The OpenPicoAmp: an open-source planar lipid bilayer amplifier for hands-on learning of neuroscience

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Abstract

Neuroscience education can be promoted by the availability of low cost and engaging teaching materials. To address this, we developed an open-source lipid bilayer amplifier, the Open-PicoAmp, which is appropriate for use in introductory courses in biophysics or neurosciences concerning the electrical properties of the cell membrane. The amplifier is designed using the common lithographic printed circuit board fabrication process and off-the-shelf electronic components. In addition, we propose a specific design for experimental chambers allowing the insertion of a commercially available polytetrafluoroethylene film. This experimental setup can be used in simple experiments in which students monitor the bilayer formation by capacitance measurement and record unitary currents produced by ionophores like gramicidin A. Used in combination with a low-cost data acquisition board this system provides a complete solution for hands-on lessons, therefore improving the effectiveness in teaching basic neurosciences or biophysics.

1 Introduction

The traditional lecture is still the standard pedagogical method for teaching science at the undergraduate level, although it has been shown that more active approaches are more efficient especially in large-enrollment courses [4]. In addition, it is now challenged by the development of massive open online course, or MOOC. This evolution should reinforce the interest in extensive hands-on learning sessions as they provide a way to improve learning which cannot be obtained by the online methods [2]. Hands-on learning sessions are also relevant in the "flipped classroom" approach which is an inverted teaching structure where instructional content is delivered outside class, and engagement with the content is done in class, under teacher guidance and in collaboration with peers. Instead of giving the same explanations over and over the teacher can capture his explanation once on video or audio, and spend energy and time individualizing instruction [11]. This provides a way to cope with large class sizes and reach students who are at varying levels of understanding and skill.

In undergraduate basic science courses, the hands-on learning sessions can take the form of laboratory sessions where of a series of challenging questions and tasks require students, divided in small groups, to practice reasoning and problem solving while provided with frequent feedback from fellow students and from the instructors. To implement this approach there is crucial need of engaging teaching materials. Entry-level neurophysiology equipment used for teaching neuroscience are in the range of thousands of euros and experiments may require the use of living animals. Here we propose an open-source lipid bilayer amplifier which is appropriate for use in introductory courses in biophysics or neuroscience. We also describe a simple experimental protocol which is currently performed by undergraduate medical students during their training in basic neuroscience at our institution. The related costs to build the amplifier and the bilayer chamber are below 300 euros and the experiments do not involve the use of animals.

The planar lipid bilayer method allows to build an artificial cell membrane and examine pore forming molecule functionally at the single-molecule level [10]. Such molecules can be isolated from

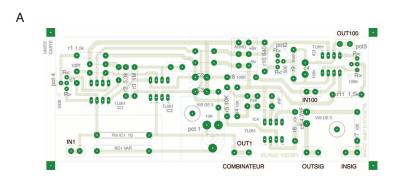




Figure 1: The OpenPicoAmp amplifier. The chosen electronic design uses a minimal number of commonly found components, like the Texas Instruments TL061 and TL071 operational amplifiers. It is therefore inexpensive and easy to build. (A) Printed circuit board for the OpenPicoAmp. (B) Implantation of the circuit and two 6 V batteries in the main box. An additional box is necessary to build the remote control allowing to switch gain and adjust the offset without producing vibrations that may compromise the stability of the lipid bilayer (not shown).

various biological resources and are reconstituted into an artificial membrane that has a defined lipid composition. The laboratory session we propose in this paper aims to help the students to understand the passive electrical properties of the cell membrane. In particular, they can directly observe the elementary currents produced by the activity of a single pore forming molecule, such ionic channels being the key players in the generation of the neuronal electrical activity.

2 Materials and Methods

2.1 Participants

The laboratory session is typically lasting four hours and starts with a brief 15-20 minutes lecture focusing on the electrical properties of the cell membrane and the voltage clamp technique. Students are reminded that an element of passive cell membrane (i.e. not producing action potentials) is electrically equivalent to an RC circuit. In addition, the operation of the voltage clamp circuit, which is an op-amp current to voltage converter circuit, is explained. The latter allow us to introduce the general concept of negative feedback which is relevant in many biological processes.

