

Severe Problems of the Voltage-Clamp Method in Concurrent Monitoring of Membrane Potentials

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Abstract

In nerve cells, the concurrent monitoring of multipoint on the axon has been conducted based on the voltage-clamp method using two long parallel electrodes inside and outside the axon. As the respective membrane potentials have been evaluated by considering the clamping potential, the local current, and the conductance, the membrane potentials were not actually evaluated. We directly measured the actual membrane potentials and local currents of the respective cells using a nerve-model-system comprising some liquid-membrane cells. It is proved that the action potential propagation is artificially facilitated or prevented by both the external electric generator and two long electrodes.

1. Introduction

In nerve cells, the change in membrane potential is generated mainly by a function of K^+ and Na^+ channels, and the action potential is propagated along the axon to the synaptic terminal [1-4]. The usual membrane potential is determined by the ratio of the concentration of K^+ inside to that of the outside, and it is called the resting potential. When neurotransmitters released by an external stimulus combine with channel-type receptors at the synapse, Na^+ mainly flows from the outside into the inside through the channels. Thus, the membrane potential shifts from the resting potential to action potential are determined by the ratio of the concentration of Na^+ inside to outside at a partial area of the synapse. The change in the membrane potential is propagated from the synapse to axon. Because voltage-gated Na^+ channels, which have short open lifetimes (approximately 1 ms), serve on the axon surface, the part indicating the action potential proceeds toward the axon terminal. The membrane potential on the axon reverts to the resting potential because of the function of delayed- K^+ channels after the hyperpolarization condition of which membrane potential is more negative than the resting potential. It has been suggested that the change in the membrane potential is propagated unidirectionally along the axon toward the synaptic terminal.

The concurrent monitoring of the membrane potentials of the multipoint on the axon has been conducted based on the voltage-clamp method [1,2,5,6]. Each membrane potential has not been measured at respective points because it is estimated based on the relationship between the constant membrane potential and potential difference transformed from the local circulating current and partial conductance. Several studies based on the directional nerve conduction using voltage-sensitive dyes have been conducted [7-9]. Although hyperpolarization has not been observed in these cases, it is usually explained because the potential response of voltage-sensitive dyes is low. Many studies on nerve conduction have been performed by applying an extracellular electric stimulus to nerve cells [1-6,10,11]. The 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 of clamping the membrane potential. However, the influence of potential-clamping by the external electric device, potentiostat, and the generation of local circulating current within two long electrodes to clamp the membrane potential on the nerve conduction has not been elucidated. On the other hand, several research groups have reported experimental artifacts in the analyzing process [12-15]. Since the Hodgkin-Huxley model is empirical and parameters are estimated based on the voltage-clamp data, parameter variation causes inaccuracies.

The authors' group has elucidated the propagation of the change in the membrane potential using a nerve-model-cell system with multiple liquid-membrane cells [16-24]. Several liquid-membrane cells were connected using a switch, multiple relay switches, and multiple timers within the electric circuit to mimic a function of K^+ and Na^+ channels [20,21]. It was shown that the change in the membrane potential from the resting potential to the action potential was caused by the external electric stimulus and that the directional propagation of the action potential could be reproduced using the model system [22,23].

In the present study, the external electric circuit corresponding to the voltage-clamping system was introduced to the model-nerve system composed of multiple liquid-membrane cells. The directional propagation of the change in the membrane potential induced by the external electric stimuli and the influence of the propagation of the action potential on the voltage-clamp method was elucidated. It is confirmed that the facilitation or prevention of the propagation of the action potential was caused by the generation of the current flow within the external potentiostat and/or the local circulating current within two long electrodes inserted to fix the membrane potential.

2. Experimental

2.1 Chemicals

Sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB) were prepared based on a previous study [25]. The TFPB⁻ salt of potassium (KTFPB) and that of tetrabutylammonium (TBATFPB) were prepared using previous procedures. All other chemicals were of analytical reagent grade and used without further purification.

2.2 Electrochemical cells

A liquid-membrane cell comprises two aqueous phases (W1 and W2) and a nitrobenzene phase (NB), as described in Figure SI-1 [23,24]. The NB solution was impregnated in a porous polytetrafluoroethylene membrane filter T100A025A (pore size: 1 μm , thickness: 75 μm , ADVANTEC TOYO KAISHA, Ltd., Japan), and the filter was used as a liquid membrane (LM). The aqueous solutions in W1 and W2 were saturated with NB to inhibit leakage of NB from the filter.

Fig. SI-1

2.3 Apparatus

Potentiometric measurements were performed on an electrometer HJE-100 (Hokuto Denko Co., Japan). The circulating current was monitored using a zero-resistance ammeter HM-103 (Hokuto Denko Co., Japan). Clamping the membrane potential in the voltage-clamp method was performed using a multichannel potentiostat HA-1010mM4A (Hokuto Denko Co., Japan). All data were recorded by an A/D converter model GL7000 (Graphtec. Co., Japan) and a personal computer.

2.4 Electrochemical Measurements

2.4.1. Directional propagation of the change in the membrane potential by an external electrical stimulus

Graphic scheme 1A indicates the nerve axon as an electric circuit. Five liquid-membrane cells (Rec cells: Rec1, Rec2, Rec3, Rec4, and Rec5) of which membrane potentials indicated the resting potential

Graphic
scheme 1

were connected in parallel, and some resistors ($R = 1 \text{ k}\Omega$ and $R' = 100 \text{ }\Omega$) were set among respective Rec cells, as shown in graphic scheme 1B. “Rec” implies a receiving side as Rec cells received the influence of the external voltage. The resistor R or R' corresponds to the resistance within the intracellular or extracellular solutions, respectively. The resistor R_{Ex} or R_{In} represents the solution resistance between the outside or inside electrode and a certain Ranvier node, respectively. The external voltage was applied using the potentiostat in two different arrangements. The first arrangement was unidirectional and applying the voltage with two electrodes placed in the electric circuit corresponding to the axon, as shown in graphic scheme 1B-a. On the other hand, only one electrode was placed close to the electric circuit corresponding to the axon in the second arrangement. In each cell, W1, W2, and LM imitated the extracellular solution, intracellular solution, and cell membrane, respectively. The Ag|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 each cell to connect each other within the electric circuit. The ionic composition of Rec cells 1 to 5 is represented in scheme 1.

(W1)	(LM)	(W2)	(1)
$1 \times 10^{-3} \text{ M KCl}$	$1 \times 10^{-4} \text{ M KTFPB}$	$2 \times 10^{-2} \text{ M KCl}$	Scheme 1
$4.95 \times 10^{-2} \text{ M MgCl}_2$	$1 \times 10^{-2} \text{ M TBATFPB}$	$4 \times 10^{-2} \text{ M MgCl}_2$	
T1-1, B1-1		T1-2, B1-2	
T2-1, B2-1		T2-2, B2-2	
T3-1, B3-1		T3-2, B3-2	
T4-1, B4-1		T4-2, B4-2	
T5-1, B5-1		T5-2, B5-2	

Herein, M denotes mol dm^{-3} . Each aqueous phase of the five cells contains the same concentration of Cl^- (0.1 M). The membrane potential of each cell was measured as a potential difference between two Ag|AgCl electrodes (B1-1, B1-2, B2-1, B2-2, B3-1, B3-2, B4-1, B4-2, B5-1, and B5-2) immersed in

W1 and W2 phases of each cell. Currents flowing through each cell were measured using the ammeters within the electric circuit. These currents were generated by the transfer of K^+ within the respective cells. It was determined that the direction of the current due to the transfer of cations from W2 (inside) to W1 (outside) is positive. The relationship between the direction of the propagation of the change in the membrane potential and that of the external electric stimulus was examined.

