Video Article

Whole-cell Patch-clamp Recordings in Brain Slices

Amir Segev[1], Francisco Garcia-Oscos[1], Saïd Kourrich[1]

[1]Department of Psychiatry, University of Texas Southwestern Medical Center

Correspondence to: Saïd Kourrich at Said.Kourrich@UTSouthwestern.edu

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Abstract

Whole-cell patch-clamp recording is an electrophysiological technique that allows the study of the electrical properties of a substantial part of the neuron. In this configuration, the micropipette is in tight contact with the cell membrane, which prevents current leakage and thereby provides more accurate ionic current measurements than the previously used intracellular sharp electrode recording method. Classically, whole-cell recording can be performed on neurons in various types of preparations, including cell culture models, dissociated neurons, neurons in brain slices, and in intact anesthetized or awake animals. In summary, this technique has immensely contributed to the understanding of passive and active biophysical properties of excitable cells. A major advantage of this technique is that it provides information on how specific manipulations (*e.g.*, pharmacological, experimenter-induced plasticity) may alter specific neuronal functions or channels in real-time. Additionally, significant opening of the plasma membrane allows the internal pipette solution to freely diffuse into the cytoplasm, providing means for introducing drugs, *e.g.*, agonists or antagonists of specific intracellular proteins, and manipulating these targets without altering their functions in neighboring cells. This article will focus on whole-cell recording performed on neurons in brain slices, a preparation that has the advantage of recording neurons in relatively well preserved brain circuits, *i.e.*, in a physiologically relevant context. In particular, when combined with appropriate pharmacology, this technique is a powerful tool allowing identification of specific neuroadaptations that occurred following any type of experiences, such as learning, exposure to drugs of abuse, and stress. In summary, whole-cell patch-clamp recordings in brain slices provide means to measure in *ex vivo* preparation long-lasting changes in neuronal functions that have developed in intact awake animals.

Video Link

The video component of this article can be found at http://www.jove.com/video/54024/

Introduction

The patch-clamp technique, an electrophysiological technique that has been developed in the late 1970s[1,2], is a primary tool for studying single or multiple ion channel functions in live tissue. Among the different patch configurations that can be achieved, whole-cell patch-clamp recordings allow the study of the electrical behavior of a substantial part of the neuron. Classically, this technique is performed *in vitro* either on brain slices, freshly dissociated neurons, or on cell culture models[3]. When performed on neurons in brain slices, this technique presents several advantages. In particular: (i) neurons are recorded in relatively preserved brain circuits that to some extent, and compared to cell culture preparations, provide an environment that is physiologically relevant[3]. This allows capturing early, or even monitoring in real time, cellular and molecular events that are triggered by any type of acute pharmacological manipulations — a temporal resolution that cannot be achieved using classical *in vivo* conditions; (ii) capability to visually identify brain regions in brain slices allows high regional specificity[3] both for the brain region studied and for specific neurons when they express fluorescent markers; (iii) access to the intracellular space of the cell by opening a significant portion of the plasma membrane (in contrast to puncturing the membrane with a sharp micropipette for intracellular recordings)[4]. In turn, this allows the content or concentration of specific ions composing the internal solution to be modified so molecular targets or cellular mechanisms can be studied under different conditions. For example, upon establishing whole-cell configuration, any specific pharmacological agent (*e.g.*, antagonists) that one can add to the recording micropipette (patch pipette) solution will directly diffuse into the cytoplasm and act on its putative intracellular targets without altering the target function in neighboring cells. Additionally, compared to sharp micropipette recording, the large opening at the tip of the patch clamp electrode provides lower resistance, less competing noise, and thus better electrical access to the inside of the cell[4]. However, note that the large opening at the pipette tip may lead to cell dialysis, and thereby the loss of intracellular molecular machinery that may be critical for the expression of the biological phenomena that are under study[5,6]. In this case, sharp electrode recordings may be more suitable. This type of recordings requires micropipettes with a pore that is much smaller than those used for whole-cell recordings, thereby preventing most of the ion exchange between intracellular space and the internal pipette solution.

