

**Title**

**Proposal of a New Mechanism for the Directional Propagation of the Action Potential  
Using a Mimicking System**

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### Abstract

A nerve conduction model is constructed by using some liquid-membrane cells that mimic the function of the  $K^+$  and  $Na^+$  channels. By imitating two types of  $Na^+$  channels (ligand-gated  $Na^+$  channel and voltage-gated  $Na^+$  channel), a new mechanism for the directional propagation of the action potential along the axon toward the axon terminal is proposed. When the nerve cell is excited by an external outer stimulus, it can be presumed that the ligand-gated channels work as a power source at the synapse to propagate the change in the membrane potential, then the voltage-gated channels locally assist the propagation at each site of the axon (nodes of Ranvier).

## Introduction

During nerve conduction, the propagation of the change in the membrane potential takes place by the action of the  $K^+$  and  $Na^+$  channels [1-6]. The membrane potential of the nerve cells is usually determined by the ratio of the concentration of  $K^+$  inside to that outside the cell and is called the resting potential. When channel-type receptors (ligand-gated  $Na^+$  channels) at the synapse combine neurotransmitters,  $Na^+$  is mainly transported from the outside to the inside of the cell in the neighborhood around the receptors. The transport of  $Na^+$  evokes a change in the membrane potential from the resting potential to the action potential, which is determined by the ratio of the concentration of  $Na^+$  inside to that outside of the cell. Subsequently, the action potential is propagated along the axon toward the axon terminal by opening the voltage-gated  $Na^+$  channels that have short open-lives (about 1 ms). Since the delayed  $K^+$  channels open one after another, it is thought that the membrane potential goes back to the resting potential via the hyperpolarization state. Thus, the propagation of the change in the membrane potential is directionally conducted along the axon toward the axon terminal.

The nerve conduction has been discussed on the basis of the voltage-clamp technique and the cable theory [5-8]. In the method, the local currents at several parts of the axon surfaces are measured under the constant membrane potential. The local currents are converted to the potential differences by considering the changes in the permeabilities. Thus the membrane potential cannot be directly recorded using this method. According to the conventional explanation on the propagation of the action potential, the action potential is propagated along the axon as a pulse. The direction of the propagation is explained by the short open-lives of the voltage-gated  $Na^+$  channels due to the inactivation and by the opening of the delayed  $K^+$  channels. However, the relation between the local membrane potential and the local current has not been directly revealed. In addition, since the hyperpolarization that appears just after the inactivation of the voltage-gated  $Na^+$  channels has been explained in terms of the transport of  $K^+$

from the inside to the outside of the cell [9,10], it is difficult to explain the hyperpolarization of which membrane potential is more negative than the formal resting potential. This means that the ratio of the  $K^+$  concentration in the inside to that in the outside becomes much higher than that former value at the resting potential and conflicts with the outflow of  $K^+$  from the inside to the outside during the excited period. Moreover, it is well known that the current that flowed due to the channel-type receptors, which mainly transport  $Na^+$  from the outside to the inside, is about ten times higher than that which occurred by the voltage-gated channels at one node of Ranvier and that the open-life of the channel-type receptor is typically much longer than 5 ms [11]. These characteristics have not yet been discussed.

In our previous studies, a new mechanism has been proposed for the propagation of the change in the membrane potential using an organic-liquid-membrane cell system [12-16]. It has been clarified that the change in the membrane potential is propagated by the generation of the circulating current through two liquid membrane sites (the potential-sending and receiving sites), and that the magnitude of the circulating current determines the threshold to produce the change in the membrane potential by considering the electroneutrality and the mass-balance [12,13]. In addition, it has been found that the ohmic drop generated by the resistor in the electric circuit decreases and slows down the propagation of the change in the membrane potential and that the charging current generated by the capacitor in the electric circuit delays the propagation analogous to the nerve transmission [13,16].

In this study, a new model system for the nerve conduction is constructed by using some liquid-membrane cells that mimic the function of the  $K^+$  and  $Na^+$  channels. We propose a new mechanism for the propagation of the action potential based on the electrochemical analysis of the model system that mimics the ligand-gated  $Na^+$  channels at the synapse and voltage-gated  $Na^+$  channels at the nodes of Ranvier.

## 2. Experimental

### 2.1 Reagents and Chemicals

Sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB) was prepared in a similar manner as previously reported.[12] The  $\text{TFPB}^-$  salt of tetrabutylammonium ( $\text{TBA}^+$ ) was obtained by mixing a 1,2-dichloroethane (DCE) solution of NaTFPB with a DCE solution of TBACl (Tokyo Kasei Chemical Co.). DCE and nitrobenzene (NB) were purchased from Wako Pure Chemical Co. All other chemicals were of analytical reagent grade and were used without further purification. A porous polytetrafluoroethylene resin (PTFE) membrane filter, T300A025A (pore size: 3  $\mu\text{m}$ , thickness: 75  $\mu\text{m}$ ) was obtained from Advantec, and was used as a liquid membrane impregnated by the NB solution.

### 2.2 Apparatus

Potentiometric measurements were performed using a potentiostat/galvanostat, HA-1010mM4A (Hokuto Denko Co.), and several electrometers, HE-106 (Hokuto Denko Co.) and HE-106A (Hokuto Denko Co.). The local current was measured by several ammeters, HM-103 (Hokuto Denko Co.) and HM-103A (Hokuto Denko Co.). All the data were recorded using two A/D converters, GL900 (Graphtec Co.). A rotary switch, MR-4-3Z (Nidec Copal Electronics Corp.), and reed relays, 291-9669 (Meder Electronic), were placed within the electric circuit of the cell system, as shown in Fig. 1. In order to imitate the open-life of the voltage-gated  $\text{Na}^+$  channel, an LMC555 timer (National Semiconductor Co.) was placed in the electric circuit to separate the cell mimicking the voltage-gated  $\text{Na}^+$  channel from the whole electric circuit.