2.4.2. Model cell system using voltage-clamp method

Graphic scheme 2 shows the model cell system mimicking the axon of the nerve cell. The membrane potential is fixed at a constant value for the experiments using the voltage-clamp method. For actual nerve conduction experiments, the change in the membrane potential from the resting potential to action potential is caused by the addition of an external electric stimulus or neurotransmitters [1-4]. In the present study, the beginning of the change in the membrane potential was caused by switching. We prepared the model cell system by assuming four Ranvier's nodes within the electric circuit. One node was a sending site where the membrane potential could be changed by switching, and the others were neighboring aligned nodes. The ionic composition of RP cell and Rec cells 1–3 is expressed by scheme 2. The ionic compositions of AP cell and V-ap cells 1–3 are expressed by schemes 3 and 4, respectively. Here, “RP” means resting potential and “Ap” or “ap” indicates action potential. Similar to scheme 1, W1, W2, and LM correspond to the extracellular solution, intracellular solution, and cell membrane, respectively.

Graphic
scheme 2

(W1)	(LM)	(W2)	(2)
$1 \times 10^{-3} \text{ M KCl}$	$1 \times 10^{-3} \text{ M KTFPB}$	$2 \times 10^{-2} \text{ M KCl}$	
$4.95 \times 10^{-2} \text{ M MgCl}_2$	$1 \times 10^{-2} \text{ M TBATFPB}$	$4 \times 10^{-2} \text{ M MgCl}_2$	
T1-1, B1-1		T1-2, B1-2	
T3-1, B3-1		T3-2, B3-2	

Scheme 2

T5-1, B5-1			T5-2, B5-2	
T7-1, B7-1			T7-2, B7-2	
(W1)	(LM)	(W2)		
2×10^{-2} M NaCl	4×10^{-3} M NaTFPB	1×10^{-3} M NaCl	(3)	Scheme 3
4×10^{-2} M MgCl ₂	1×10^{-2} M TBATFPB	4.95×10^{-2} M MgCl ₂		
	2×10^{-2} M DB18C6			
T2-1, B2-1		T2-2, B2-2		
(W1)	(LM)	(W2)		
2×10^{-2} M NaCl	1×10^{-4} M NaTFPB	1×10^{-3} M NaCl	(4)	Scheme 4
4×10^{-2} M MgCl ₂	1×10^{-2} M TBATFPB	4.95×10^{-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		

As shown in graphic scheme 2, a site comprising RP and AP cells, which mimics the synapse, is a sending site of the change in the membrane potential. Moreover, sites comprising a pair of Rec and V-ap cells, which are mimicking the Ranvier nodes, are receiving sites (I)–(III). The membrane potential between W1 and W2 was clamped using the potentiostat through two cords. Resistances R mimicking the solution resistance between each electrode (two cords) and the membrane depend on both the positions and shapes of the two electrodes. It seems that the solution resistance within the axons was negligibly small because the inner diameter of the usual axons was less than 1 mm. In the proposed model, the change in the membrane potential of each cell was monitored after shifting from the RP to AP cell, and the influence of the voltage-clamped operation was analyzed.

2.4.3. Demonstration of facilitation in the electric conductivity within the axon model due to the long

parallel electrode

Graphic scheme 3a shows the electrochemical cell mimicking the axon of the nerve cell. A polyvinyl chloride tube (length: 200 mm, inner diameter: 13 mm ϕ , outer diameter: 18 mm ϕ) was used as the cell container. Two reference electrodes were set at two points (**A** and **B**) to apply the potential difference, and two Pt counter electrodes were placed at both ends of the tube to measure the electric current. Four Ag|AgCl wires (0.3 mm ϕ , 50 mm in length) were used as reference electrodes. The electric conductivity within the cell was compared in the presence and absence of a long Ag wire (0.3 mm ϕ , 300 mm in length) coated by AgCl inside the cell. The length of the part exposed in the inner solution was 200 mm. The inner solution within the tube was 0.1 mM KCl or 1 mM KCl. The electric conductivity was evaluated by cyclic voltammetry. In addition, the electric current flowing within the long Ag wire was also detected by an ammeter, as shown in graphic scheme 3b. In this case, two long Ag wires were used instead of one long Ag wire, and two Ag wires were connected to the ammeter around the center of the tubular cell.

Graphic
scheme 3

3. Results and Discussions

3.1 Direction of the change in the membrane potential by external electrical stimulus

Figure 1A shows the change in the membrane potential of each cell. The membrane current observed at the same time is shown in Figure 1B. The membrane potentials of Rec1–Rec5 were approximately –65 mV, indicating the resting potential (–63, –61, –65, –66, and –67 mV, respectively), before switching. When an external voltage (250 mV) was unidirectionally applied to the electric circuit (at $t = 0.000$ s) along the cell system corresponding to the axon, as shown in graphic scheme 1Bb, negative currents flowed at Rec1 and Rec2 cells. At the same time, the membrane potentials of Rec1 and Rec2 shifted in the negative direction (about –150 mV). Although the potential shift of Rec 1 was larger than that of Rec 2, the magnitude of the current shift of Rec2 was larger than that of Rec1. On the

Fig. 1

other hand, the membrane potentials of Rec3, Rec 4, and Rec5 shifted in the positive direction (50 – 80 mV), and positive currents flowed at Rec3, Rec4, and Rec5 cells. Since the current flow in each Rec cell to provoke the change in the membrane potential and the total resistance within the local electric circuit are in inverse proportion, the magnitude of the potential shift at Rec3 was largest and that at Rec 5 was smallest. The circulating currents were then locally generated by the local ion transfers at each cell, as shown in graphic scheme 1Bb. The changes in the membrane potential and membrane current at a cell that was closer to a part of the application of the external voltage (Rec2 and Rec3) were larger. Although the current flows of Rec2 and Rec3 were in the opposite directions, the magnitudes of the current flows were almost equivalent. The sum of the current flows in Rec1-Rec5 was kept at 0 A. When the application of the external voltage was stopped (at $t = 5.000$ s), the reverse circulating currents were locally generated by the local ion transfers, and the membrane potentials returned to the resting potential. It is worth noting that these potential shifts were caused by the respective circulating current based on the relationship between the membrane potential and electric current within the receiving site because the circulating currents flowed as illustrated in graphic scheme 1Bb. When the membrane potential exceeded the threshold in the actual nerve conduction, voltage-gated Na^+ channels opened, and the membrane potential immediately varied with the action potential, as shown in Figure SI1. The local change in the membrane potential facilitates the further propagation of the action potential. In this case, it is predictable that the action potential unidirectionally spreads along the axon. When one electrode is placed close to the axon and another is set a long distance, it is known that the action potential is bidirectionally propagated along the axon [4]. Applying the positive charge on the electrode attached on the axon, the bidirectional propagation was observed, as shown in Figure SI2. On the contrary, it was difficult to cause the ignition of the action potential by addition of the negative charge on the axon, as shown in Figure SI3.

