Research on cellular neurophysiology education using a newly developed smartphone-enabled web-based simulation

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Contents

General introduction

Neurophysiology has become an integral part of biology curricula in both high school and undergraduate schools. Above all, understanding the phenomena and principles of cellular neurophysiology is the most fundamental and important aspect of learning neurophysiology. However, experimental methods such as intracellular recording, membrane voltage clamping, and patch clamping, which are essential to understanding them, are very difficult for high school and undergraduate student training due to both technical and cost considerations. Therefore, cellular neurophysiology is generally taught from teachers to students in a knowledge-transfer manner, using text diagrams such as membrane potentials and membrane currents that only illustrate one aspect of the principle. Students may come to rely on rote memorization, a form of passive learning that is insufficient for developing true understanding and problem-solving skills. As a result, it is becoming increasingly difficult to develop an inquisitive interest in neurophysiology. This situation in neurophysiology education must be improved. Active learning, where students conduct experiments on computer models of neurons, is an effective method for teaching core neurophysiology principles (Grisham et al., 2008; Bish and Schleidt, 2008; Rutten et al., 2012). Many cellular neurophysiology simulators have been reported so far (for example, Carnevale and Hines, 2001; AV-Ron et al., 2006;

Newman and Newman, 2013; Moore and Stuart, 2007: Stuart, 2009; Heitler, 2022; Meir, 2022). Nevertheless, learning cellular neurophysiology using computer simulations is not widespread. Japan is one of the countries where the use of information and communication technology (ICT) in education is the least advanced (Ma and Qin, 2021; Wu et al., 2023). In such countries, it is difficult to use simulations for learning. Since neurobiology instructors are not necessarily computer specialists, most of them have no experience in introducing computer simulations into practice. Therefore, it may be difficult for them to teach students how to install simulators on various models. They may not have the budget to introduce a new paid computer simulation. Other barriers are that the number of computers in the training room is not always the same as the number of students, there may be no computers available at all, or not all students necessarily own a computer. To my knowledge, there is still no simulator that covers a wide range of cellular neurophysiology, requires no installation, is free, easy to operate, and available on any device. If such simulators existed, it would greatly contribute to the education of neurophysiology.

The purpose of this thesis is to develop simulators that can be used in countries where simulation learning has been difficult until now, to actually popularize simulation learning in cellular neurophysiology, and to evaluate its effectiveness.

In Part 1, I describe research on the development of simulators to achieve this purpose. Even in countries where the use of ICT in education is less advanced, students have their own smartphones and are good at using them. Therefore, I have developed five web-based simulators that cover a wide range of neurophysiology principles, including single and whole-cell channel currents, membrane potentials and generation and conduction of action potentials using HTML5 and JavaScript. These simulators may be run free of charge on any device, regardless of the model or OS, thereby enabling schools that have no experience in introducing simulations to introduce them easily.

In Part 2, I describe research on practical training and its educational effects using the simulators developed in Part 1. These simulators were especially useful in many schools during COVID-19 restrictions. In this part, I explain the functions of the simulators I have developed and introduce some practical examples. To verify the usefulness of the simulators, I also conducted a survey in the classrooms in which the simulators were used. Understanding and motivation to learn was shown to increase significantly, indicating that these are useful for neurophysiology education.

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Part 1

Development of new smartphone-enabled, web-based simulators for cellular neurophysiology education

ABSTRACT

Since the phenomena and principles of cellular neurophysiology can be represented by mathematical models, several simulators of cellular neurobiology have been reported. However, they don't seem to be popular. Some of the reasons are that they require fees, they can only simulate some areas of cellular neurophysiology, they are confusing for beginners in neurobiology, and they are cumbersome to install. In Japan, the ICT readiness of schools is low, and the environment itself for using computers for learning is not in place. This is considered to be the biggest barrier to using simulations. But even in such countries, today's students generally have smartphones and use them well. A smartphone is also a high-performance computer, and of course it is equipped with a communication environment. A simulator that covers a wide range of cellular neurophysiology and can be run on smartphones has not yet been reported. However, by making simulators as web pages, students can run them not only on computers but also on tablets and smartphones as well. Therefore, I developed five new simulators using HTML5 and JavaScript: "Membrane Potential", "Membrane Current", "Channel Current", "Action Potential" and "Excitatory Conduction". These simulators are not only capable of accurately simulating a wide range of phenomena and principles of cellular neurophysiology but are also designed so that even beginners can easily understand and

use them. The development of these simulators has allowed anyone to use them free of charge without installation. This has made it possible to support active learning in neurobiology.

INTRODUCTION

Animals maintain their internal environments according to the situation, respond to stimuli, learn, and behave. These phenomena are caused by the nervous system. The basis of this is the nerve cell. Understanding cellular neurophysiology is an important part of neuroscience education. Neurons generate a membrane potential in their resting state, and when the potential changes due to stimulation, the membrane current changes accordingly. The membrane current is caused by channel current due to opening and closing of the channel. If the change is above a threshold, a signal action potential is generated, which is then conducted. Understanding these five areas of membrane potential, membrane current, channel current, action potential, and excitatory conduction is the most important part of cellular neurophysiology education.

If possible, high school and undergraduate students in laboratory courses may be able to do extracellular recordings of action potentials using animals such as frogs (Ferragamo and Wotton, 2006), earthworms (Kladt et al., 2010), cockroach (Ramos et al., 2007) or crickets (Dagda et al., 2013). However, they are generally unable to practice intracellular techniques such as voltage-clamp membrane current recording and patch-clamp channel current recording due to lack of facilities and technical instruction, or the cost and time limitations of training (Diwakar, 2014). Since much of this content ends up

in a lecture from instructors to the students using textual illustrations, it is difficult to motivate students to learn neuronal electrophysiology. In such cases, it has been reported that computer simulations are educationally effective (Bish and Schleidt, 2008; Rutten et al., 2012). Simulation using a simulator eliminates limitations such as location, time, cost, technology, and materials, and allows you to study various phenomena while changing parameters as many times as you like. It is difficult, however, to introduce simulations in countries including Japan whose school information and communication technology readiness factor is low (Ma and Qin, 2021; Wu et al., 2023). In such countries, it is not always the case that the number of computers installed in the training room is enough for the number of students, or that all students necessarily have computers. This is one of the biggest barriers to introducing simulations into classroom education.

How can a simulation be done in such a situation? Most students today have smartphones, which are high-performance computers that have a communication environment. If students can run simulations on their smartphones, it will greatly enhance learning neuroscience. Many computer-based simulators have been reported, including “NEURON" (Carnevale and Hines, 2001), ”SNNAP" (AV-Ron et al., 2006), “MetaNeuron" (Newman and Newman, 2013), ”Neuron in Action" (Moore and Stuart, 2007: Stuart, 2009), “Neurosim" (Heitler, 2022), and ”SimBio" (Meir, 2022). All of them,

however, are operated by personal computers only and there is no smartphone-enabled simulator yet. In this study, I developed new neuronal electrophysiology simulators to simulate features of cellular neurophysiology that (i) can be operated on smartphones and tablets as well as on computers, regardless of the type of device or OS, (ii) may be used for free and without registration, (iii) are web-based and do not require installation, (iv) can be used without confusion by instructors and students unfamiliar with simulation, and (v) can extensively simulate electrophysiological phenomena in neurons.

METHODS

Cellular neurophysiology simulators can be written in a variety of programming languages. However, in order to make it executable on all kinds of devices such as smartphones, tablets and computers, it is most realistic to create a web-based simulator. Since Java and Flash cannot be used on smartphone browsers, the simulators were created using HTML5 and JavaScript. HTML5, the language of web pages, can use various input tags such as numeric input fields, radio buttons, check boxes, etc. In addition, by using the canvas element, it is possible to create dynamic graphs with JavaScript.

Many of the electrical phenomena of a single neuron can be illustrated by the Hodgkin and Huxley model (Hodgkin and Huxley, 1952a-d). Numerous parameters are involved in cellular neurobiological phenomena. To be able to simulate using not only large-screen computers but also smartphones, it is necessary that it be designed simply and easy to understand, even on a small screen, so that the input parameters and simulation results can be viewed easily. For this purpose, I decided to create independent simulators for the five important fields of cellular neurobiology individually: membrane potential, membrane current, channel current, action potential, and excitatory conduction. As Meir (2022) notes, limiting the parameters of a simulation that can be changed can lead to more efficient and effective learning of the material for beginning students.

Therefore, I carefully selected parameters that can be changed for each simulator. For example, an action potential simulator could be designed to change the concentration of potassium and sodium ions inside and outside the cell, but in this simulator, those parameters are fixed to the standard values used by Hodgkin and Huxley (1952d). I made it possible to set up a membrane potential simulator to see how the membrane potential changes by changing the ion concentration inside and outside the cell.

Five types of simulators, “Membrane Potential”, “Membrane Current”, “Channel Current”, “Action Potential”, and “Excitatory Conduction” were created by mathematical models reported for each phenomenon. The Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin and Katz, 1949) was used for the Membrane Potential simulator. The Hodgkin and Huxley (1952d) equation was used as a voltage clamp for the Membrane Current simulator. For the Channel Current simulator, I used the state transition model of Strassberg and DeFlice (1993) with the rate constants of the Hodgkin and Huxley (1952d) equation. For the Action Potential simulator, the equation of Hodgkin and Huxley (1952d) was used as current clamp. For the Excitatory Conduction simulator, I used the model of Cooley and Dodge (1966), which combines the equations of Hodgkin and Huxley (1952d) and the cable theory. (See the optional documentation provided as a supplement.)

RESULTS

I developed web-based simulators to allow students to run simulations regardless of device model or OS. The developed html files were placed on a web server. This makes it possible to easily run simulations in a web browser for free by simply entering a URL (https://www.biol.se.tmu.ac.jp/neurobio/neuron/sim/index.html), or by reading a QR code (Figure 1).

To simplify the operation, five simulators were developed each with a single screen fitted for smartphones: “Membrane Potential,” “Membrane Current”, “Channel Current”, “Action Potential” and “Excitatory Conduction” (Figure 1). These simulators can simulate a wide range phenomenon in neuronal physiology and these are built in an independent manner, and the advantage is that you could arrange the order of use and further apply to examine. Of these, "Membrane Potential" is a simulator of the static electrical properties of neurons and the remaining four are simulators of the dynamic electrical properties of neurons. All simulators are designed to be temperature configurable, as electrophysiological phenomena in neurons are related to temperature. Except for “Membrane Potential” simulator, which simulates static properties without change over time, the size of the vertical (voltage, current and/or conductance) and horizontal (time and location) axes of the remaining four simulator graphs can be changed

according to the screen size of smartphones, tablets, or personal computers; they can be optimized for any device. The static properties simulator shows results as soon as the input values are changed. After setting the conditions in the dynamic properties simulators, they can be run when the “Simulate” button is pressed for the time selected with radio buttons. In simulators with an overwrite function, graphs can be cleared with the “CLS” button. In simulators with a “text out” function, if the check box is checked, each value over time will be displayed in text instead of being displayed as a trace. Recording a panel trace is easily possible with a screenshot. Students can also create high-resolution graphs by copying the text output and pasting it in a spread sheet, or reading it as a CSV file with an application such as GNU R.

Input of parameters specific to each simulator and display of results were shown below.

“Membrane Potential” Simulator

By inputting the intramembrane “inside” and extramembrane “outside” ion concentrations of potassium ions, sodium ions, and chloride ions and the ratio of all ion permeabilities, the value and panel of the membrane potential are displayed (Figure 2A).

The simulation results in Figure 2A show numerically and graphically the membrane potential when the temperature is 6.3℃, intercellular ion concentrations are

K^+ 400, Na^+ 50 and Cl^- 60 mM, extracellular ion concentrations are K^+ 20, Na^+ 440 and Cl^- 560 mM and the permeability constants for K^+, Na^+ and Cl^- are in the ratio 1:0.04:0.45. Figure 2B shows the ion equilibrium potential when the permeability of the membrane for potassium ions, sodium ions, and chloride ions is set to 1:0:0. Other conditions are the same as in Figure 2A.

"Membrane Current" Simulator

The voltage of the "command pulse" (clamp potential) is the stimulus input. The upper panel shows membrane potential, potassium conductance, and/or sodium conductance depending on which "box" is checked. The bottom panel displays membrane currents where only the potassium current is displayed if the tetrodotoxin (TTX), sodium channel blocker known as pufferfish poison, radio button is selected, only the sodium current is displayed if the tetraethylammonium (TEA), potassium channel blocker, radio button is selected, and the total membrane current is displayed if neither is selected (Figure 3A).

The simulation results of Figure 3A are shown below. The temperature is 6.3°C. Command pulse is 0mV. The simulation time is 16ms. Grid is checked. The three traces in the panels represent three simulations. When "V" is checked and simulated, the green line traces the membrane potential in the upper panel and the total membrane current in the lower panel. When "G_{Na}" and "TEA" are checked and simulated, the sodium

conductance is traced in the upper panel and the sodium current is traced in red in the lower panel. When "G_K" and "TTX" are checked and simulated, the potassium conductance is traced in the upper panel and the potassium current is traced in the lower panel as a blue line. In Figure3B, the simulation results are displayed as text output by checking "text out" (Figure 3B left) and pasted into a spreadsheet to create a graph (Figure 3B right). In this example, the temperature is 14.3℃. Command pulse is 0mV. The simulation time is 8ms.

"Channel Current" Simulator

Like the "Membrane Current" simulator, the voltage of the "command pulse" (clamp potential) is the stimulus input. The clamp voltage is shown in the upper panel. Potassium channel currents are displayed when the "K^+" radio button is selected, and sodium channel currents are displayed when the "Na^+" radio button is selected (Figure 4A). When the "1ch" radio button is selected, 13 random individual channel currents are displayed. If the radio button for number of channels is selected, the total channel current for the entered number of channels is displayed (Figure 4B).

The result in Figure 4A is obtained when a temperature of 15℃, a simulation time of 8 ms, a command voltage of -10 mV, a sodium ion channel selected, 1 channel (13 traces displayed), a current scale of 2 pA, and noise addition is enterd. The upper

panel is the command voltage trace, and the lower panel is the 13 trace of single channel current with added noise. The result in Figure 4B is obtained when 13 channels are selected at a temperature of 15°C, a simulation time of 8 ms, a command voltage of -10 mV, a sodium ion channel selected, 13 channels and a current scale of 2 pA. The upper panel is the command voltage trace, the lower panel is the channel current trace for the 13 channels summed.

"Action Potential" Simulator

The stimulus current magnitude and pulse duration are the stimulus inputs. Single or double stimulation can be selected with radio buttons. "Delay time" is required to be entered if "Double" stimulation is selected. Stimulation currents are displayed in the bottom panel. In the upper panel, membrane potential, potassium conductance, and/or sodium conductance are displayed depending on which box(es) is /are checked. When the potassium channel activation variable "n", sodium channel activation variable "m", and/or sodium channel inactivation variable "h" are checked, each trace is also displayed (Figure 5A).