### 2.3 Electrochemical Measurements

Liquid membrane cells were prepared in a manner similar to that in a previous study [16]. The liquid membrane cell had two aqueous phases (W1 and W2) and one NB phase (M) that

was impregnated in the PTFE porous sheet. The cell system composed of eight liquid membrane cells is illustrated in Fig.1. The membrane potential of each cell was measured against the reference electrode in W2, since W1 and W2 were mimicked the intracellular and extracellular phases, respectively. The current flowing across each cell was recorded as the local current by use of several ammeters. The cell system was divided into two parts; the sending- and receiving-sites of the change in the membrane potential in order to mimic the phenomenon that the change in the membrane potential (action potential) is propagated from the synapse to an adjacent node of Ranvier in the axon, as shown in Fig. SI1. Usually,  $K^+$  channels exist in overall membrane surface of the nerve cell. There are many channel-type receptors which mainly transport  $Na^+$  from the outside to the inside at the synapse of the nerve cell [1-4]. When the channel-type receptor combines with the transmitter, the channel opens for about 10 ms. By opening many  $Na^+$  channels at a certain domain of the synapse, the membrane potential becomes to change from the resting potential to the action potential. This area is treated as the potential-sending site. The area except for the potential-sending site at the synapse is expressed by the potential-receiving site (I). On the other hand, the axon and the presynaptic terminal are regarded as the potential-receiving site (II). In the area of the potential-receiving site (I), there are many  $K^+$  channels and channel-type receptors ( $Na^+$  channels), which cannot work by changing the membrane potential. A lot of delayed  $K^+$  channels and voltage-gated  $Na^+$  channels existed in the potential-receiving site (II). In the potential-sending site of the present model system, the RP and AP cells exhibited the resting potential and the action potential, respectively. The cell configurations of the AP and RP cells are described by Eqs. (1) and (2), respectively. The Rec1' and Rec2' cells in the potential-receiving site (I) showed the resting potential. In the potential-receiving site (II), the Rec1 and Rec2 cells indicated the resting potential and the V-ap1 and V-ap2 cells did the action potential. The ionic compositions in the Rec and V-ap cells are shown by Eqs. (3) and (4), respectively. In order to mimic the function of the voltage-gated

$\text{Na}^+$  channels, the V-ap1 and V-ap2 cells were connected to the Rec1 and Rec2 cells, respectively, in parallel with the relay switches. When the membrane potentials of the Rec1 and Rec2 cells became more positive than 0 mV corresponding to a threshold, the V-ap1 and V-ap2 cells were connected in parallel to the Rec1 and Rec2 cells, respectively. The V-ap cells were located on the potential-receiving-site (II), since the voltage-gated  $\text{Na}^+$  channels are expressed at the nodes of Ranvier in the axon.

| W1                                    | M                                    | W2                                  |     |
|---------------------------------------|--------------------------------------|-------------------------------------|-----|
| $2 \times 10^{-2} \text{ M KCl}$      | $1 \times 10^{-3} \text{ M KTFPB}$   | $1 \times 10^{-3} \text{ M KCl}$    |     |
| $4.5 \times 10^{-2} \text{ M MgCl}_2$ | $5 \times 10^{-2} \text{ M TBATFPB}$ | $5 \times 10^{-2} \text{ M MgCl}_2$ | (1) |
| (RE1)                                 |                                      | (RE2)                               |     |

| W1                                  | M                                    | W2                                    |     |
|-------------------------------------|--------------------------------------|---------------------------------------|-----|
| $1 \times 10^{-3} \text{ M NaCl}$   | $1 \times 10^{-3} \text{ M NaTFPB}$  | $2 \times 10^{-2} \text{ M NaCl}$     |     |
| $5 \times 10^{-2} \text{ M MgCl}_2$ | $5 \times 10^{-2} \text{ M TBATFPB}$ | $4.5 \times 10^{-2} \text{ M MgCl}_2$ | (2) |
|                                     | $2 \times 10^{-2} \text{ M DB18C6}$  |                                       |     |
| (RE3)                               |                                      | (RE4)                                 |     |

| W1                                    | M                                    | W2                                  |     |
|---------------------------------------|--------------------------------------|-------------------------------------|-----|
| $2 \times 10^{-2} \text{ M KCl}$      | $1 \times 10^{-4} \text{ M KTFPB}$   | $1 \times 10^{-3} \text{ M KCl}$    |     |
| $4.5 \times 10^{-2} \text{ M MgCl}_2$ | $5 \times 10^{-2} \text{ M TBATFPB}$ | $5 \times 10^{-2} \text{ M MgCl}_2$ | (3) |
| (RE5)                                 |                                      | (RE6-9)                             |     |

| W1                                  | M                                    | W2                                    |     |
|-------------------------------------|--------------------------------------|---------------------------------------|-----|
| $1 \times 10^{-3} \text{ M NaCl}$   | $1 \times 10^{-4} \text{ M NaTFPB}$  | $2 \times 10^{-2} \text{ M NaCl}$     |     |
| $5 \times 10^{-2} \text{ M MgCl}_2$ | $5 \times 10^{-2} \text{ M TBATFPB}$ | $4.5 \times 10^{-2} \text{ M MgCl}_2$ | (4) |
|                                     | $1 \times 10^{-4} \text{ M DB18C6}$  |                                       |     |

(RE10-11)

(RE12-13)

Here, DB18C6 denotes the dibenzo-18-crown-6, and it was used to facilitate the transfer of  $\text{Na}^+$  from the aqueous phase to the NB phase. TBATFPB was added as the supporting electrolyte in the membrane phase, M, to lower the solution resistance. The W1 and W2 phases of all the cells contained 0.11 M  $\text{Cl}^-$  and were electrically connected to each other by Ag|AgCl electrodes immersed in every aqueous phase. In addition, the Ag|AgCl electrodes were immersed in all the W1 and W2 phases in order to measure the membrane potential of each cell. The membrane area of all the cells were  $1.77 \text{ cm}^2$ . In the electric circuit of the potential-receiving site, resistors that mimicked the solution resistances between the two sites within the axon were placed.

