

Recording Gap Junction Current from *Xenopus* Oocytes

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Abstract

Heterologous expression of connexins and innexins in *Xenopus* oocytes is a powerful approach for studying the biophysical properties of gap junctions (GJs). However, this approach is technically challenging because it requires a differential voltage clamp of two opposed oocytes sharing a common ground. Although a small number of labs have succeeded in performing this technique, essentially all of them have used either homemade amplifiers or commercial amplifiers that were designed for single-oocyte recordings. It is often challenging for other labs to implement this technique. Although a high side current measuring mode has been incorporated into a commercial amplifier for dual oocyte voltage-clamp recordings, there had been no report for its application until our recent study. We have made the high side current measuring approach more practical and convenient by introducing several technical modifications, including the construction of a magnetically based recording platform that allows precise placement of oocytes and various electrodes, use of the bath solution as a conductor in voltage differential electrodes, adoption of a commercial low-leakage KCl electrode as the reference electrode, fabrication of current and voltage electrodes from thin-wall glass capillaries, and positioning of all the electrodes using magnetically based devices. The method described here allows convenient and robust recordings of junctional current (I_j) between two opposed *Xenopus* oocytes.

Introduction

GJs are intercellular channels that may allow the current flow and exchange of small cytosolic molecules between neighboring cells. They exist in many cell types and perform diverse physiological functions. GJs in vertebrates are formed by connexins, whereas those in invertebrates by innexins. Each GJ consists of two juxtaposed hemichannels with either 6 or 8 subunits per hemichannel, depending on whether

they are connexins or innexins^{1,2,3}. Humans have 21 connexin genes⁴, while the commonly used invertebrate models *C. elegans* and *Drosophila melanogaster* have 25 and 8 innexin genes, respectively^{5,6}. Alternative splicing of gene

transcripts may further increase the diversity of GJ proteins, at least for innexins^{7,8}.

GJs may be divided into three categories based on molecular compositions: homotypic, heterotypic, and heteromeric. A homotypic GJ has all its subunits being identical. A heterotypic GJ has two homomeric hemichannels, but the two hemichannels are formed by two different GJ proteins. A heteromeric GJ contains at least one heteromeric hemichannel. The molecular diversities of GJs may confer distinct biophysical properties that are important to their physiological functions. GJ biophysical properties are also modulated by regulatory proteins⁹. To understand how GJs perform their physiological functions, it is important to know their molecular compositions, biophysical properties, and the roles of regulatory proteins in their functions.

Heterologous expression systems are often used to study biophysical properties of ion channels, including GJs, and the effects of regulatory proteins on them. Because heterologous expression systems allow the expression of specific proteins, they are generally more amenable to dissecting protein functions than native tissues where proteins with redundant functions can complicate the analysis, and recording of I_j can be unattainable. Unfortunately, most commonly used cell lines except the Neuro-2A cell are inappropriate for studying GJ biophysical properties due to complications by endogenous connexins. Even Neuro-2A cells are not always appropriate for this kind of analysis. For example, we could not detect any I_j in Neuro-2A cells transfected with the innexins UNC-7 and UNC-9 in either the absence or the presence of UNC-1 (unpublished), which is required for the function of UNC-9 GJs in *C. elegans*^{9,10}. On the other hand, *Xenopus* oocytes are a useful alternative system for electrophysiological analyses of GJs. Although they express

an endogenous GJ protein, connexin 38 (Cx38)¹¹, potential complications can be easily avoided by injecting a specific antisense oligonucleotide¹². However, analyses of GJs with *Xenopus* oocytes require a differential voltage clamp of two juxtaposed cells, which is technically challenging. The earliest successes of double voltage clamp of frog blastomeres were reported about 40 years ago^{13,14}. Since then, many studies have used this technique to record I_j in paired *Xenopus* oocytes. However, essentially all the previous studies have been performed with either homemade amplifiers^{12,15,16} or commercial amplifiers designed for recordings on single oocytes (GeneClamp 500, AxoClamp 2A, or AxoClamp 2B, Axon Instruments, Union City, CA)^{8,17,18,19,20}. Because even the commercial amplifiers do not provide instructions for double oocyte voltage clamp, it is often challenging for new or less sophisticated electrophysiological labs to implement this technique.

Only one commercial amplifier has been developed for double oocyte voltage clamp, the OC-725C from Warner Instruments (**Table of Materials, Figure 1A**). This amplifier may be used in either a standard mode (for single oocytes) or a high side current measuring mode (for single or dual oocytes) depending on whether two sockets in its voltage probe are connected (**Figure 1B, C**). However, until our recent study⁷, there had not been a single publication describing the use of this amplifier in its high side current measuring mode. Although the amplifier has been used by another lab for dual oocyte recordings, it was used in the standard rather than the high side mode^{21,22}. This lack of reports using the amplifier in its high side current measuring mode might be due to technical difficulties. We were unable to obtain stable dual oocyte recordings using the high side mode by following instructions from the manufacturer. Over the years, we have tried three different approaches for dual

oocyte recordings, including using two OC-725C amplifiers in the high side current measuring mode, two OC-725C amplifiers in the standard mode, and two amplifiers from another manufacturer. We eventually succeeded in obtaining stable recordings only with the first approach after extensive trial and error. This publication describes and demonstrates the procedures we use to express GJ proteins in *Xenopus* oocytes, record I_j using the high side current measuring mode, and analyze the electrophysiological data using popular commercial software. Additional information about the double voltage-clamp technique may be found in other publications^{19,23}.

