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An affordable Three-Dimensional (3D) Printed Recording Chamber for Two-Electrode Voltage Clamp Electrophysiology

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Two electrode voltage-clamp (TEVC) electrophysiology in *Xenopus* oocytes is a common approach to studying the physiology and pharmacology of membrane transport proteins. Undergraduates may learn to use TEVC methodology in neuroscience or physiology courses and/or in faculty-mentored research experiences. Challenges with the methodology include the cost of commercially available recording chambers, especially when a lab needs multiple copies, and the additional time and expertise needed to use agar bridges and to stabilize solution flow and minimize noise from solution aspiration. Offering a low-cost and accessible recording chamber that overcomes these challenges would lower the barriers to success for undergraduates while also supporting publication-quality recordings. To address these issues, we developed a recording chamber using stereolithography, a 3D printing process. The physiology (PhISio) recording chamber features two options for solution aspiration that allow for individual preferences, optimizes placement of pre-made agar bridges to achieve laminar flow and reduce the time delays in initiating daily experiments, and minimizes the challenges of changing solution height and aspiration noise during perfusion. We compared the functionality of the PhISio chamber with a commercially available Warner Instruments RC-1Z chamber in electrophysiological recordings of inwardly rectifying potassium channels expressed in *Xenopus* oocytes. The PhISio chamber produced equivalent results to the RC-1Z chamber with respect to time-dependent solution changes and has several operational advantages for both new and experienced electrophysiologists, providing an affordable and convenient alternative to commercially available TEVC recording chambers.

Key words: two-electrode voltage clamp (TEVC); recording chamber; stereolithography; 3D printing; electrophysiology; oocyte

Two electrode voltage-clamp (TEVC) electrophysiology using *Xenopus* oocytes is a common approach to studying the physiology and pharmacology of membrane transport proteins such as ion channels (Wagner et al., 2000). Undergraduates may learn the approach in laboratory courses and/or may develop greater mastery of the approach in mentored research experiences. A limitation to undergraduate success with the methodology is the cost and complications of using commercially available recording chambers that require experience to optimize their use and may require daily maintenance. Even experienced electrophysiologists benefit from greater efficiency in the preparatory work needed at the start of an experiment.

To address these issues, we produced and tested a new recording chamber design that we optimized for lab teaching and publication-quality recordings. The chamber was constructed by stereolithography (SLA) using 3D printing, a manufacturing process in which a photosensitive resin is deposited or fused in layers (Hyde et al., 2014) and polymerized and hardened by ultraviolet light. This results in a high-resolution product with a smooth surface and watertight properties (Hyde et al., 2014).

We tested the new "PhISio" chamber by comparing TEVC recordings to those obtained under the same conditions with a commercially available and commonly used recording chamber. The name PhISio refers to a chamber for physiology recordings, designed by Ian Shogren.

The PhISio chamber features two alternatives for solution aspiration that allow for experimenter preferences, optimize ground well and agar bridge placement to achieve laminar flow, and minimize instabilities that can arise from changing solution height during perfusion. The functionality, ease of use, and low cost of this 3D printed chamber make it a new and improved alternative to a commercially available TEVC electrophysiology recording chamber.

MATERIALS AND METHODS

3-D Printing of PhISio Chamber

The PhISio chamber was designed using the free software, TinkerCAD (Autodesk, San Rafael, CA). The object files were exported in the STL format and imported into the latest version of PreForm from Formlabs.

The chamber (v1.3) was printed on a Formlabs Form 3 printer (Somerville, MA) using the Formlabs Build Platform (0.1 mm print resolution) and Formlabs Gray Resin V4. The gray resin was selected for its sturdy material and watertight properties. After printing, the chamber was vigorously washed with a cloth in 90% isopropyl alcohol to remove any resin in the lower trough before being cured at 60°C for 30 minutes on a Formlabs Form Cure UV chamber. Once cured, the plastic supports were broken with pliers and the chamber's bottom surface was made smooth with fine sandpaper.