### 3. Results and Discussion

#### 3.1 Generation of the Action Potential at Synapse and Passive Response in the Axon

Figure 2 shows the time-courses of the changes in the membrane potential (A) and the current (B) of the Rec cells in the presence of the resistor ( $R = 3 \text{ k}\Omega$ ) and without the V-ap cells. Since the cross sectional area of the axon is very small ( $10^{-8}$ - $5 \times 10^{-6} \text{ cm}^2$ ) [17], the solution resistance between two adjacent nodes of Ranvier seems to be very high ( $\gg 1 \text{ k}\Omega$ ). The membrane potential ( $E_{\text{W1-W2}}$ ) of each Rec cell in the potential-receiving-sites (I) and (II) initially showed the resting potential, and the  $E_{\text{W1-W2}}$  values of the Rec1, Rec1', Rec2 and Rec2' cell were  $-76$ ,  $-75$ ,  $-75$  and  $-80 \text{ mV}$ , respectively. On the other hand, the  $E_{\text{W1-W2}}$  values of the RP and the AP cells were  $-76$  and  $+75 \text{ mV}$ , respectively. When the Rec cells were connected to the RP cell, the membrane potential of each cell remained almost close to the resting potential, and the local currents were negligibly low. Upon the switching of the sending site from the RP cell to the AP cell at  $t = 0 \text{ s}$ , the local current was generated and the membrane potential of every cell was changed.  $\text{Na}^+$  was transported from W2 to W1 in the AP cell, while  $\text{K}^+$  was transported from W1 to W2 in the Rec cells. Since every cell was connected in parallel, the sum of the currents flowing through the

Rec cells was equivalent to the current flowing through the AP cell in accord with Kirchhoff's current law. The peak current observed just after the switching from the RP to the AP cell was composed of the charging currents and the current for the ion transfer at each interface. The magnitude of the peak current decreased as the resistance between the receiving site and the sending site increased. Thus, the change in the membrane potential of the Rec2 (Rec2') cell was delayed in comparison with the Rec1 (Rec1') cell due to suppression of the current flowing through the respective cell. On the other hand, both the membrane potential and the current of each cell became almost constant after 5 s. The limiting current of every Rec cell was determined by the transport of  $K^+$  from M to W2, since its magnitude did not depend on the total value of the resistors between the objective Rec cell and the AP cell. The values of the current flowing through the Rec1, Rec1', Rec2 and Rec2' cells at  $t = 10$  s were 4.3, 4.2, 4.2 and 3.6  $\mu A$ , respectively. Similarly, the  $E_{W2-W1}$  values of the Rec1, Rec1', Rec2 and Rec2' cells at  $t = 10$  s were +31, +33, +20 and +18 mV, respectively. Moreover, the difference in the membrane potential between the Rec1 and the Rec2 cells (or the Rec1' and the Rec2' cells) satisfies the relation expressed by Eq (5).

$$E_{W1-W2, Rec1} - E_{W1-W2, Rec2} = i_{Rec2} R \quad (5)$$

where  $E_{W1-W2, Rec1}$  and  $E_{W1-W2, Rec2}$  represent the membrane potentials of the Rec1 and Rec2 cells, respectively, and  $i_{Rec2}$  denotes the current flowing through the Rec2 cell. Finally, upon switching of the Rec cells from the AP cell to the RP cell at 11 s, the opposite currents were generated through all the Rec cells, and the membrane potentials gradually went back to the initial resting potentials. As for the change in the currents, the charging currents were mainly observed just after switching from the AP cell to the RP cell. The faradaic currents due to the transport of  $K^+$  from W2 to W1 seems to be negligibly low. Although the hyperpolarization has been frequently reported in nerve conduction studies [5,6,9,10], such hyperpolarization did not appear in the present case. Since the local current is directly converted to the membrane potential using the

change in the permeability, it is thought that the hyperpolarization is accidentally generated during analysis by the voltage-clamp method [9,10,18,19], as shown in Fig. SI2. Figure 3 indicates the relation between the membrane potential and the membrane current by considering the transports of  $K^+$  and  $Na^+$  across the membrane. The  $i_{W1-W2}-E_{W1-W2}$  curves on the transports of  $K^+$  and  $Na^+$  across the membrane were derived by using the Goldman-Hodgkin-Katz equation [20]. Since a part of  $K^+$  channels always open, the resting potential is determined by the transport of  $K^+$ . When the  $Na^+$  channels begin to open, the positive current due to the transport of  $Na^+$  from W2 (extracellular side) to W1 (intracellular side) flows and the membrane potential changes in the positive direction. Since the negative current due to the transport of  $K^+$  from W1 to W2 belatedly occurred, the change in the total current of the given area is expressed as Fig. SI2. Although the negative current due to the transport of  $K^+$  from W1 to W2 flows after closing the  $Na^+$  channels, the membrane potential is more positive than the resting potential by considering the relation indicated in Fig. 3. If such a hyperpolarization actually occurs at a given node of Ranvier, excess amounts of  $K^+$  have to be transported from the cell outside to the inside against the concentration ratio. This condensation of  $K^+$  from the outside to the inside was explained by the function of the electrogenic  $Na^+/K^+$  pump [21-23]. However, a detailed mechanism of the coupling of the pumps remain unclear, then the reason why the membrane potential goes back to the resting potential cannot be clearly explained. By considering the rate of the influx of  $Na^+$  to the outflux of  $K^+$  during the action potential propagation [23], the possibility to inhibit the propagation may arise by the pump and the efficiency seems to have decreased.