Any form of experience (acute or chronic), including learning[7-10], exposure to drugs of abuse[11,12], stress[13,14], *etc.*, can alter various aspects of neuronal function in specific brain regions. Because these alterations often require time to develop (hours to days), whole-cell recordings in brain slices from animals that have undergone a specific experience allow researchers to identify these changes. Basically, many (if not all) components that participate in neuronal functions (*e.g.*, ligand-activated ion channels, voltage-gated ion channels, neurotransmitter transporters), and thereby brain circuit activity and behavior, can be altered by experience (experience-dependent plasticity)[10,15-17]. At the neuronal level, brain circuit activity emerges from constant interactions between synaptic (*e.g.*, glutamate transmission) and intrinsic cellular excitability factors (*e.g.*,

axosomato-dendritic ion channels: sodium, Na^+; potassium, K^+; and calcium, Ca^{2+}). Under specific conditions using whole-cell patch-clamp electrophysiological techniques, signal alterations originating specifically from changes in synaptic vs. intrinsic excitability can be isolated.

In most cases, synaptic excitability is assessed using the whole-cell voltage-clamp technique. This recording mode allows the measurement of ion currents [*e.g.*, mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA receptors) and N-Methyl-D-aspartic acid receptors (NMDA receptors)] through the neuronal plasma membrane while holding the membrane potential at a set voltage. Here, experimenters use internal micropipette solutions that contain cesium (Cs^+), a broad blocker of K^+ channels (key intrinsic excitability factors). Upon establishment of whole-cell configuration, the diffusion of Cs^+ in intracellular space will block K^+ channels, and thereby will allow both a relatively efficient space-clamp and prevent influence of intrinsic excitability factors on other measurements. Space-clamp issues, *i.e.*, the difficulty to voltage-clamp the whole cell, arise when recording irregular shaped cells (*e.g.*, neurons), and particularly neurons with a vast and complex dendritic arbor[18,19]. Because somatic voltage clamp poorly controls voltage in the dendritic tree of neurons, various aspects of dendritic electrical signals under study are distorted in a dendritic distance-dependent manner. Combined with pharmacological tools such as picrotoxin (gamma-Aminobutyric acid, $GABA_A$ receptor antagonist) or kynurenic acid (broad blocker of glutamate receptors) dissolved in the extracellular solution (artificial cerebro-spinal fluid, ACSF), this technique allows the measurement of glutamate receptor- and $GABA_A$R-mediated currents respectively.

In contrast, intrinsic excitability is usually assessed in current-clamp recording mode. As opposed to voltage-clamp recording, this recording mode allows the measurement of variations in membrane potentials induced by ion currents flowing through the neuronal plasma membrane. Typically, alteration in intrinsic excitability is assessed through changes in the capability for neurons to generate action potentials, which requires both Na^+ and K^+ channels. Therefore, when performing current-clamp recordings, micropipettes are filled with an internal solution that contains K^+ instead of Cs^+. Combined with pharmacological agents that block glutamate and $GABA_A$ receptor-mediated currents dissolved in the ACSF, this experimental design allows the measurement of the contribution of intrinsic factors (*e.g.*, K^+ channels) to neuronal firing without being contaminated by potential changes in synaptic excitability factors.

This article will describe the basic necessary procedural steps to (i) prepare healthy brain slices; (ii) achieve whole-cell configuration, and (iii) monitor basic parameters to assess synaptic and intrinsic excitability.

Protocol

All experiments were carried out in accordance with protocols approved by the UT Southwestern Institutional Animal Care and Use Committee, and were chosen so as to minimize stress, discomfort, and pain experienced by the experimental animals.

1. Solutions

Note: Prepare micropipette internal solutions in advance. For most basic experimental purposes, two kinds of solutions should suffice: Cs^+-based and K^+-based solutions.

