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The OpenPicoAmp: An Open-Source Planar Lipid Bilayer Amplifier for Hands-On Learning of Neuroscience

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Abstract

Understanding the electrical biophysical properties of the cell membrane can be difficult for neuroscience students as it relies solely on lectures of theoretical models without practical hands on experiments. To address this issue, we developed an open-source lipid bilayer amplifier, the OpenPicoAmp, which is appropriate for use in introductory courses in biophysics or neurosciences at the undergraduate level, dealing with 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. We provide a complete documentation allowing to build the amplifier and the experimental chamber. The students hand-out giving step-by step instructions to perform a recording is also included. Our experimental setup can be used in basic experiments in which students monitor the bilayer formation by capacitance measurement and record unitary currents produced by ionic channels like gramicidin A dimers. 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.

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 [1,2]. 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 [3]. 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 [4]. 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 200 euros and the experiments do not involve the use of animals.

One way to show that you understand how something works is to build it. As we are able reconstitute a neuronal cell membrane at the molecular level using the so-called black lipid membrane technique (BLM) [5], we can say that we understand how neurons produce electrical signals. The planar lipid bilayer method allows to build an artificial cell membrane and examine pore forming molecule functionally at the single-molecule level. The laboratory session we propose in this paper allows the students to observe directly these 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.

Materials and Methods

Participants

The laboratory session lasts typically 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 a *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 allows 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 students is an optimal configuration. Lab sessions at our institution are run with ten bilayer setups working simultaneously.

All educational research testing was done anonymously, on a voluntary basis and therefore does not require informed consent. This study was approved by the Institutional Ethical Committee of the School of Medicine of the Université Libre de Bruxelles.

Equipment

Amplifier. We have developed a low cost open-source bilayer amplifier (Files S1, S2, S3 and S4). 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 commonly found electronic components and kept their number to a minimum (Figure 1A). The amplifier is designed using the lithographic printed circuit board fabrication process and off-the-shelf electronic components (Figure 1B), 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 two built-in gains of 1 mV/pA and 100 mV/pA, low-passed from 3400 to 16 Hz respectively. The OpenPicoAmp at its highest gain has a noise level of 0.03 pA rms (0.1–10 kHz) which makes it suitable for the recording of the unitary current produced by the opening of a single channel molecule. All commercially available bilayer amplifiers have a full bandwidth at least in the 10 kHz range, allowing to digitally record single channels openings with dwell times in the *ms* range, with a low-pass filtering in the 1 kHz range. In the proposed configuration, the OpenPicoAmp head stage has a more limited 2.4 kHz bandwidth and the signal, at the 100 mV/pA gain, is low-pass filtered with a fixed cutoff frequency of 16 Hz. Therefore it will not be suitable to study ion channels with dwell times shorter than 50–100 *ms*. As a consequence, using this amplifier for research purpose is only possible for ion channels which display slow gating events, such as for example channels formed by some antibiotics and toxins. Thus it is well adapted to perform the experiments proposed here. To allow recording of faster events, the fixed cutoff frequency can be increased by adjusting the components values for the low-pass filter. But careful testing will be needed to evaluate the signal to noise ratio in any new configuration and, in particular, its sensitivity to interferences from line voltages. The experimental chamber and the amplifier circuit are enclosed in an aluminium box that acts as a Faraday cage minimizing electric noise (Figure 2A).

The electronics design is 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.

Bilayer chamber and electrical recording. The bilayer chamber is composed of two compartments milled in two blocks of polyoxymethylene (Delrin) allowing the insertion of a commercially available polytetrafluoroethylene (PTFE or Teflon)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 (Files S5 and S6). 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 (Figure 2B). This design choice allows an easy replacement of the Teflon film which is sometimes torn during the manipulations by the students. Due to the fact that lipid bilayer are very sensitive to the presence of membrane soluble chemicals, the chambers used to make bilayers must be cleaned very carefully and this motivates the choice of an hydrophobic material for their implementation. Polyoxymethylene is an hydrophobic material similar to polytetrafluoroethylene but has greater physical durability. Furthermore, these two materials display low electrical conductivity which is a desirable property in the context of single channel recording.