### 3.2 Electrochemical Conception of the Voltage-gated $Na^+$ Channels

Figure 4 illustrates the time-courses of the changes in the membrane potential (A) and in the current (B) in the presence of the V-ap cell ( $R = 5 \text{ k}\Omega$ ). The V-ap1 or V-ap2 cell that mimics a

function of the voltage-gated  $\text{Na}^+$  channels at the receiving site (II) was connected to the relay switch when the membrane potential of the Rec1 or Rec2 cell exceeded 0 mV. In this case, 0 mV is regarded as the threshold. As mentioned in Sec. 3.1, by switching from the RP cell to the AP cell at  $t = 0$  s, a change in the membrane potential of each cell and the local current were generated. Since the membrane potential of the Rec1 cell exceeded the threshold ( $= 0$  mV) at  $t = 0.65$  s, the V-ap1 cell was connected to the circuit by the relay switch. Simultaneously, the local current was also generated between the Rec1 cell and the V-ap1 cell, and it altered the change in the membrane potential of the Rec1 cell from 0 mV to +31 mV within 0.3 s. In addition, according to the connection of the V-ap1 cell, the current for the ion transport at the Rec2 cell also increased, and the change in the membrane potential of the Rec2 cell also increased. Although the change in the membrane potential of the Rec2 cell becomes lower than that of the Rec1 cell because of the  $iR$  drop, the generation of the current at the Rec2 cell causes the change in the membrane potential of the Rec2 cell. Similarly, when the membrane potential of the Rec2 cell also exceeded 0 mV at  $t = 1.20$  s, the membrane potential of the Rec2 cell was altered from 0 mV to +38 mV within 0.3 s by the connection of the V-ap2 cell. Thus, the change in the membrane potential is only enhanced in the receiving site (II). On the other side (the receiving site (I)) of the electric circuit, the membrane potentials and the currents of the Rec1' and Rec2' cells, which were not connected to the V-ap cells, were hardly affected by the connection of the V-ap cells at the receiving site (II). In every cell, both the membrane potential and the current became almost constant at  $t = 9$  s. The  $E_{w1-w2}$  values of the Rec1, Rec1', Rec2 and Rec2' cells at  $t = 9$  s were +48, +31, +44 and +18 mV, respectively. On the other hand, the value of the local currents flowing through Rec1, Rec1', Rec2 and Rec2' cells at the same time were 2.5, 3.0, 2.6 and 2.7  $\mu\text{A}$ , respectively.

### 3.3 Proposal of a New Mechanism for the Action Potential Propagation

Figure 5 illustrates a proposed mechanism for propagation of the change in the membrane potential from the potential-sending site (synapse) to the potential-receiving sites (axon and the end of the cell). There are 4 Rec cells (1, 2, 3, 4) and 3 V-ap cells (1, 2, 3) in the electric circuit. In each cell couple (Rec/V-ap 1, 2, 3), the corresponding V-ap cell was connected 500 ms after the coupling of the Rec cell. This period means the open time imitating the mean value of the open life of the voltage-gated  $\text{Na}^+$  channel at the node of Ranvier. By considering the results mentioned above, we propose a new mechanism for the propagation of the action potential. First, when the ligand-gated  $\text{Na}^+$  channels are opened by the combination of the channel-type receptors with the transmitters at the synapse, the membrane potential become to change from the resting potential to the action potential. If the membrane potential exceeds the threshold, the membrane potential varies from the threshold to the action potential. This behavior corresponds to the switching from the RP to the AP cell in the present study. After the membrane potential at the adjacent nodes of Ranvier in the axon then exceeds the threshold, the membrane potential is changed to the action potential. This action corresponds to the connection of the V-ap cell to the electric circuit through the relay switch in this study. Thus the propagation of the change in the membrane potential is assisted by an increase in the local current flowing between the area indicating the action potential and the nodes of Ranvier removed from the synapse. On the other hand, the change in the membrane potential decreases and delays as the resistor corresponding to the distance from the synapse increases, namely the solution resistance in the actual nerve cell. The action potential is propagated along the axon by an increase in the circulating current generated by opening the ligand-gated  $\text{Na}^+$  channels.

When the membrane potential in the nodes of Ranvier exceeds the threshold, the voltage-gated Na channels begin to open in the nodes of Ranvier. The circulating current generated by the voltage-gated  $\text{Na}^+$  channels at a certain node of Ranvier also affects the circumstance and the current flows in the nearby node in the direction to the axon terminal. The

membrane area indicating the action potential spreads to the axon terminal. If the voltage-gated  $\text{Na}^+$  channels are immediately closed, the membrane potential does not go back to the resting potential at once and maintains a more positive potential than the initial resting potential, as shown in Fig. 2. Because multiple ligand-gated  $\text{Na}^+$  channels remain open for about 10 ms during the excited condition [11], it seems reasonable to assume that the membrane potential of the area where the voltage-gated  $\text{Na}^+$  channels have already closed remains more positive than the initial resting potential in the case of actual nerve systems.