The results in Figure 5A are the traces obtained when the temperature is 6.3°C, the simulation time is 20ms, and the single stimulus is applied with a stimulus intensity of 10μA and a stimulus duration of 1.5ms. A grid is shown in all the panels. Since the

check boxes for “V”, “GNa”, and “GK” are checked, traces of membrane potential (black line), sodium conductance (red line), and potassium conductance (blue line) are displayed in the top panel. Since the check boxes of “n”, ”m”, ”h” are checked, the potassium channel activation variable "n" (blue line), the sodium channel activation variable "m" (red line), and the sodium channel inactivation variable "h" (yellow line) traces are shown in the middle panel. Traces in the bottom panel shows the stimulation current. Figure 5B shows the membrane potential (upper panel) and stimulation current (lower panel) when double stimulation is applied. The temperature is 6.3℃ and the simulation time is 20ms. The first pulse is the stimulus intensity of 10μA and the stimulus duration of 1.5ms. The paired second pulse is the stimulus The paired second pulse is the stimulus The paired second pulse is the stimulus —intensity of 30μA, the stimulus duration of —1.5ms, and the delay time of 8ms. By applying this double stimulation, the refractory period can be investigated.

“Excitatory Conduction” Simulator

Like the “Action Potential” simulator, the stimulus current magnitude and pulse duration are the stimulus inputs. It is required to enter the location (distance from the stimulation electrode) of the recording electrode on the 100 mm long axon, shown by the filled triangle in the axon diagram above the graph. The upper panel shows potentials at 0.5 mm

intervals on the axon. The bottom panel displays the intracellular potential at the location of the recording electrode. Each time the “play” button is pressed, the excitation site, where the membrane potential is indicated by color intensity, moves along the axon in slow motion at a speed of 1/1000th, and the graph changes are displayed (Figure 6A).

Figure 6A shows the results obtained when a temperature was 10℃, a stimulus intensity, 30μA, a stimulus duration, 0.1ms, a simulation time, 5ms, and a recording electrode position, 20mm. The upper panel is the membrane potential trace of each segment along the axon at 5ms from the start. The diagram of the axon shows the excitatory area in red at 5ms from the start. The bottom panel is a trace of the membrane potential from start to 5ms at a distance of 20 mm from the stimulating electrode. In Figure 6B, the temperature is 20°C and the other conditions are the same as in Figure 6A.

These results indicate that for the first time, simulation learning can be easily introduced into any school.

DISCUSSION

Convenience of web-based simulator in student education

Web pages generally look the same on smartphones, tablets, and computers, regardless of device, model, operating system, or browser. Granlund et al. (2000) introduced the use of some web-based simulations written in Java and described that web-based simulation could be a powerful tool in education and training. In the past, web browsers did not have the ability to display dynamic graphs, so it was common to make it possible by executing Java programs (for example, Elias and Kohn, 2013). However, in order to run Java in a web browser, it became necessary to install and configure the Java execution environment in a different way for each OS and browser. This is a rather cumbersome task, and it may have been quite difficult for non-computer-savvy instructors to teach students how to install and configure the Java runtime environment for the various types of browsers on their own personal computers. Above all, it is not possible to create a Java execution environment in a smartphone web browser.

HTML5, a language for describing WWW hypertext, which was published as a W3C (World Wide Web Consortium) Recommendation in 2014, added a new Canvas element to which graphics can be added. The cellular neurophysiology simulator newly developed in this thesis can display waveforms such as membrane current and membrane

potential even on a web page by using this HTML5 Canvas element with JavaScript. Browsers on smartphones were replaced early by browsers that support HTML5. However, a browser (Internet Explorer 8) that does not support HTML5 has often been used on personal computers using Windows 7 as the OS. In the 2020s, almost all the browsers on personal computers owned by Japanese students have finally been replaced with HTML5-compatible, making it possible to practice cellular neurophysiology using this simulator on their own personal computers in addition to smartphones.

Features of the newly developed simulators

Since these simulators do not use image files and are all text-based, the file size of any of the five simulators is less than 50k bytes. It's smaller than a small photo, so it takes less time and less money to download. For simulators other than Web-based ones, reinstallation is required when files are changed due to bug fixes or updates. In the case of the web-based simulator, I only need to change the file placed on the web server, and the next time students access the web file, the new one will automatically be executed.

With HTML5 web-based simulator, once the web file of the simulator is loaded by accessing the URL, the simulation is executed by the web browser of the client device. The rapid improvement in the processing speed of smartphones and computers is also considered to be a major factor in the practical application of this simulator. Conversely,

in an age when device processing speed was only a tenth of what it is now, it would have been impractical for students to practice. As described above, it can be said that the simulator of this thesis, which can be used by students without installation and regardless of device, have finally become feasible due to the present situation.

The superiority of these simulators in student training in cellular neurophysiology

The differences between the newly developed simulators and many other educational simulators of cellular neurophysiology that have been reported so far are not only that the formers are smartphone-enabled, web-based, require no installation, and are provided free of charge. In this thesis, I was able to develop a simulator that can be practiced in a wide range of important fields for cellular neurophysiology, such as membrane potential, membrane current, channel current, action potential, and excitatory conduction. This is considered very important in the light of the objective of providing student education. In contrast, “SNNAP” (Av-Ron et al., 2006) does not have membrane potential simulator, channel current simulator, or excitatory conduction simulator functions. “MetaNeuron” (Newman and Newman, 2013) does not have channel current simulator or excitation conduction simulator functions. “Neurosim” (Heitler, 2022) does not have channel current simulator functionality. “ Neuron in Action” (Moore and Stuart, 2007) implements the five functions of these simulators. But Newman and Newman (2013) said:

there is not easily accessible to the beginning neuroscience student as it has a complex interface, must be installed on students' computers via a DVD, and must be purchased for a significant fee. "NEURON" is a free and advanced simulator, but as Northcutt (2021) stated, "Approximately half of the students in the undergraduate class had problems either downloading NEURON or running simulations once it downloaded."

In summary, I was able to develop these simulators with the following characteristics through this thesis; which do not require installation, are free of charge, are easy to use, and cover a wide range of cellular neurophysiology, regardless of smartphone or personal computer model, OS, or browser. By developing these simulators, even in countries like Japan, where ICT implementation in schools is low and simulations have hardly been introduced in the field of cellular neurophysiology, simulations can now be introduced easily.

In this study, various simulators were limited to phenomena within a single neuron. Furthermore, it is considered, by developing a simulator that incorporates ligand-gated channels into the HH model, important phenomena between neurons, such as excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs), can be easily simulated.

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FIGURE LEGENDS

Figure 1.

Smartphone screens for each simulator (A-E) and QR code (A-E: each simulator; F: simulators homepage with link to short instruction guide)

The examples show (A) “Membrane Potential” simulator - resting membrane potential (red line) at user-set intracellular and extracellular ion concentrations;

(B) “Membrane Current” simulator - sodium and potassium membrane conductance (upper panel) and current (lower panel) after a command voltage input;

(C) “Channel Current” simulator - single channel currents (sodium in this example) after a command voltage input;

(D) “Action Potential” simulator - membrane potential, potassium conductance, and sodium conductance (upper panel) and channel activation/inactivation (lower panel) during an action potential; and

(E) “Excitatory Conduction” simulator - propagation (upper panel) and point measurement (lower panel, set at blue line and filled triangle location) of excitatory conduction.

(F) QR code of the URL of the top page of these simulators

Figure 2.

A. Membrane Potential simulator annotations (by smartphone)

B. A sample of Membrane Potential simulator results (by computer)

Figure 3.

A. Membrane Current simulator annotations (by smartphone)

B. Simulator text output screen (left) and screen where these values were pasted into Excel and graphed (right) (by computer).

Figure 4.

A. Channel Current simulator annotations (by smartphone)

B. A sample of Channel Current simulator results (13 channels summation example) (by computer)

Figure 5.

A. Action Potential simulator annotations (by smartphone)

B. A sample of Action Potential simulator results ("Gating values panel" hidden) (by computer)

Figure 6.

A. Excitatory Conduction simulator annotations (by smartphone)

B. A sample of Excitatory Conduction simulator results (by computer)

Figure 1.

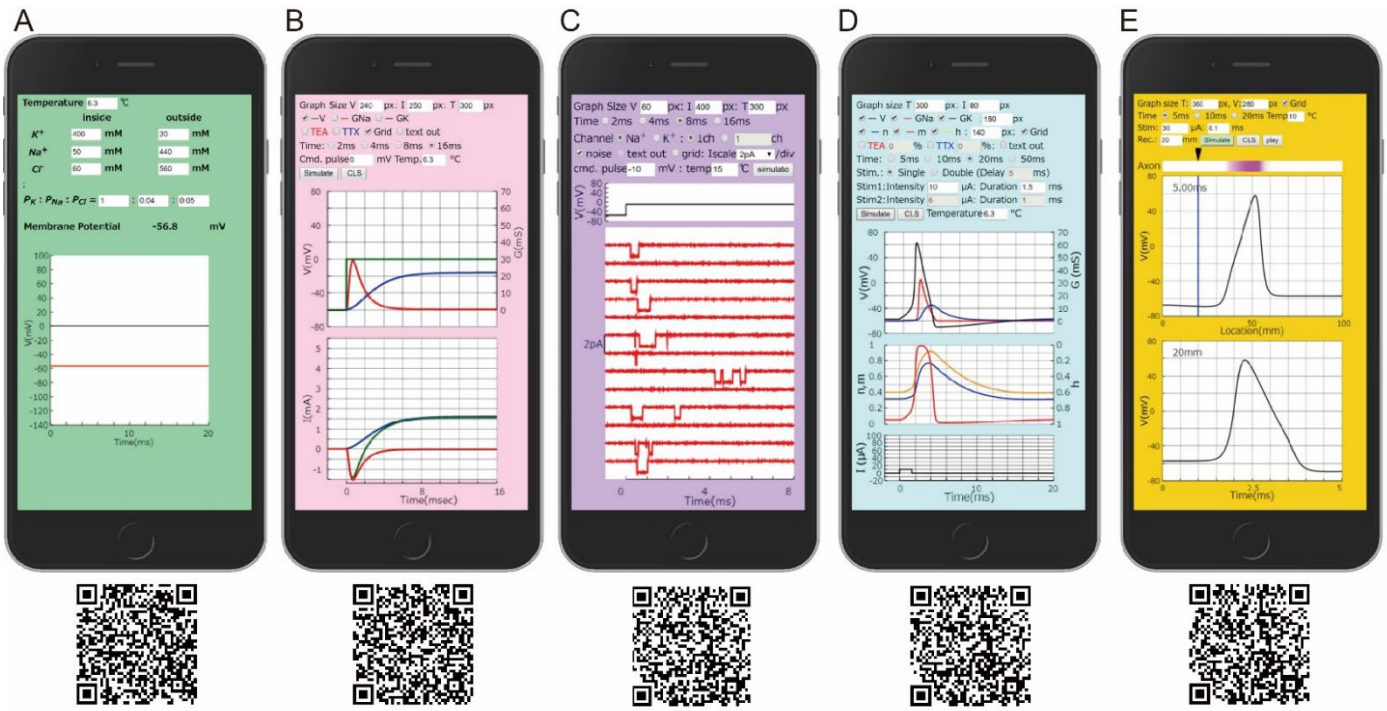
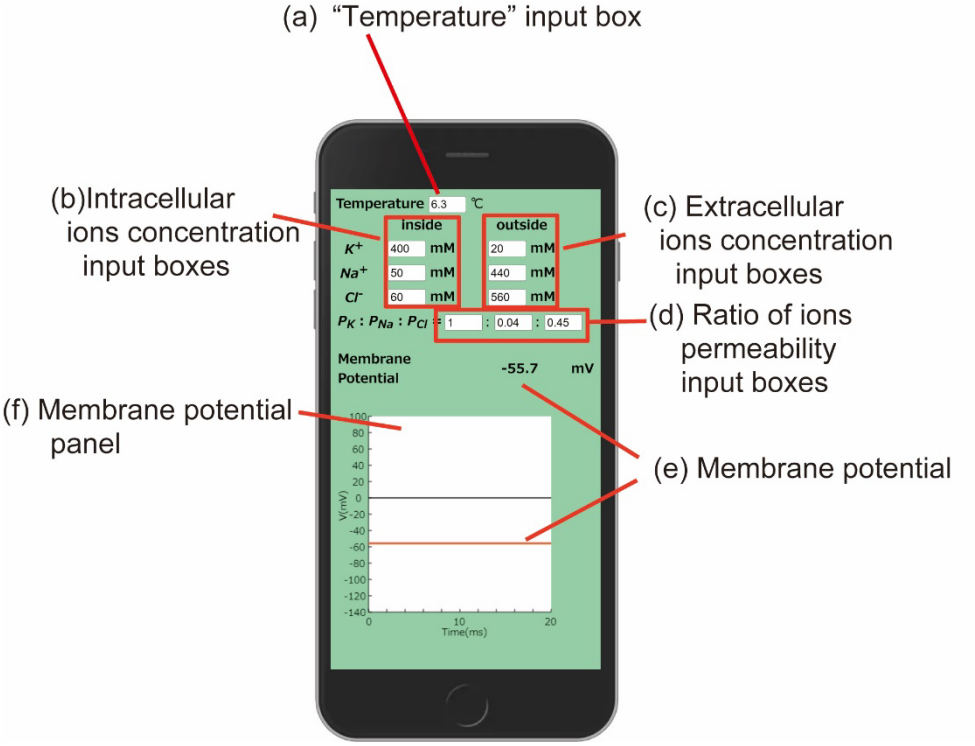

A
B
C
D
E
F
https://www.biol.se.tmu.ac.jp/neurobio/neuron/sim/index.html

Figure 2.