Sessions are coordinated by adjuncts instructors and older students which have been previously trained. Usually one group of two students is assigned per bilayer setup. To allow the session to run smoothly and maximize the interactions with the students, one instructor for two groups of



Figure 2: Bilayer chamber. (A) The bilayer chamber is sitting in an metallic box that, when closed, acts as a Faraday cage minimizing electric noise. The chamber is composed of two compartments milled in two blocks of polyoxymethylene which are pressed together by a pair of screws. Each compartment is connected to the amplifier though an Ag-AgCl electrode and a salt bridge. (B) the Teflon film is sealed with silicon grease between the two blocks. Before insertion of the film in the chamber, a circular 200 μm aperture is first etched in the film (visible at the centre of the film separating the two compartments).

students is an optimal configuration. Lab sessions at our institution are run with ten bilayer setups working simultaneously.

2.2 Equipment

2.2.1 Amplifier

We have developed a low cost open-source bilayer amplifier (fig. 1). The amplifier is designed using the lithographic printed circuit board fabrication process and off-the-shelf electronic components, like the TL071 and TL061 operational amplifiers (Texas Instruments, USA). The amplifier head stage is a current to voltage converter equipped with a 1 $G\Omega$ feedback resistor. The two-stage amplifier has a two built-in gains of 1 mV/pA and 100 mV/pA, low-passed from 1000 to 16 Hz. The amplifier at its highest gain has a sensitivity in the pA range which makes it suitable for the recording of the unitary current produced by the opening of a single channel molecule. The design choices for this amplifier were driven by the following conditions: it should be easy to build and inexpensive. Therefore we have chosen to use easy to find electronic components and kept their number to a minimum. As a consequence this amplifier may not be suitable to be used for research purpose due to bandwidth limitations, but is well adapted to perform the experiment proposed here. The electronics design can be found in the supporting information section of this paper. These design files are licensed under a Creative Commons Attribution Share-Alike license, which allows for both personal and commercial derivative works, as long as this paper is credited and the derivative designs are released under the same license.



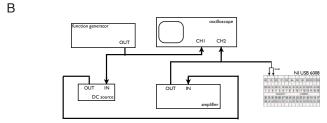


Figure 3: The complete planar bilayer setup as implemented at our institution. (A) On this picture, the different parts of the setup can be seen: the amplifier and its remote control, a waveform generator, a switchable DC source and an oscilloscope. In addition, we use a NI USB6008 for data acquisition with the lab computers. The recording chamber, connected to the main box of the amplifier, is placed on a heavy granite slab resting on four tennis balls. (B) The corresponding block diagram of the setup.

2.2.2 Bilayer chamber and electrical recording

The bilayer chamber is composed of two compartments milled in two blocks of polyoxymethylene (DelrinTM) allowing the insertion of a commercially available polytetrafluoroethylene (PTFE or TeflonTM) film (Norton T-100 Premium Grade skived PTFE film, Saint-Gobain, Germany). The films used for this experiment have a thickness of 50 μm . Before insertion of the film in the chamber, a circular 200 μm aperture is first etched in the PTFE film using a modified syringe needle. The experimental chamber consists of the Teflon film sealed with silicon grease between the two Delrin blocks which are pressed together by a pair of screws (fig. 2). This design choice allows an easy replacement of the Teflon film which is sometimes teared during the manipulations by the students. The two compartments of the experimental chamber are called *cis* and *trans*. The *trans* side is always defined as zero voltage, corresponding to the usual convention in electrophysiology where the reference potential is on the extracellular side. Thus the voltage signal is applied on the cis side while the trans side is held at 0 V by the current to voltage converter acting as a virtual ground. The experimental chamber and the amplifier circuit are enclosed in an aluminium box that acts as a Faraday cage minimizing electric noise. Mechanical vibrations are damped by placing the experimental chamber on a heavy granite slab resting on four tennis balls. Each compartment is connected to the amplifier though an Ag-AgCl electrode and a salt bridge (1 M KCl, 2% Agar). The complete setup consists of the amplifier, a waveform generator, a switchable DC source and an oscilloscope. Alternatively, low cost USB interfaces, like the NI USB6008 or the NI MyDAQ (National Instruments, USA), can be used to provide a solution for data acquisition and signal generation using the lab computers (fig. 3).