In contrast, according to the conventional mechanism for the action potential propagation, it is interpreted that the membrane area where the voltage-gated  $\text{Na}^+$  channels have been closed shows the resting potential or a more negative potential than the initial resting potential (hyperpolarization) [9-11]. This is due to the disregard of the existence of the ligand-gated  $\text{Na}^+$  channels and due to the erroneous conversion from the current to the membrane potential, as we previously concluded [16]. On the other hand, the circulating currents for the ion transport and the charging current are generated whenever the other voltage-gated  $\text{Na}^+$  channels open. Thus the change in the current seems to be propagated like a pulse along the axon during the nerve conduction. In addition, taking into account the open lives of the voltage-gated  $\text{Na}^+$  channels, another model system about the nerve conduction that can control the open lives of the V-ap cells was constructed with the relay switches and timers. Our proposed mechanism for the action potential propagation was supported in this model, as indicated in Fig. 6.

#### 4. Conclusions

In this study, a new nerve-cell model that mimics the functions of the  $\text{K}^+$  and  $\text{Na}^+$  channels was constructed, and the directional propagation of the change in the membrane potential was elucidated. New interpretation of the hyperpolarization is found in the analysis based on the present model system. Considering the function of the ligand-gated  $\text{Na}^+$  channels at the synapse

(AP cell) and voltage-gated  $\text{Na}^+$  channels at the nodes of Ranvier (V-ap cells), we proposed a new mechanism for the directional propagation of the action potential on the basis of the analysis regarding the relation between the membrane potential and the circulating current. That is to say, the ligand-gated  $\text{Na}^+$  channels at the synapse serve as a power supplying devices to propagate the action potential along the axon, and the voltage-gated  $\text{Na}^+$  channels at the nodes of Ranvier work as supporting power supplying devices to assist the directional propagation of the action potential to the end of the axon at the same time.

## References

1. I. B. Levitan and L. K. Kaczmarek, *The Neuron: Cell and Molecular Biology*, Oxford University Press, New York, 3rd edn., 2002.
2. R. Phillips, J. Kondev and J. Theriot, *Physical Biology of the Cell*, Garland Science, New York, 2nd edn., 2009.
3. G. J. Kress and S. Mennerick, *Neuroscience*, 2009, 158, 211-222.
4. W. Schwarz and J. Rettinger, *Foundations of Electrophysiology*, Shaker, Germany, 2000.
5. B. Hille, *Ion channels of Excitable Membrane*, Sinauer Associates, Sunderland, USA, 3rd edn., 2001.
6. A. L. Hodgkin and A. F. Huxley, *J. Physiol.*, 1952, 117, 500-544.
7. I. Tasaki and G. Matsumoto, *Bull. Math. Biol.*, 2002, 64, 1069-1082.
8. R. Appali, U. van Rienen and T. Heimburg, *Advances in Planar Lipid Bilayers and Liposomes*, Vol. 16, 275-299, 2012.
9. J. A. Connor and C. F. Stevens, *J. Physiol.*, 1971, 213, 1-19.
10. B. Frankenhaeuser and A. F. Huxley, *J. Physiol.*, 1964, 171, 302-315.
11. E. R. Kandel and S. A. Siegelbaum, *Principles of Neural Science*, Chap. 11, McGraw-Hill, New York, 4th edn., 2000.
12. N. Ueya, O. Shirai, Y. Kushida, S. Tsujimura and K. Kano, *J. Electroanal. Chem.*, 2012, 673, 8-12.
13. Y. Kushida, O. Shirai, Y. Kitazumi and K. Kano, *Bull. Chem. Soc. Jpn.*, 2014, 87, 110-112.
14. Y. Kushida, O. Shirai, Y. Kitazumi and K. Kano, *Electroanalysis*, 2014, 26, 1858-1865.
15. Y. Kushida, O. Shirai, Y. Takano, Y. Kitazumi and K. Kano, *Anal. Sci.*, 2015, 31, 677-683.
16. Y. Takano, O. Shirai, Y. Kitazumi and K. Kano, *Phys. Chem. Chem. Phys.*, 2016, 18, 12689-12695.

17. M. K. Bear, B. W. Connors and M. A. Paradiso, *Neuroscience: Exploring the Brain*, Lippincott Williams&Wilkins Inc., USA, 3rd edn., 2007.
18. C. Gold, D. A. Henze, C. Koch and G. Buzsaki, *J. Neurophysiol.*, 2006, 95, 3113-3128.
19. C. Koch and I. Segev, *Nature Neuroscience*, 2000, 3, 1171-1177.
20. S. G. Schultz, *Basic Principles of Membrane Transport*, Cambridge Univ. Press, USA, 1980.
21. G. A. Kerkut and R. C. Thomas, *Biochem. Physiol.*, 1965, 14, 167-183.
22. M. Feletou and P. M. Vanhoutte, *Br. J. Pharmacol.*, 1988, 93, 515-524.
23. R. M. Spanswick, *Ann. Rev. Plant Physiol.*, 1981, 32, 267-289.
24. M. H. P. Kole, S. U. Ilshner, B. M. Kampa, S. R. Williams, P. C. Ruben and G. J. Stuart, *Nat. Neurosci.*, 2008, 11, 178-186.

## Figure captions

Fig. 1 Overview of the nerve cell (A), a model system constructed with some liquid membrane cells (B) and the structure of each cell (C)

Fig. 2 The time-courses of the membrane potentials (A) and of the currents (B) of the Rec cells in the presence of the AP cell

Solid line: Rec1 cell, dashed line: Rec1' cell, one-dot chain line: Rec2 cell, two-dot chain line: Rec2' cell, gray solid line: AP cell.

Fig. 3 The relation between the membrane potential ( $E_{W1-W2}$ ) and the membrane current ( $i_{W1-W2}$ ) based on the Goldman-Hodgkin-Katz equation

Black solid line: The  $i_{W1-W2}$ - $E_{W1-W2}$  curve on the transport of  $K^+$  (electrophysiological concept), black dashed line: the  $i_{W1-W2}$ - $E_{W1-W2}$  curve on the transport of  $Na^+$  (electrophysiological concept), gray solid line: the  $i_{W1-W2}$ - $E_{W1-W2}$  curve on the transport of  $K^+$  (electrochemical concept), gray dashed line: the  $i_{W1-W2}$ - $E_{W1-W2}$  curve on the transport of  $Na^+$  (electrochemical concept).