A


(a) “Temperature” input box
(b)Intracellular ions concentration input boxes
(c) Extracellular ions concentration input boxes
(d) Ratio of ions permeability input boxes
(e) Membrane potential
(f) Membrane potential panel
Temperature 6.3 ℃
inside
outside
K+ 400 mM 20 mM
Na+ 50 mM 440 mM
Cl- 60 mM 560 mM
PK : PNa : PCl = 1 : 0.04 : 0.45
Membrane Potential -55.7 mV
V(mV)
Time(ms)

B

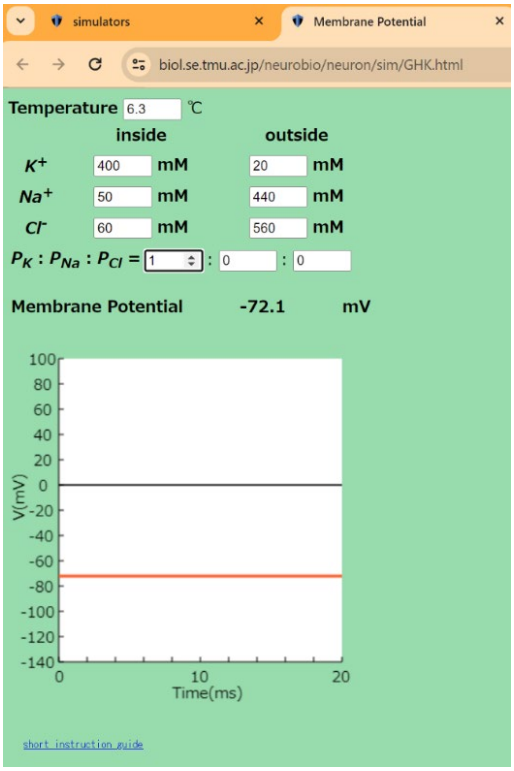
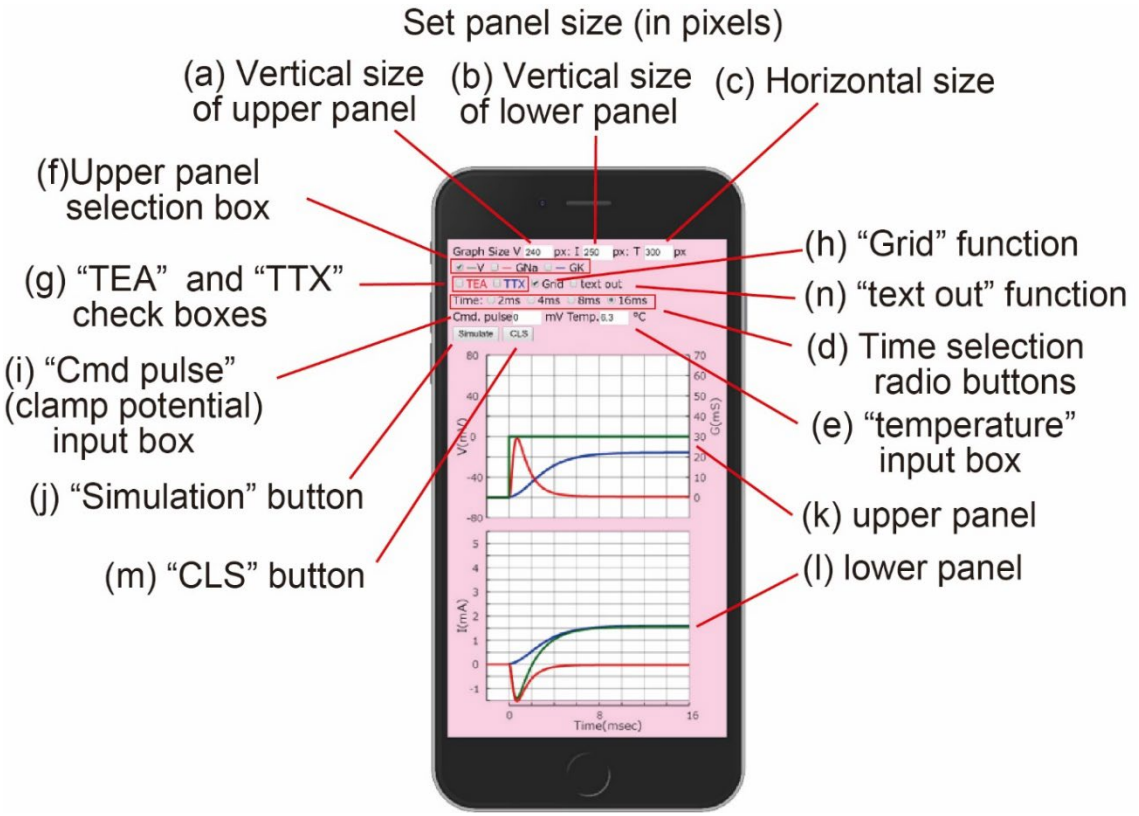

simulators
Membrane Potential
biol.se.tmu.ac.jp/neurobio/neuron/sim/GHK.html
Temperature 6.3 ℃
inside
outside
K+ 400 mM 20 mM
Na+ 50 mM 440 mM
Cl- 60 mM 560 mM
PK : PNa : PCl = 1 : 0 : 0
Membrane Potential -72.1 mV
V(mV)
Time(ms)
short instruction guide

Figure 3.

A


Set panel size (in pixels)
(a) Vertical size of upper panel
(b) Vertical size of lower panel
(c) Horizontal size
(f)Upper panel selection box
(g) “TEA” and “TTX” check boxes
(i) “Cmd pulse” (clamp potential) input box
(j) “Simulation” button
(m) “CLS” button
(h) “Grid” function
(n) “text out” function
(d) Time selection radio buttons
(e) “temperature” input box
(k) upper panel
(l) lower panel
Time(msec)

B

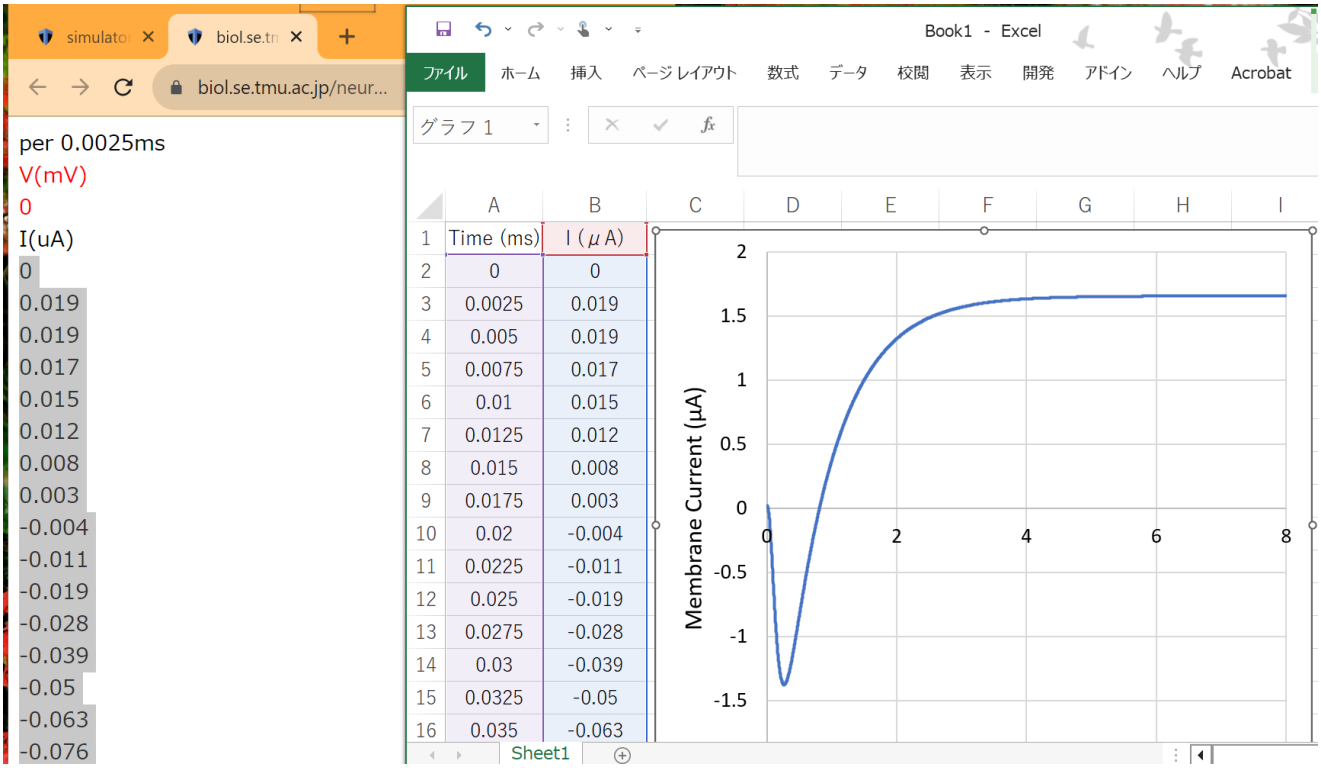
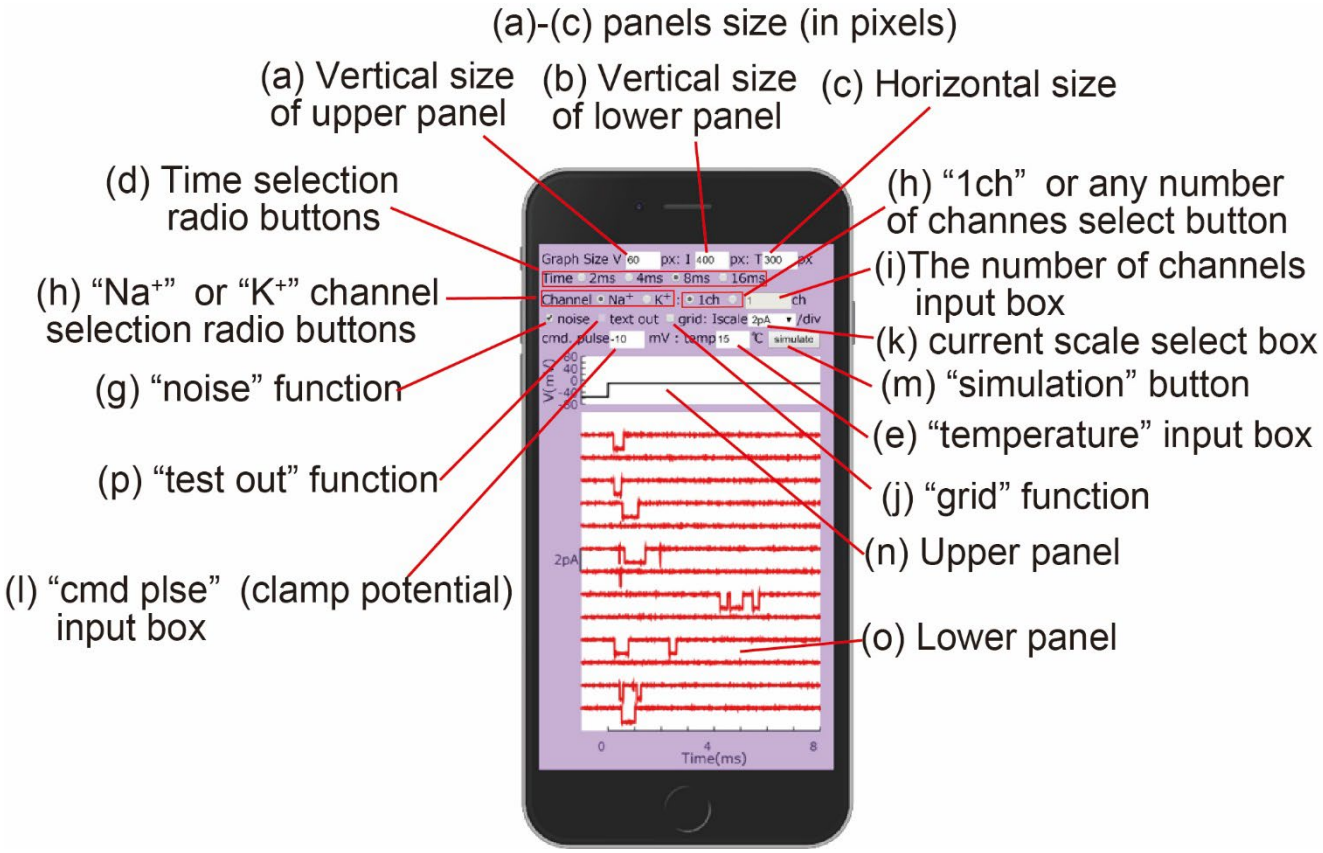

biol.se.tmu.ac.jp/neur...
per 0.0025ms
V(mV)
0
I(uA)
0
0.019
0.019
0.017
0.015
0.012
0.008
0.003
-0.004
-0.011
-0.019
-0.028
-0.039
-0.05
-0.063
-0.076
Book1 - Excel
ファイル ホーム 挿入 ページ レイアウト 数式 データ 校閲 表示 開発 アドイン ヘルプ Acrobat
グラフ 1
Time (ms) I (μA)
Membrane Current (μA)
Sheet1

Figure 4.

A


(a)-(c) panels size (in pixels)
(a) Vertical size of upper panel
(b) Vertical size of lower panel
(c) Horizontal size
(d) Time selection radio buttons
(h) "Na+" or "K+" channel selection radio buttons
(g) "noise" function
(p) "test out" function
(l) "cmd plse" (clamp potential) input box
(h) "1ch" or any number of channes select button
(i)The number of channels input box
(k) current scale select box
(m) "simulation" button
(e) "temperature" input box
(j) "grid" function
(n) Upper panel
(o) Lower panel
Time(ms)

B

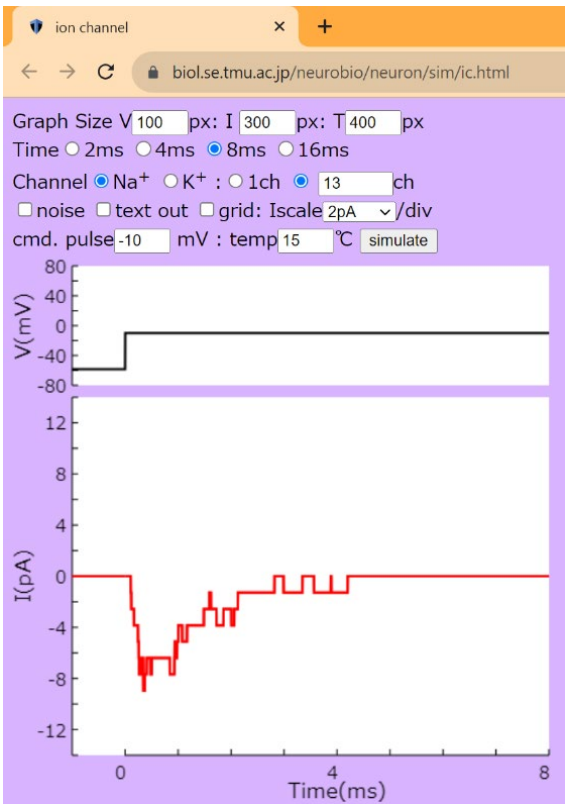
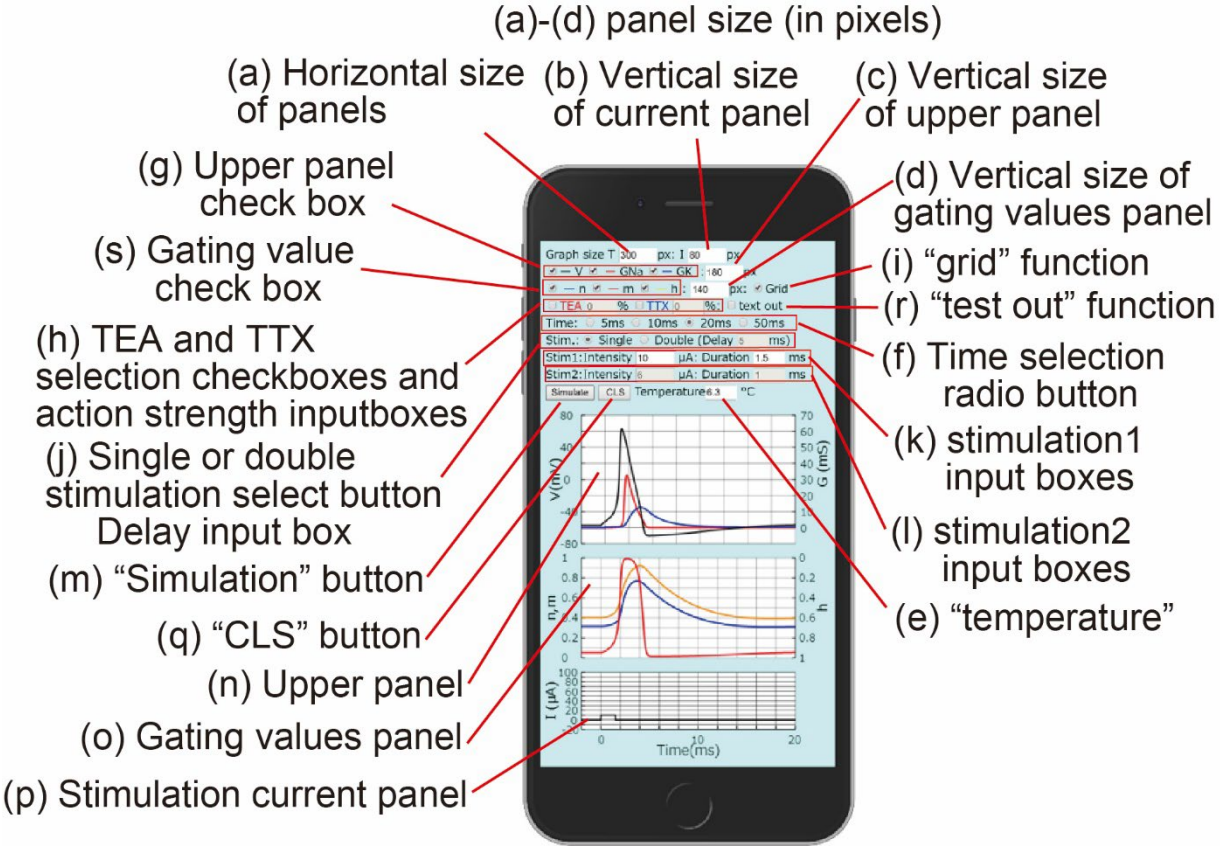