Fig. SI2

Fig. SI3

In an actual nerve propagation, the change in the membrane potential unidirectionally propagates

from a synapse to an axon terminal. This is because the action potential generated at the synapse propagates to the axon terminal along the axon [1-4,22,23].

3.2 Propagation of the change in the membrane potential using voltage-clamp method

The influence of the voltage-clamp method on the nerve conduction was estimated using the model cell system presented in graphic scheme 4. In the electric circuit, electric resistances were placed between the respective sites mimicking Ranvier's nodes (receiving sites 1-3). The electric circuit mimicking two long electrodes of the nerve cell inside and outside to clamp the membrane potential corresponded to the respective solution resistances. Herein, 100 Ω of electric resistances (R_S) were selected as the solution resistance between the neighboring sites inside the nerve cell because the inside diameter of a general myelinated axon is small (1 to 20 μm), and the distance between neighboring Ranvier's nodes is 0.3 to 2 mm [28]. Figure 2 shows the change in the membrane potential of each cell observed between -0.01 s and 0.14 s. The data between -5 s and 10 s were presented in Fig. SI4. The membrane current of each cell recorded between -0.01 s and 0.14 s is shown in Figure 3. Similarly, the data between -5 s and 10 s were presented in Fig. SI5. Here, the electric resistances R_S ($= R_{\text{Ex}} = R_{\text{In}}$) were changed ($R_S = 10 \text{ k}\Omega$, 2 $\text{k}\Omega$, 1 $\text{k}\Omega$, 510 Ω , and 0 Ω).

Results obtained in the case of $R_S = 2 \text{ k}\Omega$ were first investigated. The potential difference between W1 (extracellular) and W2 (intracellular) was set at -75 mV by two electrodes for voltage clamping. The membrane potentials of RP at the sending site and Rec cells 1-3 (Rec1, Rec2, and Rec3) at the receiving site before switching (-76, -77, -76, and -77 mV, respectively) were approximately -76 mV, indicating the theoretical resting potential. Therefore, the clamping potential was settled at -76 mV. Similarly, the membrane potentials of AP at the sending site and V-ap cells 1-3 (V-ap1, V-ap2, and V-ap3) at the receiving site before switching (75, 70, 78, and 75 mV, respectively) were approximately 75 mV. Just after switching from the RP cell to the AP cell within the electric circuit (at

Graphic
scheme 4

Fig. 2

Fig. 3

Fig. SI4

Fig. SI5

$t = 0.000$ s), the circulating currents were locally generated by the local ion transfers, as shown in graphic scheme 4b and Figure 3. The V-ap1 cell was connected to the circuit when the membrane potential of the Rec1 cell exceeded the threshold, which was set at 0 mV (at $t = 0.046$ s), as presented in Figure 2. Since the currents of Rec2 and Rec3 increased, this connection accelerated the changes in the membrane potentials of Rec2 and Rec3 in the receiving site. When the membrane potentials of Rec2 reached the threshold at 0.052 s, the V-ap2 cell was connected to the electric circuit. The current of Rec3 cell then increased in the same manner, and the V-ap3 cell connected to the electric circuit at $t = 0.056$ s. Thus, the action potential was unidirectionally propagated. When we switched from AP to RP cell in the electric circuit (at $t = 5.000$ s), the reverse circulating currents were locally generated by the local ion transfers, and the membrane potentials returned to the resting potential (at $t = 9.000$ s). The hyperpolarization was not observed in all the cells because the resting potential was equal to the theoretical value determined by the ratio of K^+ concentration only [24]. Similar results were obtained when the membrane potential was not clamped and was clamped with $R_S = 1$ or 10 k Ω . The change in the membrane potential after switching in the case of non-clamping and clamping with $R_S = 10$ k Ω was larger than the case of clamping with $R_S = 2$ k Ω . In the case of clamping with $R_S = 1$ k Ω , the change in the membrane potential after switching was smaller than that observed with $R_S = 2$ k Ω . Although the potential changed in the same way until V-ap1, V-ap2, and V-ap3 cells connected to the electric circuit, the time required for the connection changed. The connection using the cell system with $R_S = 2$ or 10 k Ω was faster than that of non-clamping cell system. To the contrary, the connection using the cell system with $R_S = 1$ k Ω was slower than that of the non-clamping cell system. The membrane potentials of Rec1, Rec2, and Rec3 cells were kept less than 0 mV in the case of clamping with $R_S = 510$ and 0 Ω . Thus, V-ap1, V-ap2, and V-ap3 cells were not connected to the electric circuit.

For the time-course of the membrane current, the absolute values of the electric currents within the potentiostat (the voltage-clamping electric circuit) and AP cell decreased with an increase in the

R_S value, as illustrated in Figure SI6. As the R_S value increased, the influence of the voltage clamping on the change in the membrane potential weakened because of the contribution of iR drop. Therefore, the conduction behaviors in the case of $R_S = 1, 2$ or $10\text{ k}\Omega$ is similar to the case of non-clamping. Here, the smaller the R_S value was, the smaller the current flowed within Rec1, Rec2, and Rec3 cells at the receiving site. In addition, the current flow within the potentiostat increased with a decrease in the R_S value. Most of the current generated by connection with AP cell flowed within the potentiostat at $R_S = 0\text{ }\Omega$. Similarly, the current flowed within the potentiostat at $R_S = 510\text{ }\Omega$, $1\text{ k}\Omega$, and $2\text{ k}\Omega$. Accordingly, it is thought that the current flow within the potentiostat prevented the propagation of the action potential. On the other hand, the propagation of the action potential in the case with $R_S = 2$ or $10\text{ k}\Omega$ is faster than that in the case of the non-clamping cell system. Since the current flows of Rec2 and Rec3 were larger than that of the non-clamping cell system until V-ap1, V-ap2, and V-ap3 cells connected to the electric circuit, the local circulating current across two long electrodes seemed to be caused. Usually, the current flow within the potential-clamping system has not been paid attention. Therefore, generating an electric current with the voltage-clamping method directly affects nerve conduction. Moreover, there is another severe problem using the voltage-clamping method. The conduction velocity will be hastened in the presence of the long electrode because the electric current tends to flow easily within the long electrode along the axon. Considering the increase in the conductivity within the axon due to the existence of the long electrode (Ag wire coated with AgCl), the electric current flowing within the axon increases and the propagation of the action potential can be facilitated, as shown in Figure SI7. The influence of an electric conductor within the axon on the electrical conductivity inside the axon was estimated. An electrochemical cell composed of a polyvinyl chloride tube, Ag|AgCl electrodes, Pt wire electrodes, silicon caps, polypropylene tips, etc. was prepared to mimic the axon, as illustrated in graphic scheme 3. Cyclic voltammograms were obtained by scanning the potential differences between two Ag|AgCl electrodes (distance, **A**: 20 cm, **B**: 10 cm)