Fig. 4 The time-courses of the membrane potentials (A) and of the currents (B) of the Rec cells in the presence of the V-ap cells

Solid line: Rec1 cell, dashed line: Rec1' cell, one-dot chain line: Rec2 cell, two-dot chain line: Rec2' cell, gray solid line: AP cell.

Fig. 5 The time-courses of the membrane potentials of the Rec cells (1, 2, 3, 4)

Each V-ap cell (1, 2, 3) was connected for 500 ms (1: 0.5-1.0 s, 2: 0.8-1.3 s, 3: 1.2-1.7 s).  $R =$

1.5 k $\Omega$

Fig. 6 A new mechanism for the directional propagation of the action potential

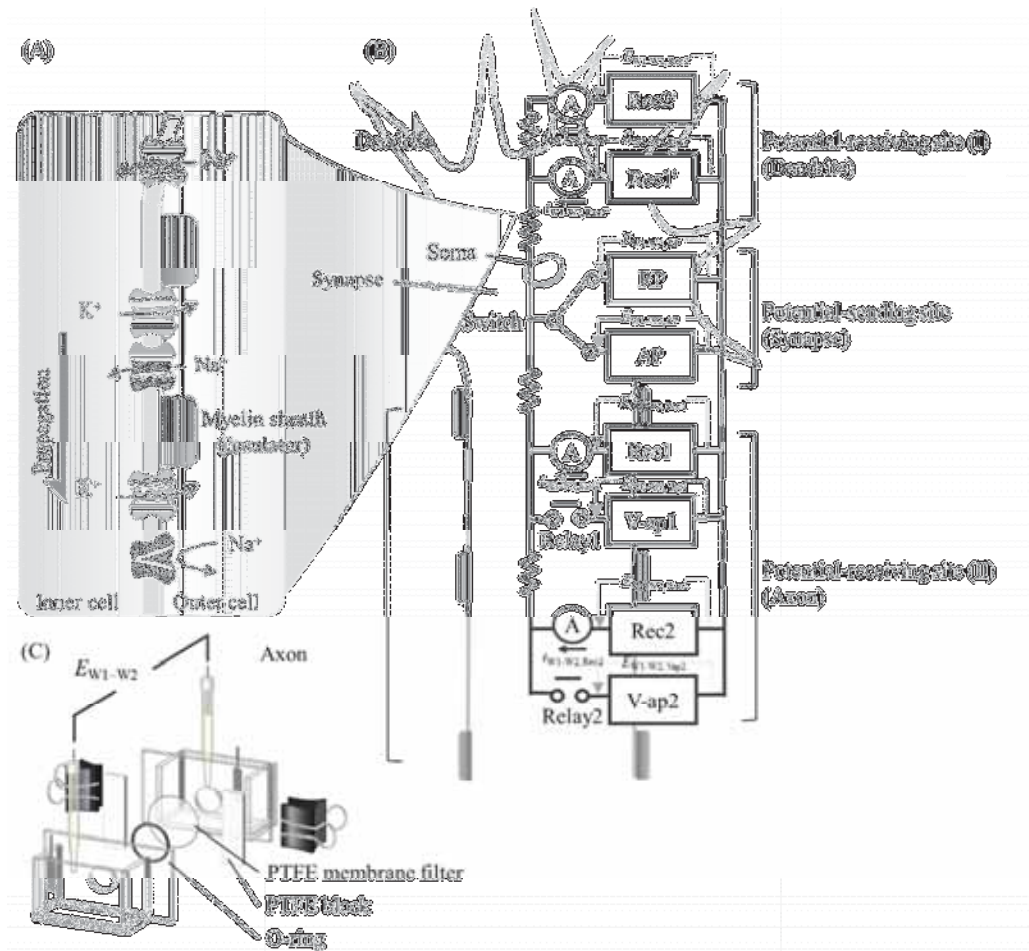


Fig. 1 Y. Takano, et al.