2.2.3 Chemicals and solutions

Commercially available phospholipids can be used to form planar lipid bilayers. We selected 3-sn-phosphatidylcholine (Sigma-Aldrich, ref. 61758 FLUKA). The lipids were dissolved in n-octane (Sigma-Aldrich, ref. O2001 SIGMA) to a final concentration of $20 \ mg/ml$. This lipid solution has to be prepared shortly before performing the experiments. The salt or electrolyte solutions used in most planar lipid bilayer experiments are often KCl or NaCl. In our case the recordings were made in ionic symmetrical conditions with $1 \ M$ NaCl in both compartments or in asymmetrical ionic condition with $1 \ M$ NaCl in the cis compartment and $0.1 \ M$ NaCl in the trans compartment.

Gramicidin A is a small peptide that forms ionic channels in lipid bilayers [9, 5, 3]. When two gramicidin A molecules link transiently, they form an open ionic channel which is selective for monovalent cations allowing the passage of millions of ions per second through the lipid bilayer. The corresponding unitary current is therefore in the pA range and can be detected with the proposed amplifier. The duration of a channel opening corresponds to a survival time of the complex between two gramicidin molecules. For the sake of simplicity, we are going to refer to these single-channel events as openings of the channel. These openings typically last several hundreds of ms and are thus also easy to resolve temporally. Gramicidin A is available commercially and inexpensive (Sigma-Aldrich, ref. 50845 SIGMA). A 1 nM stock solution is prepared by successive dilution with ethanol. The stability of gramicidin A in this solution appears very high (more than 4 years).

2.3 Experiments

The laboratory session is divided in three successive steps corresponding to a progressive approach of the different aspects of the topic. The corresponding student hand-out can be found in the in the supporting information section of this paper.

2.3.1 Step 1

The cell membrane is considered to be electrically equivalent to leaky capacitor. Guiding question : How does such a RC circuit reacts when submitted to a variable or continuous voltage signal? Learning goals : Students will understand that (1) the total current is essentially produces by the capacitive current when the circuit is submitted to a high frequency voltage signal and (2) that the total current is purely resistive when the circuit is submitted to a constant voltage. Therefore these two conditions can be used to actually measure the capacitance and resistance of the circuit elements. Activity: Students have to compute and measure the capacitive and resistive currents for a circuit composed of a resistance with a capacitor in parallel when it is submitted (1) to a high frequency triangular signal and (2) to a constant voltage.

2.3.2 Step 2

Guiding question: How does a lipid bilayer reacts when submitted to similar variable or continuous voltages? Learning goals: Students will understand that a planar lipid bilayer is electrically equivalent to a RC circuit. Activity: Students have to obtain a lipid bilayer and measure the current first when it is submitted (1) to to a high frequency triangular signal and (2) to a constant voltage. This allow them to measure the bilayer capacitance and resistance.

2.3.3 Step 3

Guiding question: What is an ionic channel? Learning goals: Students will observe that certain molecules can form pores in the lipid bilayer allowing transiently the selective passage of ions and therefore act as electrical switches at the molecular level. Activity: Students have to obtain a lipid bilayer in presence of an ionophore and measure the current when it is submitted to a range of constant voltages.

2.4 Survey methodology

At the beginning of the laboratory session, students were given ten minutes to fill out a short answer survey. An additional survey was then administered at the end of session. The pre- and post-surveys consisted of True/False questions about membrane biophysics. The answers were scored as `0 = incorrect' and `1 = correct'). A "knowledge score" was determined as the sum of correct answers. A paired samples t-test was conducted to compare knowledge scores before and after the demonstration.

3 Results

3.1 Experiments

In a typical four hours session, all students were able to obtain the lipid bilayer formation and study its electrical properties. In addition, we found that routinely more than 50 % of attendees could actually complete all the steps of the proposed experimental protocol and observe the single channel currents. We consider this as a good performance, as it is a rather delicate experiment and for many students this was their first experience in such a laboratory environment.

