

Generating change in membrane potential by external electric stimulation and propagating the change by using nerve model cell systems

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Abstract

A change in the membrane potential in nerve cells is thought to be generated and propagated mainly by a function of K^+ and Na^+ channels. The concurrent monitoring of multipoints on the axon has been generally conducted on the basis of the voltage-clamp or current clamp method. Given that the respective membrane potentials have been evaluated by considering the applied potential, local current, and conductance, the propagation of the change in the membrane potential was measured. By using a nerve model system composed of some liquid membrane cells, we directly measured the actual membrane potentials and the local currents of the respective cells. We demonstrated that the local membrane current caused by an external voltage induced a change in the membrane potential and that the change was propagated by connecting the liquid membrane cells and mimicking voltage-gated Na^+ channels. It has been proved that hyperpolarization hardly occurs on the occasion of existence of the flux of K^+ and Na^+ only in the present model system and that the change in the membrane potential corresponding to the action potential is directionally propagated.

1. Introduction

A change in the membrane potential in nerve cells is generated and propagated mainly by the function of K^+ and Na^+ channels [1–4]. The usual membrane potential is determined by the ratio of the concentration of K^+ inside the nerve cell to that outside the cell; this ratio is called the resting potential. When neurotransmitters combine with channel-type receptors at the synapse by an external stimulus, Na^+ mainly flows from the outside of the cell into the inside of the cell around the receptors. Thus, the membrane potential shifts from resting potential to action potential, which is mainly determined by the ratio of Na^+ concentration inside the cell to that outside the cell at the synapse or at a partial area of the synapse. The change in the membrane potential is immediately propagated from the synapse to the axon. Considering that voltage-gated Na^+ channels that have short open lifetimes (approximately 1 ms) serve on the axon surface, the part indicating the action potential runs toward the axon terminal. Subsequently, the membrane potential on the axon goes back to the resting potential because of the function of delayed- K^+ channels after the hyperpolarization condition in which the membrane potential is more negative than the resting potential. Thus, it has been considered that a change in the membrane potential is propagated directionally along the axon toward the synaptic terminal.

The concurrent monitoring of the membrane potential at the multipoints on the axon has been performed on the basis of the voltage-clamp method [1, 2, 5]. Thus, the direct membrane potential has not been measured at the respective points because it is estimated by taking into account both the constant membrane potential and the potential difference converted from both the local current and membrane conductance. Several studies on nerve conduction using voltage-sensitive dyes have been conducted; however, the hyperpolarization has not been clearly observed yet because of the low time resolution of CCD cameras [6, 7].

The authors elucidated the propagation of the change in membrane potential by using organic

liquid membrane (LM) cells as a model of the nerve system. Ueya et al. [8] constructed an LM cell first to investigate the ways of propagating change in the membrane potential. Kushida et al. [9] made cells to mimic the resting potential and action potential by introducing a switch system. Furthermore, Takano et al. [10] developed an LM system with a function of K^+ and Na^+ channels to mimic actual nerve cells. Takano et al. [11] improved the cells to mimic voltage-gated Na^+ channels by using relay switches in the electric circuit and succeeded in reproducing the directional propagation of the change in the membrane potential. On the basis of these results, Shirai et al. [12] explained a new conduction mechanism based on the propagation of the change in the membrane potential using the liquid-membrane model system.

Several studies on nerve conduction have been performed by applying extracellular electric stimulus to nerve cells [1, 2, 5]. Action potential is locally generated between two extracellular points on the axon surface and is propagated to the nerve terminal along the axon under the condition clamping of the membrane potential. However, details on the mechanism responding to the extracellular electric stimulus have not been elucidated.

In this study, a model system mimicking the nerve cell was constructed to elucidate a way of responding to the external electric stimulus. The change in the membrane potential was generated by applying the potential gradient between two outside points, and the mechanism of change in the membrane potential was discussed by considering the effect of resistance within the circuit that was elucidated.