1. Use Cs^+-based solutions (*e.g.*, Cs^+ gluconate solution, see **Materials**) for voltage-clamp experiments. Prepare at RT.
    1. Prepare 117 mM Cs-Gluconate solution by mixing 4.62 g D-gluconic acid (~3.696 ml) with 3.54 g CsOH (~2.01 ml).
    2. Add ddH_2O to 90 ml and let it equilibrate for 30 min.
    3. Add the solid ingredients (20 mM HEPES = 0.476 g; 0.4 mM EGTA = 15.2 mg; 2.8 mM NaCl = 16.4 mg; 5 mM tetraethylammonium (TEA) chloride = 83 mg).
    4. Add ddH_2O to ~97 ml.
    5. Adjust the pH of the solution with 50% CsOH to 7.2 - 7.3.
    6. Check osmolarity and correct if needed with ddH_2O.
    Note: A good range is ~280 - 285 mOsm. Optimal osmolarity should be 15 - 20 mOsm below the osmolarity of standard ACSF (usually 300 - 310 mOsm, 300 mOsm in our laboratory). Osmolarity may vary depending on solutions specific compositions.
    7. Aliquot to 1,000 µl and store at -20 °C or below.
    8. Prepare, aliquot, freeze, and add ATP/GTP to the internal solution on the day of recording.
        1. Add 64.63 mg ATP to 10 mg GTP and dissolve in 637.11 µl of ddH_2O.
        2. Prepare 10 µl aliquots and store at -20 °C or below. Mix each 100x aliquot with 1,000 µl of internal solution on the day of the experiment. Once ATP/GTP is added to the internal solution, maintain it on ice to prevent ATP/GTP degradation.

2. Use K^+-based solutions (*e.g.*, K-Gluconate solution, see **Materials**) for both current- and voltage-clamp experiments where K^+ conductances remain functional so that neuronal firing can be assessed. Prepare at RT.
    1. Weigh all materials according to the desired final volume. For preparing 90 ml of solution, 120 mM K-Gluconate = 2.81 g; 20 mM KCl = 0.149 g; 10 mM HEPES = 0.238 g; 0.2 mM EGTA = 0.008 g; 2 mM $MgCl_2$ = 0.021 g.
    2. Use enough ddH_2O to reach 90% of the final solution volume. This should ensure that enough room is left for pH and osmolarity adjustment.
    3. After adding and mixing all the ingredients, make sure the solution is clear before measuring the pH.
    4. While constantly stirring the solution, adjust pH to 7.2 - 7.3 using K^+ hydroxide (KOH).
    5. After adjusting the pH, use the osmometer and adjust osmolarity to 280 - 285 mOsm.
    Note: Optimal osmolarity should be 15 - 20 mOsm below the osmolarity of standard ACSF (usually 300 - 310 mOsm, 300 mOsm in our laboratory). Osmolarity may vary depending on solutions specific compositions.
    6. Aliquot to 1,000 µl and store at -20 °C or below.
    7. Prepare, aliquot, freeze, and add ATP/GTP to the internal solution on the day of recording (see step 1.1.8).

3. Prepare 1 L of standard ACSF (see **Materials**).

Note: We use this recipe in our laboratory when recording medium spiny neurons (MSNs) in brain slices, however, recipes may differ between laboratories, and therefore, we recommend the experimenter use a recipe that is routinely used when recording the brain region of interest.

4. Prepare the dissection ACSF (slicing solution, ~125 ml. Note: Exact volume will depend on the size of the slicing chamber as it should fully submerge the brain) for use in steps 2.2 - 2.8.
    1. Prepare 5 mM kynurenic acid (to block glutamate receptor-induced excitotoxic processes) in standard ACSF in a sufficient volume to submerge the brain during slicing. Use a sonicator to help dissolve the kynurenic acid.
    Note: The length of sonication may vary depending on volume and amount of solids in the solution. The solutions must be clear by the end of the process (around 1 - 2 min in our conditions).
    2. Cool down while bubbling with 95% O_2, 5% CO_2 gas in a bucket of ice until temperature reaches 0 - 2 °C.

5. Prepare ACSF for recording.
    1. Take 1 L of standard ACSF (or whatever left from the solution prepared in step 1.3) to which appropriate pharmacological agents may be added depending on planned experiments.
        1. For example, add 100 µM picrotoxin when recording excitatory post-synaptic currents or potentials (EPSCs or EPSPs), glutamate receptor antagonists (kynurenic acid, 2 mM; or a combination of D-APV 50 µM with CNQX 10 µM) when recording inhibitory post-synaptic currents or potentials (IPSCs or IPSPs), and both picrotoxin and glutamate receptor antagonists when assessing neuronal firing in the absence of any influence from synaptic events.