The cumulative time to print and cure one chamber was four hours, however, multiple chambers may be printed simultaneously to increase time efficiency. Each chamber

required 20 mL of resin at a cost of $3 (US dollars) based on the current market price for 1 L of Gray Resin V4 ($149). Our PhISio chamber design is accessible for free on GrabCAD at https://grabcad.com/library/phisio-chamber-1 (Figure 1).

Oocyte Injection and Maintenance

Published procedures were used to prepare rat Kir2.1 channel RNA (GenBank accession number NM_017296) and inject oocytes (Boland et al., 2009; Tang et al., 2015). Oocytes from *Xenopus laevis* were prepared from ovarian lobules purchased from Xenopus-1 (Dexter, MI) or were purchased ready for injection from Ecocyte Bio Science (Austin, TX). Stage V/VI oocytes were microinjected with 32 ng of cRNA dissolved in diethyl pyrocarbonate-treated water and maintained for 2 days at 16-17 °C in a solution containing (in mM): 96 NaCl, 1 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, and 2 sodium pyruvate, pH 7.4, with 50 units/ml penicillin G and 50 µg/ml streptomycin.

Electrophysiology

Inwardly rectifying potassium currents were recorded from oocytes using standard electrophysiological methods (Boland et al., 2009; Tang et al., 2015) using OC-725C amplifiers (Warner Instruments, Hamden, CT). More detailed methods on electrophysiology may be useful to those initiating two-electrode voltage clamp recordings (Stühmer, 1992) or seeking information about vendors and product numbers for commonly used materials for these recordings (Wang et al., 2017). We constructed the voltage-measuring and current-passing electrodes from G150TF-4 glass from Warner Instruments using a PC-10 Narishige puller (Amityville, NY); they were backfilled with 1 M KCl and had resistances between 0.3 and 0.8 MΩ. Currents were sampled at 5-10 kHz and filtered at 1-2 kHz. All recordings were done at room temperature (about 22-23°C) and the bath was perfused continuously during recordings at a rate of ~ 1 mL/min. The standard external solution contained (in mM): 80-95 NaCl, 5-20 KCl (for a total of 100 mM chloride salts), 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, pH 7.3-7.4. Data were recorded on computers equipped with Digidata 1320A and pClamp software (Molecular Devices, San Jose, CA). GraphPad Prism (San Diego, CA) was used to plot and analyze data.