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 (Figure 3A). A lipid bilayer, formed in the aperture of the Teflon film is electrically equivalent to a leaky capacitor (Figure 3B). Mechanical vibrations are damped by placing the experimental chamber on a heavy granite slab resting on four tennis balls (Figure 4A). Each compartment is connected to the amplifier through 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 (Figure 4B).

Chemicals and solutions. Commercially available phospholipids can be used to form planar lipid bilayers. We selected 3-sn-phosphatidylcholine (ref. 61758 FLUKA, Sigma-Aldrich, Belgium) as soy lipid extracts are the less expensive. The lipids were dissolved in n-octane (ref. O2001 SIGMA, Sigma-Aldrich, Belgium) 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 [6–8]. It is active against gram-positive bacteria and used for clinical application as an antibiotic. 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 (File S7). The duration of this elementary step of current is determined by the life time of the gramicidin dimer extending through the membrane. For the sake of simplicity, we are going to refer to these events as channel openings. These openings typically last several hundreds of *ms* and are thus also easy to resolve temporally. Gramicidin A is available commercially and inexpensive (ref. 50845 SIGMA,
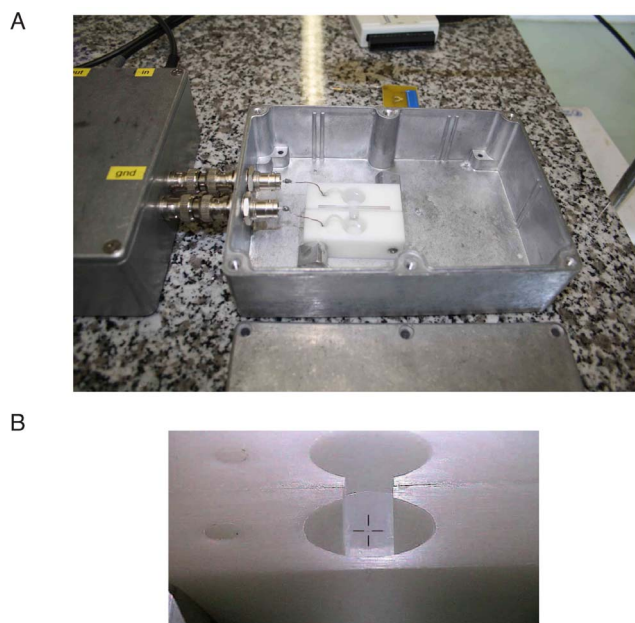
Figure 1. The OpenPicoAmp amplifier. The chosen electronic design uses a minimal number of commonly found components, like the Texas Instruments TL061 and TL071 operational amplifiers. It is therefore inexpensive and easy to build. (A) Printed circuit board layout for the OpenPicoAmp. (B) Implantation 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).
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Sigma-Aldrich, Belgium). A 1 nM stock solution is prepared by successive dilution with ethanol. The stability of gramicidin A in this solution appears very high when stored at $-20^\circ C$ (more than 4 years).

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 (File S8).

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 cross superimposed on the image). doi:10.1371/journal.pone.0108097.g002

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 fixed voltage signal? Learning goals: Students will understand that (1) the total current is essentially produced 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.

Step 2. Guiding question: How does a lipid bilayer reacts when submitted to similar variable or fixed 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 a high frequency triangular signal and (2) to a constant voltage. This allow them to measure the bilayer capacitance and resistance.

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 gramicidin A and measure the unitary currents when the bilayer is submitted to a range of constant voltages.

Survey methodology

At the beginning of the laboratory session, students were given ten minutes to fill out a short answer survey (File S9). An additional survey was then administered at the end of session. These survey were anonymous. 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.