2. Slice Preparation

1. Construct or obtain a slice recovery chamber.
Note: The principle for a recovery chamber is straightforward and can be made in the laboratory (**Figure 1**). Briefly, the chamber is a receptacle in which a basket is inserted to hold the brain slices at a level that is lower than the surface of ACSF. Various scientific companies also sell slice recovery chambers.
    1. As an example, obtain four rings (4 - 6 mm high) (**Figure 1A**, side view; **B**, top view) by cutting a 30 cc syringe. Then, glue stretched nets (*e.g.*, cut from a nylon hose) to one side of the rings to hold the brain slices (**Figure 1B**) and glue the rings together.
    Note: A glue gun can be used.
    2. Once the four rings are glued, glue a curved isosceles trapezoid-shaped plastic wall to two of the rings (**Figure 1A** and **B**) to divert oxygen bubbles from the recovering brain slices (**Figure 1C** and **D**). As shown in **Figure 1D**, insert an oxygen diffusing system (here, a gas dispersion tube) on the same side as the plastic walls.

2. Prior to slicing, oxygenate (95% O_2/5% CO_2) and cool down the slicing solution (see step 1.4) to 0 - 2 °C.
3. Fill the custom recovery chamber with standard ACSF at RT. Ensure that the ACSF is well oxygenated (20 - 30 min, time may vary according to the chamber volume) before placing slices in the recovery chamber. Ensure that gas bubbles do not come in direct contact with the slices or disrupt them.
4. Line the vibratome ice tray with ice and fill with cold water so that one third to a half of the slicing chamber is submerged. Carefully place an oxygen delivery system (*e.g.*, gas diffusing stone) and a temperature probe in the slicing chamber so neither item interferes with the blade movement or slice manipulation.
5. Prepare the dissection area and tools necessary for extracting the brain and dissecting the desired brain region.
Note: The exact dissection performed will depend on the specific brain region studied as different brain structures will require slicing at different planes (*e.g.*, coronal, sagittal, or horizontal slices).
    1. Place the following tools on an underpad: decapitation scissors, scalpel, small straight sharp tip scissors, vessel cannulation forceps (or any surgical tool with a wide tip, such as rongeurs, which is more suitable for rat skulls), curved hemostatic forceps, tweezers, spatula, scooping spatula, filter paper, Petri dish, single edge razor blade, and cyanoacrylate glue.

6. When temperature reaches 0 - 2 °C, transfer the slicing solution to the slicing chamber (buffer tray).
7. Anesthetize the mouse in a desiccation chamber using isoflurane. Exact amount may vary according to the size of the chamber used, but for a small shoebox cage use a few drops (~3 - 4). Leave the mouse in the cage until rendered immobile (not responding to tactile stimuli; around 15 sec for the conditions described here). Perform tail and foot pinch tests to ensure the animal is deeply anesthetized, then decapitate before the heart stops beating (enhances cell viability).
Note: With appropriate justification, some laboratories obtain the authorization to perform live decapitation in order to minimize as much as possible excitotoxic processes and enhance cell viability.
8. Perform the dissection.
Note: The brain must be extracted rapidly (<45 sec).
    1. Using the scalpel cut the superficial skin on top of the skull from rostral to caudal.
    2. Peel the scalp on each side of the head.
    3. Using small straight sharp tip scissors, cut the interparietal plate along the lambdoid suture to remove the cerebellum. Remove the occipital bone.
    4. Using the same scissors, cut the sagittal suture.
    5. Slide the vessel cannulation forceps (or rongeurs if breaking a rat skull) below each parietal bones and pull to expose the brain.
    6. Using the curved hemostatic forceps, pinch the frontal bones to break them, then use tweezers or the vessel cannulation forceps to remove the broken bones. Cut and remove the dura mater as gently as possible as it can interfere with the dissection.
    7. Slide the spatula below the brain and gently pull the brain out of the skull to place it in the slicing chamber (buffer tray) previously filled with ice-cold ACSF. Let the brain cool down for 1 - 2 min.
    8. Prepare the dissection platform by filling a Petri dish with ice and some ice water to allow greater surface contact, cover it with its lid and place a filter paper on top. Wet the filter paper with cold ACSF.
    9. Once the brain is cooled down, place the brain on the ice-filled Petri dish, and quickly perform the appropriate dissection for obtaining the desired plane of slicing.