Protocol

The surgeries are performed following a protocol approved by the institutional animal care committee of the University of Connecticut School of Medicine.

1. Frog surgery and preparation of defolliculated oocytes

1. Anesthetize an adult female African clawed frog (*Xenopus laevis*) (**Table of Materials**) by immersing in a cool (with ice) tricaine solution (~300 mg/L).
2. Wait (~15 min) until the frog shows little or no response to squeezing its webbed feet. Lay the frog on an operating table with its belly facing up.
3. After a longitudinal incision (8-10 mm long) on either the left or the right lower abdominal region, gently pull out a small piece of ovary tissue with a pair of forceps, and cut free the ovary tissue with a pair of small scissors.
4. Immediately immerse the free ovary tissue in a Ca^{2+} -free ND96 solution (NaCl 99 mM, KCl 2 mM, MgCl_2 1 mM, HEPES 5 mM, pH 7.5) inside a 60-mm Petri dish.
5. After closing the incision by simple interrupted sutures using a 5-0 silk suture (**Table of Materials**), put the frog back into a shallow water tank for recovery.

NOTE: The incision is closed in two steps: first the peritoneal and muscle layers, and then the skin layer. Each frog is subjected to 5 surgeries with at least a 4-week interval between two consecutive surgeries.
6. Transfer the isolated ovary tissue to a 50-mL centrifuge tube containing 10 mL of Ca^{2+} -free ND96 solution with 20 mg of collagenase (**Table of Materials**) and 20 mg of hyaluronidase (**Table of Materials**).
7. Shake the tube on an orbital shaker at room temperature (RT) until all the oocytes have become isolated (solitary).
8. Thereafter, transfer 30-50 oocytes to a Petri dish using a glass Pasteur pipette and examine the oocytes frequently under a stereomicroscope ($\geq 25\times$ highest magnification power) to determine whether they are defolliculated.

NOTE: The Pasteur pipette should be "cut" to a wider tip opening using a diamond scriber (**Table of Materials**) and flamed to smooth the cutting edge.
9. As soon as 70%-80% of the oocytes are defolliculated, decant the enzyme solution by gently tilting the tube. Wash the oocytes 5 times by filling up the tube with ND96 solution (NaCl 96 mM, KCl 2 mM, CaCl_2 1.8 mM, MgCl_2 1 mM, HEPES 5 mM, pH 7.5) and decanting the solution.
10. After the final wash, transfer the oocytes to a 60-mm Petri dish containing ND96 solution. Pick and transfer large and healthy-looking oocytes to a 60-mm Petri dish containing ND96 solution supplemented with sodium pyruvate (2 mM) and penicillin-streptomycin (100 U/mL) using a clean glass Pasteur pipette.

NOTE: "large and healthy-looking oocytes" are those of stages V and VI showing no sign of over-digestion by the enzymes.

- Place the Petri dish containing the picked oocytes inside an environmental chamber (15-18 °C).

2. GJ protein expression

- Synthesize complementary RNA (cRNA) of a specific connexin or innexin *in vitro* using an RNA transcription kit (**Table of Materials**) following the manufacturer's protocol.
- Precipitate the cRNA using a lithium chloride method described in the user manual of the RNA transcription kit.
- Wash the pellet with 70% ethanol, dissolve it in 20 μL of nuclease-free H_2O , and add 1 μL of ribonuclease inhibitor (40 U, **Table of Materials**).
- Measure the concentration of the cRNA using a spectrophotometer (**Table of Materials**).
- Mix the cRNA with a Cx38 antisense oligo so that the final concentrations are 200-1,000 ng/ μL cRNA and 100 ng/ μL oligo.

NOTE: The oligo sequence is 5'-GCTTTAGTAATTCCCATCCTGCCATGTTTC-3', which corresponds to the nucleotides from -5 to +25 in *Xenopus laevis* Cx38 mRNA (NCBI Accession: NM_001088018)¹¹. The oligo is kept as a stock solution (2.0 mg/mL) prior to being mixed with the cRNA.
- Eliminate particles in the cRNA by spinning in a microcentrifuge (~16,000 x g) for 2 min, and quickly transfer the entire supernatant to a new tube by pipetting (avoid disturbing the bottom of the microcentrifuge tube).

- Divide the cRNA into aliquots (2.5 μL /vial), and store the aliquots in a -80 °C freezer.
- Prepare an oocyte injection dish by gluing a small piece (~1 cm x 1 cm) of nylon mesh (**Table of Materials**) to the bottom of a 35-mm Petri dish using a quick-cure epoxy adhesive.

NOTE: The oocyte injection dish is reusable. Wash it with 70% ethanol after each use.

- Fill the oocyte injection dish approximately halfway with ND96 solution (supplemented with pyruvate and penicillin-streptomycin).
- Place 25-30 oocytes in rows inside the Petri dish.
- Prepare glass micropipettes for oocyte injections by following instructions in the user manual of the automated nanoliter injector (**Table of Materials**).