3.1.1 Calibration

Students have to compute the capacitive and resistive currents for a circuit composed of a 1 $G\Omega$ resistance with a 220 pF capacitor in parallel when it is submitted (1) to a 500 Hz 10 mV peak to peak triangular signal and (2) to a constant 120 mV voltage. When the circuit is submitted to the triangular signal, which has a slope of \pm 10 V/s, the capacitive current is a square signal with an amplitude of 2200 pA. The resistive current is a triangular signal of 10 pA peak to peak amplitude, which is therefore negligible. The total current measured by the amplifier with the 1 mV/pA gain appears therefore a square signal of 2.2 V of amplitude on the oscilloscope. When submitted to the constant 120 mV voltage, the capacitive current is zero and there is only a constant resistive current of 120 pA appearing as a 120 mV on the oscilloscope.

Subsequently, they connect the corresponding RC circuit to the amplifier and do the actual measurements using the oscilloscope. This also provides a calibration of the amplifier allowing to subsequently evaluate the bilayer capacitance during its formation by simply measuring the capacitive current when it is submitted to the triangular signal, as each pF of capacitance will contribute $10\ mV$ to the amplitude of the signal read on the oscilloscope with amplifier at the $1\ mV/pA$ gain.

3.1.2 Bilayer formation

The two compartments of the experimental chamber are first filled with electrolyte solution (1 MNaCl). Subsequently 2 μl of a 20 mq/ml solution of 3-sn-phosphatidylcholine diluted with n-octane are injected over the aperture. The bilayer is formed by spontaneous thinning. If the lipid bilayer does not form within a few minutes after adding the lipids, the electrolyte solution is agitated to speed up the thinning process. The agitation can be achieved by producing small air bubbles near the aperture with a 10 μl pipette (see movie in the supplementary information). The bilayer formation is monitored by measuring the capacitive current during the application of a triangular voltage signal (500 Hz, 10 my peak to peak). As a the planar lipid bilayer is forming in the central part of the aperture, the capacitive current will increase and then stabilize (fig. 4.A). A stable lipid bilayer formed inside the 200 μm aperture in the teflon film will typically produce a capacitance in the range of hundreds of pF. The capacitance of the bilayer is the first criteria for proceeding with an experiment. Low capacitance reflects a bilayer that has not completely formed or that is overly thick. In that case further agitation is needed to speed up the thinning process. The final bilayer stability measurement is the amount of current flowing across a channel-free bilayer in response to test potentials in the voltage range that is to be used in the experiment. If test potentials generate substantial currents across the membrane in the absence of ionic channels, the bilayer is deemed

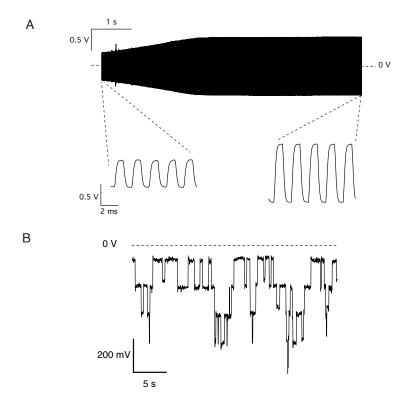


Figure 4: Experimental recordings obtained with the OpenPicoAmp. (A). The lipid bilayer formation is monitored by measuring the increase in the capacitive current during the application of a triangular voltage signal ($500\ Hz$, $10\ mV$ peak to peak). In this particular example, the lipid bilayer forms and stabilizes in less than $10\ s$ with a final capacitance around $70\ pF$. (B) After addition of gramicidin A, the students are able to record unitary currents when the bilayer is submitted to constant voltage. In the presented recording, obtained at -120 mV, a maximum of four gramicidin A pores are open at the same time. All the presented data has been acquired by students using a National Instruments USB6008 A/D converter. All currents traces appears as voltage deflections as the amplifier acts as a current to voltage converter with a gain of $1\ mV/pA$ (panel A) or $100\ mV/pA$ (panel B).

unstable and should be broken and reformed before beginning an experiment. In our case, the total current at a constant 120 mV voltage is measured and should be below 2 pA.

Knowing the specific capacitance of a lipid bilayer $(0.5 \ \mu F/cm^2)$ and the intensity of the capacitive current, the students are also able to estimate the bilayer diameter which should be slightly lower than the aperture diameter due to the presence of the annulus at the periphery of the bilayer [12]. In addition, by measuring the membrane current while applying a constant holding potential and knowing that the typical thickness of a planar lipid bilayer is around 10 nm, they can provide a estimate of the bilayer resistivity and demonstrate that lipids act as an insulator as the typical value is around $10^3 \ G\Omega.cm$. This experiment allows the students to understand that the electrical response of a passive cell membrane is well described by an equivalent RC circuit. There is no difference in the behaviour of electrons in the model circuit and ions in the bilayer chamber.