10. To obtain sagittal slices containing the nucleus accumbens (NAc), use a single edge razor blade to cut and remove the olfactory tubercles and the cerebellum if they are still present. Then, perform a sagittal cut of 2 - 3 mm from the lateral border of the right hemisphere to obtain the flat surface that will be glued on the specimen holding plate (see step 2.8.11).
    Note: Cutting only 2 - 3 mm from the lateral border of the hemisphere will allow the collection of slices containing the NAc from both hemispheres. The appropriate dissection will depend on the brain region that is investigated. Here, the dissection is performed so NAc neurons can be recorded in sagittal brain slices.
11. Rapidly glue (using cyanoacrylate glue applied to the specimen holding plate) the flat cut surface of the brain onto the plate according to the desired plane of slicing. To obtain sagittal brain slices see step 2.8.10.
12. Immediately place and secure the specimen holding plate in the slicing chamber so the brain is sliced rostro-caudally (for safety, set up the blade holder only when the specimen plate is secured).
13. Set the vibratome with appropriate slicing parameters (parameters used in the lab for the vibratome mentioned in **Materials**: speed 3 - 4, vibration 9-10, and slice thickness 250 µm).
14. Upon slicing, use a plastic-trimmed transfer pipette to transfer the brain slices to the recovery chamber (at RT) (see step 2.3). Recovery time may vary depending on the neuronal type that is under study (typically 30 - 90 min).

3. Recording Micropipettes and Rig Preparation

1. Refer to the specific guidelines of the puller user's manual to obtain the desired micropipette properties.
    Note: For MSNs, we use a pipette resistance range of 3.2-4.0 MΩ.
2. Oxygenate the ACSF and adjust the flow to 2 ml/min. Vacuum ACSF using a peristaltic pump or vacuum lines installed in the facility.
3. Turn on the perfusion heater controller and adjust the temperature settings in order to obtain the desired temperature (*e.g.*, 31.8 - 32.2 °C).
    Note: Temperature stability depends upon having both a constant ACSF level and constant flow velocity in the chamber. Since several biophysical properties of neurons (*e.g.*, input resistance, R_i, also called membrane resistance, R_m) are temperature-sensitive, maintaining a stable temperature is important.
4. Turn on computer-controlled amplifier, camera, micromanipulator, and microscope background light. If performing an experiment that requires electrical stimulation of the tissue, turn on stimulus controller and the isolation unit.
    Note: Some amplifiers from other manufactures recommend a "warm-up" before use, so it is recommended to consult the manual for the exact operating procedure.
5. Start camera capture, signal acquisition and amplifier software.
6. **Slice Placement and Visualization:**
    1. Using a plastic trimmed-tip transfer pipette, gently draw in one brain slice from the recovery chamber.
    2. Place the transfer pipette in the recording chamber and gently squeeze the slice out of the pipette onto the coverslip lining the bottom of the chamber.
        Note: As long as no overflow is occurring, it is harmless to have some ACSF from the recovery chamber spilling into the bath.
    3. Use forceps to alter the position of the slice so the desired area will be placed exactly in the center of the recording chamber. Use the microscope low power (4X) objective lens and the eyepiece for assistance in positioning.
    4. After the desired position has been achieved, secure the brain slice position with a slice hold-down (also known as a "harp") in the chamber.
    5. Switch to high power (40X) objective lens and lower it gently until contact is formed with the ACSF in the chamber.
    6. Use the fine adjustment wheel to bring the tissue into focus. While in contact with the ACSF, do not use the coarse adjustment wheel on the microscope as lowering the objective lens excessively can crush the slice or even break the cover slip lining the bottom of the chamber, which can cause ACSF to spill onto the condenser and damage it.
    7. When the focus is at tissue level, observe cells in the targeted region for shape. Dead cells are easily identifiable by their swelled plasma membrane and nucleus (**Figure 1E**). Healthy cells should appear as round, ovoid, or elliptical homogenous structures (**Figure 1E**).
    8. Look for a target cell. Mark it on the computer screen in order to help guide the recording micropipette. If using software such as QCapture, draw a square around the target cell by holding the left mouse click.
    9. Raise the objective lens so there will be sufficient space in the cone formed by the objective lens being in contact with the ACSF to place and move the recording micropipette.