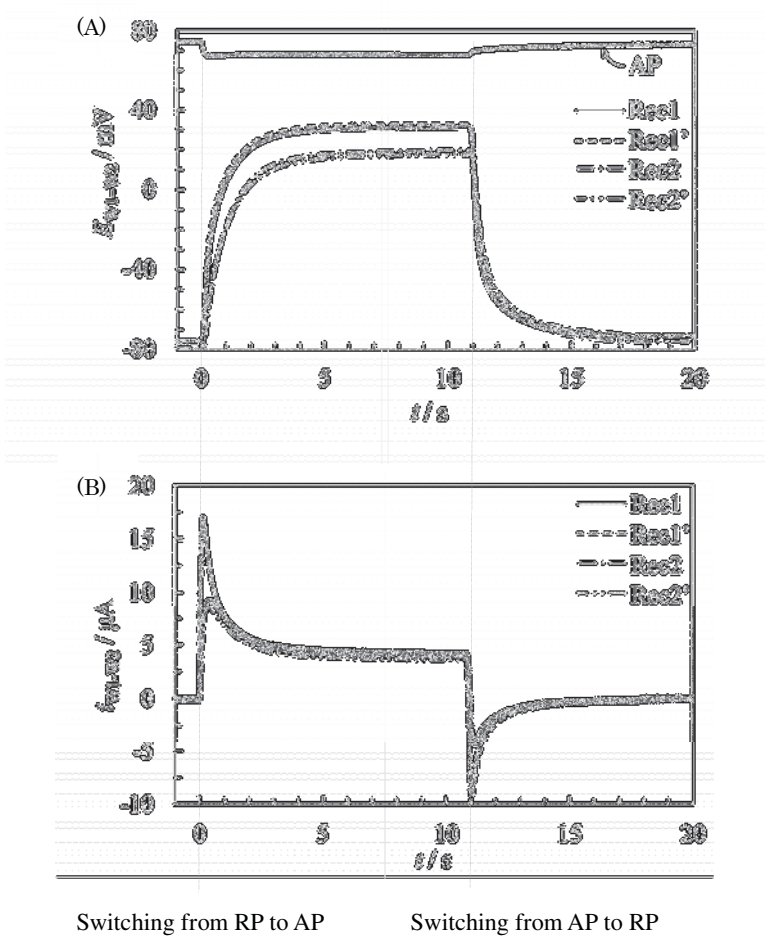


Fig. 2 Y. Takano, et al.

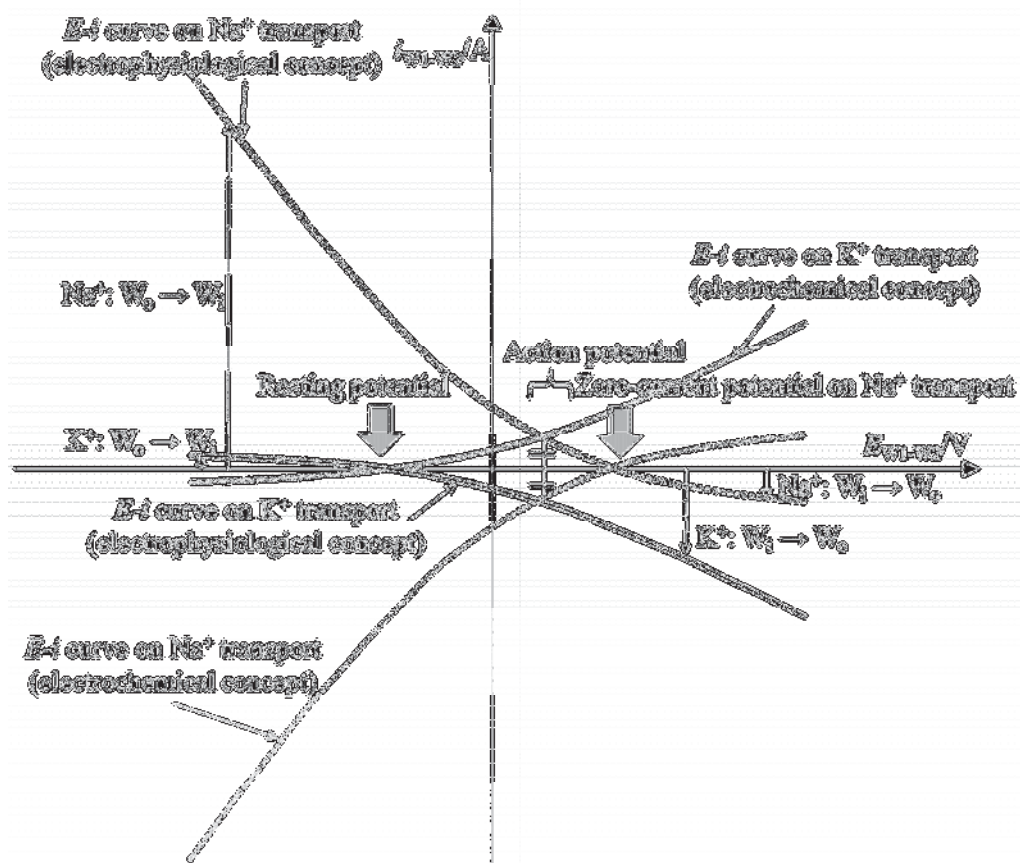
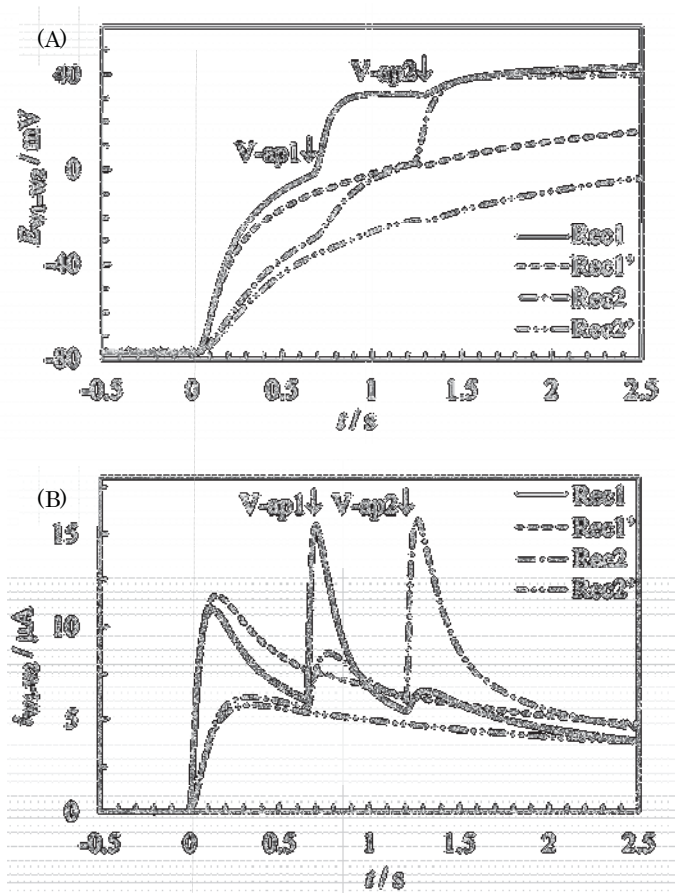


Fig. 3 Y. Takano, et al.