and recording the current between two Pt wire electrodes. Figure 4 indicates cyclic voltammograms in the aqueous solution containing 1 mM KCl. The electrical conductivities in the presence of the long electrode within the tube was 8-100 times larger than those in the absence of the long electrode. It is easily predictable that the insertion of the electric conductor such as long electrodes facilitates the nerve conduction. The voltage-clamp method is usually used to measure the change in the membrane potential at multiple points, and the long electrode is inserted into the axon to clamp the membrane potential. Because local circulating currents generated on the long electrode increase the conductance of the inside of the axon. The conduction velocity increased when the resistors between two sites mimicking Ranvier's nodes were small. Thus, the usual voltage-clamp method has a risk of producing such an artifact. In the present cell system, the influence of the capacitance components on the propagation of the action potential in the voltage-clamp method is not considered. Since the addition of capacitors to the electric circuit caused the delay in the propagation [17], the influence of various electric components on the propagation should be evaluated.

4. Conclusion

The directional propagation of the action potential caused by the external electric stimulus was elucidated using the nerve-model system mimicking the function of K^+ and Na^+ channels. It was observed that the local circulating currents caused the propagation of the action potential according to the application of an external electric stimulus. In the voltage-clamp method, it has been pointed out that artifacts appear due to various empirically derived parameters of the Hodgkin-Huxley model by several research groups. Moreover, we found that the voltage-clamp method has severe problems in the accurate analysis of the nerve conduction because the electric current flows within the external electric circuit (the voltage-clamping device) to keep the membrane potential constant and the electric conductivity of intracellular and extracellular fluids increases by inserting two long electrodes. In

particular, there is a fear that the inner electrode facilitates the conduction within the axon.

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Caption of Graphic Schemes

Graphic scheme 1 Schemes of a nerve-model cell system as an electric circuit in the case of applying an external electrical stimulus (A) and simple nerve-model cells composing five liquid-membrane cells by applying an external electric stimulus (B). A nerve-model system by applying the unidirectional voltage along the axon (a) and the illustration indicating the direction of current flow (b), respectively.

Graphic scheme 2 Overview of a nerve-model cell for voltage-clamp experiments.

Graphic scheme 3 Schemes of electrochemical cells mimicking the axon of the nerve cell in the presence of a long electrode inserting in the axon. The electrochemical cell to evaluate the influence of the long electrode on the conductivity within the tube (a) and that to measure the current flow within the long electrode (b).

Graphic scheme 4 Cell construction in the presence of the voltage-clamp system as the electric circuit. Initial condition of the cell system as the electric circuit (a); electric circuit when the potential difference between two long electrodes is fixed by the potentiostat (b).

Figure Captions

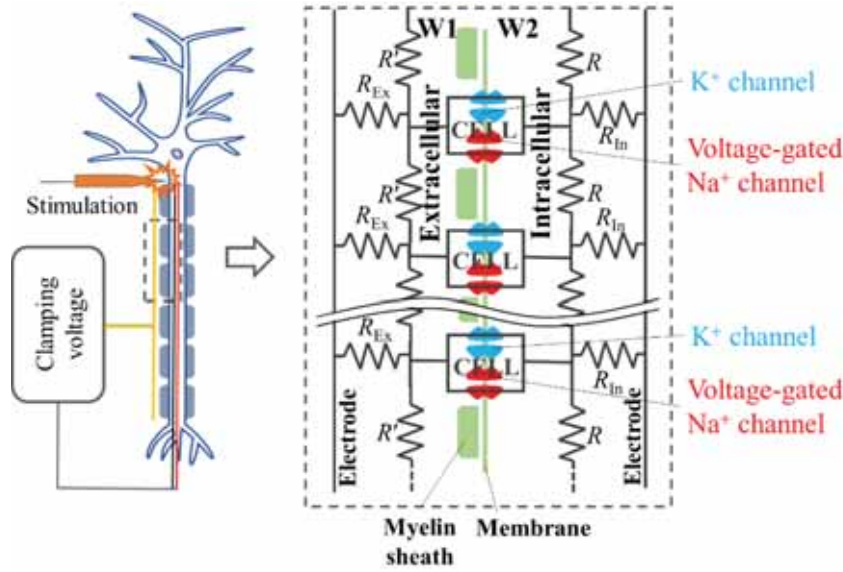
Fig. 1 Time courses of the membrane potential (a) and the membrane current (b).

Fig. 2 Time courses of the membrane potential recorded under the variable values of R_S . Time: – 0.01 s to 0.14 s.

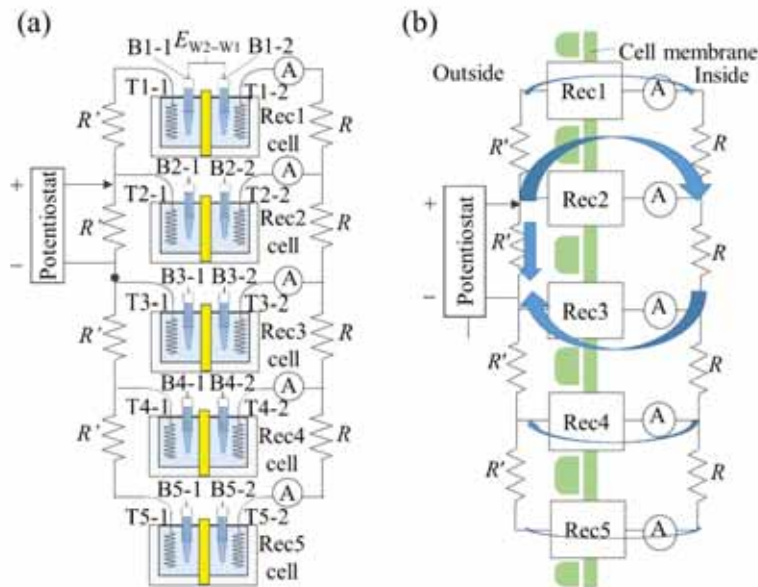
Fig. 3 Time courses of the membrane current recorded under the variable values of R_S . Time: –0.01 s to 0.14 s.

Fig. 4 Cyclic voltammograms between two Ag|AgCl electrodes within the tube in the presence and absence of a long Ag wire. Distance between two Ag|AgCl electrodes: (a) 10 cm, (b) 20 cm. Aqueous solution: 1 mM KCl. Potential scanning rate: 100 mV s^{-1} . bg: absence of the inner long electrode, Ag: presence of the inner long electrode, Ag-ammeter: the current flowed in ammeter is used as i instead of the current flow between two Pt wire electrodes.