Results

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 a majority of attendees could
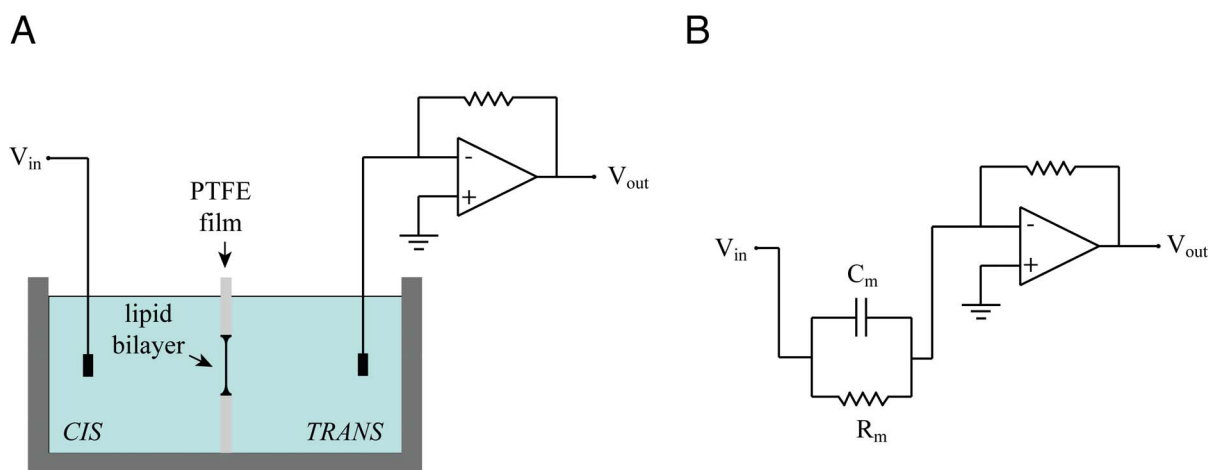