ion channel
biol.se.tmu.ac.jp/neurobio/neuron/sim/ic.html
Graph Size V 100 px: I 300 px: T 400 px
Time 2ms 4ms 8ms 16ms
Channel Na+ K+ : 1ch 13 ch
noise text out grid: Iscale 2pA /div
cmd. pulse -10 mV : temp 15 ℃ simulate
V(mV)
I(pA)
Time(ms)

Figure 5.

A


(a)-(d) panel size (in pixels)
(a) Horizontal size of panels
(b) Vertical size of current panel
(c) Vertical size of upper panel
(d) Vertical size of gating values panel
(g) Upper panel check box
(s) Gating value check box
(h) TEA and TTX selection checkboxes and action strength inputboxes
(j) Single or double stimulation select button Delay input box
(m) “Simulation” button
(q) “CLS” button
(n) Upper panel
(o) Gating values panel
(p) Stimulation current panel
(i) “grid” function
(r) “test out” function
(f) Time selection radio button
(k) stimulation1 input boxes
(l) stimulation2 input boxes
(e) “temperature”
Time(ms)

B

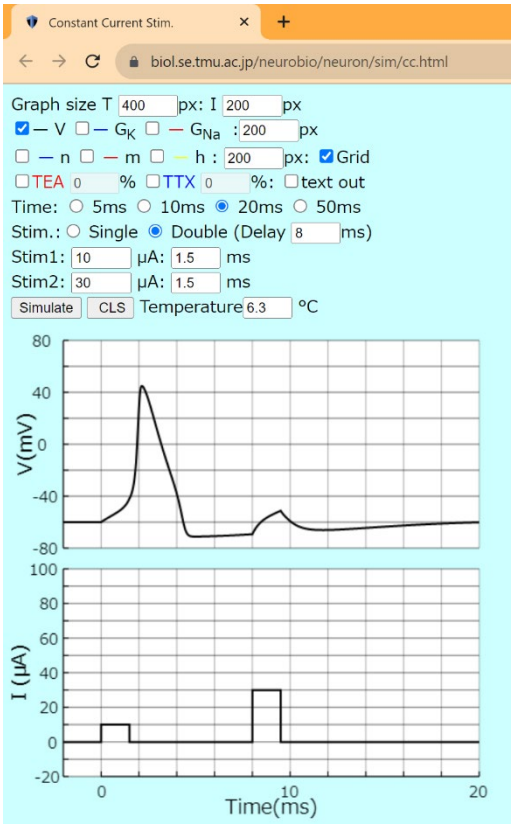
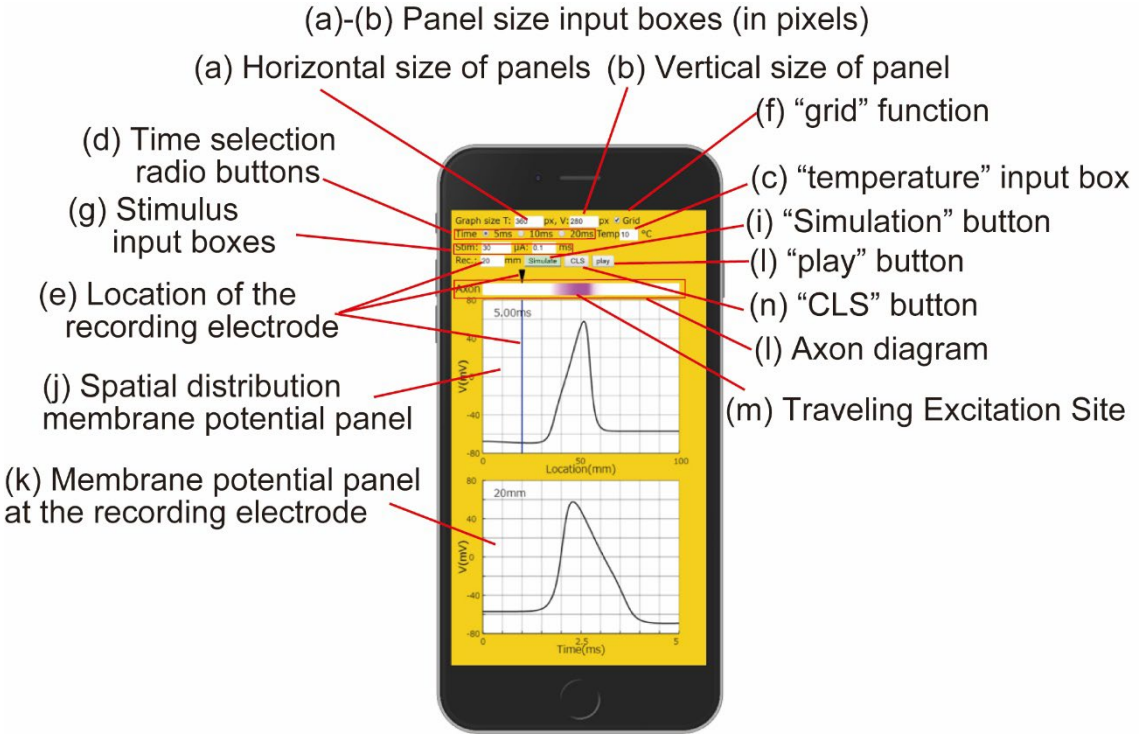
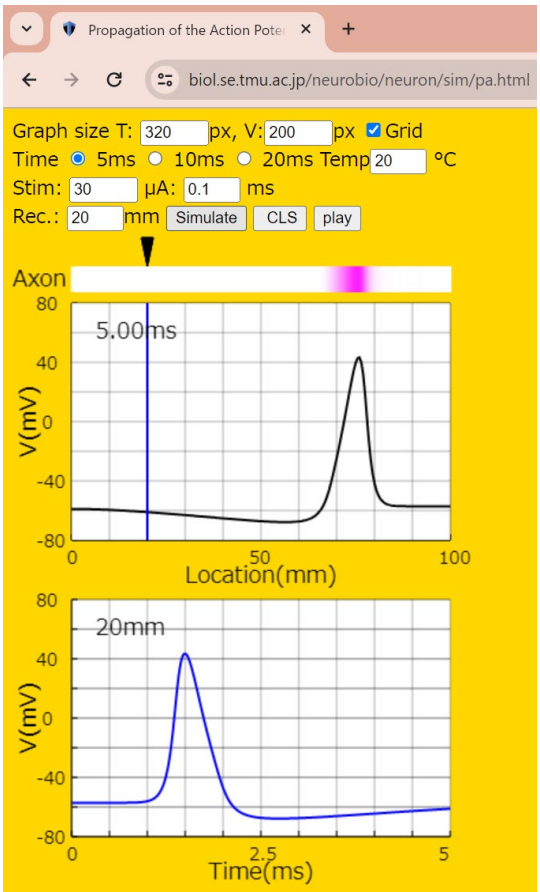

Constant Current Stim.
biol.se.tmu.ac.jp/neurobio/neuron/sim/cc.html
Graph size T 400 px: I 200 px
— V — GK — GNa : 200 px
— n — m — h : 200 px: Grid
TEA 0 % TTX 0 %: text out
Time: 5ms 10ms 20ms 50ms
Stim.: Single Double (Delay 8 ms)
Stim1: 10 μA: 1.5 ms
Stim2: 30 μA: 1.5 ms
Simulate CLS Temperature 6.3 °C
V(mV)
I (μA)
Time(ms)

Figure 6.

A


(a)-(b) Panel size input boxes (in pixels)
(a) Horizontal size of panels
(b) Vertical size of panel
(f) “grid” function
(d) Time selection radio buttons
(c) “temperature” input box
(g) Stimulus input boxes
(i) “Simulation” button
(l) “play” button
(e) Location of the recording electrode
(n) “CLS” button
(l) Axon diagram
(j) Spatial distribution membrane potential panel
(m) Traveling Excitation Site
(k) Membrane potential panel at the recording electrode
5.00ms
Location(mm)
20mm
V(mV)
Time(ms)

B


Propagation of the Action Pote
biol.se.tmu.ac.jp/neurobio/neuron/sim/pa.html
Graph size T: 320 px, V: 200 px Grid
Time 5ms 10ms 20ms Temp 20 °C
Stim: 30 μA: 0.1 ms
Rec.: 20 mm Simulate CLS play
Axon
5.00ms
V(mV)
Location(mm)
20mm
Time(ms)

Part II

Student practical training using the developed simulators

and evaluation of their educational effect

ABSTRACT

I newly developed five types of simulators: Membrane Potential, Membrane Current, Channel Current, Action Potential, and Excitatory Conduction, which can be easily used even in schools that have no experience in introducing simulations.

In 2020, due to the COVID-19 pandemic, not only lectures but also laboratory classes were conducted remotely at most universities. Under these circumstances, these simulators that I developed were used for student practical training in cellular neurobiology at many schools that had no experience with simulation learning. And they continued to be used even after face-to-face training resumed. Thus, I have found that it is possible at many schools to conduct student practical training covering a wide range of cellular neurobiology using the five simulators I developed.

In order to verify the educational effectiveness of practical training using these simulators, I conducted several surveys among students regarding their understanding, motivation for learning, and usefulness of the simulators. As a result, I found that these simulators are useful for improvement of understanding and motivation for learning. This suggests that these simulators are effective tools for supporting the education of cellular neurophysiology.

INTRODUCTION

In the neurology section of high school biology and undergraduate neurobiology, the main learning objective is to understand the phenomena and principles of membrane potentials, membrane currents, channel currents, action potentials, and excitatory conduction.

Computer simulation is one effective way to achieve this learning goal (Bish and Schleidt, 2008; Stuart, 2009). However, the use of simulation is generally not widespread in high school and undergraduate education in the field of cellular neurobiology. The reasons for this are that cellular neurobiology simulators can only simulate some phenomena, are expensive, and are difficult to install and operate. Japan is one of the countries where the use of ICT in education is the least advanced (Ma and Qin, 2021; Wu et al., 2023). The biggest hurdle in these countries is the inability to properly prepare computers to run simulations. Therefore, I developed five web-based smartphone-enabled simulators that solve problems such as these (see Part 1). Following this, I distributed these simulators to be used in practical training to evaluate their efficacy.

In 2020, after developing these simulators, the COVID-19 pandemic occurred. Regarding the changes in neurobiology education resulting from this, Ramos (2020) described: “Beginning in early March, US colleges and universities moved to online and virtual education. Such a rapid and drastic change in educational delivery method has had

profound effects on students and educators." In Japan, where the environment for using ICT had not been well established, the effects were even greater. Most Japanese neurobiology educators had no experience in the educational use of computer simulations for practical training. Generally, educators do not know what kind of computers their students master or even own. Both educators and students may be unfamiliar with using computers. However, even under such circumstances, the five simulators developed through this research can be introduced without any problems. Additionally, these simulators can simulate a wide range of neuronal phenomena, making them suitable for week-long training sessions.

The primary purpose of this research is to improve educational effectiveness by introducing the developed simulators into classes. Therefore, I called for a wide range of universities that would be willing to provide practical training using these simulators. In response, I received applications from several universities. At these universities, various student practical trainings in neurobiology were conducted using the developed simulators. I then conducted several surveys regarding their understanding, motivation for learning and usefulness of the simulations in the classrooms in which the simulators were used to verify their educational usefulness of practical training.

METHODS

The five simulators were presented at the annual meeting of the Japanese Society for Comparative Physiology and Biochemistry in 2018 (Yamamoto and Kurokawa, 2019) and 2019 (Yamamoto and Kurokawa, 2020). The URL has been published so that anyone can use it for free without registration since 2018. I also created the activities to be carried out using each of the membrane potential, membrane current, channel current, action potential, and excitatory conduction simulators. These were provided to anyone who intended to use the simulators in their classrooms if they wanted to get them.

In 2020, I asked instructors of undergraduate education for cooperation in implementing practical training using these simulators and conducting comprehension surveys for their students. Two national universities, two public universities, two private universities, and one private high school in Japan cooperated with the survey in total. A total of 1146 students (1055 undergraduate students and 91 high school students) responded to the survey for experiments using five types of simulation experiments. Of these, 199 students used Membrane Potential simulator; 156, Membrane Current simulator; 150, Channel Current simulator; 403, Action Potential simulator and 238, Excitatory Conduction simulator. The simulators were used in a laboratory neurobiology course where most of the undergraduates were biology majors. In high

school, the simulators were used in biology classes.

The contents of the survey were as follows:

・Degree of understanding of training content before and after simulation training.