NOTE: Use of a microelectrode beveler (**Table of Materials**) can help produce sharp injection micropipettes that cause minimum damage to oocytes.
- Backfill an injection micropipette with lightweight mineral oil, and insert it into the micropipette holder of the injector.
- Transfer the cRNA from one of the stored aliquots to the inside surface of a Petri dish cover or bottom by pipetting.
- Aspirate the cRNA droplet into the tip of the micropipette by pressing the FILL button in the injector controller.
- Inject the cRNA (~50 nL/oocyte) by pressing the **INJECT** button.

NOTE: The injection volume may be set by the dip switches in the injector controller.
- Transfer the injected oocytes to a new Petri dish containing ND96 solution (supplemented with pyruvate and penicillin-streptomycin), and keep them inside the environmental chamber (15-18 °C) for 1-3 days

(depending on GJ protein expression speed and level).

Replace the solution daily by transferring the oocytes to a new Petri dish.

3. Oocyte pairing

1. Transfer a few of the injected oocytes into a 35-mm Petri dish containing ND96 solution.
2. Use two fine tweezers (**Table of Materials**) to gently peel off the transparent vitelline membrane that wraps the oocyte.

NOTE: It may be necessary to sharpen the tweezer tips before the first use and from time to time, using a piece of fine (600 Grit) sandpaper.

3. Place 2 oocytes per well in an oocyte pairing chamber (**Figure 1D**) containing ND96 solution (supplemented with pyruvate and penicillin-streptomycin).

NOTE: The oocyte pairing chamber is constructed by gluing a small piece of a Microwell Minitray (**Table of Materials**) to the bottom of a 35-mm petri dish (**Table of Materials**) using a quick-cure epoxy adhesive. The Minitray is cut into small pieces using a hot wire cutter (**Table of Materials**). Use of the Minitray for oocyte pairing was originally described by others²⁴. Although a GJ protein might distribute non-uniformly in the oocyte cell membrane depending on charge properties of amino acid residues in its cytosolic domains²⁵, random pairing (without discriminating between the animal and vegetal poles of the oocyte) appears to be sufficient in general.

4. Keep the Petri dish containing paired oocytes at RT on the isolation table to be used for electrophysiological recordings.

NOTE: The pairing duration can vary from a few hours to one day. A convenient practice is to pair oocytes in the late afternoon and record from them on the following day.

4. Acquisition system preparation

1. Configure two OC-725C oocyte clamp amplifiers (**Table of Materials**) to the high side current measuring mode by adjusting an internal dip switch (**Figure 1B**).
2. Ground the amplifiers by first interconnecting the Ground Circuit sockets on the rear panels and then connecting to the Faraday cage and the fiber light used for illuminating the recording chamber.
3. Connect the amplifiers to an analog-to-digital signal converter (**Table of Materials**), and configure them in the Clampex module of the pClamp software (**Table of Materials**) following instructions from the amplifier manufacturer.
4. Test the acquisition system with the model cell supplied with the amplifier.
 1. Connect the V_{DIFF} probe and current (I) cables to the model cell, connect the Red and Black sockets in the V_{DIFF} probe with the included jumper, connect either leg of the jumper to either pin in the model cell, and connect the ground wire in the model cell to the cable from the **GROUNDING CIRCUIT** socket of the amplifier (**Figure 1E**).
 2. Zero the Voltage Electrode (V_m) and Bath Electrode (I_m) meters of the amplifier by turning the **V_m OFFSET** and **V_e OFFSET** knobs, respectively, switch Clamp from **OFF** to either **Fast** or **SLOW** and turn the **GAIN** dial clockwise to a level that allows proper voltage clamp (**Figure 1A**). The D.C. GAIN switch may be at either the **OUT** or the **IN** position.

- Run a simple acquisition protocol containing a few voltage steps to confirm that the voltage displayed in the V_m meter changes according to the acquisition protocol and that voltage and current traces are displayed properly in Clampex.

NOTE: Only one amplifier may be tested each time using this approach.

5. Recording I_j between paired oocytes

- Create acquisition protocols for recording I_j in the pClamp software.

NOTE: Create two acquisition protocols. In one of them, amplifier # 1 is used to produce a series of membrane voltage (V_m) steps (e.g., -150 to +90 mV at 10-mV intervals), whereas amplifier #2 is used to maintain a constant V_m (e.g., -30 mV). In the other protocol, amplifier #2 is used to produce the V_m steps, whereas amplifier # 1 is used to maintain the constant V_m . The positive and negative V_m steps should be applied alternatively (e.g., -150, +90, -140, +80), which can be programmed in Clampex using the **User List** feature in the acquisition protocol.

- Set up the recording stage

- Drop one Petri dish containing paired oocytes into the Petri dish receptacle in the recording platform (**Figure 2A**)
- Select a pair of oocytes and rotate the Petri dish if necessary so that the two oocytes are in the left and right direction, and lock the stage in position by its magnetic base.

- Fix the magnetic stands holding the current and voltage probes at appropriate locations on the isolation table.

NOTE: Make sure that the current and voltage probes from one amplifier are located on the left side, whereas those from the other amplifier are on the right side, and that the voltage probe is in front of the current probe on each side (**Figure 2B, C**).