2. Experiments

2.1. Chemicals

Sodium (Na) tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (TFPB) (i.e., NaTFPB) was prepared in a similar manner as a previous study [13]. The TFPB⁻ salt of potassium (KTFPB) and that of tetrabutylammonium (TBATFPB) were obtained as follows. The methanol solution of KTFPB and

that of TBATFPB were prepared by mixing the methanol solution of KCl (Wako Pure Chemical Co.) and that of TBABr (Tokyo Kasei Chemical Co.) with that of NaTFPB. After adding ultrapure water to the methanol solutions of KTFPB and TBATFPB and after cooling them, the precipitations of KTFPB and TBATFPB were formed. KTFPB and TBATFPB were purified by repeating this recrystallization method. All other chemicals were of analytical reagent grade and were used without further purification.

2.2. Electrochemical cells

An LM cell made of glass was composed of two aqueous phases (W1 and W2) and a nitrobenzene phase (NB), as illustrated in Fig. SI-1. Each aqueous phase was saturated with nitrobenzene. A porous polytetrafluoroethylene membrane filter T100A025A (pore size: 1 μm ; thickness: 75 μm ; Advantec Tokyo Ltd.) was used as a structural material, and the filter impregnated with the NB solution was utilized as an LM.

2.3. Apparatus

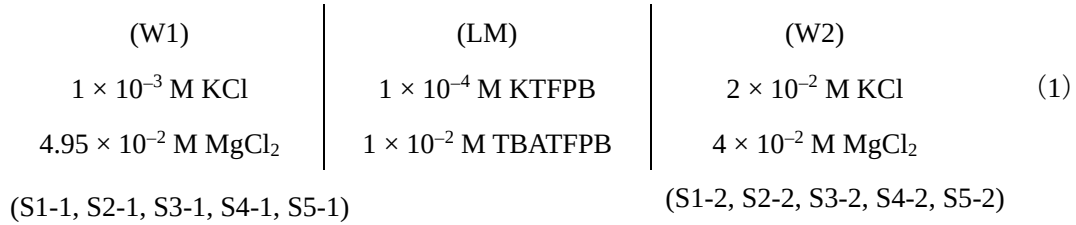
Potentiometric measurements were performed on an electrometer (HJE-100; Hokuto Denko Co.). The circulating current was measured by an ammeter (HM-103; Hokuto Denko Co.). Applications of the external voltage were performed by a multichannel potentiostat (HA-1010mM4A; Hokuto Denko Co.). All data were recorded using an A/D converter model (GL7000; Graphtec Co.) and a personal computer.

2.4. Electrochemical Measurements

2.4.1. Explanation for the response to the external electric stimulation

As illustrated in Fig. 1 (A), the cell system mimics the nerve axon. Five LM cells (Rec cells:

Rec1, Rec2, Rec3, Rec4, and Rec5) with membrane potentials indicating a resting potential (assuming to be approximately -70 mV) were connected in parallel, and some resistors ($R = 1$ k Ω and $R' = 100$ Ω) were placed between the respective Rec cells. Since these cells recognize the respective local membrane potentials, these cells are named Rec cells (potential-receiving cells). As shown in Fig. 1 (B), the resistor R or R' mimicked the resistance within the intracellular or extracellular solutions, respectively. The external voltage was then applied by the potentiostat. In each cell, the aqueous phase 1 (W1), aqueous phase 2 (W2), and organic phase (LM) mimicked the extracellular solution, intracellular solution, and cell membrane, respectively. The Ag|AgCl electrodes (S1-1, S1-2, S2-1, S2-2, S3-1, S3-2, S4-1, S4-2, S5-1, and S5-2) were immersed in the W1 and W2 of every cell to connect each other. The ionic composition of Rec cells 1 to 5 is written by Eq. (1). NB was used as a solvent of LM.



Each W1 or W2 phase of the five cells contained an identical concentration of Cl⁻ (0.1 M: M = mol dm⁻³). The membrane potential of each cell was measured as a potential difference between two Ag|AgCl electrodes immersed in the W1 and W2 phases of each cell. The current flowing through each cell was measured by the ammeter within the electric circuit. The current flowing through the respective LM cell was generated by the transfer of K⁺. Here, it is determined that the direction of the current flowing because of the cations transfer from W2 (inside) to W1 (outside) is positive.