・ Motivation before and after simulation training

・The usefulness of the simulator used in the training

These were examined on a five-point Likert scale. A comment section was included in all surveys. Before preparing the understanding survey, I asked the instructors to send me the details of the lesson plan in order to know which simulator the instructor used and what kind of training was carried out. Comprehension surveys were prepared according to the description (for example, if a refractory period simulation experiment was done, I included the survey item, "understanding of the refractory period"). The surveys were created using Google form, and the surveys were conducted anonymously and voluntarily. The students accessed the URL after the simulation training. Scores pre- and post-survey were compared by paired t-test.

RESULTS

Practice examples

Using each of the five simulators, that have high flexibility, instructors were able to teach various contents in cellular neurophysiology. Examples of simulation practice using each simulator are shown below.

"Membrane Potential" Simulator

The equilibrium potential of an ion is calculated using by the Nernst equation and the resting membrane potential by the Goldman-Hodgkin-Katz (GHK) equation (Goldman, 1943; Hodgkin and Katz, 1949). That is, the equilibrium potentials of potassium, sodium, and chloride ions can be obtained from concentrations inside and outside the membrane. These concentrations in squid giant axons are given as default. The equilibrium potentials of potassium ions, for example, can be obtained by input of values of the permeability ratio P_K:P_{Na}:P_{Cl} =1:0:0, and so forth. The resting potential can be determined from the permeability ratio of all ions.

Bernstein (1902) proposed the hypothesis that the resting potential is caused by the concentration ratio of potassium ions inside and outside the membrane, and equal to the equilibrium potential of potassium ions. —This is mostly correct. Depending on the hypothesis, students can obtain a linear-relationship curve of the membrane potential

versus log extracellular K^+ concentration by changing the value of extracellular K^+ concentration (Figure 1). It is known, however, that a real cell membrane has a permeability to sodium that ranges between 1 and 10% of its permeability to potassium (Nicholls et al., 2001). By drawing a curve of the resting potential depending on the GHK equation, P_K:P_{Na}:P_{Cl}=1:0.04:0.45 (Hodgkin and Katz, 1949), it can be seen that as concentrations of potassium decrease, the membrane potential depolarizes away from the equilibrium potential of potassium ions (Figure 1), as shown experimentally by Hodgkin and Keynes (1955). At the peak of an action potential there is an instant in time when membrane potential does not change and the GHK equation uses the following permeability rate, P_K:P_{Na}:P_{Cl}=1:20:0.45 (Hodgkin and Katz, 1949). Students also learn that higher sodium ion permeability results in more depolarized membrane potential, which simulates "overshoot" potential of impulses.

"Membrane Current" Simulator

Using this simulator, whole-cell voltage-clamp experiments can be simulated. Larger biphasic current responses can be obtained to the more depolarizing command pulses. Potassium current, which is a late steady current, is displayed when tetrodotoxin (TTX), which inhibits voltage-gated sodium channels, is applied (i.e. the TTX button is chosen). Applying tetraethylammonium (TEA) by choosing the TEA button, which inhibits

voltage-gated potassium channels, displays sodium current, which is an early transient current. A current-voltage (I-V) curve (Hodgkin, Huxley and Katz, 1952: Raman and Ferster, 2022) can be obtained by reading the peak value of each current change against the step voltage (Figure 2). Under voltage-clamp conditions, the current response to voltage steps reflects changes in membrane conductance. A conductance-voltage curve can also be drawn by reading the peak value of each conductance change in response to the command voltage pulse (Hodgkin and Huxley, 1952a). Students can learn much about properties of voltage-dependent ion channels through discussions about why the sodium and potassium current in the I-V curve appear different, while their conductance changes are similar.

"Channel Current" Simulator

This simulator allows the user to observe the commonalities and differences in the gating properties between sodium and potassium ion channels involved in the generation of action potentials, including voltage dependency, inactivation only in sodium channels, and delayed response of only potassium channels. In a single-channel simulation, all 13 channels displayed on one screen have different aperture patterns. The I-V curve of each single ion channel can be compared with the curve obtained using the "Membrane Current" simulator. As shown by Strassberg and DeFlice (1993), as the channel population is

increased, for both the potassium channel current (left) and the sodium channel current (right), the sum of discrete channel currents converges to the continuous whole-cell membrane current (Figure 3).

"Action Potential" Simulator

Hodgkin and Huxley (1952b) showed the time course of action potentials and conductance. Inward current stimulation depolarizes the membrane potential.

Depolarization in membrane potential increases the open probability of voltage-gated sodium and potassium channels, as simulated in "Channel Current" simulator, that causes an increased conductance of each ion. Conductance changes of each ion are simulated during generation of action potentials, in which activation of sodium conductance begins earlier than that of potassium conductance (Figure 4 left).

Action potentials are generated when positive feedback of depolarization and sodium channel activation occurs before repolarization by sodium channel inactivation and potassium channel activation. Stimulus threshold can be inquired by stimulus intensity, which is the minimum intensity required for positive feedback. An all-or-none action potential can be seen by varying the intensity of the stimulus (Figure 4, middle and right).

The stimulation threshold is determined by the strength and duration of the

stimulation current. Guttman (1966) showed that the product of threshold intensity and duration is nearly constant, while rheobase increases with increasing temperature. The temperature dependency of the strength-duration curve can also be simulated (Figure 5).

The stimulus threshold changes during the refractory period of a previous action potential. The refractory period can be found using double stimuli on the simulator (Figure 6). During the absolute refractory period, the threshold intensity is infinity (i.e., when a second stimulation cannot generate an action potential no matter how strong the stimulation). During the relative refractory period, the threshold is higher, but can be reached. This can be understood by drawing a graph of stimulus thresholds after varying stimulus delays. Some students may find that just after the period, the threshold becomes lower than that of the first stimulation, referred to as the super normal period (Khurana and Khurana, 2020).

"Excitatory Conduction" Simulator

In excitatory conduction, the membrane potential changes not only temporally but also spatially, making action potential conduction sometimes difficult for students to understand. Cooley and Dodge (1966) performed this simulation used an IBM7094 mainframe computer, but now this can be achieved with a smartphone (Figure 7 left). By changing the position of the recording electrode, reading the arrival time of the action

potential, and graphing it, the conduction velocity is indicated as the slope of the graph (Figure 7, right). When the play button is pressed, the movement of the excitable region and changes in the membrane potential are displayed in animation (after numerical calculation); this helps students grasp visually how an action potential propagates. By changing the position of the recording electrode, reading the arrival time of the action potential, and graphing it, the conduction velocity is indicated as the slope of the line (Figure 7, right). Temperature dependency of the conduction velocity can also be explored.

Survey

Changes in Understanding due to Simulation Experiments

The understanding of the phenomenon before the simulation experiment (pre) and after the simulation experiment (post) were graphed on a five-level Likert scale (Figure 8).

In the graph, the ratio of "understand very well" and "understand somewhat" was indicated as a numerical value. The results show that in simulation experiments using every type of simulator, the combined ratio of "understand very well" and "understand somewhat" was less than 50% before the simulation experiments but increased to over 90% after the simulation experiments. Significant differences were shown using paired t-test in all five simulators with $p<0.01$.

Changes in Motivation through Simulation Experiments

The motivation to learn before the simulation experiment (pre) and the motivation after the simulation experiment (post) were graphed on a five-level Likert scale (Figure 9). In the graph, the ratio of "strongly have" and "somewhat have" was indicated as a numerical value. The results show that in simulation experiments using every type of simulators, the combined ratio of “strongly have” and “somewhat have” was around 50% before the simulation experiment but increased around 90% after the simulation experiments. Significant differences were shown using paired t-test in all five simulators with $p<0.01$.

Usefulness of These Simulators

Survey results for the rate of usefulness of five simulators were graphed on a five-level Likert scale, in which the ratio of "extremely useful" and "somewhat useful" was indicated as a numerical value (Figure 10). The results show that in simulation experiments using all five types of simulators, the combined ratio of “extremely useful” and “useful to somewhat” were over 80%.

DISCUSSION

It has been reported that computer simulations are educationally effective, especially in neuroscience (Bish and Schleidt, 2008). Computer simulations, however, have not been introduced effectively in neuroscience education in Japanese high schools and undergraduate institutions since there are often not enough computers for all students. Nevertheless, the simulators I developed were proactively introduced in many schools in 2020 and 2021, when the COVID-19 pandemic forced most schools to implement distance learning urgently and for the first time.

Several factors made these easy to implement. Unlike commercial packages such as Neurons In Action (Moore and Stuart, 2007), Neurosim (Heitler, 2022), and Action Potentials (Meir, 2022), these simulators are provided free for anyone to use without registration. These simulators are web-based, can be run by simply accessing a URL, and are very easy to operate. Since the web-based simulators do not require installation and can be run simply by visiting a URL, I avoided download problems. Most simulators, such as SNNAP (AV-Ron et al., 2006) and MetaNeuron (Newman and Newman, 2013), can only be used on personal computers, but these new simulators are smartphone-enabled in addition to computer-enabled. Students are good at using smartphones, so even instructors who were not familiar with computers had no problem in teaching operation.

These simulators could demonstrate a wide range of neuronal phenomena depending on what educational content was taught. Instructors could incorporate their own contents into remote experiments using any simulator.

The most striking impetus for introduction of the simulators was the COVID-19 pandemic. Since the simulators were considered to be educationally effective in each simulation experiment, these continued to be used even after face-to-face experiments resumed. At these schools, there were cases where simulation experiments were conducted instead of animal experiments, and where simulation experiments were conducted in combination with animal experiments. In the case of simulations combined with animal experiments, many students commented that the simulation experiments helped them to better understand and run experiments on animals. Therefore, it can be said that the simulators reduce or even replace the need for experimental animals.

Most of the student comments on the simulators were positive. Many said that they were able to deepen their understanding through the lectures on the basic principles of cellular neurophysiology before or after simulation experiments. This indicates that this simulator is an educational tool, but not a textbook. Because these simulators are simple, in many cases it is necessary for students to think about the conditions, repeat the simulation, and create graphs based on the values. Some commented that activities such

as creating logarithmic graphs based on the values that students read from simulations were useful in improving their scientific skills. In addition, there were many comments such as, "I deepened my understanding by doing it in a group." Indeed, the portability of smartphones and tablets allow flexible and easy student-student and student-teacher interaction. The ability to share information over the Internet using smartphones and conduct group experiments while communicating with each other was also thought to have contributed to enhancing the educational effect.

Due to lack of comparison of groups taught with and without simulators in the survey, objectivity may be insufficient in verification of educational effects of the simulators. It is obvious, however, that at least the students thought that their understanding had deepened subjectively. Many commented that these simulation experiments had increased their interest in the phenomena of cellular neurophysiology. The usefulness of these simulators was also rigorously evaluated, indicating that these were actually easy for students to understand and use. It appears that the motivation of most of students increased by actively conducting these simulation experiments due to ease of operation. It is possible that the ease of operation in actively conducting these simulation experiments contributed to the increased motivation of most students to learn about neurophysiological science.

One of the advantages of web-based simulators is that, unlike simulators that require installation, when I update the simulator, these updates are seamless for users who do not need to download a new program. These simulators I developed are licensed under the MIT license and are free for anyone to use, copy, modify, etc.

I believe the smartphone-based web-based simulators that I have developed are useful for high schools and undergraduates in cellular neurophysiology education. Discuss in future plan to obtain objective not only subjective evaluation of improvement by the usage of simulators.

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FIGURE LEGENDS

Figure 1.

The effects of extracellular K^+ concentration on the membrane potential. The orange curve is given by the GHK equation for K^+, Na^+, and C^{l-}, and the blue line is given by the Nernst equation for K^+ in a semi-logarithmic graph.

Figure 2.

Simulated current-voltage curve obtained using the “Membrane Current” simulator. Potassium current-voltage curve (blue line) and sodium current-voltage curve (red line) are drawn.

Figure 3.

Simulated voltage-clamp step response of channel currents. Potassium current (left) and sodium current (right) at command potential, 80 mV. The top trace is the whole-cell membrane current, and numbers on the waveform in the graph below indicates the number of channels summed are indicated in the lower three traces. These traces were drawn with a CSV file created using the simulator's "text out" function of the “Membrane Current” (top traces) and the “Channel Current” simulators.

Figure 4.

Action potential simulation screen. Left: Relationship between conductance change and action potential. In the upper panel, the black trace is the membrane potential, the red trace is the sodium conductance, and the blue trace is the potassium conductance. Lower panel shows stimulation current. Middle: Action potential with varying stimulus intensity. Right: Stimulation below threshold intensity does not evoke action potential. Stimulus threshold can be inquired by stimulus intensity, which is the minimum intensity to cause the positive feedback. An all-or-none action potentials can be seen by varying the intensity of the stimulus (Figure 5, middle and right).

Figure 5.

Threshold stimulation strength-duration curve in a double-logarithmic graph. Blue, red and green traces are obtained at 0°C, 15°C and 25°C respectively.

Figure 6.

Simulation of refractory period. Left: Overlaid waveform of action potential (upper traces) and stimulation current (lower traces). Right: delay-strength curve.

Figure 7.

Simulation of excitatory conduction. Left: The top trace shows the membrane potential along the axon at various times (5 ms in this graph) and the bottom traces show the membrane potential over time at four different recording electrode positions, which are indicated by filled triangles. Right: Relationship between the distance from the stimulation site to the recording electrode and the arrival time of excitation.

Figure 8.

Survey results for understanding before and after the simulation experiments with a five-level Likert scale. Likert scale: 1, I do not understand at all.; 2, I do not understand much.; 3, I understand a little.; 4, I understand somewhat.; 5, I understand very well. Pre: Survey response obtained before simulation experiments, Post: after simulation experiments. MP, Membrane Potential; MC, Membrane Current; CC, Channel Current; AP, Action Potential; EC, Excitable Conduction simulator experiments. Numbers indicate the percentages replying, “I understand very well” and “I understand somewhat.” Significant differences were shown using paired t-test in all simulators.

Figure 9.

Survey results for motivation before and after the simulation experiments with a five-level Likert scale. Likert scale: 1, I have no motivation to learn.; 2, I do not have much motivation to learn.; 3, I have a little motivation to learn.; 4, I have some motivation to learn.; 5, I have strong motivation to learn. Pre: Survey response obtained before simulation experiments, Post: after simulation experiments. MP, Membrane Potential; MC, Membrane Current; CC, Channel Current; AP, Action Potential; EC, Excitable Conduction simulator experiments. Numbers indicate the percentages replying, "I have strong motivation to learn" and "I have some motivation to learn." Significant differences were shown using paired t-test in all simulators.

Figure 10.

Survey results for usefulness with a five-level Likert scale. MP, Membrane Potential, 199 students; MC, Membrane Current, 156 students; CC, Channel Current, 150 students; AP, Action Potential, 403 students; EC, Excitable Conduction, 238 students. Numbers indicate the percentages replying, "Extremely useful" and "Useful to somewhat."

Figure 1.