7. **Micropipette Placement and Positioning**
    1. Using a 1 ml syringe, a nonmetallic microsyringe needle, and a dedicated filter, fill a micropipette with the internal solution prepared in advance according to the planned experiment (K^+-based or Cs^+-based internal solution, see steps 1.1., 1.2, and **Materials** for composition). Use enough solution so the internal solution comes into contact with the chloride-coated silver wire electrode within the micropipette holder.
        Note: The silver wire electrode can be chlorinated by soaking it in household bleach. Nucleoside triphosphates (ATP & GTP) can be added to the internal solution prior to use. Keep the syringe containing the solution on ice to prevent ATP/GTP degradation.
    2. Make sure there are no air bubbles in the micropipette as they can come out while the micropipette is in the tissue and obscure the slice.
    3. Place the micropipette in the electrode holder so the solution comes in contact with the silver chloride coated wire electrode.
    4. Tighten the pipette cap so that the cone washer will form a seal around the micropipette.
    5. Apply positive pressure before immersing the micropipette in the ACSF to prevent debris from entering the pipette.
    6. Place the headstage in the locked position (facing the chamber), and using the micromanipulator, guide it down towards the chamber so it is roughly under the center of the immersed objective.
    7. While moving the micropipette with the micromanipulator (set at medium to high speed), use the computer screen to locate the micropipette and guide it toward the location of the cell on the X-Y axis.
    8. Measure the micropipette resistance by applying a voltage step (*e.g.*, 4 mV for 100 msec), which can be accomplished manually or automatically via specific software such as 'bath' mode if using "Membrane Test" in Clampex software (see also step 4). In order to

make sure no air bubbles or any other foreign objects block the micropipette, apply positive pressure using the air-filled syringe (*e.g.*, 30 cc syringe) connected to the micropipette holder with polyethylene tubing.

9. After clearing the micropipette, perform a voltage offset to reduce pipette current to zero, which can be accomplished manually or via specific software such as 'pipette offset' on the computer-controlled amplifier commander.
   Note: This function will compensate for any voltage caused by concentration differences between the bath and the micropipette solutions (*i.e.*, liquid junction potential[20]).

4. Membrane Test

Note: This step applies to the amplifier mentioned in the **Materials**.

1. When using a computer-controlled amplifier commander, always set it on voltage-clamp mode to perform the membrane test.
   Note: When membrane test is set in "Bath" mode, the membrane test allows the measurement of the micropipette resistance and the seal resistance when the seal is formed.
2. Once the membrane is ruptured (see step 5.8), switch the membrane test to "Cell" mode so that series resistance (R_s) (also called access resistance, R_a), R_i and membrane capacitance (C_p) can be obtained.

5. Final Approach, Seal Formation, and Obtaining the Whole-cell Configuration

1. Using the fine focus wheel, start focusing down while lowering the micropipette gradually. Always focus down first and then lower the micropipette down to the plane of focus. This will ensure that the micropipette tip will not abruptly penetrate into the slice.
2. When the micropipette comes in touch with the surface of the slice, slow down the micromanipulator speed to medium-low mode.
3. Gently apply light positive pressure with the air-filled syringe connected to the pipette holder to clear any debris from the approach path.
4. Approach the cell either by alternating with the X-Y-Z control knobs, or by approaching diagonally (if the micromanipulator model allows it) where both X-Z axes are changed with the rotation of the Z axis knob. The latter method will prevent vertical compression of tissue.
   Note: Here, the goal is to approach the cell by inflicting minimal damage to the slice. When the micropipette is close enough to the cell a dimple appears (a round discoloring of the cell surface caused by the positive pressure applied through the tip of the micropipette) (**Figure 2**).
5. When the dimple appears (**Figure 2-1**), apply a weak and brief suction through the tube that is connected to the pipette holder suction tube in order to create the seal (**Figure 2-2**). Keep monitoring the membrane test.
   Note: If a partial seal is formed (<1 GΩ), injecting negative currents by lowering the holding potential (on the computer-controlled amplifier commander) can facilitate seal formation and reach gigaohms resistance ("gigaohm seal" or "gigaseal" >1 - 5 GΩ). The high resistance of the seal (>1 GΩ) will both limit noise contamination to the recorded signal and contribute to the mechanical stability of the patch.
6. While a gigaseal is forming, use the computer-controlled amplifier commander to bring the cell's holding potential as close as possible to physiological resting potential (V_{rest}) in order to prevent sudden changes once the membrane is ruptured. For example, MSNs are usually voltage-clamped at -70 or -80 mV (physiological V_{rest}: -70 to -90 mV).
7. After the gigaseal has formed, compensate for fast and slow capacitance manually or automatically. If using a computer-controlled amplifier commander such as Multiclamp commander, press 'Auto' for 'Cp Fast' and 'Cp Slow".
8. If the seal remains stable and above 1 GΩ (or injecting less than 10 - 20 pA to hold the cell at the desired membrane potential), apply a brief and strong suction through the same tube as in 5.5 to rupture the plasma membrane (**Figure 2-3**).
   Note: This might take several trials. A good membrane rupture is achieved when suction is performed strongly enough so that ruptured membrane does not clog the micropipette (which may lead to an increase in R_s during recording), but weakly enough in order to not draw in a large portion of the membrane or the cell.
9. After achieving a successful whole-cell configuration, regularly monitor the micropipette location to assess and correct for significant drift as it may lead to loss of the patch. Drift amplitude may vary according to several factors, *e.g.*, the quality of the rig installation and pulling forces on the headstage. Ideally, drift should be almost non-existent.
10. By switching to "Cell" mode in the membrane test, view different parameters of the cell such as R_i, R_s and C_p. Monitor these parameters during recording.
    Note: All these parameters can help assess initial health status of the cells and cell types (see "Membrane test" section, step 4).
11. Once the above steps are completed, remain in voltage-clamp mode to measure currents (*e.g.*, EPSCs, IPSCs), or switch to current-clamp mode if planning to measure changes in membrane voltage (*e.g.*, action potential firing). For the latter, inject either positive or negative current to hold the cell at the desired membrane voltage (to perform this step, refer to amplifier manual guide).