Switching from RP to AP

Fig. 4 Y. Takano, et al.

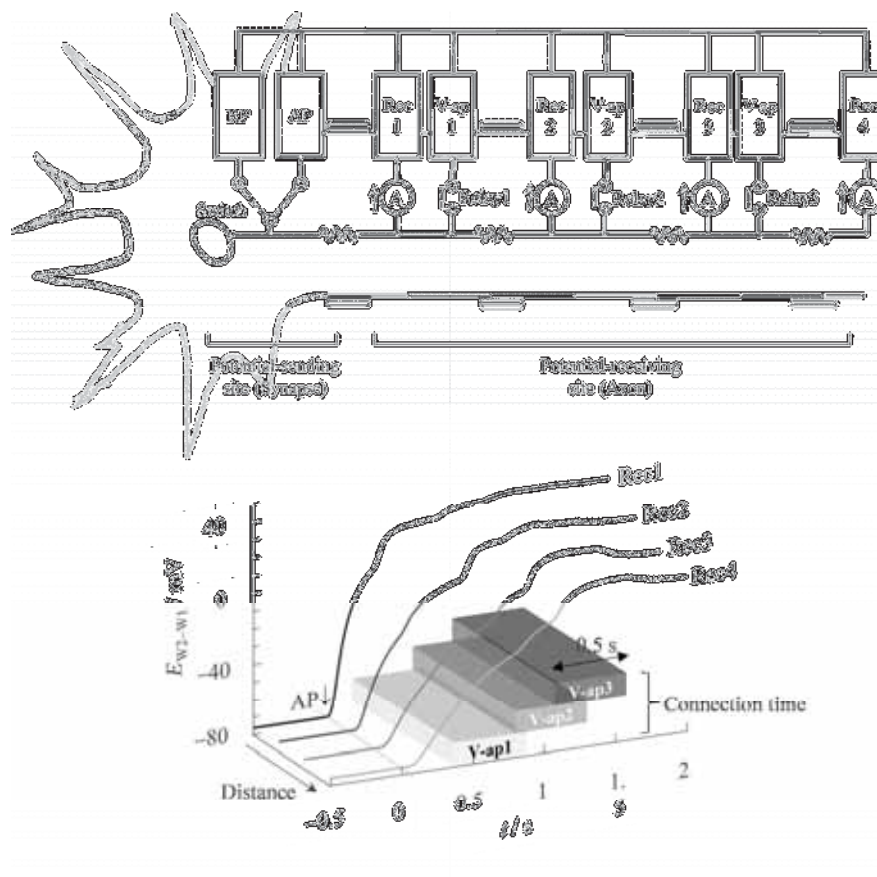


Fig. 5 Y. Takano, et al.

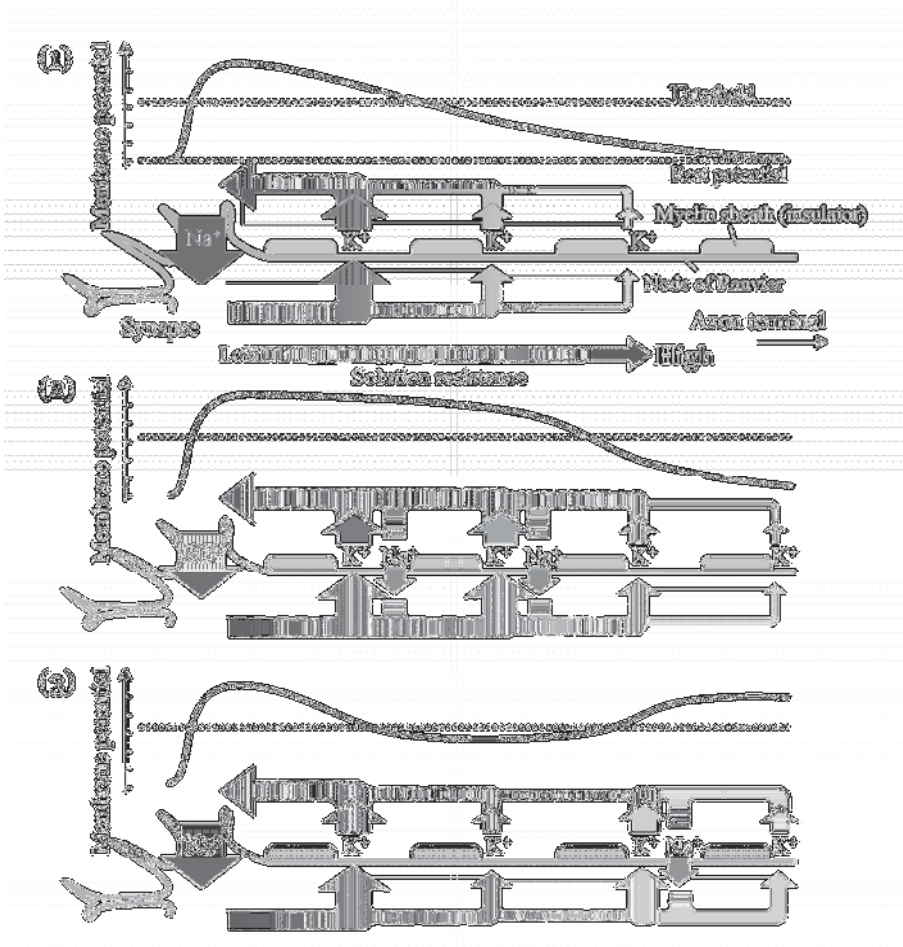


Fig. 6 Y. Takano, et al.