Figure 3. The planar lipid bilayer technique. (A) Experimental setup for the electrical recording. The two compartments of the experimental chamber are filled with a 1 M NaCl solution. A small volume of the lipid solution is injected over the aperture in the teflon film and the bilayer is formed by spontaneous thinning. 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. (B) A lipid bilayer is electrically equivalent to leaky capacitor. With the proposed design for the experimental chamber, typical values for the resistance (R_m) of a channel-free lipid bilayer are in the 100 $G\Omega$ range with a capacitance (C_m) in the range of hundreds of pF. doi:10.1371/journal.pone.0108097.g003
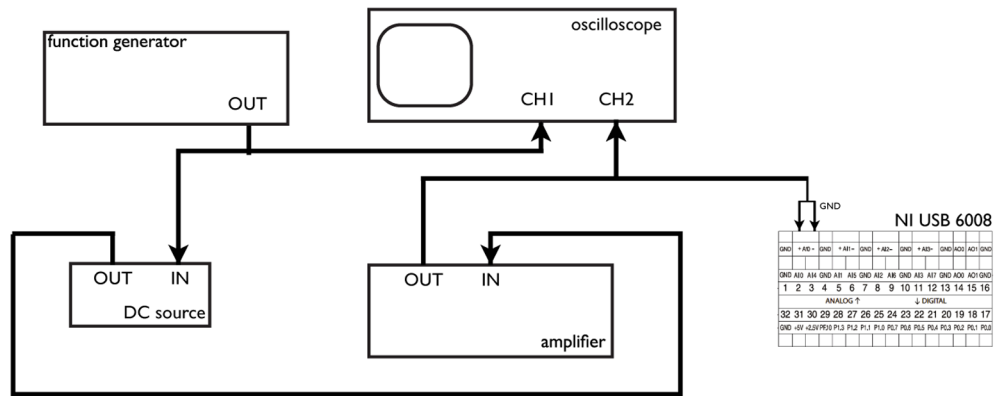
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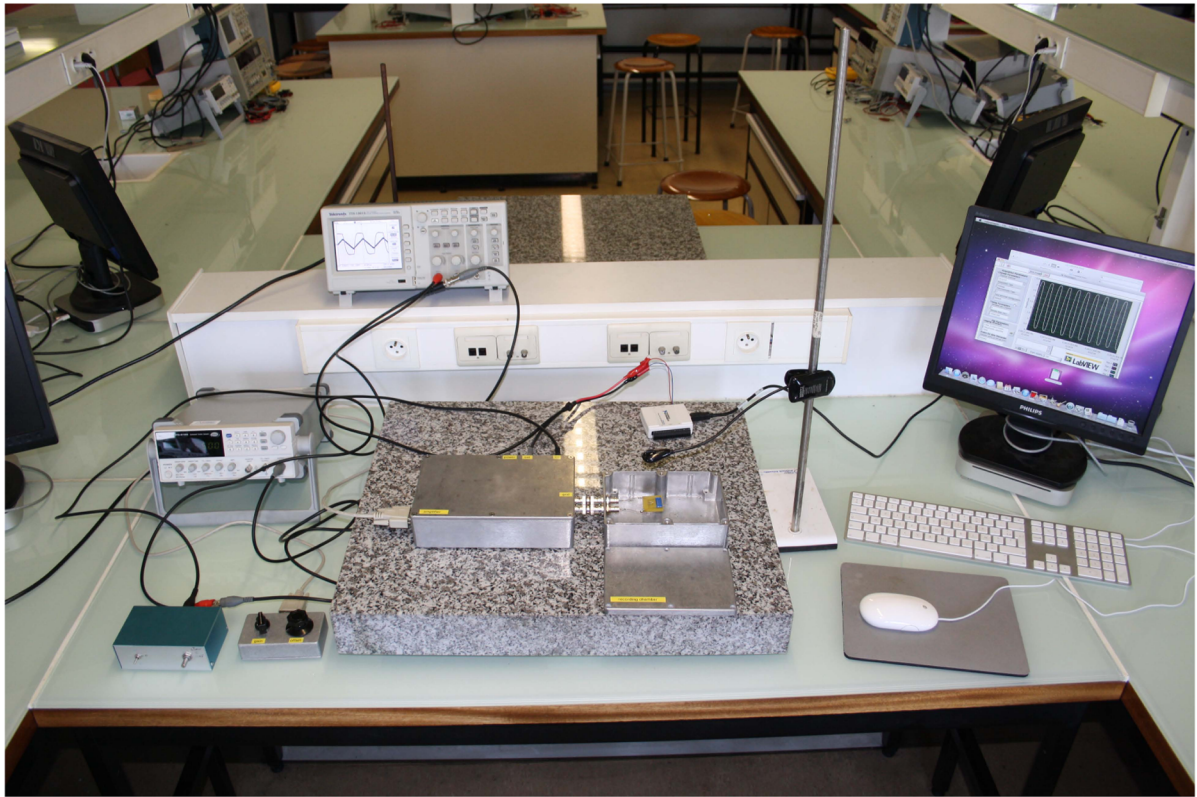
B

Figure 4. The complete planar bilayer setup as implemented at our institution. (A) 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. A model circuit composed of a 1 $G\Omega$ resistance with a 220 pF capacitor in parallel is used for calibration. (B) The corresponding block diagram of the setup.

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actually succeed to 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.

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 fixed 120 mV voltage. When the circuit is submitted to the triangular signal, which has a slope of ± 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 which is essentially the steady state capacitive current. 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 model RC circuit to the amplifier and measure the total current flowing in the circuit using the oscilloscope, when it is submitted to a 500 Hz 10 mV peak to peak triangular signal and to a fixed 120 mV voltage. All currents appear as voltage deflections as the amplifier act as a current to voltage converter. This step also provides a calibration of the amplifier allowing to evaluate the bilayer capacitance during its formation by measuring the capacitive current when the bilayer is submitted to the triangular signal (each pF of capacitance will contribute 10 mV to the amplitude of the signal read on the oscilloscope with amplifier set at the 1 mV/pA gain).