- Set up the reference electrode

- Place a reference electrode (**Table of Materials**) near the edge of the Petri dish toward the user side (**Figure 2B, C**).
- Connect the reference electrode to the black socket (Circuit Ground) in only one of the two V_{DIFF} probes.

- Set up the V_{DIFF} electrodes

- Pull a pair of glass micropipettes, break off a bit of the tip with the diamond scribe (**Table of Materials**), and smooth the tip edge by fire polishing.

NOTE: Tip resistance should be less than 150 k Ω (measured with ND96 in the pipette). Typically micropipettes with tip resistance of 20-150 k Ω are used.

- Hold the micropipette above the flame of an alcohol burner to bend it to a smooth angle ($\sim 130^\circ$) at a position ~ 1 cm away from the tip.

NOTE: Fabricated micropipettes are reusable. Rinse them with water after each use.

- Backfill the glass pipette completely with ND96 solution, insert it into a prefilled (with ND96) microelectrode holder (**Table of Materials, Figure**

- 2D**), and ensure no air bubbles are present in the system.
4. Insert the 2-mm pin of the microelectrode holder into the 2-mm socket of a *V_{DIFF}* electrode connection wire (**Figure 2D**).
 5. Clamp the 2-mm socket on a magnetically based clamper (**Figure 2D**), and aim the tip of the *V_{DIFF}* electrode toward one of the two oocytes (**Figure 2E**).
NOTE: Adjust the position and angle of the clamper so that the tip of the electrode is very close to the oocyte
 6. Insert the 1-mm pin of the *V_{DIFF}* electrode connection wire into the red socket (*V_{DIFF}* Input) in the *V_{DIFF}* probe on the same side.
 7. Prepare and connect a *V_{DIFF}* electrode for the other oocyte and amplifier following similar procedures.
NOTE: A close-up view of a pair of oocytes and all the electrodes are shown in **Figure 2E**.
5. Set up the current and voltage electrodes
 1. Prepare voltage and current electrodes from glass capillaries, backfill them (about halfway) with a KCl solution (KCl 3.0 M, EGTA 10 mM, HEPES 10 mM, pH 7.4 with KOH), and insert them into the electrode holders provided with the amplifiers.
NOTE: The electrodes should have a resistance of ~1 MΩ. Suitable electrodes may be obtained from a type of thin-wall glass capillaries (**Table of Materials**) using a specific set of pulling parameters (**Table 1**). Such tips can easily penetrate the oocyte cell membrane without the need to press either the *V_m* or the *V_e* BUZZ button on the amplifier.
 2. Lower the electrodes into the bath solution, zero the *V_m* and *I_m* meters by turning the ***V_m* OFFSET** and ***V_e* OFFSET** dials and check electrode resistance by pressing ***V_m* Electrode Test** and ***V_e* Electrode Test**.
 3. Insert the current and voltage electrodes into the oocytes, and observe negative membrane potentials (typically -20 to -50 mV).
NOTE: The *V_m* and *I_m* meters of the same amplifier should display two identical or very similar values.
 6. Data acquisition
 1. In the Clamp section of the amplifier, confirm that D.C. GAIN is at the **IN** position, turn the **GAIN** knob clockwise to a level that allows proper voltage clamp (typically one-third to half of the full range), and turn the Clamp switch from **OFF** to **FAST**.
NOTE: Upon turning on the clamp, the *V_m* and *I_m* meters will display the holding voltage and the holding current, respectively. Although the holding current may vary somewhat depending on oocyte conditions, it is generally small and stable at the holding *V_m* (e.g., -30 mV).
 2. Run an acquisition protocol.
NOTE: Four traces will be displayed on the screen. The voltage and current traces from one amplifier indicate the *V_m* steps applied to oocyte #1 and the current needed to produce the *V_m* steps, whereas those from the other amplifier indicate the constant *V_m* (-30 mV) of oocyte #2, and the current injected to maintain this constant *V_m*. The current injected into oocyte #2 represents the *I_j*.