Representative Results

Temperature, a factor that is easily controlled by the experimenter, influences the biophysical properties of ion channels and receptors, and thereby the waveform of post-synaptic currents (PSCs) (EPSC and IPSCs) and the capability of neurons to elicit spikes. **Figure 3** and **Figure 4** show the effect of temperature on neuronal firing and the slope of evoked EPSCs (eEPSCs) respectively. The firing pattern (**Figure 3**) (*i.e.*, latency to the 1st spike, spike number, frequency, and action potential waveform) is shaped by a timed and coordinated opening and closing of specific voltage-gated ion channels (Na^+, Ca^{2+}, and K^+), a process sensitive to temperature. **Figure 3** shows how the mean spike number increases with temperature. Note that in the experimental conditions described here (MSNs recordings) although spike frequency does not seem to be altered at subphysiological temperature (28 °C), it significantly increases when temperature reaches physiologically relevant level (32 °C). **Figure 4A** shows an example of how the slope of eEPSCs, a parameter that is commonly used to assess synaptic strength, increases with temperature.

Although R_s can be somewhat controlled by the experimenter, *i.e.*, through an efficient membrane opening when transitioning from seal state to whole-cell configuration, R_s usually slowly increases during recording. This can be the result of various uncontrollable events, *e.g.*, membrane re-closing or debris clogging the pipette tip during the recording. An attempt to re-open the membrane by applying a slight suction, although it might compromise the patch, can sometimes help maintain a stable R_s. In all cases, because R_s changes can alter the waveform of the electrical signal under study, it must be carefully monitored, and particularly when recording PSCs (voltage-clamp mode). **Figure 4** shows that when R_s increases (**Figure 4B**), amplitude of glutamate receptor-mediated currents (eEPSCs) decreases (**Figure 4C, D**). Typically, experimenters discard the data when changes in R_s exceed 15% (*e.g.*, this laboratory), however some laboratories do so from a 20% change. This criterion must be indicated in the article's method section.

For a defined neuron, R_i can be influenced by several factors, including temperature, cell health, and quality of patch. Specifically, when R_i decreases, PSC amplitudes or capability of neurons to generate spikes also decreases. For example, **Figure 4E** shows that when R_i does not vary significantly, the number of spikes remain relatively stable (Neuron 1); and when R_i increases, the number of spikes increases as well (Neuron 2). Therefore and similarly to R_s, R_i must be carefully monitored, as 10% changes are sufficient to bias data.

As described above, it is critical to control or monitor temperature, R_s, and R_i during recordings. For example, observed changes in the signal that is under study (PSCs or firing) may be due to changes (or a lack of control) of these factors rather than the effect of experimental manipulations, *e.g.*, pre- *vs.* post-effects of drug bath application.