2.4.2. Effects of the solution resistance and the applied external voltage on the change in the membrane

potential

In nerve systems, the propagation of a change in the membrane potential along the axon is affected by the solution resistance because of the small cross-sectional area of the nerve axon. In usual nerve axons, the intracellular solution resistance often cannot be ignored because usual nerve axons are very thin (1–500 μm) [14]. Given that the distance between two adjacent nodes of Ranvier is 0.2–2 mm, its resistance is estimated to be more than 1 $\text{M}\Omega$ [14]. In this study, different resistors ($R = 1, 10, 30 \text{ k}\Omega$) were selected as resistors of intracellular solution to compare the influence of the resistors on the propagation, since the influence is remarkable in the case that the resistance between neighboring cells is smaller than 100 $\text{k}\Omega$. $R' = 100 \Omega$ was selected as a resistor for the extracellular solution. The magnitude of the applied voltage also affects the propagation of the change in the membrane potential along the axon. Thereafter, an external voltage of 100 and 200 mV was applied.

2.4.3. Directionality of the action potential propagation by applying the external voltage

As illustrated in Fig. 1 (C), V-ap cells 1–5 were connected to Rec cells 1–5 in parallel. Here, these mimicked the function of the voltage-gated Na^+ channel (V-ap cell). The voltage-gated Na^+ channel opens when the membrane potential exceeds the threshold. Since the voltage-gated Na^+ channels serve as additive electric sources and the voltage is applied to the electric circuit, this type cell was named V-ap cell. By using relay switches, each V-ap cell was connected to the present model system; furthermore, when the membrane potential of each Rec cell exceeded 0 mV, that cell was selected as the threshold. The $\text{Ag}|\text{AgCl}$ electrodes (T1-1, T1-2, T2-1, T2-2, T3-1, T3-2, T4-1, T4-2, T5-1, and T5-2) were immersed in the W1 and W2 of every cell to connect each other. The ionic composition of Rec cells 1 to 5 is written by Eq. (2). The ionic composition of V-ap cells 1 to 5 is written by Eq. (2). Dibenzo-18-crown-6 (DB18C6) is served as an ionophore to stabilize Na^+ in the membrane phase. The membrane potentials of V-ap cells 1–5 were approximately +70 mV.

(W1)	(LM)	(W2)	(2)
2×10^{-2} M NaCl	1×10^{-4} M NaTFPB	1×10^{-3} M NaCl	
4×10^{-2} M MgCl ₂	5×10^{-2} M TBATFPB	4.95×10^{-2} M MgCl ₂	
	1×10^{-4} M DB18C6		
(T2-1, T4-1, T6-1, T8-1, T10-1)		(T2-2, T4-2, T6-2, T8-2, T10-2)	

3. Results and discussion

3.1. Generation and propagation of action potential by applying an external voltage

When an external voltage was applied in the case of $R = 10 \text{ k}\Omega$ and $R' = 100 \text{ }\Omega$, the membrane potential and membrane currents of Rec cells 1–5 were changed, as shown in Fig. 2. The membrane potential and membrane current of each cell (Rec 1–5) indicated almost the same values before the application of the external voltage (Fig. 3 (A)). The membrane potentials (E_{W2-W1}) of Rec cells 1–5 were approximately -70 mV (-72.6 , -73.0 , -70.5 , -71.8 , and -72.2 mV , respectively). The W1, W2, and M phases included K^+ , and both interfaces were depolarized by the transfer of K^+ . Therefore, membrane potentials were determined by the ratio of the concentration of K^+ in W1 to that in W2, and they indicated approximately 72 mV . When the external voltage ($V = 200 \text{ mV}$) was applied by the potentiostat (at $t = 0 \text{ s}$), the membrane potentials began to change, and the circulating currents were locally generated. Figure 3 (B) shows the directions of the circulating currents. The membrane currents (I_{W2-W1}) of Rec cells 1, 2, 3, 4, and 5 were -2.4 , -4.0 , 1.9 , 3.2 , and $0.4 \text{ }\mu\text{A}$, respectively. The membrane potentials of Rec cells 1, 2, 3, 4, and 5 became almost constant values of -89.5 , -117.0 , $+12.0$, -38.0 , and -54.0 mV , respectively, at $t = 10 \text{ s}$ after the voltage application. Just after the external voltage application was stopped (at $t = 14 \text{ s}$), the opposite currents were immediately generated, and the respective currents were close to 0 A . Simultaneously, the respective membrane potential was gradually restored to its initial resting potential. Thus, hyperpolarization was not observed in the

potential change in the case of Rec cells 3–5. On the other hand, the E_{W2-W1} values of Rec cells 1 and 2 show hyperpolarization and negative current flow. The largest current was observed at Rec2, which was the closest to the partly applied voltage, and the magnitude of the current decreased with increasing the distance. A change in the membrane potential became larger as the total resistance within the electric circuit became smaller.