6. Data analysis

1. Analysis with Clampfit.
 1. Open a recorded *abf* file in the Clampfit module of pClamp.
 2. Use cursors 1 and 2 to enclose a segment of the baseline before the I_j traces.
 3. Click on the icon **Adjust Baseline** to bring out a Baseline window. Select **Subtract Mean of Cursors 1..2** for Method, and confirm that **All Visible Signals** and **All Visible Traces** are selected for Trace Selection.
 4. Upon clicking **OK**, the Baseline window closes, the baselines of all the current and voltage traces converge at the zero level. Note that the voltage steps change to new levels that are equal to the original voltage minus the holding voltage (e.g., -30 mV).
 5. To plot the I_j and V_j relationship using steady-state I_j , enclose a desired segment of the I_j traces representing steady-state I_j with cursors 1 and 2, place cursor 3 anywhere within the time window of the voltage steps.
 6. Go to **Analyze/ Quick Graph/ I-V** to open an *I-V* window.
 7. Under **X-Axis** (Voltage), select **Cursor 3 from Signal**, specify the voltage step signal (e.g., Voltage 1) in the dropdown menu, and check the box **Invert**.
NOTE: The Invert box is checked to convert the V_m to values equivalent to V_j , which is defined as V_m of oocyte #2- V_m of oocyte #1.
 8. Under **Y-Axis** (Current), select the source of the I_j signal (e. g., *Current 0*) from the dropdown menu next to **Signal**, define **Region** as **Cursors 1..2** from the dropdown menu, and select **Mean**.
NOTE: To plot peak I_j and V_j relationships, cursors 1 and 2 should enclose the segment of I_j traces containing the peak I_j in step 6.1.5 and select **Peak** (instead of **Mean**) in step 6.1.8.
 9. Under **Destination** option, select either **Replace** or **Append**.
 10. Upon clicking **OK** in the *I-V* window, an $I_j - V_j$ relationship is displayed on the screen, and the corresponding I_j and V_j values may be found by clicking **Window/Result**.
2. Plot and fit the G_j - V_j relationship in OriginPro (**Table of Materials**)
 1. Copy the two columns containing V_j and I_j values from the Results window of Clampfit (step 6.1.10) to a new Workbook in Origin. Make sure that the V_j and I_j values are under X and Y columns, respectively.
 2. Add four new columns in the Workbook by clicking **Column/ Add New Columns**.
 3. Name the first new column as G_j , fill it by entering the equation "column I_j /column V_j * 1,000" in the **Set Column Values** window, and create a scatter plot of the G_j - V_j relationship.
NOTE: The multiplication by 1,000 (optional) is to avoid the inconvenience of dealing with very small numbers in subsequent steps.
 4. Fit the G_j data points over the negative and positive V_j ranges independently to the Boltzmann function.

Calculate G_j at $V_j = 0$ mV by entering the G_{jmax} , G_{jmin} , A , and V_0 values from the fitting into the Boltzmann equation, which can be done in a spreadsheet. The G_j thus obtained will be used as G_{jmax} in the next step.

NOTE: The equation to fit the Boltzmann function is: $G_j = (G_{jmax} - G_{jmin}) / \{1 + \exp[A(V_j - V_0)]\} + G_{jmin}$, in which V_0 is the V_j at which the conductance is half-maximal, G_{jmax} is the maximal conductance, G_{jmin} is the V_j -insensitive residual conductance²³. To perform the fitting, first add the Boltzmann equation as a new fitting function into OriginPro, and then select this function for fitting. Enter approximate seed values for the G_{jmax} , G_{jmin} , V_0 , and A before executing the fitting function. Make sure that the G_{jmax} parameter is not fixed for this fitting.

5. Name the second and third new columns as nGj-L and nGj-R ("n" for "normalized"), and fill them by dividing the G_j column by the G_{jmax} values derived from the left and right $G_j - V_j$ curves, respectively (step 6.2.4).
6. Name the fourth new column as nGj-LR, and fill it by copying values from the negative and positive V_j ranges of the nGj-L and nGj-R columns, respectively.

7. Create a scatter plot for nGj-LR over V_j , and fit the data points over the negative and positive V_j ranges to the Boltzmann function independently. Make sure that the G_{jmax} parameter is fixed at 1.0 for the fitting.
8. Keep records of the fitting results (e.g., the fitted graph and the G_{jmin} , V_0 , and A values).

Representative Results

UNC-7 and UNC-9 are innexins of *C. elegans*. While UNC-9 has only one isoform, UNC-7 has multiple isoforms that differ mainly in the length and amino acid sequence of their amino terminals^{7,8}. These innexins may form homotypic as well as heterotypic (of UNC-7 and UNC-9) GJs when expressed in *Xenopus* oocytes^{7,8}. Representative I_j traces and the resulting normalized $G_j - V_j$ relationships of UNC-7b and UNC-9 homotypic GJs are shown in **Figure 3**. In these experiments with paired oocytes, V_m of Oocyte 1 were clamped to different levels from the holding voltage (-30 mV), whereas that of Oocyte 2 was kept constant at -30 mV to monitor the I_j . The results show that these two types of GJs differ in the V_j -dependent I_j inactivation rate, V_j dependence (indicated by the slope of the $G_j - V_j$ curve), and the amount of the residual G_j . Many other examples of UNC-7 and UNC-9 GJs, including rectifying GJs, may be found in our recent publication⁷.