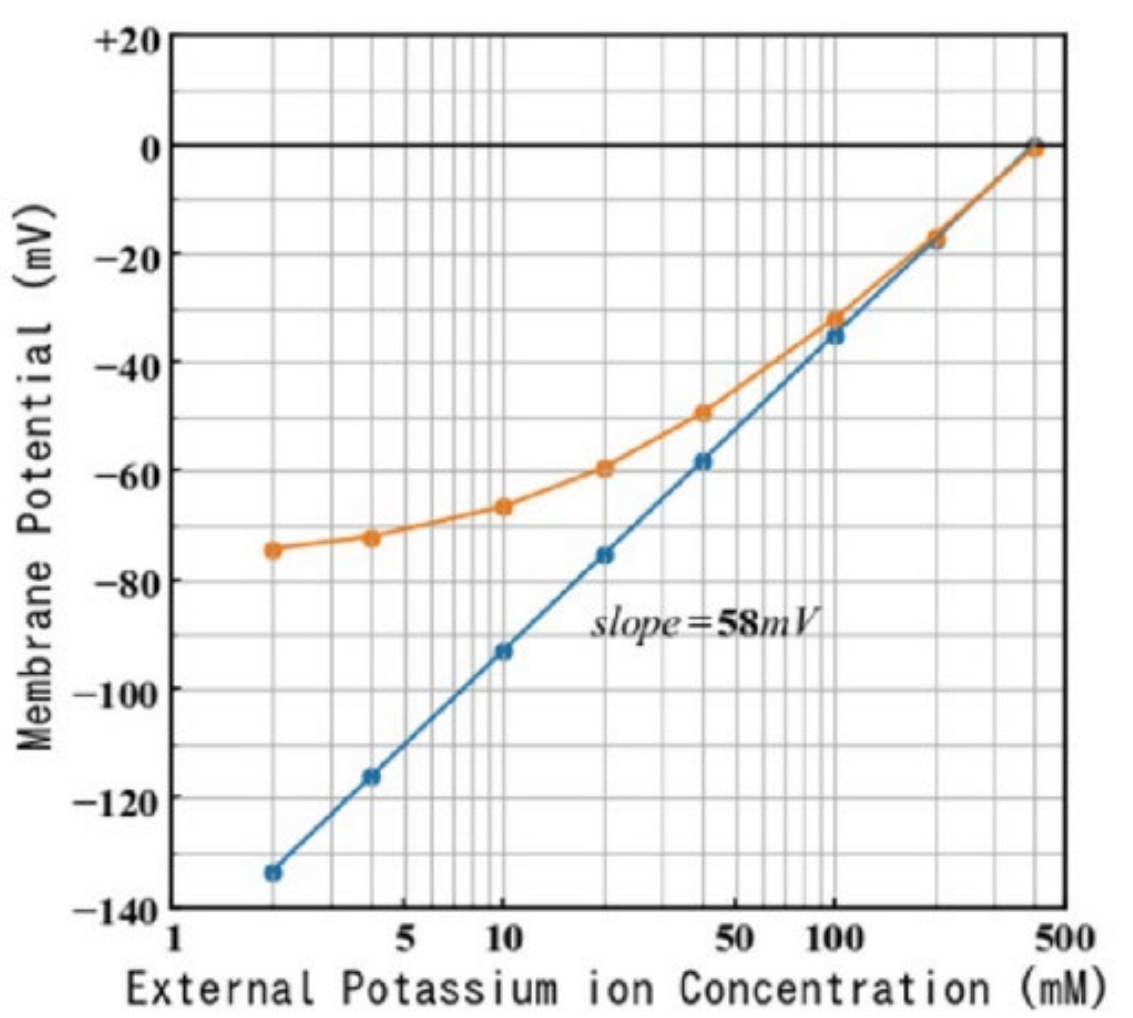

+20
0
−20
−40
−60
−80
−100
−120
−140
Membrane Potential (mV)
slope = 58mV
1
5
10
50
100
500
External Potassium ion Concentration (mM)

Figure 2.

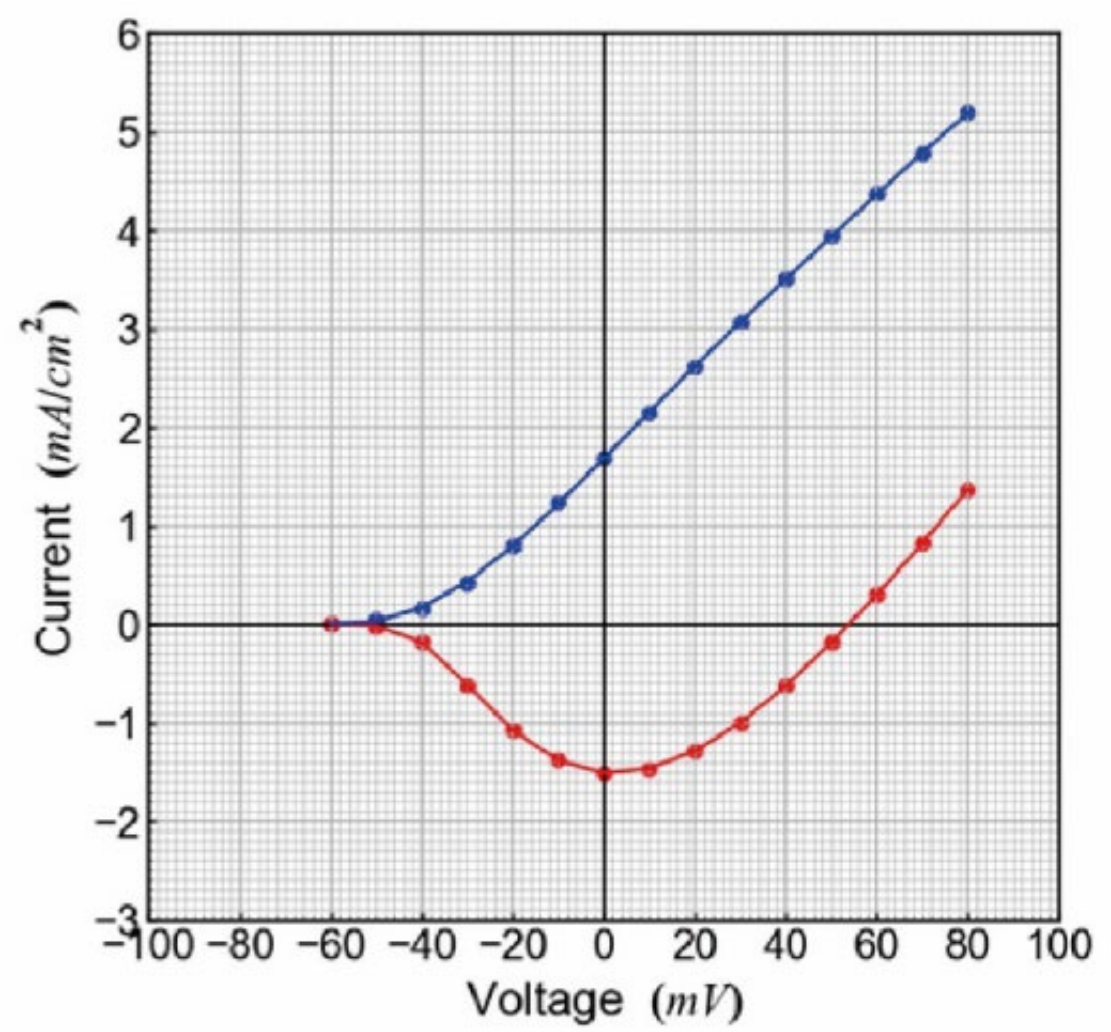

Current (mA/cm^2)
Voltage (mV)
6
5
4
3
2
1
0
−1
−2
−3
−100 −80 −60 −40 −20 0 20 40 60 80 100

Figure 3.

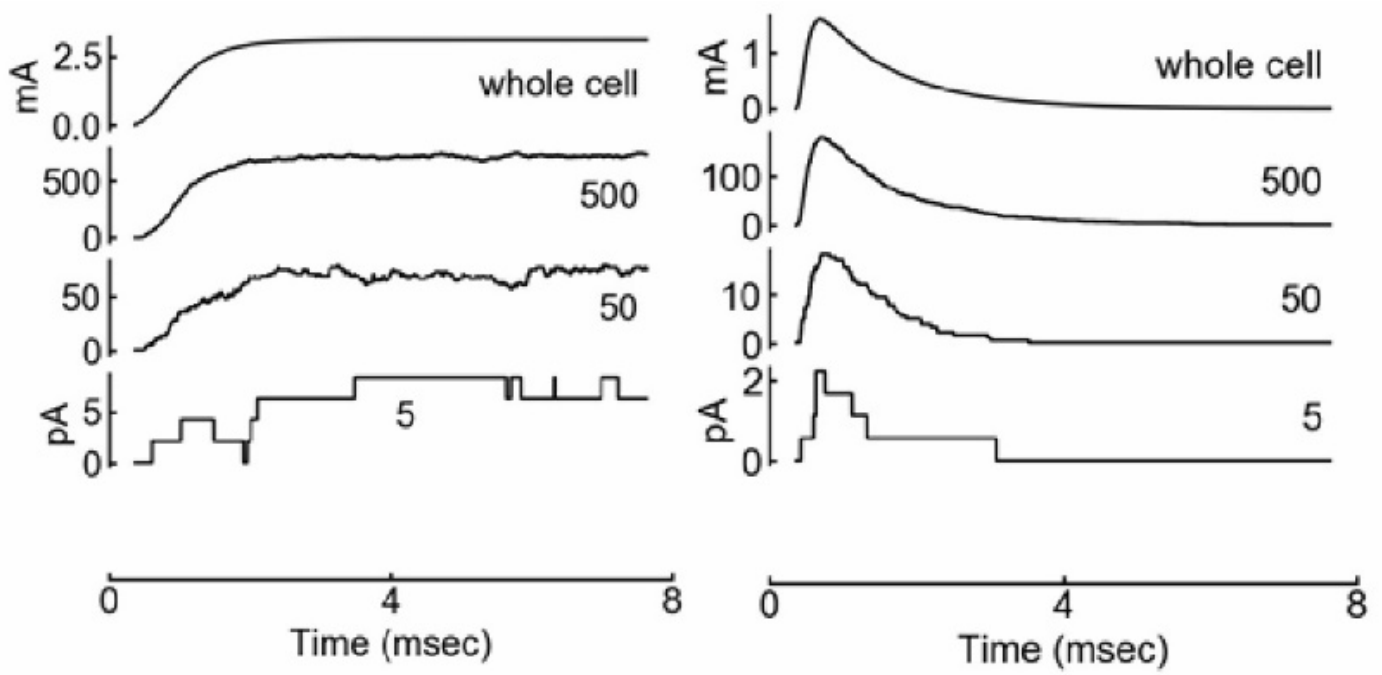

mA
2.5
0.0
whole cell
500
0
500
50
0
50
pA
5
0
5
0
4
8
Time (msec)
mA
1
0
whole cell
100
0
500
10
0
50
pA
2
0
5
0
4
8
Time (msec)

Figure 4.

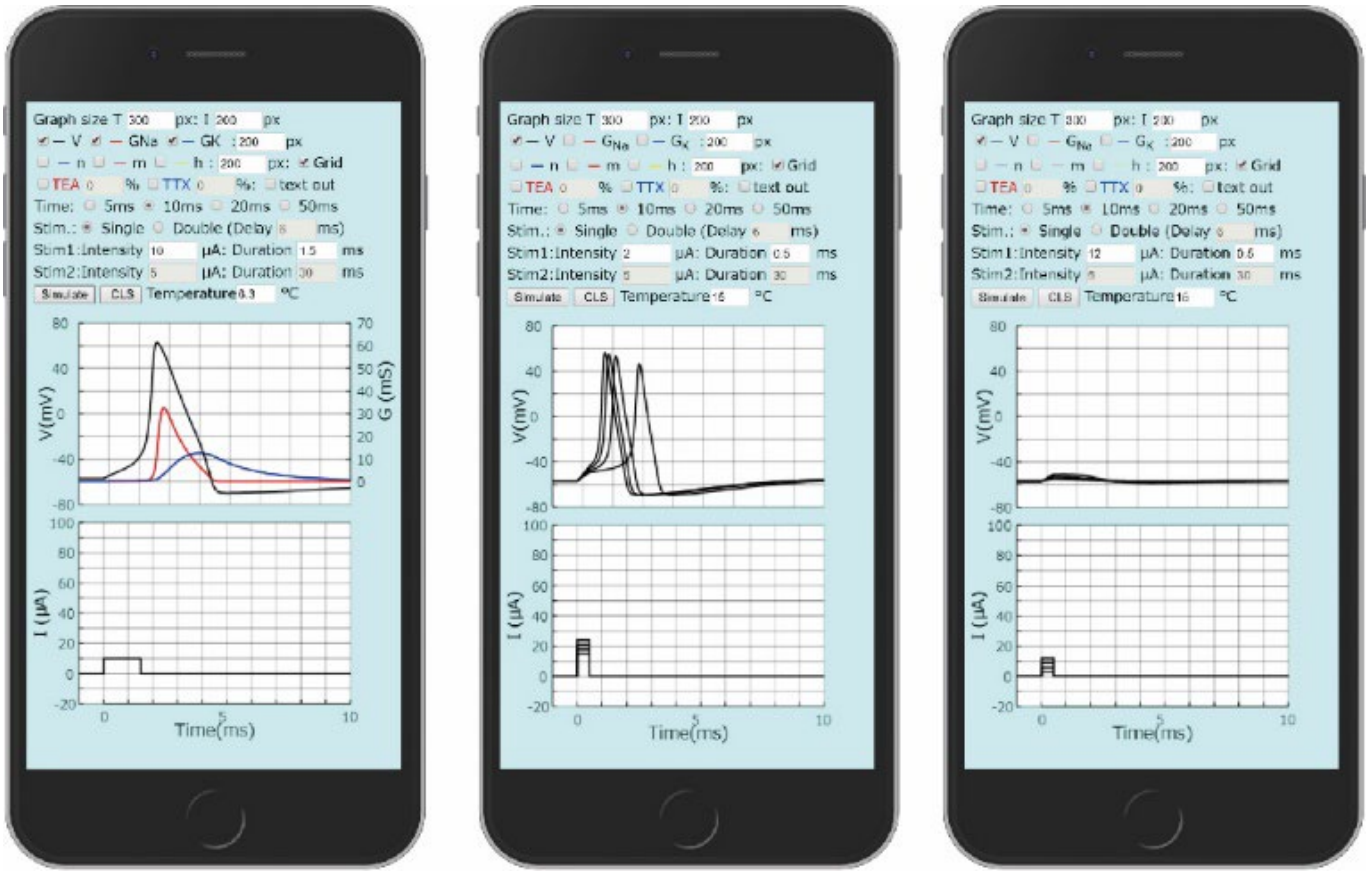

Graph size T 300 px: I 200 px
— V — GNa — GK : 200 px
— n — m — h : 200 px: Grid
TEA 0 % TTX 0 %: text out
Time: 5ms 10ms 20ms 50ms
Stim.: Single Double (Delay 6 ms)
Stim1:Intensity 10 µA: Duration 1.5 ms
Stim2:Intensity 5 µA: Duration 30 ms
Simulate CLS Temperature 8.3 °C
V(mV)
G (mS)
I (µA)
Time(ms)
Graph size T 300 px: I 200 px
— V — GNa — GK : 200 px
— n — m — h : 200 px: Grid
TEA 0 % TTX 0 %: text out
Time: 5ms 10ms 20ms 50ms
Stim.: Single Double (Delay 6 ms)
Stim1:Intensity 2 µA: Duration 0.5 ms
Stim2:Intensity 5 µA: Duration 30 ms
Simulate CLS Temperature 6 °C
V(mV)
I (µA)
Time(ms)
Graph size T 300 px: I 200 px
— V — GNa — GK : 200 px
— n — m — h : 200 px: Grid
TEA 0 % TTX 0 %: text out
Time: 5ms 10ms 20ms 50ms
Stim.: Single Double (Delay 6 ms)
Stim1:Intensity 12 µA: Duration 0.5 ms
Stim2:Intensity 5 µA: Duration 30 ms
Simulate CLS Temperature 16 °C
V(mV)
I (µA)
Time(ms)

Figure 5.