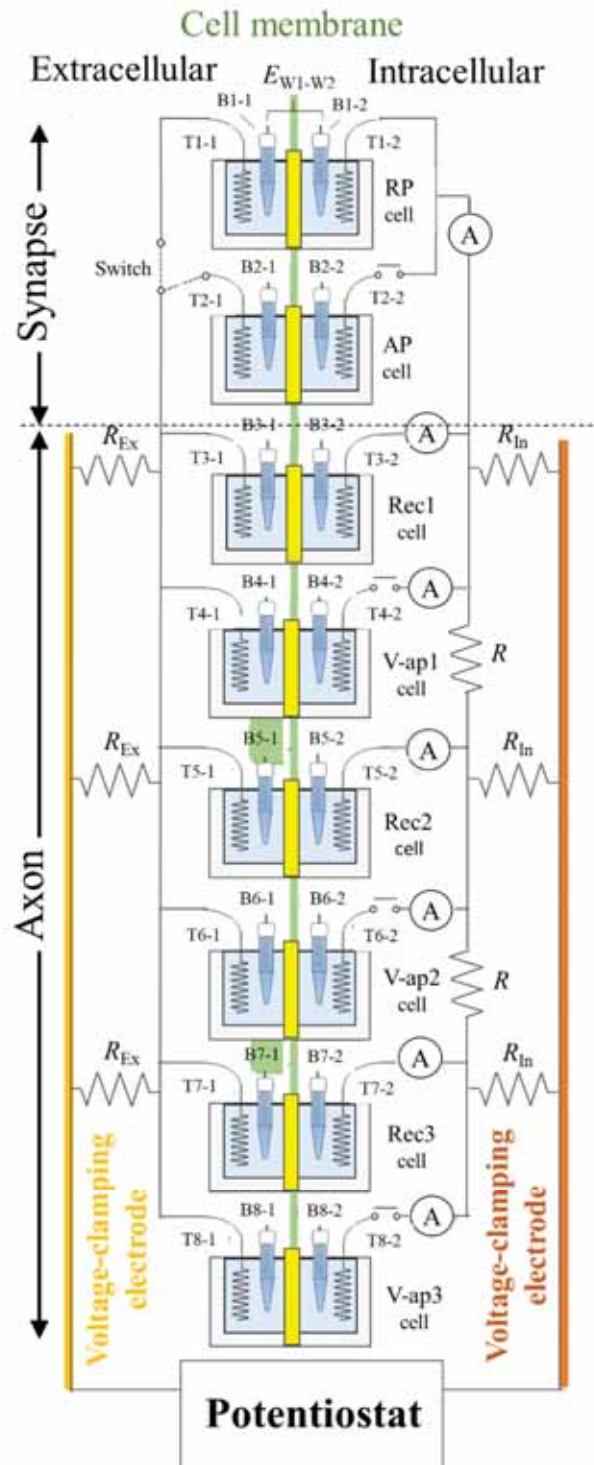
A)



B)

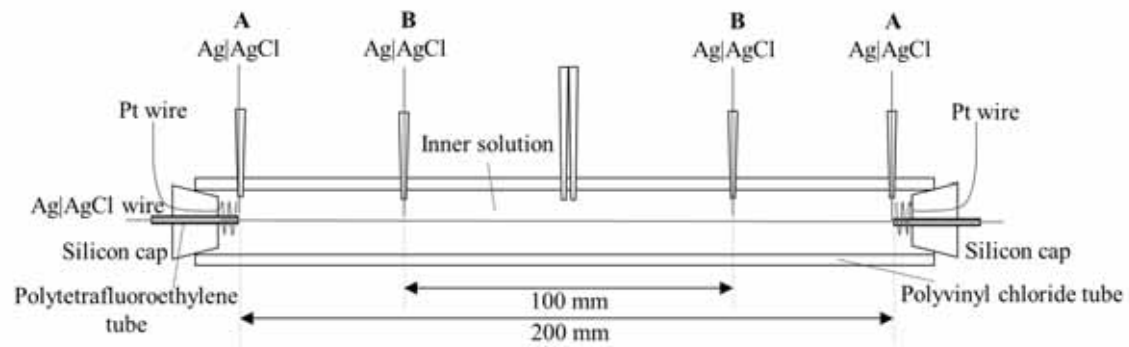


Graphic scheme 1 Schemes of a nerve-model cell system as an electric circuit in the case of applying an external electrical stimulus (A) and simple nerve-model cells composing five liquid-membrane cells by applying an external electric stimulus (B). A nerve-model system by applying the unidirectional voltage along the axon (a) and the illustration indicating the direction of current flow (b), respectively.

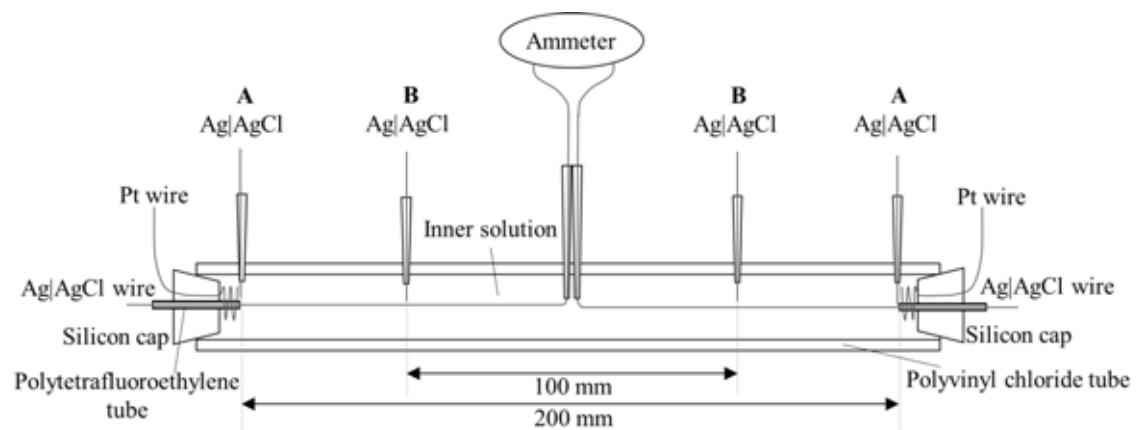


Graphic scheme 2 Overview of a nerve-model cell for voltage-clamp experiments.