3.2. Effects of the magnitude of the resistance of the intracellular solution and that of the applied external voltage on the change in the membrane potential

Figure 4 expresses the time courses of E_{W2-W1} and I_{W2-W1} of each Rec cell using three types of resistors ($R = 1, 10, 30 \text{ k}\Omega$) within the electric circuit. Although the resistance between two neighboring nodes of Ranvier seems to be larger than $1 \text{ k}\Omega$, three resistors were selected to realize the influence of the solution resistance. The absolute value of the change in E_{W2-W1} of all Rec cells was decreased and delayed. In particular, changes in Rec1, Rec4, and Rec5 cells were remarkable. The E_{W2-W1} values of Rec1 cells using $R = 1, 10$, and $30 \text{ k}\Omega$ at 10 s were about $-130, -90$, and -75 mV , respectively. Similarly, the E_{W2-W1} values of Rec4 cells at 10 s were about $+20, -35$, and -60 mV , respectively. In addition, E_{W2-W1} values of Rec5 cells at 10 s were about $+15, -55$, and -70 mV , respectively. For I_{W2-W1} , not only the current due to the ion transport but also the charging current appeared just after the switch was turned on and off. Similarly, the circulating currents also decreased with increasing total resistance, as represented in Fig. 4 (B). It is thought that these characteristics were caused by the loss of the potential difference in generating the change in the membrane potential. The decrease in the change in the membrane potential caused by the resistors was in agreement with the results reported by Kushida et al. [10] and Takano et al. [15]. Similarly, changes in I_{W2-W1} were also observed.

Figure 5 indicates the time courses of E_{W2-W1} and I_{W2-W1} in each Rec cell by applying two

different voltages ($V = 100, 200$ mV). The change in the membrane potential increased and accelerated at every Rec cell with an increase in applied voltage. The magnitude of the circulating current also increased at the same time. In this case, it is clear that the facilitation of the change in the membrane potential and the increase of the membrane current were attributed to the increase of the potential difference because iR decreases and because the solution resistance in the model is constant.

3.3. Directional propagation of action potential by applying an external voltage

When V-ap cells 1–5 that mimicked the functions of the voltage-gated Na^+ channels were connected to the electric circuit, the propagation of the change in the membrane potential was investigated. Figure 7 shows the time courses of the membrane potential E_{W2-W1} and the membrane current of each Rec cell. At first, each cell (Rec1–5) showed the resting potential. The membrane potential of each cell (E_{W2-W1}) was $-74.4, -74.3, -74.3, -74.3$, and -74.5 mV, respectively, before the voltage application. Similarly, the membrane potential (E_{W2-W1}) of V-ap cells 1, 2, 3, 4, and 5 in open circuit was $+70.7, +71.8, +72.4, +65.5$, and $+68.5$ mV, respectively (Fig. 6 (A)). By using relay switches, each V-ap cell was connected to the electric circuit when the membrane potential of each adjacent Rec cell exceeded 0 mV. When the external voltage ($V = 200$ mV) was applied at $t = 0$ s, the membrane potential and the circulating current began to change (Fig. 6 (B)). The membrane potentials of Rec3, Rec4, and Rec5 changed in a positive direction, and the positive membrane currents flowed at the same time. When the membrane potential of Rec3 cell reached 0 mV at $t = 0.37$ s, V-ap3 cell was connected to the electric circuit through the relay switch. The change in the membrane potential was facilitated by the connection of V-ap3 cell at approximately 0.37 s. Thereafter, the membrane potential of the Rec4 and Rec5 cells began to change in the positive direction ($t = 0.37$ s), and the V-ap4 cell was connected to the electric circuit through the relay switch. Therefore, the change in the membrane potential was more facilitated at approximately 0.91 s. Furthermore, the membrane