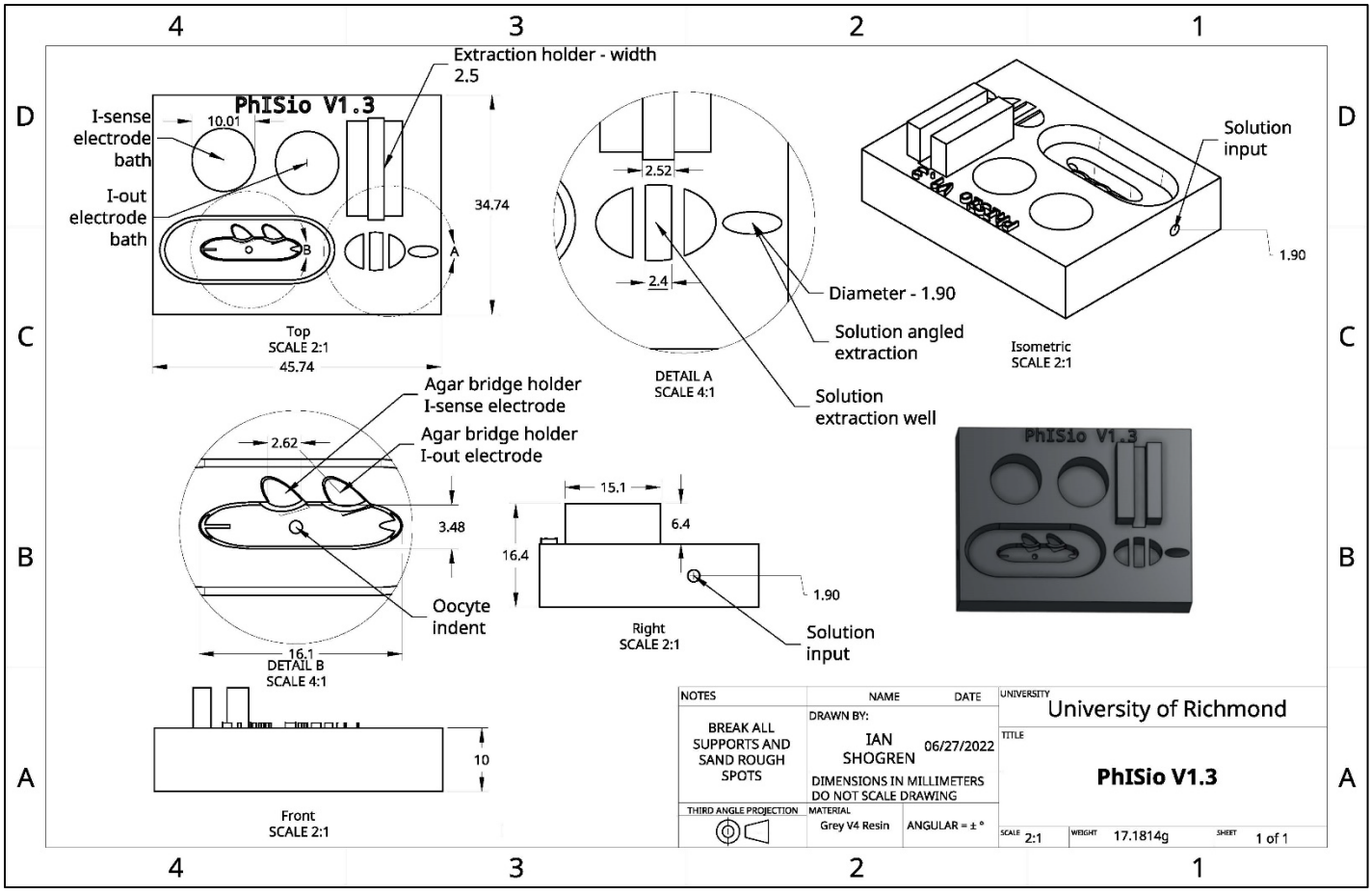

Figure 1. Technical drawing of the PhISio chamber with dimensions. The chamber is 45.74 mm x 34.74 mm x 16.4 mm. The solution input is 1.9 mm in diameter which creates a tight seal around 1 mm Teflon tubing; a small amount of Vaseline was used around the tubing to prevent leakage. The solution extraction tunnel is angled at 25° and is 1.9 mm in diameter allowing for a 14-gauge hypodermic needle to be inserted. The agar bridge holders contain a plastic bridge to restrict movement. They are angled away from the solution flow to avoid interference with laminar flow. We constructed glass agar bridges bent into a U-shape under a flame (see placement in Figure 2) and they must be longer than 6 mm to span the gap between grounding wells and the recording chamber.