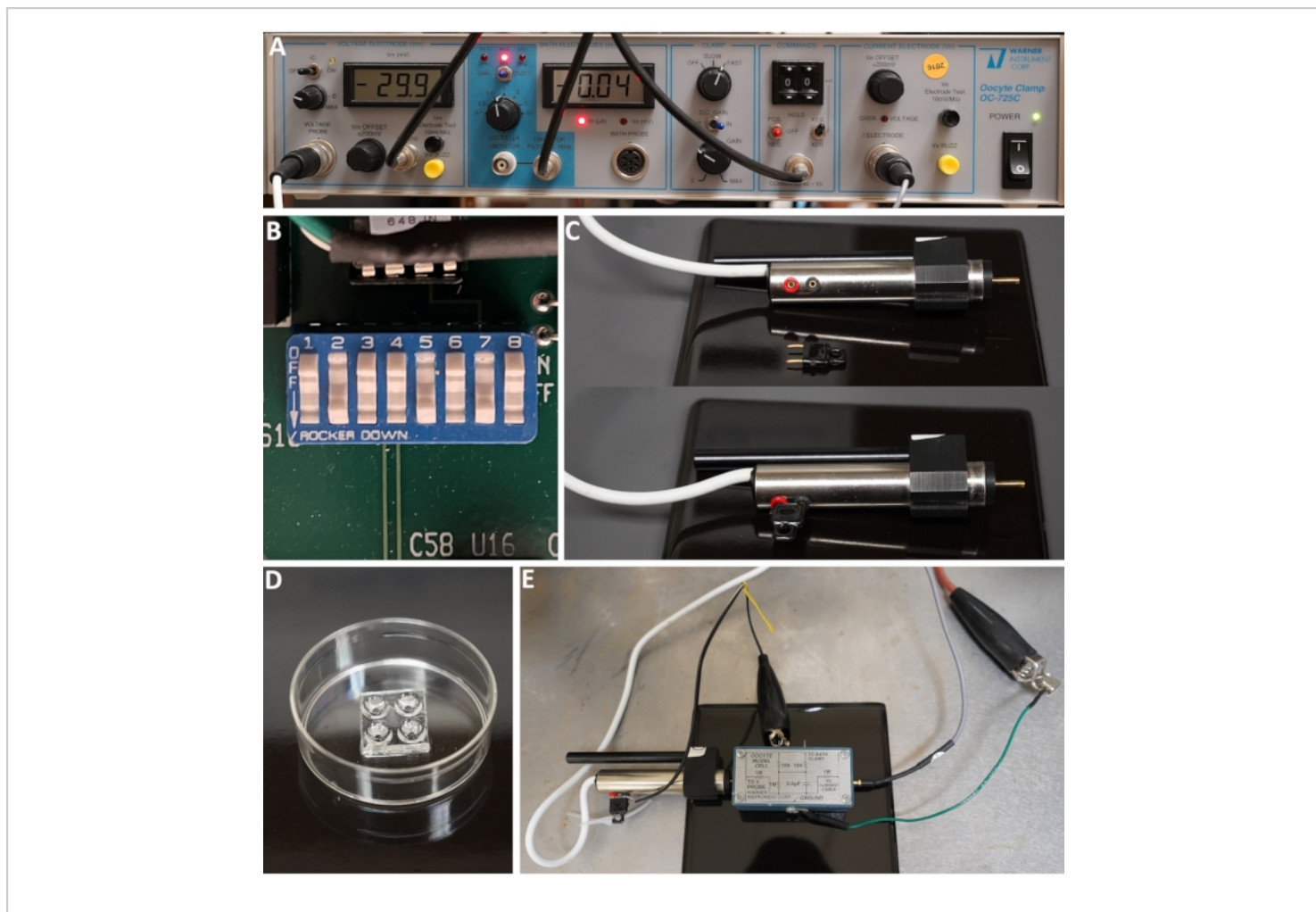


Figure 1: Oocyte pairing chamber and amplifier setup. (A) Front panel of the oocyte clamp amplifier OC-725C. (B) A DIP switch inside the amplifier configured for the high side current measurement. All the toggle switches except 2, 5, and 7 are in the OFF position to use the amplifier in the high side current measuring mode. (C) A V_{DIFF} probe with the red socket (for V_{DIFF} input) and black socket (for Circuit Ground) either unconnected or connected. The probe may be used for the standard voltage-clamp mode when the two sockets are connected. (D) An oocyte pairing chamber. (E) A model cell with connections for testing the acquisition system with the amplifier in the high side current measuring mode. [Please click here to view a larger version of this figure.](#)