Bilayer formation. The two compartments of the experimental chamber are first filled with electrolyte solution (1 M NaCl). Subsequently 2 μl of a 20 mg/ml solution of 3-sn-phosphatidylcholine dissolved in n-octane are injected over the aperture using a 10 μl pipette (see movie in File S7). The bilayer is formed by spontaneous thinning. The bilayer formation is monitored by measuring the capacitive current during the application of a triangular voltage signal (500 Hz, 10 mV peak to peak). As the planar lipid bilayer is forming in the central part of the aperture, the capacitive current will increase and then stabilize (Figure 5A). 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. 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. 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 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 solvent-containing 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 [9]. 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 an estimate of the bilayer resistivity and demonstrate that lipids act as insulators 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 electrical response between the model circuit and the lipid bilayer.

Unitary currents. After breaking the previously obtained channel-free bilayer, students are asked to reconstitute 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 simultaneous individual channel openings, allowing the resolution of the elementary current produced by the transient dimerization of two gramicidin A molecules. After addition of

gramicidin A to the aqueous phase it may require up to 15 minutes for the molecules to diffuse to the bilayer and insert. The students are able to record unitary currents when the bilayer is submitted to constant voltage (Figure 5B). This further allows the study of the biophysical properties of the gramicidin pores in symmetric ionic condition (1 M $NaCl$ cis/1 M $NaCl$ $trans$) by measuring the unitary currents, i, at different values of the holding potential (Figure 6A). The current mediated by each channel is proportional to the applied voltage, demonstrating the ohmic behavior of the gramicidin channels:

$$i = \gamma(V_{hold} - V_{Nernst}) \tag{1}$$

where γ is the conductance of a single gramicidin channel, V_{hold} the applied transmembrane potential and V_{Nernst} the reversal potential for Na^+ ions, which is given by the Nernst equation:

$$V_{Nernst} = \frac{RT}{F} ln \frac{[Na^+]_{trans}}{[Na^+]_{cis}} \tag{2}$$

where R is universal gas constant, T the absolute temperature, F the Faraday constant, $[Na^+]_{trans}$ and $[Na^+]_{cis}$ the Na^+ concentrations in the $trans$ and cis compartments respectively. Therefore when these measures are repeated in asymmetric ionic conditions (1 M $NaCl$ cis/0.1 M $NaCl$ $trans$), the reversal potential of the unitary current is shifted by approximatively 60 mV compared to the symmetric ionic condition (Figures 6B and 6C).

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 (p = 0.0015, n = 121) in the pre-test (M = 7.12, SD = 1.79) and the post-test knowledge (M = 7.85, SD = 1.77), suggesting that the proposed material improved their knowledge of core concepts of membrane biophysics.