(a)

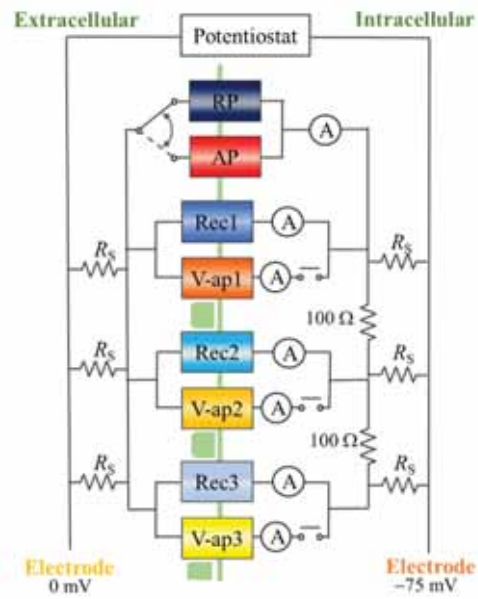


(b)

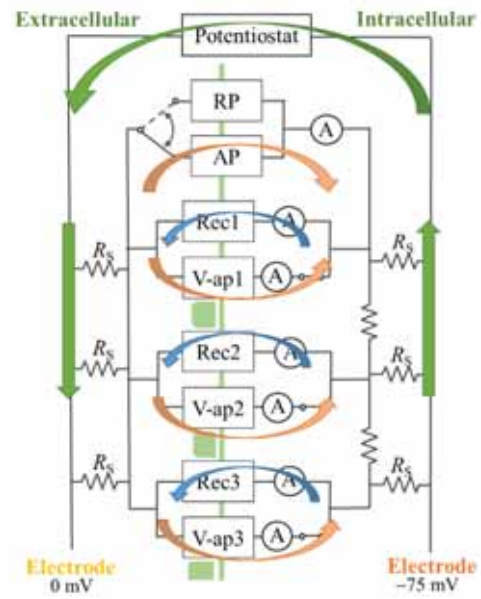


Graphic scheme 3 Schemes of electrochemical cells mimicking the axon of the nerve cell in the presence of a long electrode inserting in the axon. The electrochemical cell to evaluate the influence of the long electrode on the conductivity within the tube (a) and that to measure the current flow within the long electrode (b).

(a)



(b)



Graphic scheme 4 Cell construction in the presence of the voltage-clamp system as the electric circuit. Initial condition of the cell system as the electric circuit (a); electric circuit when the potential difference between two long electrodes is fixed by the potentiostat (b).

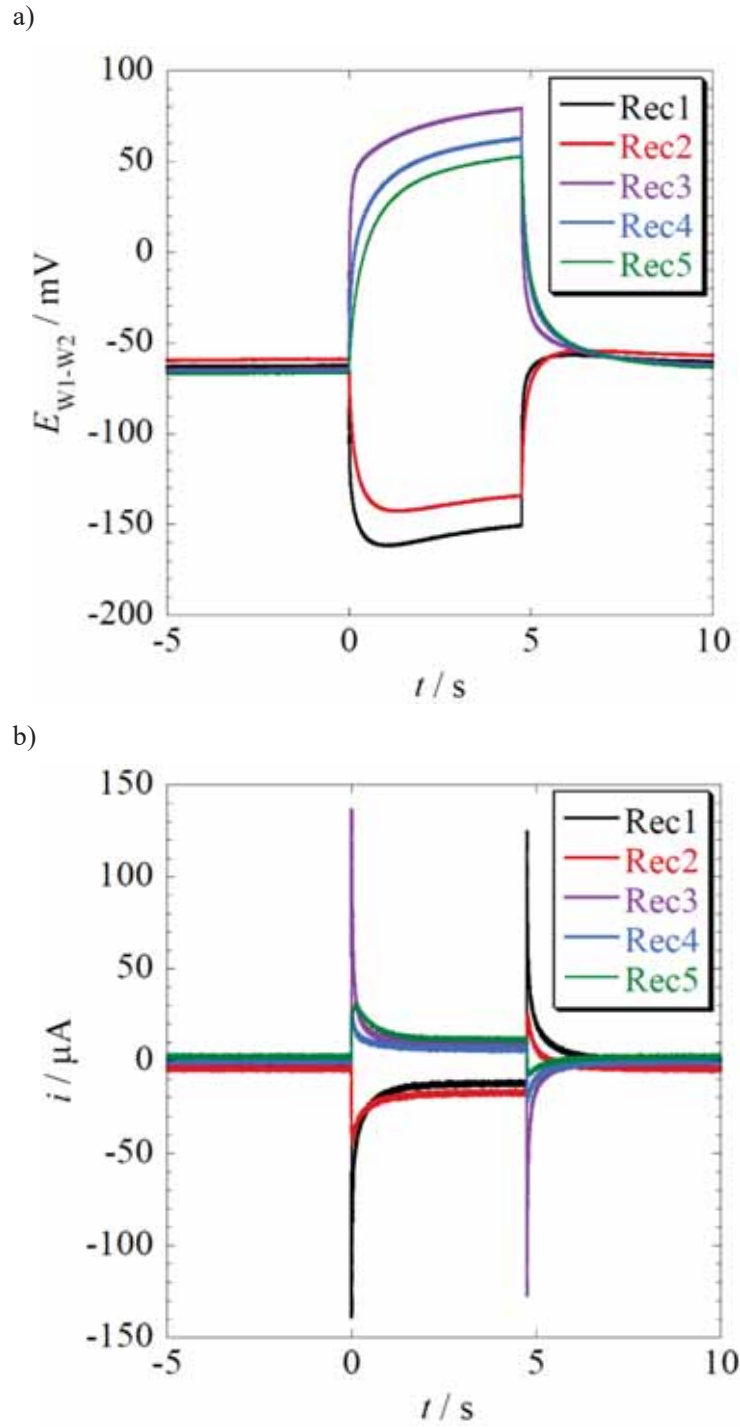
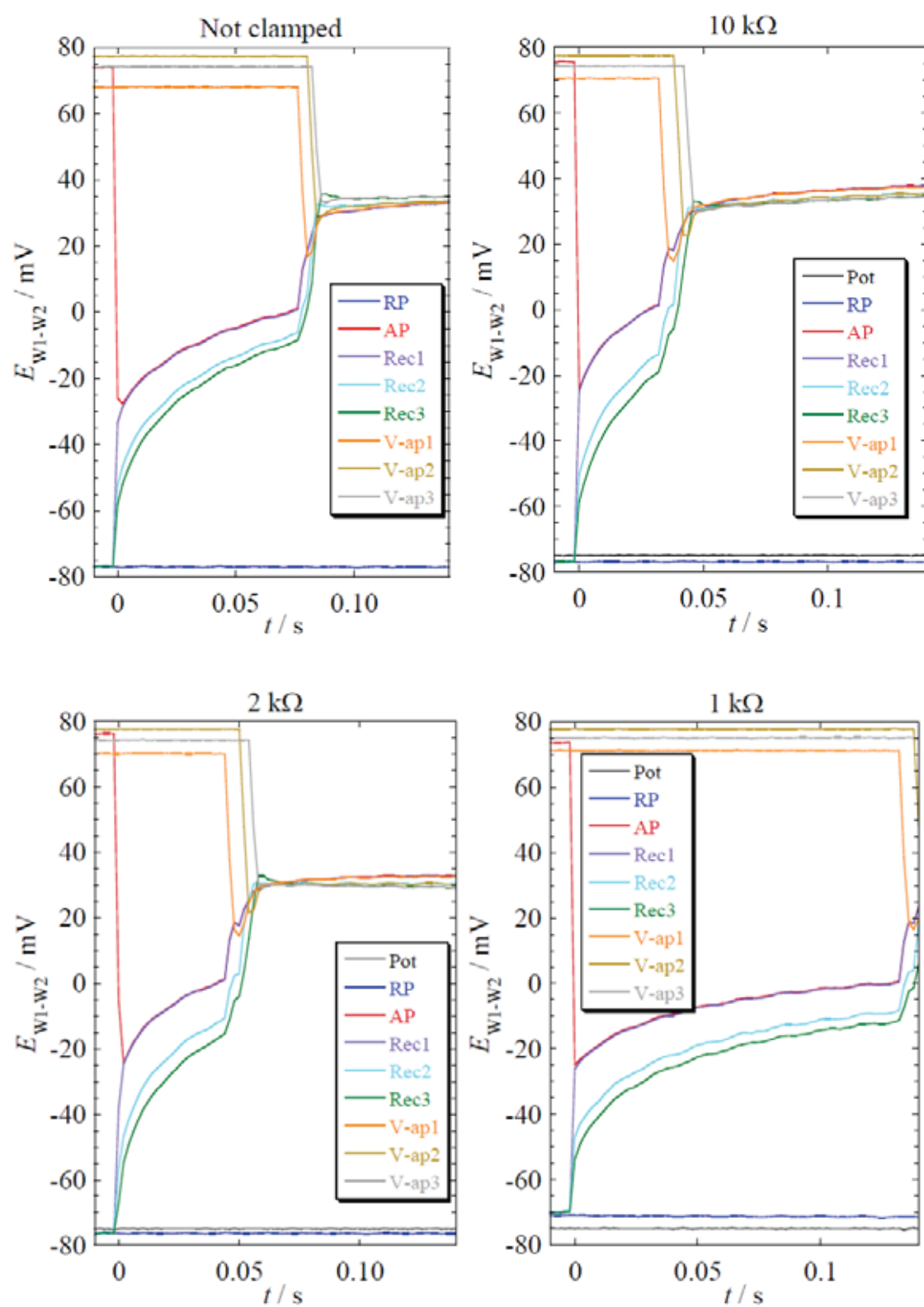