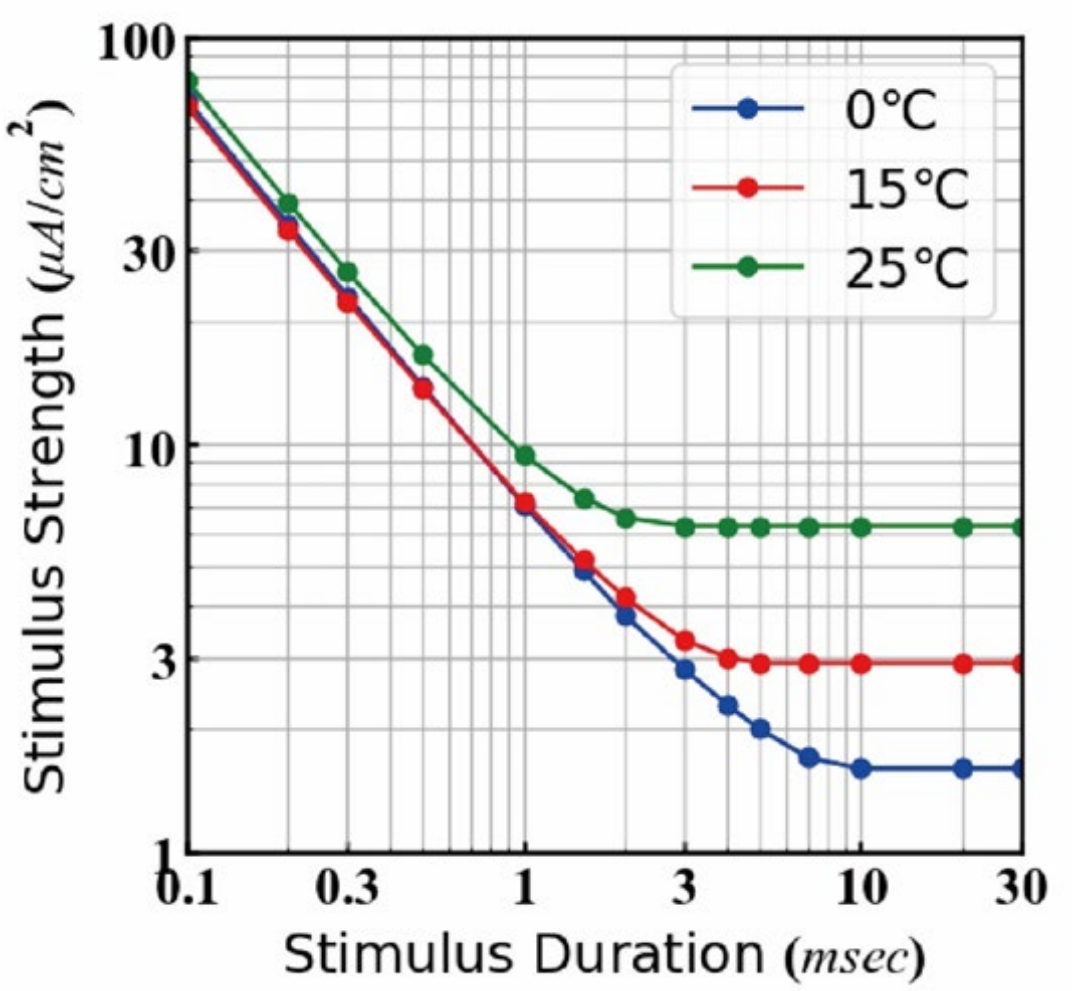

100
30
10
3
1
0.1
0.3
1
3
10
30
Stimulus Strength ($\mu A/cm^2$)
Stimulus Duration (*msec*)
0°C
15°C
25°C

Figure 6.

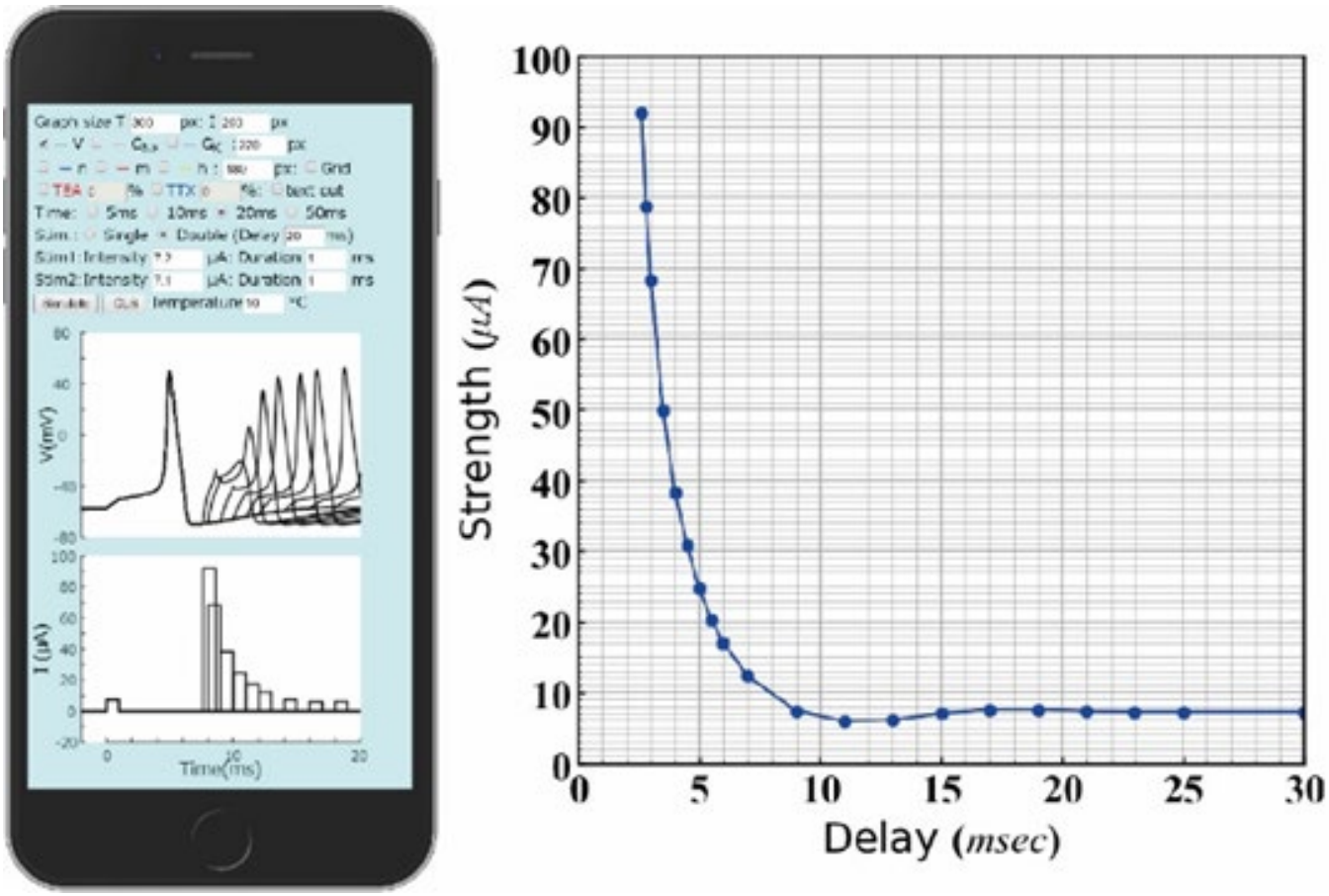
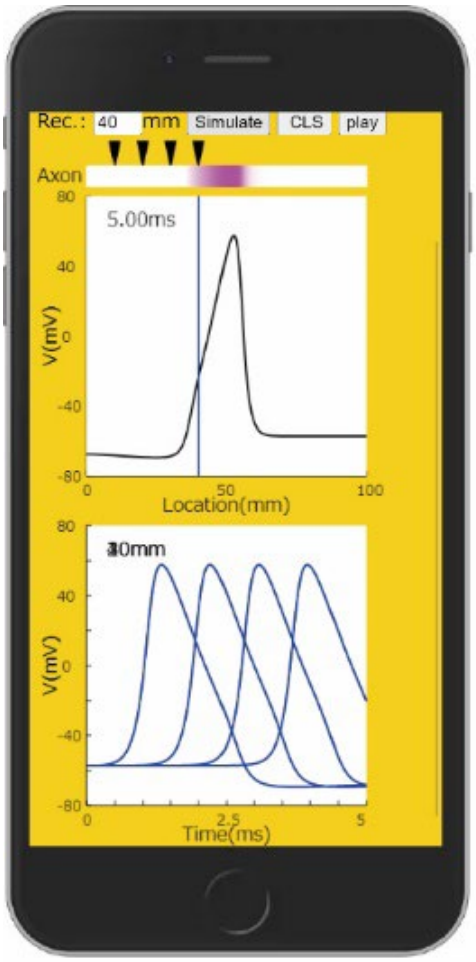

Time: 5ms 10ms 20ms 50ms
Sim1: Intensity
Sim2: Intensity
Temperature
V(mV)
I (μA)
Time(ms)
Strength (μA)
Delay (msec)

Figure 7.


Rec.: 40 mm
Simulate
CLS
play
Axon
5.00ms
V(mV)
Location(mm)
Time(ms)

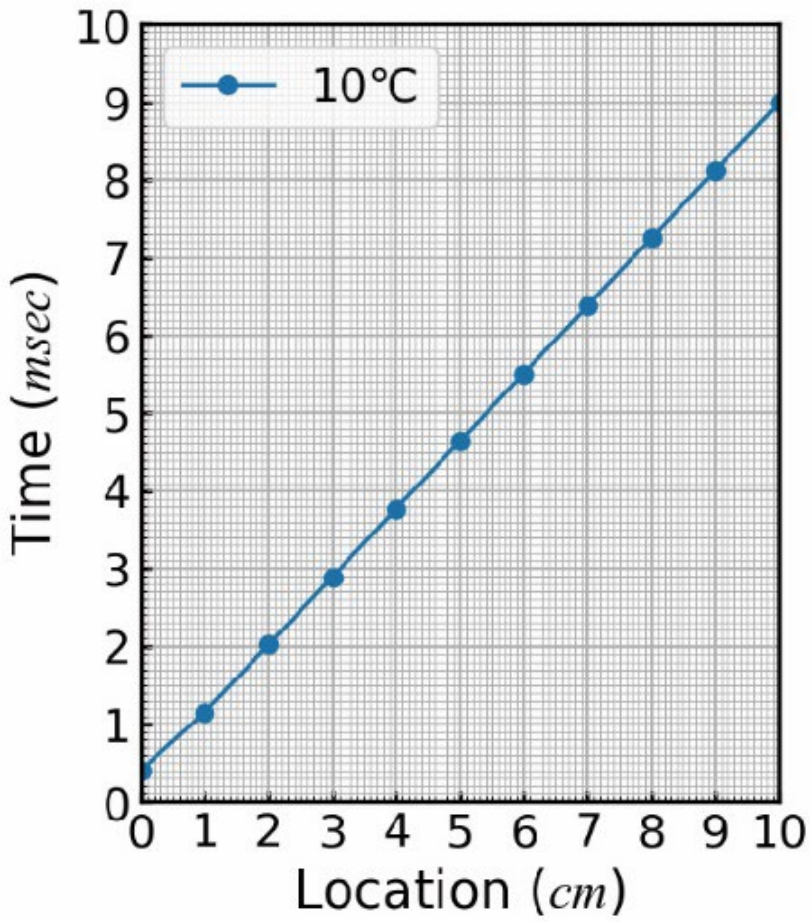

10°C
Time (msec)
Location (cm)

Figure 8.

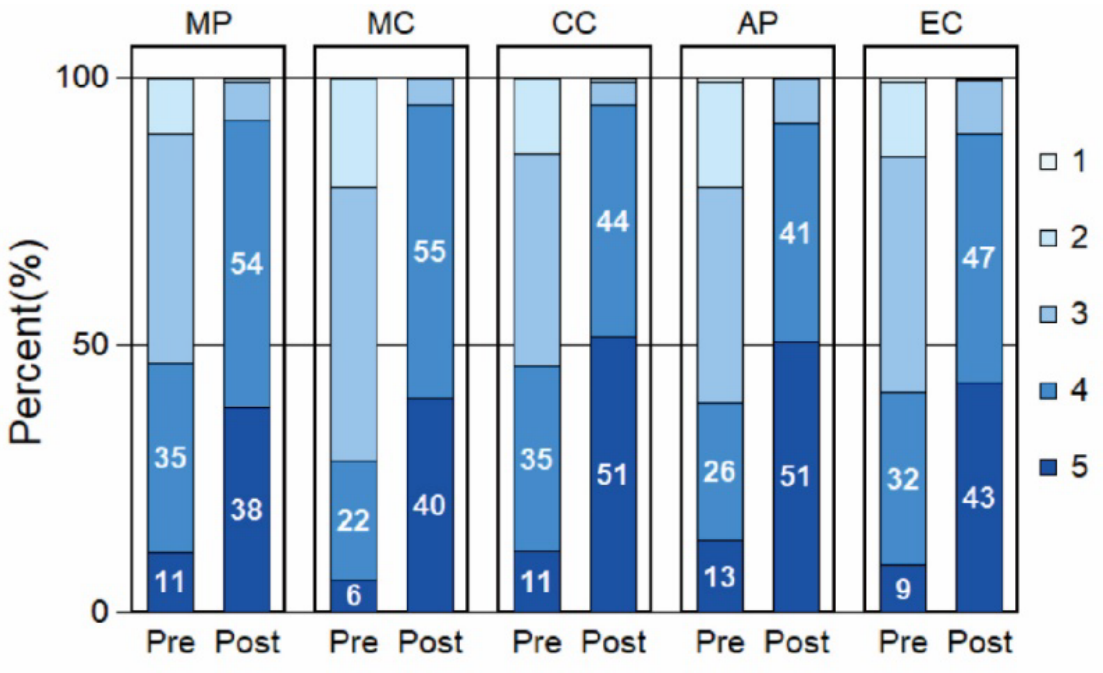

MP
MC
CC
AP
EC
100
50
0
Percent(%)
Pre Post Pre Post Pre Post Pre Post Pre Post
11
35
38
54
6
22
40
55
11
35
51
44
13
26
51
41
9
32
43
47
1
2
3
4
5

Figure 9.