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 administrations Brain Initiative and the European Unions 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 they need to accomplish their goals. 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 basic 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. Commercial bilayer amplifiers, like the Warner Instruments BC-535, cost more than 8000 euros. The Open-PicoAmp can be build for less than 200 euros but is, of course, less versatile as it was specifically designed in the framework of the proposed experimental protocol. Such a low cost, open source and
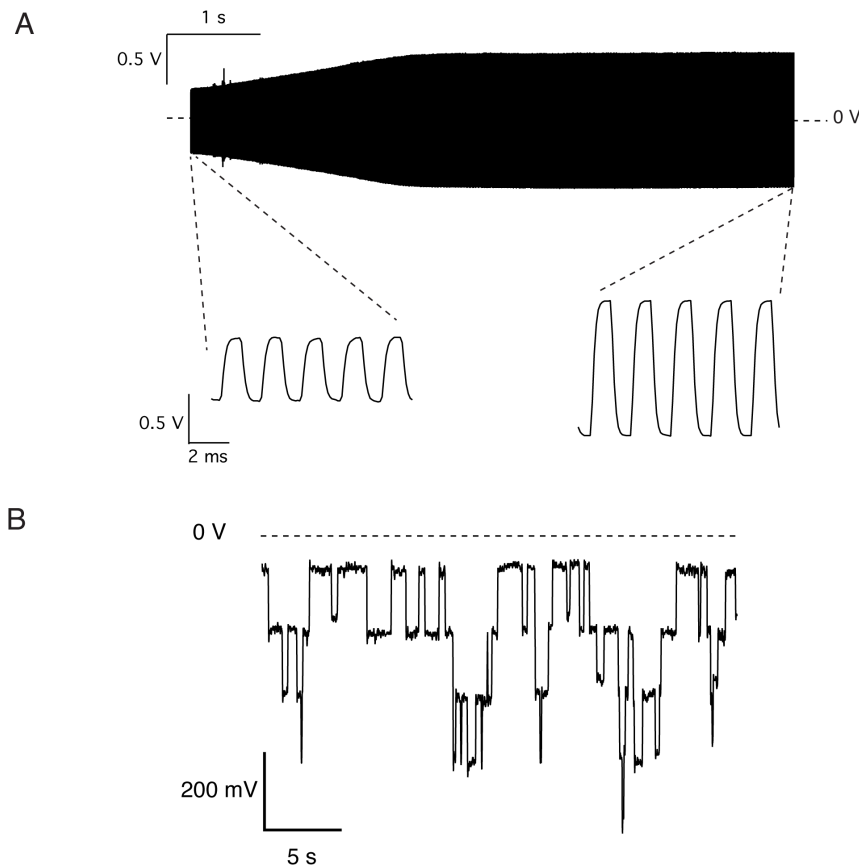
Figure 5. Experimental recordings obtained with the OpenPicoAmp. (A). The lipid bilayer formation is monitored by measuring the increase in the capacitive current during the application of a triangular voltage signal (500 *Hz*, 10 *mV* peak to peak). In this particular example, the lipid bilayer forms 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 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). All the presented data has been acquired by students using a National Instruments USB6008 A/D converter using a sampling frequency of 10 *kHz* and a low-pass filter cutoff at 3.4 *kHz* (panel A) or a 100 *Hz* sampling frequency with a low-pass filter cutoff at 16 *Hz* (panel B).
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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 [10,11]. This type of open-source hardware projects will without doubt encourage collaborative tinkering, promote science education and may even prove to be useful as research tools [12,13].

The proposed experimental protocol provides a simple example and can be further refined if the students attend more laboratory sessions. For example, the single channel recordings with gramicidin can be repeated in the presence of pharmacological agents affecting the gating of the channels, e.g. 50 mM fluoxetine [14]. 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. More advanced subjects can also be introduced, like the determination of single channel current and mean channel opening times using autocorrelation function analysis of current noise [15]. The design of the bilayer chamber could also be adapted to allow the simultaneous optical monitoring of the bilayer formation. It is possible to view the phospholipid film using a lens with 10 x magnification and a light source. The thin film interference of the reflected light at the water/membrane and membrane/water interfaces produces colorful fringes when the membrane thickness is on the order of