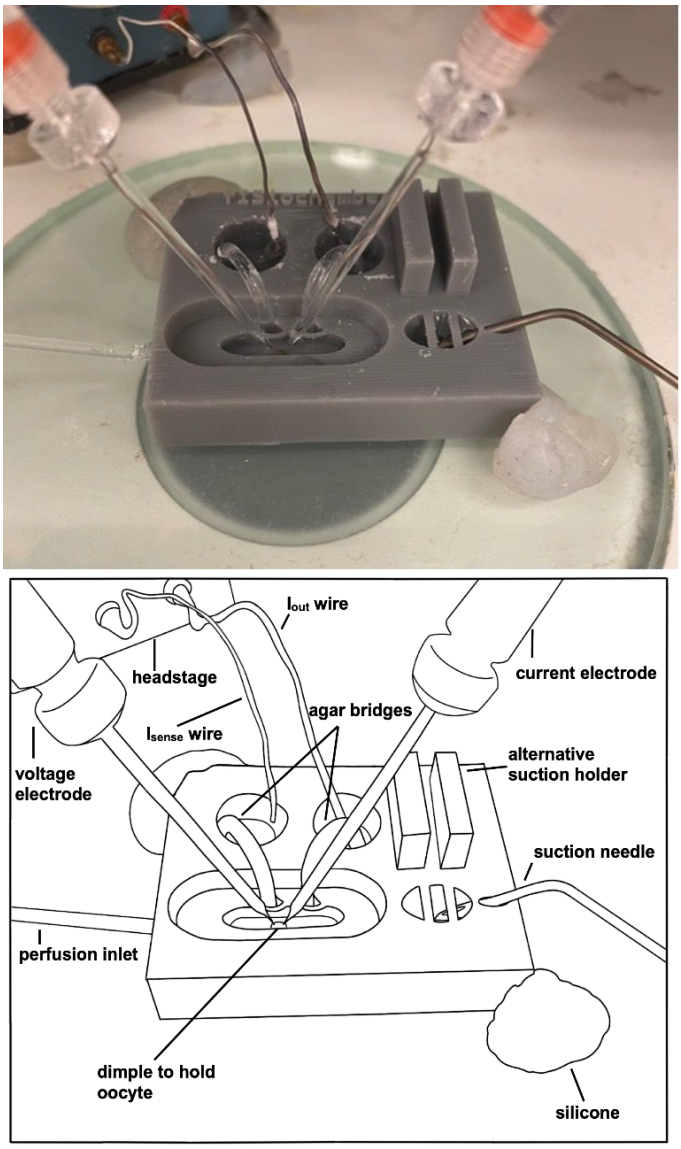

Figure 2. (*Top*) Photograph of the PhISio chamber (V1.3) during a TEVC oocyte recording with (*bottom*) a labeled sketch to identify the objects in the image. The placement of the agar bridges is secured by chamber design. The hypodermic suction needle was bent at about 75 degrees and petroleum jelly (Vaseline) was placed on the needle tip to reduce noise. Molded silicone (Walgreens Pharmacy soft silicone ear plugs) was placed on two external corners of the chamber to position it on the microscope stage.

RESULTS

The PhISio chamber, shown in operation in Figure 2, has two key design features. First, the chamber has two agar bridge wells for wires that maintain the bath reference potential (I-sense and I-out electrodes; see Figure 1, "D4") that each align with indents for agar bridge holders (see Figure 1, "B4") in the recording area of the chamber. We prepared U-shaped glass tubing filled with 3% agar in 1 M KCl which functions as an agar salt bridge to electrically connect to the recording (bath) solution while minimizing the transfer of ions or solute. The indents for the agar bridges are angled at 45° opposite to the direction of solution flow and the bridges are secured there by small plastic connectors that prevent accidental displacement of the bridges. The design prevents the agar bridges from interfering with the optics, solution flow, or recording electrodes during an experiment. The location of the indent for the agar bridge connecting to the I-sense bath electrode well was offset to the center of the chamber to obtain optimal performance, per instructions in the operating manual for the OC-725C voltage clamp amplifier.

Second, the PhISio chamber allows for multiple methods of extracting the perfusate. A bent hypodermic suction needle can be placed on the side of the extraction well (as shown in Figure 2) or a tightly fitted glass tube may be placed in the back of the chamber, a location that is adaptable to a more space-constrained microscope stage. Two small plastic connectors at the top of the solution extraction well (see Figure 1, "C2-C3") keep the needle or tubing in place during recordings and optimize laminar flow through the chamber. Having two options for aspiration provides built-in flexibility for individual needs or preferences without modification of the chamber.

We chose for comparison with the PhISio chamber, the polycarbonate RC-1Z slotted bath oocyte recording chamber from Warner Instruments (Hamden, CT). The RC-1Z creates one agar bridge via a trough between the ground electrode well and the main chamber that must be filled with a molten agarose solution on the underside of the chamber and then sealed to create a water-tight base using a coverslip and Vaseline. The second agar bridge mechanism is through a well that is electrically connected to the oocyte recording solution via an agar bridge formed from U-shaped glass tubing, as we do for both bridges in the PhISio chamber. Some users of the RC-1Z may simply put the I-sense electrode directly into the aspiration well. Another structural difference between the chambers is that the RC-1Z has only one option for solution aspiration from the chamber during perfusion.