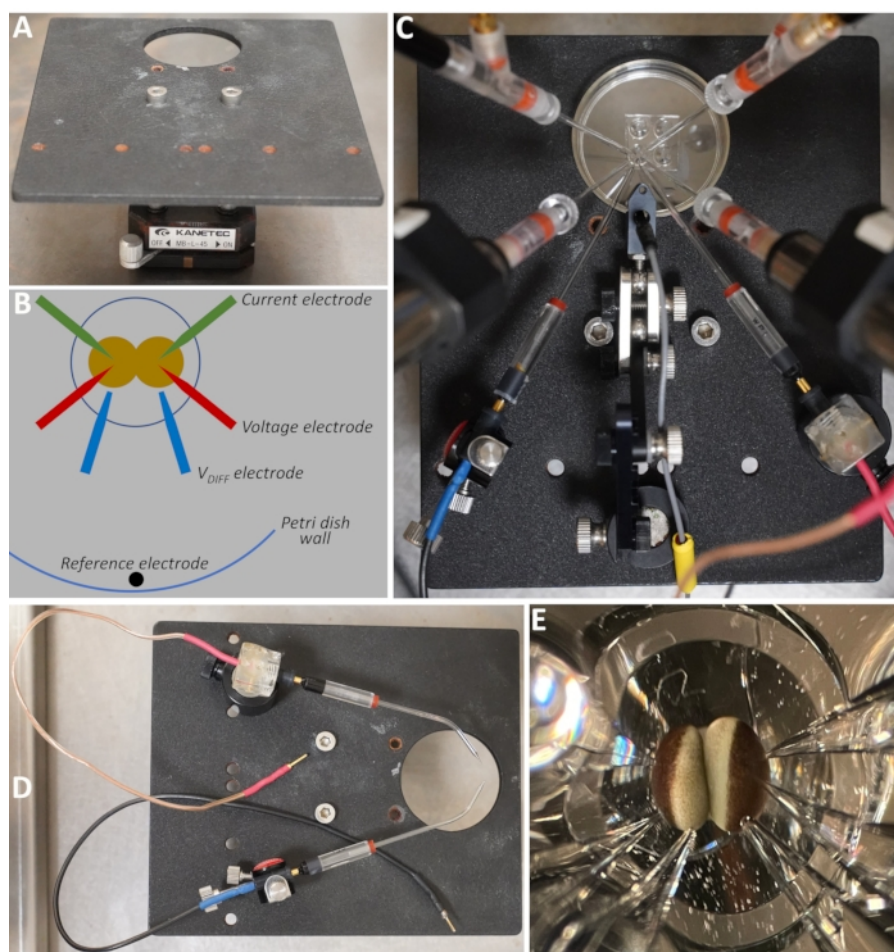


Figure 2: Oocyte and electrode setup. (A) The recording stage. The circular hole has a diameter of 36 mm. (B) Diagram showing the positions of the various electrodes. (C) Actual layout of the various electrodes. (D) Two V_{DIFF} electrodes with their holders and connection cables clamped on the recording stage by two different magnetic clamps, including a modified Agar Bridge Magnetic Holder (**Table of Materials**) (top) and a tube clamp (**Table of Materials**) (bottom). The former is more stable in maintaining the electrode position because of its larger magnetic base but requires modification. The glass micropipettes are rotated 90° from their operating positions in order to show the bending angles. (E) A close-up view of a pair of oocytes and the electrodes. [Please click here to view a larger version of this figure.](#)