Fig. 1 Time courses of the membrane potential (a) and the membrane current (b).



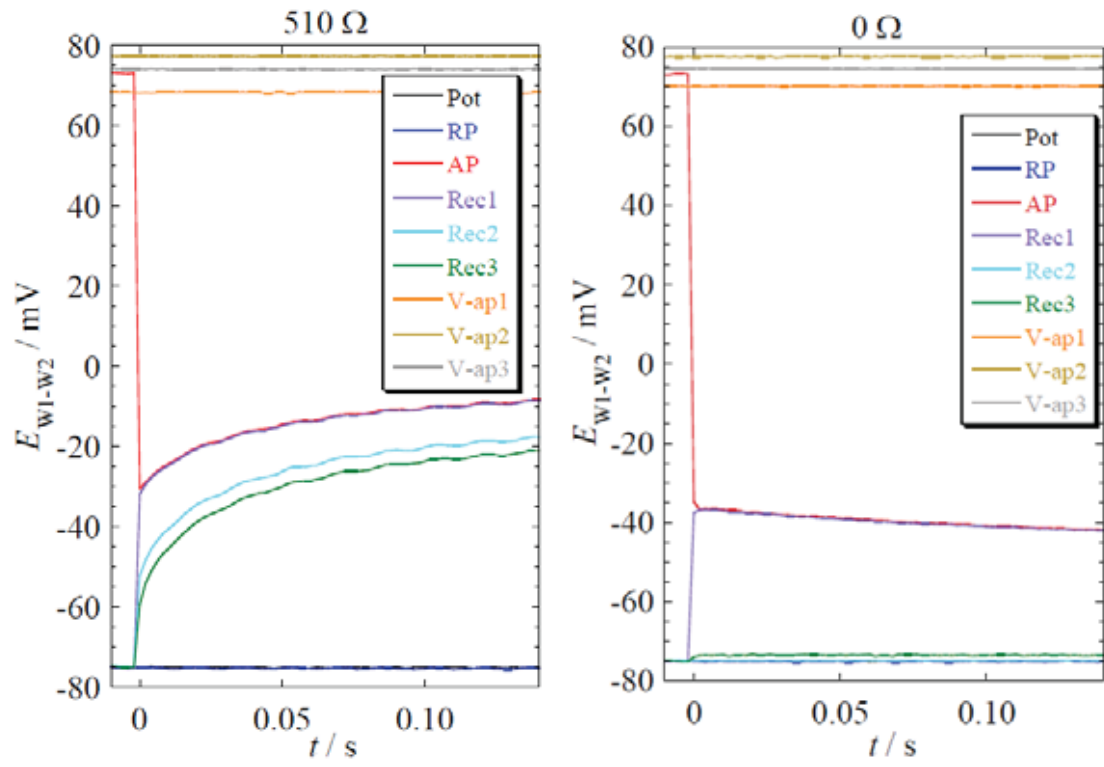
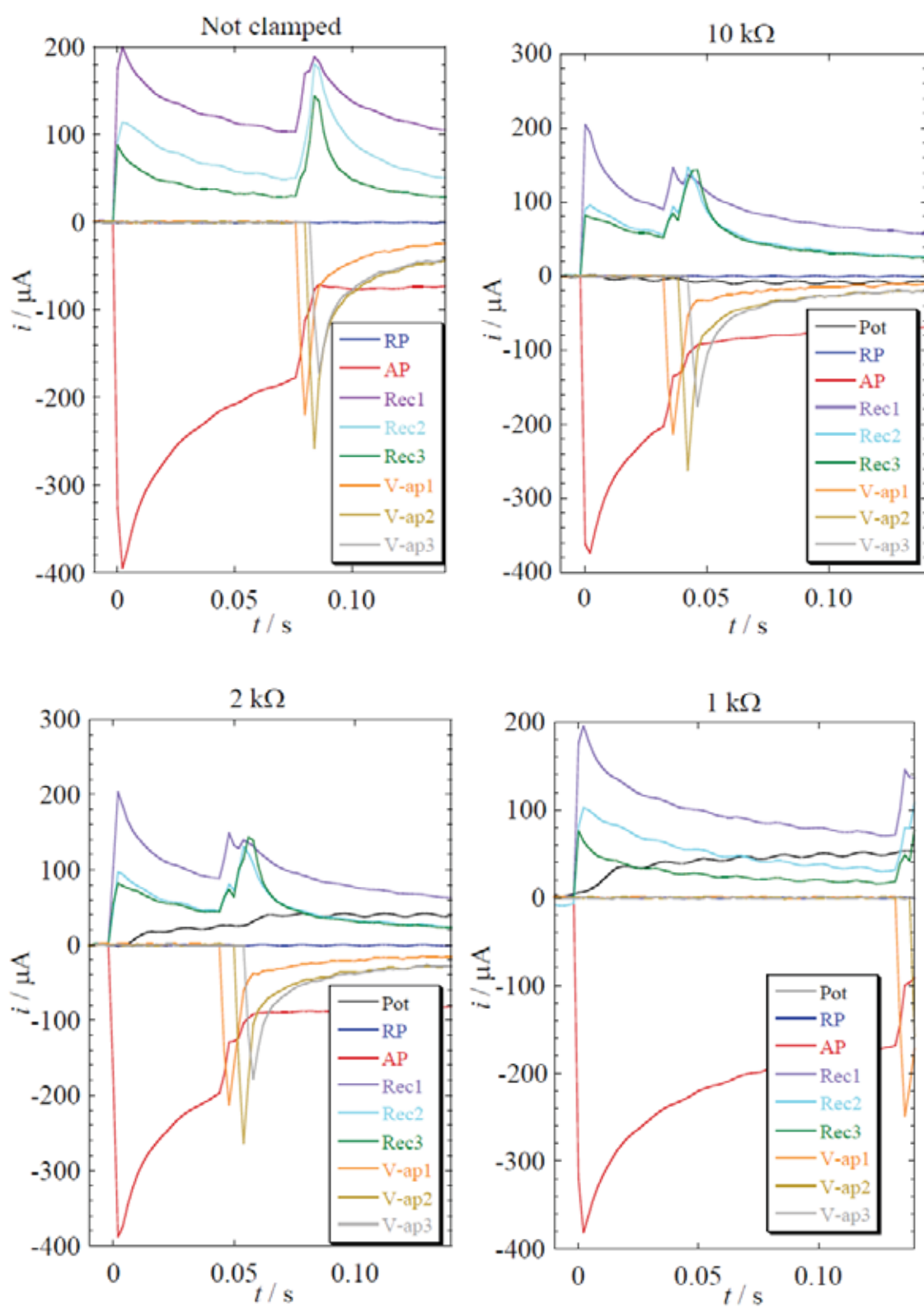