To compare the functionality of the PhISio chamber to the RC-1Z chamber, we performed identical TEVC experiments measuring inwardly rectifying (Kir) potassium currents. We selected Kir channels for this study because external ion solution changes can be used to increase or decrease the current amplitude and we wanted to measure the time courses of both changes in the same recordings. Thus, we measured the rate of decrease of Kir2.1 current amplitude when exchanging from higher to lower external potassium concentrations (Figure 3B, C) and the rate of current increase when changing from lower to higher external potassium concentrations (Figure 3D). The time constants for changes in current amplitude upon solution exchange were not significantly different in the PhISio and RC-1Z chambers (Figure 3E; two-tailed t-test). We also compared the rate of current inhibition and recovery from inhibition when exchanging to a solution containing barium, a well-known blocker of Kir2.1 channels (Alagem et al., 2001). The onset of block (Figure 4C) and recovery from block when returning to a barium-free solution (Figure 4D) were not significantly different in recordings done in the two chambers (Figure 4E; two-tailed t test). We also observed

that the PhISio chamber supported low noise recordings that were similar to those recorded in the RC-1Z chamber (Figure 3A, 4A and 4B), indicating that the design features for flow and aspiration (which may add noise to recordings) did not produce observable functional differences in the two chambers.

DISCUSSION

The purpose of this article is to (1) share with neuroscience educators and researchers a design that facilitates the teaching of TEVC electrophysiology with a focus on budget constraints and (2) improve recording chamber design to assist both new and experienced electrophysiologists seeking to perform publication-quality electrophysiology recordings. An important consideration during the design process was making the setup easier to operate without compromising functionality.

The PhISio chamber is a fully functional and optimized replacement for commercially available TEVC recording chambers. In direct comparisons, the Kir2.1 recordings done using the PhISio chamber showed equivalent results for solution exchange times when compared to the RC-1Z chamber and the PhISio chamber supported low noise recordings (Figures 3 and 4) due to reliable placement of the suction device (Figure 2). The features of the current traces recorded in the PhISio chamber were similar to the recordings in the RC-1Z chamber, although the PhISio chamber provides two ways for suction devices to access the external solution that is continually removed during perfusion.

One of the improvements in the PhISio chamber is the design for positioning the agar bridges. The commercially available RC-1Z chamber requires an agar bridge to be formed on each recording day by cleaning out the old agar and replacing it in the trough and then sealing the underside of the chamber with a coverslip. This is a tedious step and one that slows down the start of an experiment; for undergraduate research or for teaching labs, it is an inefficient process. In the new PhISio chamber, agar bridges can be pre-fabricated from U-shaped glass tubing (stored in 1 M KCl in the refrigerator) and then simply placed in the chamber at the start of an experiment; this reduces the setup time before data collection may begin. Teaching lab environments are limited on time, so having a chamber design that is easy to setup and use maximizes students' time for recordings; even experienced electrophysiologists benefit from the time saved.

We designed a way for the PhISio chamber to secure the position of the glass tubing that creates the agar bridge. In our design, two indents for the agar bridge holders have a nearly elliptical shape (see Figure 1, "B4") and are continuous with the bath solution used for the oocyte recordings; a narrow bridge of plastic secures the location of the glass bridges that are placed in these indents. This prevents the glass bridges from displacing during the experiment and thus avoids interference with the optics, solution flow, or the recording pipettes while also minimizing time lost in optimizing their position each time a new recording is initiated. Standardizing their location is helpful to new experimentalists as there are many procedural steps to attend to during a TEVC experiment. Another design improvement was to increase the volume of the wells for the I-sense and I-out electrodes (see Figure 1, "D4") so that evaporation of KCl from these wells during long duration experiments would not interrupt recordings.