the wavelength of light in the membrane. Once the film reaches nanometer-scale thickness, the membrane turns black because light reflected off the two interfaces interferes destructively for all wavelengths; hence the name black lipid membrane (BLM) [16]. Finally, at this stage, we do not provide any open source software for data acquisition and visualization. But such software exists [17] 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. In the framework of this project, the lab equipment was built by a lab technician as medical students do not have a training in basic electronics in their curriculum. Students from other disciplines, like physics or engineering, have this prerequisite training. Therefore, they could be directly involved in the building of the equipment and this would provide an additional valuable learning experience. We hope that the proposed experiment will be used as a teaching tool in other institutions, allowing fruitful exposure of undergraduate students to biophysics or neurosciences.
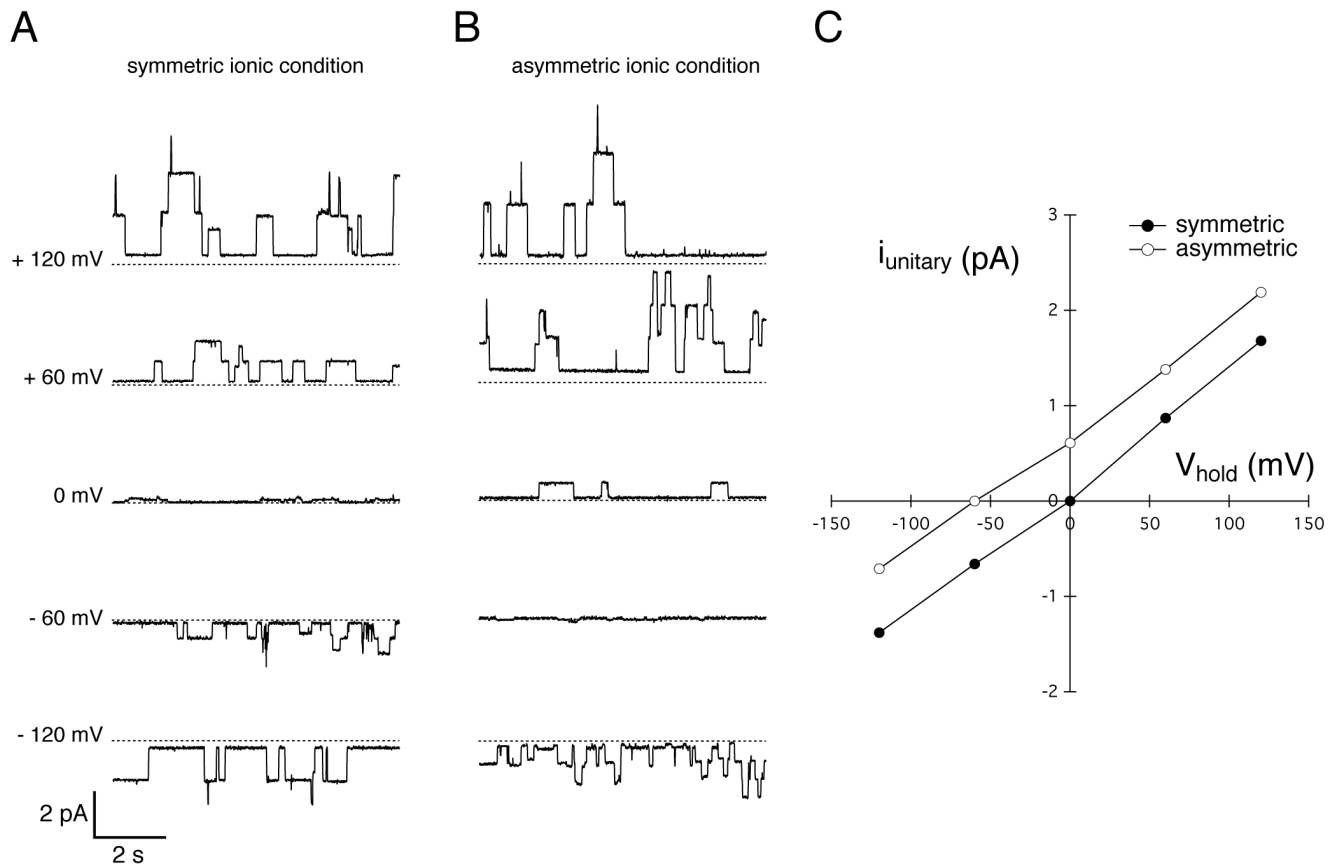
Figure 6. Effect of ionic gradients on gramicidin A unitary currents, recorded with the OpenPicoAmp. (A) Gramicidin A unitary currents in symmetric ionic conditions (1 *M NaCl cis*/1 *M NaCl trans*) at different holding potentials. The dotted line on each individual recording corresponds to 0 *pA*. (B) Gramicidin A unitary currents in asymmetric ionic conditions (1 *M NaCl cis*/0.1 *M NaCl trans*) at different holding potentials. (C) Gramicidin channels are ohmic conductors. The unitary conductance observed in these recordings is around 13 *pS*. The reversal potential of the unitary currents is shifted from 0 *mV*, in symmetric ionic condition, to approximatively −60 *mV*, in asymmetric ionic condition. All data was acquired using a NI USB 6008 A/D converter with a 100 Hz sampling frequency with the amplifier gain at 100 *mV/pA* (low-pass filter cutoff at 16 *Hz*).
doi:10.1371/journal.pone.0108097.g006

Supporting Information

File S1   CadSoft Eagle file giving the board layout for printing amplifier circuit. The CadSoft Eagle software Light Edition (freeware) can be downloaded from http://www.cadsoftusa.com/.
(BRD)