Fig. 2 Time courses of the membrane potential recorded under the variable values of R_s . Time: – 0.01 s to 0.14 s.



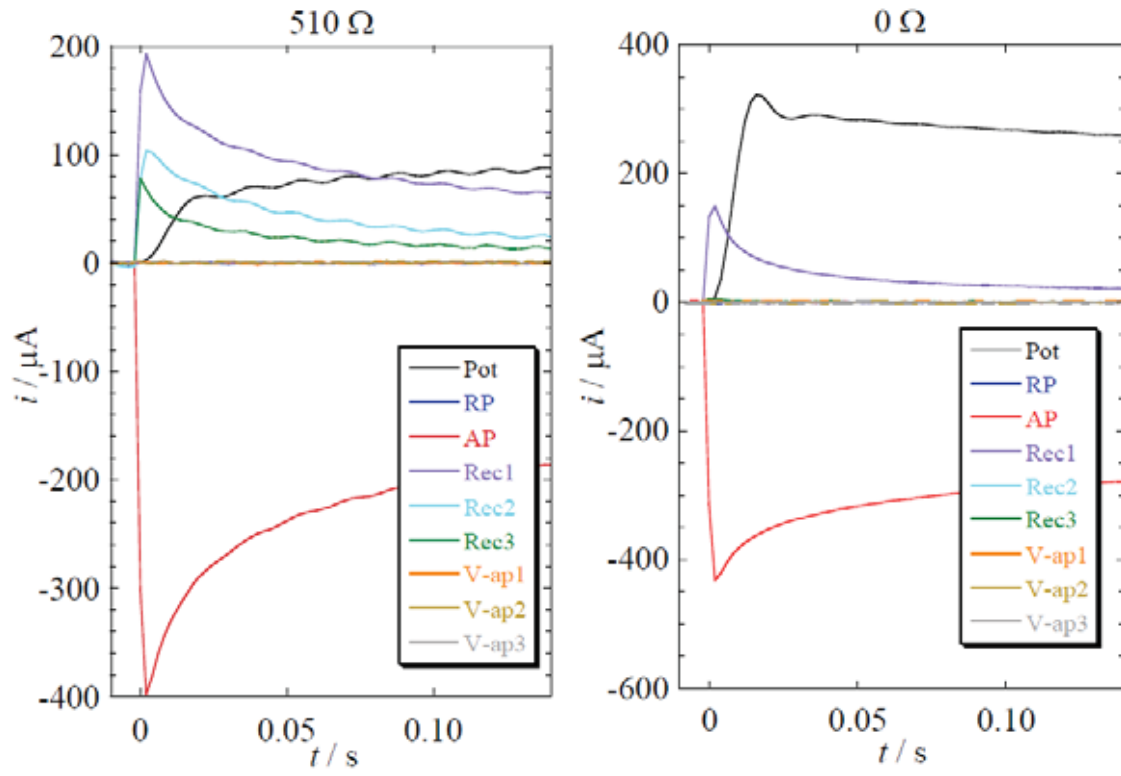


Fig. 3 Time courses of the membrane current recorded under the variable values of R_s . Time: -0.01 s to 0.14 s.

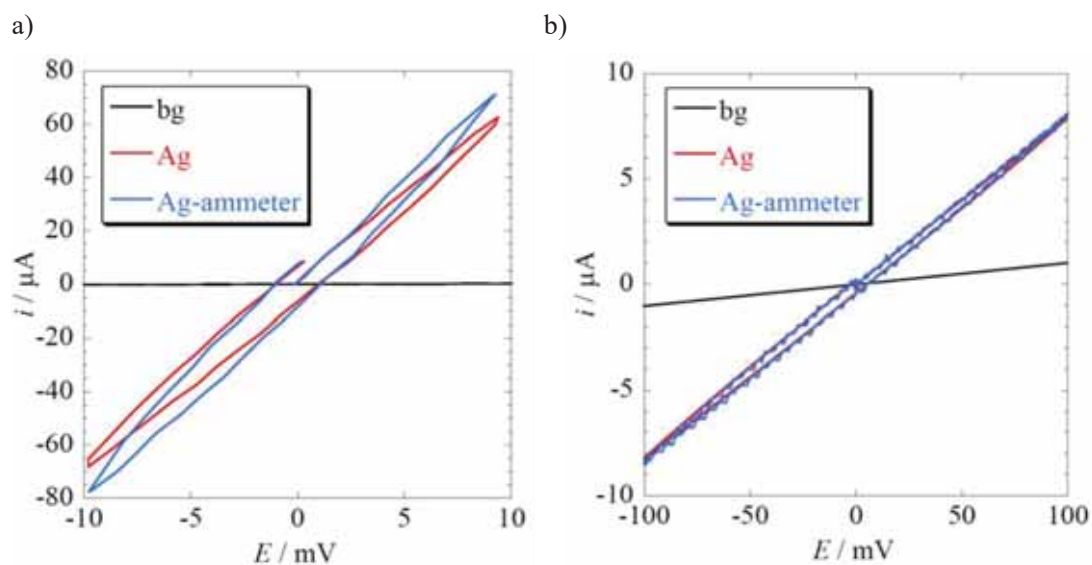


Fig. 4 Cyclic voltammograms between two Ag|AgCl electrodes within the tube in the presence and absence of a long Ag wire. Distance between two Ag|AgCl electrodes: a) 10 cm, b) 20 cm. Aqueous solution: 1 mM KCl. Potential scanning rate: 100 mV s^{-1} . bg: absence of the inner long electrode, Ag: presence of the inner long electrode, Ag-ammeter: the current flowed in ammeter is used as i instead of the current flow between two Pt wire electrodes.