We compared the operation of the PhISio chamber to one commercially available recording chamber; notably, other chambers (Warner Instruments RC-3Z and RC-26Z) also utilize the agar trough and coverslip method and would

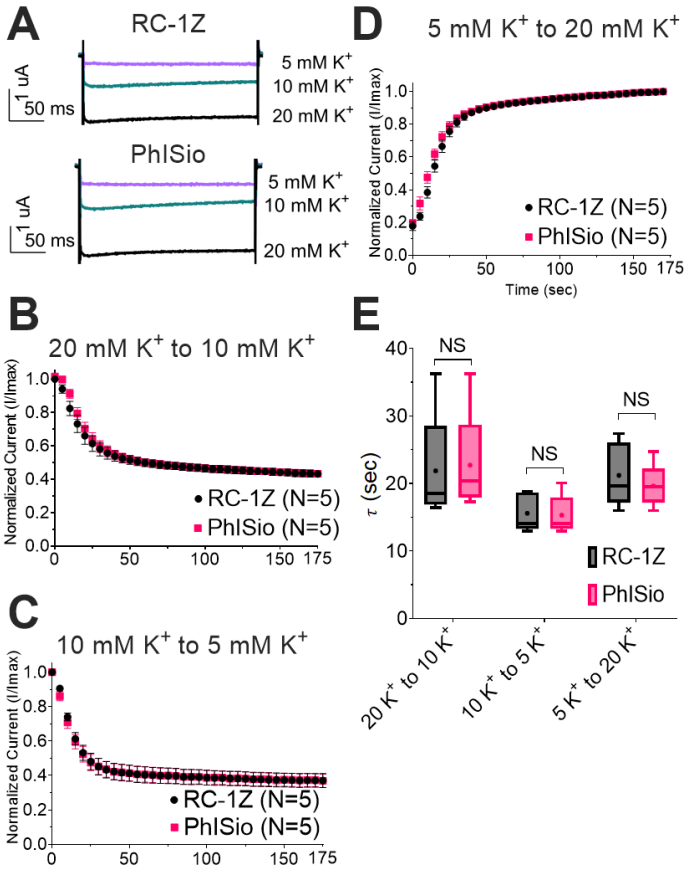

Figure 3. Comparison of rates of external solution exchange in two recording chambers. (*A*) Representative inwardly rectifying (Kir2.1) current traces recorded in the RC-1Z and PhISio chambers when extracellular K^+ was exchanged from 20 to 10 to 5 mM K^+ (black, teal, lavender traces, respectively). The time course of change in Kir2.1 current amplitudes (normalized to the maximum current at the time solution change was initiated) was measured upon exchanging the extracellular bath solution from (*B*) 20 mM to 10 mM K^+, (*C*) 10 mM to 5 mM K^+, and (*D*) 5 to 20 mM K^+. Recordings done in the RC-1Z chamber (black) were compared to those done in the PhISio chamber (pink) using the same preparations of solutions and oocytes. Data points are the mean ± SEM for the normalized current amplitudes. (*D*) Data from B, C, and D were fitted to single exponential functions and the time constants (□) determined. (*E*) Box plots show the rates of solution exchange in RC-1Z (black) and PhISio (pink) chambers; the maximum and minimum values are shown as whiskers and the box bounds the 25th and 75th percentile with the median (line inside the box) and the mean value (+ sign inside the box) depicted. There were no significant differences in the time constants based on chamber type (two-tailed t-test; NS = not significant).