3.1.3 Unitary currents

After breaking the previously obtained channel-free bilayer, students are asked to reformed it in the presence of 1 pM of the ionophore gramicidin A in each compartment. This low concentration

is chosen to obtain less than ten individual open ionic channels allowing the resolution of the elementary currents produced by each channel opening. After addition of gramicidin A to the aqueous phase it may require up to 15 minutes for the channels to diffuse to the bilayer and insert. The students are able to record unitary currents when the bilayer is submitted to constant voltage (fig. 4.B). The current mediated by each channel is proportional to the applied voltage; dividing the current flowing through each channel by the applied voltage gives the conductance of the channel which is around 15 pS in these experimental conditions. This further allows the study of the biophysical properties of the gramicidin pores by measuring the unitary currents at different values of the holding potential demonstrating their ohmic behavior. Finally these measures can be repeated in asymmetric ionic conditions to show that the reversal potential of the unitary current follows Nernst equation.

3.2 Survey results

We assessed the improvement in the student knowledge of basic biophysics by comparing the knowledge scores (total points) that were collected both before and after the demonstration. There was a significant difference in the pre-test (M = 7.12, SD = 1.79) and the post-test knowledge (M = 7.85, SD = 1.77; p = 0.0015, n=121), suggesting that the proposed material improved their knowledge of core concepts of membrane biophysics.

4 Discussion

Understanding the human brain is one of the greatest challenges facing contemporary science. Through the progress of neuroscience, we can gain profound insights into our inner workings, develop new treatments for brain diseases and build revolutionary new computing technologies. Besides the Obama administration's 100 million Brain Initiative and the European Union's 1 billion, decadelong Human Brain Project, there are numerous private and public research efforts in Europe and abroad, focusing on the human brain. This will undoubtedly provide a surge of activity in brain research as scientists try to build the tools and knowledge to explain how brains work. Therefore it is of particular importance to promote neuroscience education to fuel this initiatives by providing inspiration to future scientists.

In this paper, we provide a simple experimental protocol allowing university undergraduate students to build artificial cell membranes and examine ionic channels properties at the single-molecule level. In this framework, we have developed an open-source lipid bilayer amplifier, the OpenPicoAmp, which is appropriate for use in introductory courses in biophysics or neurosciences. Such a low cost, open source and well documented solution to build a complete planar lipid bilayer setup was not available so far, although similar projects exist for extracellular recordings of neural electrical activity [8, 7].

The proposed experimental protocol provides a simple example and can be further refined if the students attend more lab sessions. For example, the single channel recordings with gramicidin can be repeated in the presence of different ionic gradients or pharmacological agents affecting the gating of the channels, e.g. 50 mM fluoxetine [6]. In addition, other ionic channels could be studied but, in this case, the electronics of the amplifier may need to be adapted to allow the recordings of faster events. Finally, at this stage, we do not provide any open source software for data acquisition and visualization. But such software exists [1] and we are currently developing a solution that will be tailored to the experimental protocol proposed here.

The reactions of the students to the lab sessions are positive and their learning outcomes are clearly improved by this hands-on approach. We hope that the proposed experiment will used as a teaching tool in other institutions, allowing fruitful exposure of undergraduate students to biophysics or neurosciences.

5 Supporting information

5.1 Electronics

- File S1: CadSoft Eagle .brd file giving the board layout for printing amplifier circuit.
- File S2: CadSoft Eagle .sch file including the circuit diagram for the amplifier circuit.
- File S3: A PDF document detailing the practical implementation of the amplifier, the parts and costs of components with associated part numbers from electronics supplier Farnell Element 14.

5.2 Bilayer chamber

- **File S4**: An PDF document detailing the design of our bilayer chamber (all dimensions given in mm).
- File S5: A PDF document describing the preparation of the Teflon film and the assembly of the bilayer chamber.

5.3 Video and hand-outs

- File S6: A short video .avi showing the actual experiment (download link: http://homepages.ulb.ac.be/~dgall/S6_BLM.avi)
- File S7: The student hand-out for the experiments (PDF)
- File S8: The student survey questions (PDF).

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