potential of the Rec5 cell reached 0 mV at approximately 0.93 s, and the V-ap5 cell was connected to the electric circuit. The membrane potential then changed and the membrane current was grown ($t = 0.93$ s). On the other hand, the membrane potentials of Rec1 and Rec2 changed in the negative direction, and the negative membrane current flowed. The currents were generated to maintain electroneutrality and mass balance in every phase. Just after the external voltage application was stopped, the opposite current was generated. The current was gradually restored to zero 2 or 3 s after turning off. However, the membrane potential was gradually restored to the initial resting potential. Thus, hyperpolarization was not observed in the present model system in a similar manner as previous studies [10-12].

Thus, because only the V-ap3, V-ap4, and V-ap5 cells were connected to the electric circuit by relay switches, the change in the membrane potential was directionally propagated. Given the membrane potential of Rec1 or Rec2 at the part above the voltage application where the point changed to hyperpolarization and did not reach the threshold, neither V-ap1 nor V-ap2 was connected to the circuit. In this way, the action potential propagated only to the part below the voltage application point, and that is similar to the firing and propagation of the action potential in neurons by applying external voltage (12).

4. Conclusion

We constructed a nerve model system mimicking the functions of K^+ and Na^+ channels. The circulating currents were locally generated at all cells by applying an external voltage to hold electroneutrality within the cell system. The membrane potentials of all cells were mutually changed following the polarity of the external voltage to maintain the total current between the inside and outside of the membrane. The changes in the membrane potentials were propagated to the adjacent cells, and they were decreased and delayed according to the total electric resistances at all cells. The

magnitude and speed of the change in the membrane potential depend on the magnitude of the applied external voltage. In the present model system, when we incorporated V-ap cells mimicking the voltage-gated Na^+ channel in the nerve model system, the action potential was promoted to be directionally propagated and became faster and stronger. In addition, it is easy to affect the conduction behavior by the external electric stimuli. When the nerve conduction is elucidated by the voltage-clamp or current-clamp method, the data should be carefully analyzed.

Hyperpolarization was not observed for the change in membrane potential in the present model system. Since the resting potential is a mixed potential due to the flows of plural ions such as K^+ , Na^+ , Ca^{2+} , Cl^- , and H_2PO_4^- in the actual nerve cells, it seems that the difference between the actual nerve conduction and the conduction in the model system is generated.

5. References

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Figure captions

Fig. 1 Electrochemical cells.

(A) Overview of a neuron and a model system.

(B) The LM model system used to explain the mechanism responding to the external electric stimulation.

(C) The LM model system including V-ap cells mimicking the voltage-gated channels.

Fig. 2 Time courses of the membrane potential (E_{W2-W1}) (A) and the membrane current (I_{W2-W1}) (B).

The applied potential ($t = 0-14$ s): 200 mV. The respective resistors (R and R'): $R = 10$ k Ω (intracellular), $R' = 100$ Ω (extracellular).

Fig. 3 The cell system as the electric circuit.

(A) Initial condition of the cell system as the electric circuit.

(B) The electric circuit during the voltage application.

Fig. 4 Time courses of the membrane potential (E_{W2-W1}) (A) and the membrane current (I_{W2-W1}) (B).

The applied potential ($t = 0-14$ s): 200 mV. The respective resistors (R and R'): $R = 1, 10, 30$ k Ω (intracellular), $R' = 100$ Ω (extracellular).

Fig. 5 Time courses of E_{W2-W} (A) and I_{W2-W} (B).

The applied potential ($t = 0-14$ s): 100, 200 mV. The respective resistors (R and R'): $R = 10$ k Ω (intracellular), $R' = 100$ Ω (extracellular).

Fig. 6 Electric circuit as the model system before the voltage application (A) and after the voltage application (B).

Fig. 7 Time courses of the membrane potential ($E_{w_2-w_1}$) and enlarged views (A) and the membrane current and enlarged views (B).

The applied potential ($t = 0-14$ s): 200 mV. The respective resistors (R and R'): $R = 10$ k Ω (intracellular), $R' = 100$ Ω (extracellular).