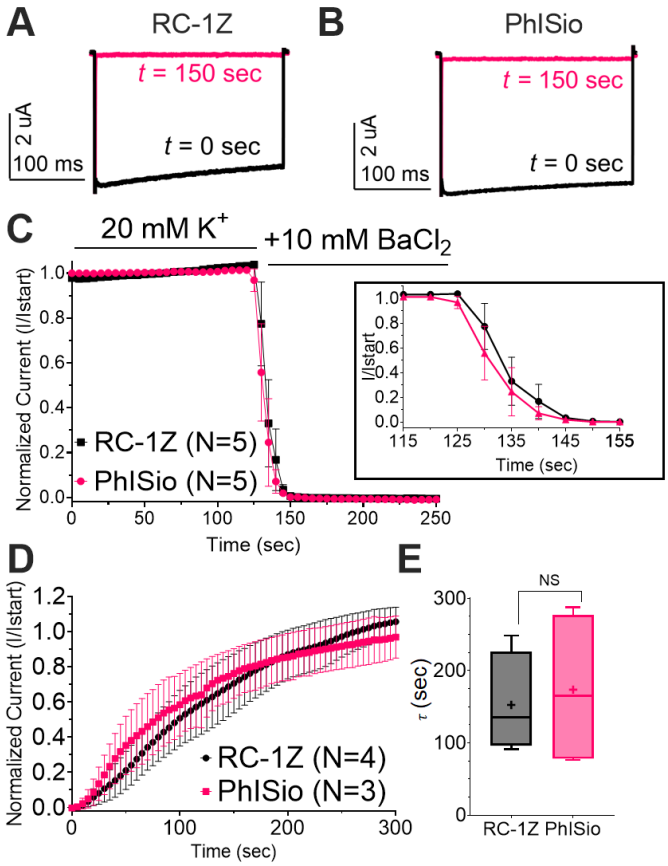

Figure 4. Comparison of rates of barium block of Kir2.1 currents recorded in the RC-1Z and PhISio chambers. Representative inwardly rectifying current traces recorded in (*A*) RC-1Z and (*B*) PhISio chambers before (0 sec; black traces) and during (150 sec; pink traces) barium block. (*C*) Kir2.1 currents were recorded in 20 mM external K^+ and blocked by addition of 10 mM $BaCl_2$; the inset (right) magnifies the time course of current inhibition, showing nearly 100% block within 20 sec for both chambers. (*D*) The time course of recovery from barium block, compared in the two chambers. For (*C*) and (*D*), data points are mean ± SEM for the current amplitudes, normalized to the maximum current at the time of solution change). (*E*) The time course of recovery from barium block was fitted to a single exponential (□) and compared for the two chambers (two-tailed t-test; NS = not significant). Box plots were constructed as in Figure 3.

have encountered the same limitations we found with the use of the RC-1Z chamber. Instead, the PhISio chamber allows for the use of pre-fabricated agar bridges which can be removed and replaced with ease. Figure 2 depicts the placement of the glass agar bridges.

A key advantage of the PhISio chamber is its low cost compared to commercial models; the cost of materials for printing the PhISio chamber was about $3 whereas the RC-1Z chamber currently lists for $301. The savings in using the PhISio chamber are multiplied for teaching or research labs that have more than one TEVC recording station. In addition, some investigators may wish to have multiple chambers available to prevent the unknown possibility of chamber contamination by drugs used in studies of membrane transport protein function. 3D printing of multiple PhISio chambers is cost-effective and the printing, curing, sanding, and cleanup process takes only 4 hours. Many universities and colleges now provide access to 3D printing technology. If institutional access to a 3D printer is a barrier, our free STL file may be uploaded to a company such as Xometry (https://www.xometry.com/) and printing the chamber will cost $50 to $60. We have made the chamber design freely accessible (see Methods) which also provides a template for educators and researchers to adjust the design to their specific needs. And, although advanced computer-assisted design software such as Autodesk Inventor and Fusion 360 exist, we used the free web-based service TinkerCAD to demonstrate that a quality chamber design can be produced with no added expenses for software. Future design modifications could similarly be done with TinkerCAD.

TEVC recordings in oocytes are useful in studying the function and regulation of ion channel proteins of many types (Stühmer, 1992; Wang et al., 2017) and the method can be modified to study action potentials as well (Corbin-Leftwich et al., 2018). The methodology is accessible for undergraduate neuroscience research and teaching labs although it requires a significant investment of resources. Our work established a way to make an affordable, easy to use, and functional TEVC recording chamber that should facilitate teaching and research on ion channels in undergraduate and other research labs.

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