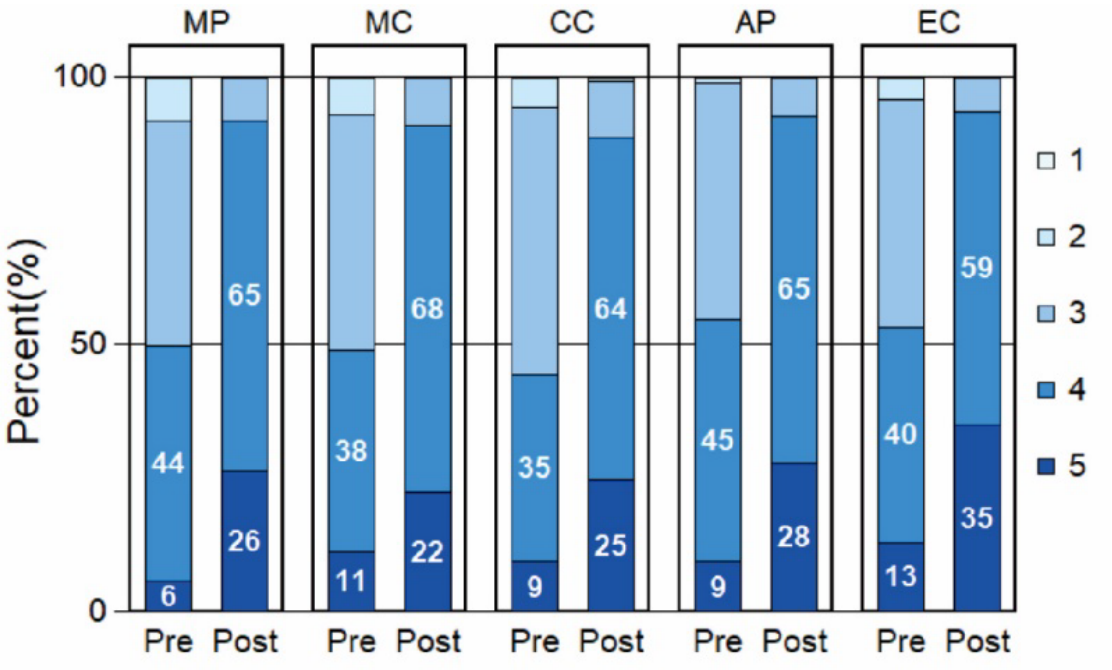
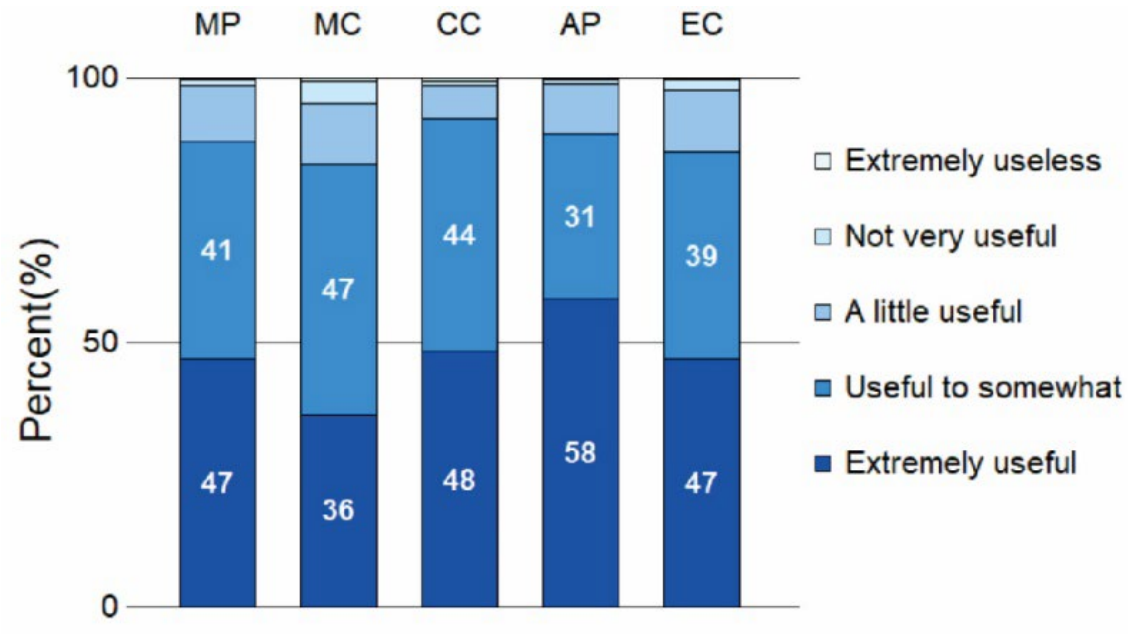

MP
MC
CC
AP
EC
100
50
0
Percent(%)
Pre
Post
44
65
6
26
38
68
11
22
35
64
9
25
45
65
9
28
40
59
13
35
1
2
3
4
5

Figure 10.


MP
MC
CC
AP
EC
100
50
0
Percent(%)
41
47
47
36
44
48
31
58
39
47
Extremely useless
Not very useful
A little useful
Useful to somewhat
Extremely useful

CONCLUSIONS

1. With the aim of deepening students' understanding and interest in the phenomena and principles of cellular neurophysiology, I have developed unprecedented Smartphone-based Web-based simulators that can be run in any environment.

2. By developing five types of simulators, Membrane Potential, Membrane Current, Channel Current, Action Potential, and Excitatory Conduction, it has become possible to deal with all fields of cellular neurophysiology. By using these simulators, various cellular neurophysiological training that was not possible before has become possible.

3. During the COVID-19 pandemic, these simulators were introduced into many schools and utilized as new cellular neurophysiology practices. In order to investigate the educational effects and usefulness of those simulators, questionnaire surveys were conducted for the students who participated in the practical training. As a results, it were demonstrated that the five types of simulators developed in this research significantly increase the understanding of the phenomena and principles of cellular neurophysiology and the motivation for learning, and are useful for learning.

ACKNOWLEDGEMENTS

I would like to thank Dr. Adam Weitemier and Dr. Makoto Kurokawa for useful advice and encouragement throughout this study, Dr. Naoki Yamamoto for his helpful discussions and comments on this study.

Supplementary Material

The mathematical model of each simulator is described below.

"Membrane Potential" Simulator

This simulator uses the Goldman-Hodgkin-Katz voltage equation.

$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_{out} + P_{Na}[Na^+]_{out} + P_{Cl}[Cl^-]_{in}}{P_K[K^+]_{in} + P_{Na}[Na^+]_{in} + P_{Cl}[Cl^-]_{out}} \quad (1)$$

V_m: membrane potential, R: gas constant, T: absolute temperature, F: Faraday constant

P_{ion}: ion permeability, $[ion]_{in}$: intracellular ion concentration, $[ion]_{out}$: extracellular ion concentration

"Membrane Current" Simulator

This simulator uses the Hodgkin-Huxley (HH) model for squid giant axons. The HH model expresses the behavior of a total of six variables, total membrane current (Im), membrane potential (Vm), and time (t), sodium conductance activation parameter (m), sodium conductance inactivation parameter (h) and potassium conductance activation parameter (n), using four differential equations and six relational expressions.

$$I_m = C_m \frac{dV_m}{dt} + \overline{g_{Na}} m^3 h(Vm - E_{Na}) + \overline{g_K} n^4 (Vm - E_K) + \overline{g_L}(Vm - E_L) \quad (2)$$

$$\frac{dm}{dt} = \alpha_m(1 - m) - \beta_m m \quad (3)$$

$$\frac{dh}{dt} = \alpha_h(1 - h) - \beta_h h \quad (4)$$

$$\frac{dn}{dt} = \alpha_n(1 - n) - \beta_n n \quad (5)$$

$$\alpha_m = \frac{0.1\varphi(25 - Vm)}{\exp\left(\frac{25 - Vm}{10}\right) - 1} \quad (6)$$

$$\beta_m = 4\varphi \exp\left(-\frac{Vm}{18}\right) \quad (7)$$

$$\alpha_h = 0.07\varphi \exp\left(-\frac{Vm}{20}\right) \quad (8)$$

$$\beta_h = \frac{\varphi}{\exp\left(\frac{-Vm + 30}{10}\right) + 1} \quad (9)$$

$$\alpha_n = \frac{0.01\varphi(-Vm + 10)}{\exp\left(\frac{-Vm + 10}{10}\right) - 1} \quad (10)$$

$$\beta_n = 0.124\varphi \exp\left(\frac{-Vm}{80}\right) \quad (11)$$

Cm: membrane capacitance, $\overline{g_{Na}}$: maximum sodium conductance, E_{Na}: equilibrium potential of sodium ions

$\overline{g_K}$: maximum potassium conductance, E_K: equilibrium potential of potassium ions

$\overline{g_L}$: leak conductance, E_L: equilibrium potential of leak ions

α_i and β_i (i=m, h, n): rate constants

$\varphi\left(3^{\frac{T-6.3}{10}}\right)$: temperature coefficient (T: degree Celsius)

Since this simulator uses a voltage clamp method, $\frac{dVm}{dt} = 0$. Also, since the current due to leak conductance is small, it is ignored and only the sodium current and potassium current are simulated.

"Channel Current" Simulator

Like the membrane current simulator, this simulator also simulates sodium or potassium currents due to voltage clamp method. However, whereas the membrane current simulator simulates current across the membrane, this simulator simulates channel-by-channel ionic current due to patch clamp method.

In the potassium channel, as shown in Figure 1, five discrete states are considered, and the transition probability between each state is expressed using the rate constants α_n and β_n for changes in n in the HH model. The rate constants from left to right fall in the sequence $4\alpha_n$, $3\alpha_n$, $2\alpha_n$, α_n, and those from right to left fall in the sequence $4\beta_n$, $3\beta_n$, $2\beta_n$, β_n. C_0 to C_3 indicate states in which 0 to 3 of the four subunits of the potassium channel are activated and the channel is closed. "O" indicates a state in which all four subunits are activated and the channel is open, and only this state conducts ions.

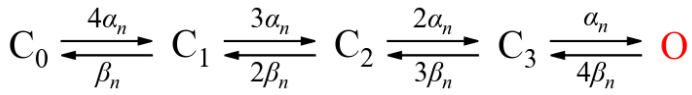


Figure 1. Kinetic states of the HH model potassium channel

Eight major gating states result when the logic used Figure 1 is applied to sodium channels in the HH model. Only state "O" is where all subunits are activated, the gates are open, and ions conduct (Figure 2).

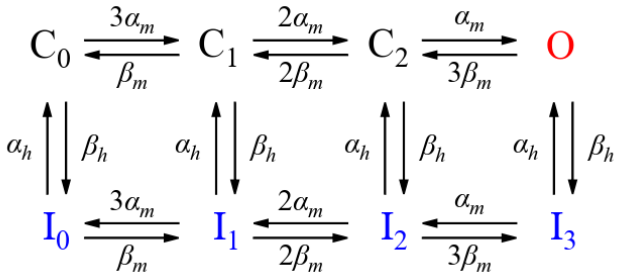


Figure 2. Kinetic states of the HH model sodium channel

This simulator can add noise to the channel current waveform to mimic real recordings. This involves adding white noise to the channel current waveform. After performing fast Fourier transform on it to remove low-frequency components and high-frequency components, it is returned to a waveform using inverse fast Fourier transform.

"Action Potential" Simulator

This simulator uses the HH model based on equations 2 to 11. In this simulator that simulates changes in membrane potential, equation 2 is used in current clamp mode. In other words, Im is the stimulation current during stimulation and is 0 otherwise.

"Excitatory Conduction" Simulator

This simulator simulates using nonlinear partial differential equations that combine the HH model and cable theory.

$$\frac{1}{2\pi ar}\frac{\partial^2 Vm}{\partial x^2} = C_m\frac{\partial V_m}{\partial t} + \overline{g_{Na}}m^3h(Vm - E_{Na}) + \overline{g_K}n^4(Vm - E_K) + \overline{g_L}(Vm - E_L) - Im \quad (12)$$

Where a is the axon radius, and r is resistance per unit length of the intracellular fluid. Rearranging equation 12, the capacitor current is written in terms of the ion and membrane currents and the spatial "diffusion" of the membrane potential:

$$\frac{\partial V_m}{\partial t} = \frac{1}{2\pi arCm}\frac{\partial^2 Vm}{\partial x^2} - \frac{1}{Cm}[\overline{g_{Na}}m^3h(Vm - E_{Na}) + \overline{g_K}n^4(Vm - E_K) + \overline{g_L}(Vm - E_L) - Im] \quad (13)$$

This equation can be solved using the finite difference method. First change the notation so Vm becomes V_j^i, where i indicates the present time and j represents a discrete location along the axon. The time and space derivatives are approximated by the finite differences

$$\frac{\partial Vm}{\partial t} = \frac{\partial V_j^i}{\partial t} \approx \frac{V_j^{i+1} - V_j^i}{\Delta t} \quad (14)$$

$$\frac{\partial^2 Vm}{\partial x^2} = \frac{\partial^2 V_j^i}{\partial x^2} \approx \frac{V_{j+1}^i - 2V_j^i + V_{j-1}^i}{(\Delta x)^2} \quad (15)$$

Substituting these approximations into equation 13 gives:

$$\frac{\partial V_j^i}{\partial t} \approx \frac{V_j^{i+1} - V_j^i}{\Delta t} = \frac{1}{2\pi arCm}\left[\frac{V_{j+1}^i - 2V_j^i + V_{j-1}^i}{(\Delta x)^2}\right] - \frac{1}{Cm}[\overline{g_{Na}}m^3h(Vm - E_{Na}) + \overline{g_K}n^4(Vm - E_K) + \overline{g_L}(Vm - E_L) - Im] \quad (16)$$

Which can be solved for the new value of Vm at location j for the next time step, i+1:

$$V_j^{i+1} = V_j^i + \Delta t\frac{1}{2\pi arCm}\left[\frac{V_{j+1}^i - 2V_j^i + V_{j-1}^i}{(\Delta x)^2}\right] - \frac{\Delta t}{Cm}[\overline{g_{Na}}m^3h(Vm - E_{Na}) + \overline{g_K}n^4(Vm - E_K) + \overline{g_L}(Vm - E_L) - Im] \quad (17)$$