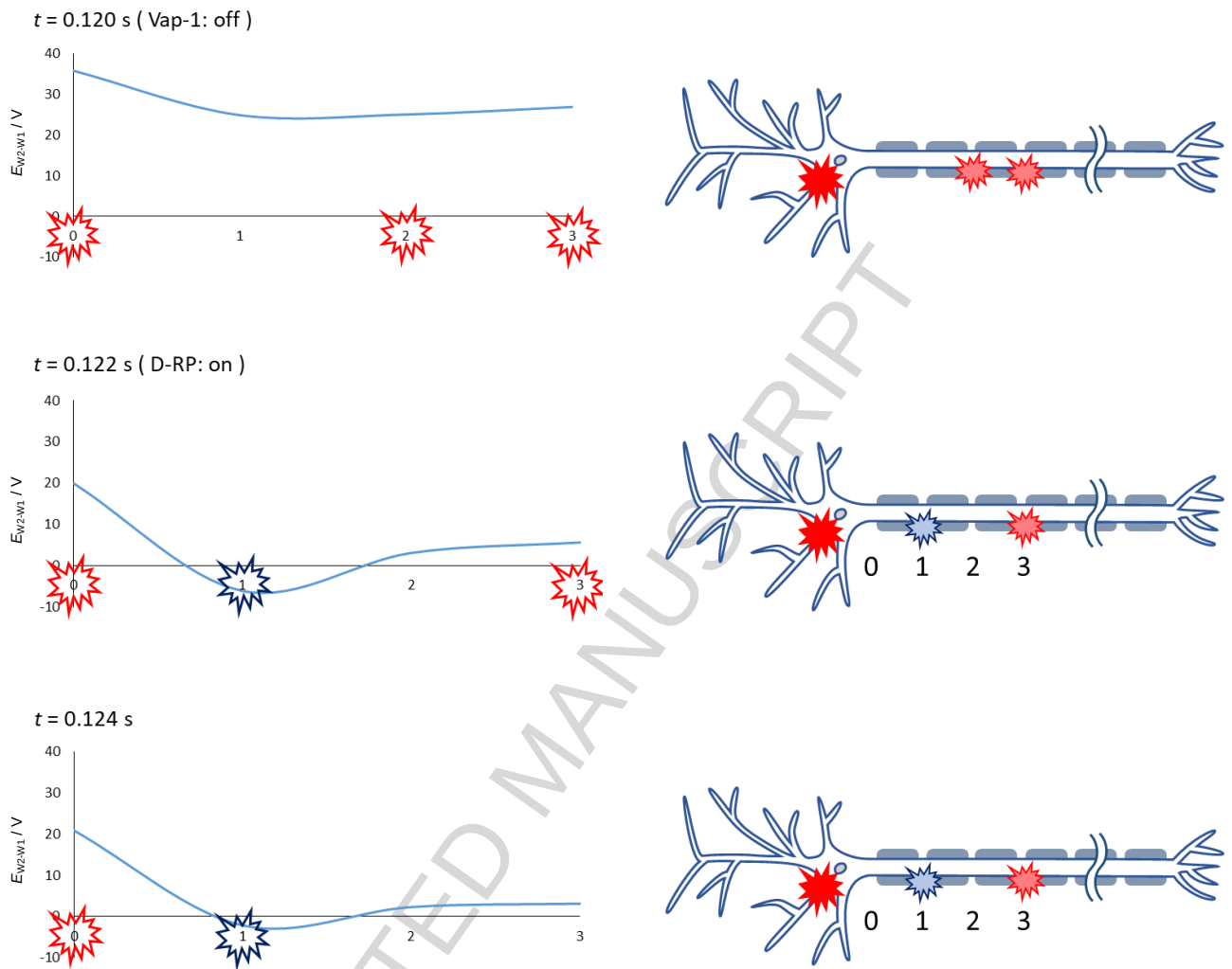


Fig. 4. M. Kaji, et al.

Highlights

- Membrane potentials and currents were directly observed at multipoints in a model.
- Action potentials spread by synaptic ligand and voltage-gated Na⁺ channels.
- Hyperpolarization was not caused by delayed-K⁺ or voltage-gated Na⁺ channels.