File S2   CadSoft Eagle file including the circuit diagram for the amplifier circuit and the linked board layout.
(GZ)

File S3   Document detailing the practical implementation of the amplifier and a ready to print layout for the printed-circuit board (scale 1:1).
(PDF)

File S4   Spreadsheet giving the bill of materials for the OpenPicoAmp.
(ODS)

File S5   Document detailing the design of our bilayer chamber (all dimensions given in *mm*) and a STL file allowing the 3D printing of the parts.
(GZ)

File S6   Document describing the preparation of the Teflon film, the assembly of the bilayer chamber and the making of the electrodes.
(PDF)

File S7   A short video showing the proposed experiment.
(MOV)

File S8   The student hand-out for the experiments.
(PDF)

File S9   The student survey questions. The lecture material related to the laboratory session can be obtained upon request by contacting the corresponding author.
(PDF)

Author Contributions

Conceived and designed the experiments: VS DG. Performed the experiments: VS DG. Analyzed the data: VS DG. Contributed reagents/materials/analysis tools: FD. Wrote the paper: VS DG.

References

1. Haak DC, HilleRisLambers J, Pitre E, Freeman S (2011) Increased structure and active learning reduce the achievement gap in introductory biology. Science 332: 1213–1216.
2. Freeman S, Eddy SL, McDonough M, Smith MK, Okoroafor N, et al. (2014) Active learning increases student performance in science, engineering, and mathematics. Proc Natl Acad Sci U S A 111: 8410–8415.
3. Deslauriers L, Schelew E, Wieman C (2011) Improved learning in a large-enrollment physics class. Science 332: 862–864.
4. Prober CG, Heath C (2012) Lecture halls without lectures–a proposal for medical education. N Engl J Med 366: 1657–1659.
5. Mueller P, Rudin DO, Tien HT, Wescott WC (1962) Reconstitution of cell membrane structure in vitro and its transformation into an excitable system. Nature 194: 979–980.
6. Mueller P, Rudin DO (1967) Development of k+-na+ discrimination in experimental bimolecular lipid membranes by macrocyclic antibiotics. Biochem Biophys Res Commun 26: 398–404.
7. Hladky SB, Haydon DA (1972) Ion transfer across lipid membranes in the presence of gramicidin a. i. studies of the unit conductance channel. Biochim Biophys Acta 274: 294–312.
8. Finkelstein A, Andersen OS (1981) The gramicidin a channel: a review of its permeability characteristics with special reference to the single-file aspect of transport. J Membr Biol 59: 155–171.
9. White SH (1972) Analysis of the torus surrounding planar lipid bilayer membranes. Biophys J 12: 432–445.
10. Marzullo TC, Gage GJ (2012) The spikerbox: A low cost, open-source bioamplifier for increasing public participation in neuroscience inquiry. PLoS One 7: e30837.
11. Land BR, Wyttenbach RA, Johnson BR (2001) Tools for physiology labs: an inexpensive high-performance amplifier and electrode for extracellular recording. J Neurosci Methods 106: 47–55.
12. Pearce JM (2012) Materials science. building research equipment with free, open-source hardware. Science 337: 1303–1304.
13. (2013) Sharing blueprints for better research. Nat Methods 10: 591.
14. Kapoor R, Kim JH, Ingolfson H, Andersen OS (2008) Preparation of artificial bilayers for electrophysiology experiments. J Vis Exp. 20: 1033.
15. Kolb HA, Bamberg E (1977) Influence of membrane thickness and ion concentration on the properties of the gramicidin a channel. autocorrelation, spectral power density, relaxation and single-channel studies. Biochim Biophys Acta 464: 127–141.
16. Tien H, Carbone S, Dawidowicz E (1966) Formation of black lipid membranes by oxidation products of cholesterol. Nature 212: 718–719.
17. Dempster J (2001) The Laboratory Computer: A Practical Guide for Physiologists and Neuroscientists (Biological Techniques Series). Academic Press.