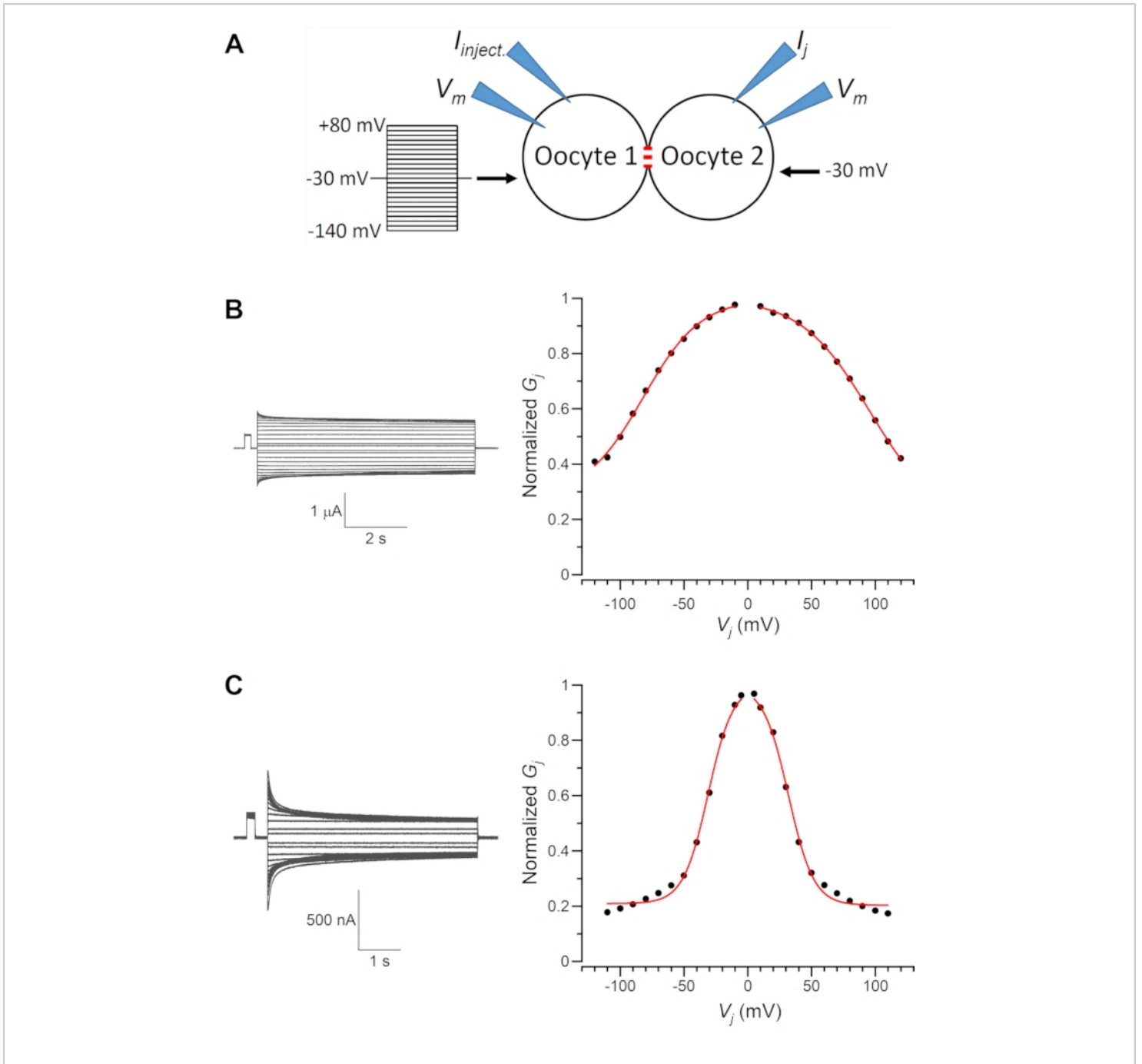


Figure 3: Representative recording traces. (A) Diagram showing the oocyte experiment. Negative and positive membrane voltage (V_m) steps are applied to Oocyte 1 from a holding V_m of -30 mV whereas Oocyte 2 is held at a constant V_m of -30 mV. The transjunctional voltage (V_j) is defined as V_m of Oocyte 2 - V_m of Oocyte 1. (B) Sample I_j traces and the resulting normalized junctional conductance (G_j) - V_j relationship of UNC-9 homotypic gap junctions. (C) Sample I_j traces and the resulting normalized G_j - V_j relationship of UNC-7b homotypic gap junctions. The G_j - V_j relationships are fitted by a Boltzmann function (red lines). [Please click here to view a larger version of this figure.](#)

| Step No. | Heat | Pull | Velocity | Time |
|----------|------|------|----------|------|
| 1 | 260 | ... | 40 | 200 |
| 2 | 240 | ... | 40 | 200 |
| 3 | 240 | 60 | 40 | 200 |
| 4 | 245 | 100 | 60 | 200 |

Refer to the user manual at the manufacturer's website for definitions of the pulling parameters (<https://www.sutter.com/MICROPIPETTE/p-97.html>).

Table 1: Electrode pulling parameters. These parameters are based on thin-wall glass capillaries (**Table of Materials**) and a ramp temperature of 258 at a P-97 micropipette puller (**Table of Materials**). They need to be adjusted according to the ramp temperature for this glass on your puller. For example, if the ramp temperature on the puller is 20° higher, add 20° to each step and make necessary adjustments. Generally, tip size may be optimized by adjusting the velocity of the last step. Please refer to the user manual at the manufacturer's website for meanings of the pulling parameters (<https://www.sutter.com/MICROPIPETTE/p-97.html>).

Discussion

System optimization appears to be necessary for dual oocyte voltage-clamp experiments. Without it, recordings can be highly unstable, and the amplifiers may have to inject an excessive amount of current to reach the target V_m , resulting in oocyte damage and recording failures. Several factors are critical to obtaining stable dual oocyte recordings with the high side current measuring method. First, the current and voltage electrodes must have appropriate resistance (~1 M Ω), and their holders must be clean. Second, the V_{DIFF} electrodes must have low resistance (<150 k Ω) and be close to the oocytes. Third, all the electrodes for the same oocyte (voltage, current, and V_{DIFF}) must be positioned on the same side (left or right), and the order of the electrodes (from back to front) should be current, voltage, and V_{DIFF} . Lastly, the reference electrode should be located near the edge of the 35-mm Petri dish toward the user.

We have modified a few recommended procedures from the manufacturer and made some other improvements. Among them are: 1) ND96-filled micropipettes instead of KCl-loaded agar bridges to serve as V_{DIFF} electrodes. The glass electrodes are easy to construct, reusable, and non-harmful to oocytes; 2) a low leakage KCl electrode as the reference electrode. This electrode has low resistance (~2.7 k Ω) and stable potential, and leaks little electrolytes (~5.7 x 10⁻⁸ mL/h); 3) a custom-designed and constructed recording platform that allows stable, convenient, and precise positioning of oocytes and the various electrodes. This stage also provides ample access to a stereomicroscope, a fiber light with dual goosenecks, and the four magnetic stands used to mount and position the current and voltage electrodes; and 4) fabrication of current and voltage electrodes from a type of thin-wall glass capillaries. These electrodes have the desired tip resistance (0.5-1.4 M Ω), can penetrate the oocyte cell membrane very easily, and cause minimum damage to oocytes.

Occasionally, the recording system does not work properly, as indicated by an unusually large or continuously increasing holding current, development of a white spot in the cell membrane around the current electrode, and unstable V_m traces in response to voltage commands. The possible causes are 1) a V_{DIFF} electrode system has a small air bubble or the tip of the V_{DIFF} electrode is not aimed properly toward the oocyte; 2) a voltage or current electrode has high resistance (e. g. $>2\text{ M}\Omega$) or its holder is dirty from salt deposit; 3) the connection wire for a V_{DIFF} electrode is broken; 4) the D.C. gain is not set to IN during voltage clamp.

Here we have described a method for recording I_j from *Xenopus* oocytes. It allows a stable voltage clamp of two opposed oocytes. This method is easy to implement and appears to have no obvious limitations for analyzing the biophysical properties of GJs. However, we are not in a position to tell how it compares with other published methods. We hope that labs that are newly interested in setting up the double oocyte voltage-clamp technique will find this method worth considering.

Disclosures

The authors have no conflict of interest.

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