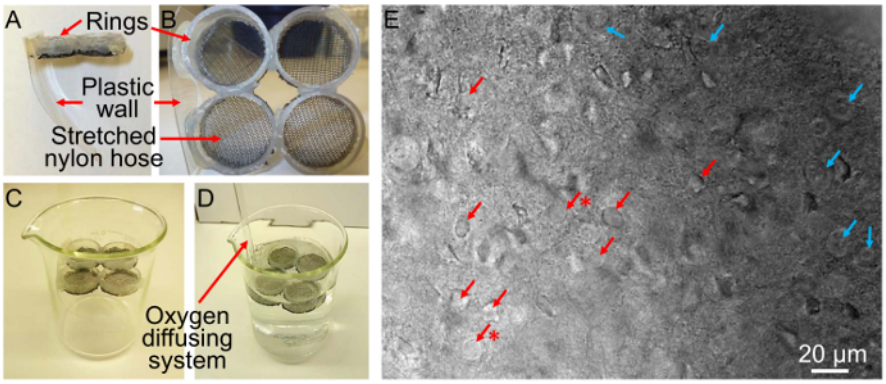

Figure 1. Custom-made Recovery Chamber (A-D) and a Picture of a Brain Slice at 400X Showing Healthy and Dead Neurons (E). A-D) The procedure to make a custom recovery chamber is described in step 2.1. **E)** Picture of NAc medial shell MSNs in a brain slice at 400X showing examples of healthy (red arrows) *vs.* dead neurons (blue arrows). Note that although some cells are indicated as healthy, their spherical aspect indicate that they may not be as healthy as desired (red arrows with asterisks). Final health status is assessed based on V_{rest} and R_i after achieving whole-cell configuration. Please click here to view a larger version of this figure.

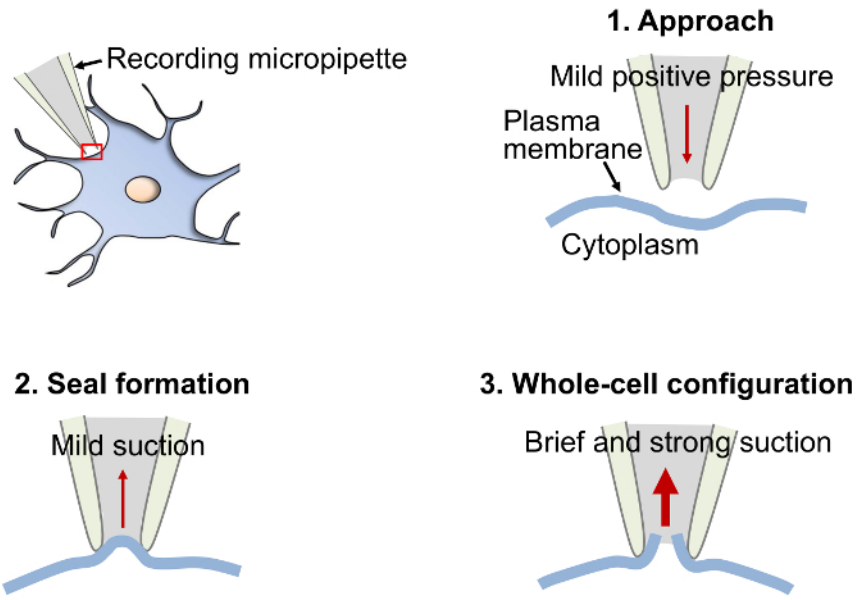

Figure 2. Diagram Depicting the Basic Procedural Steps to Obtain a Gigaseal and Establish the Whole-cell Configuration. When the micropipette is close enough to the cell to create a dimple in the plasma membrane (step 1, Approach), apply a brief and gentle suction to create a tight contact between the micropipette and the plasma membrane. If performed properly, the contact will strengthen and the resistance will increase and reach 1 GΩ (gigaseal) or more (step 2, Seal formation). Once the seal is stable and above 1 GΩ, apply a brief and strong suction to rupture the plasma membrane (step 3, Whole-cell configuration). Achieving the whole cell configuration will allow continuity between the cytoplasm and the micropipette interior. For details, see protocol step 5.1-5.8. Please click here to view a larger version of this figure.

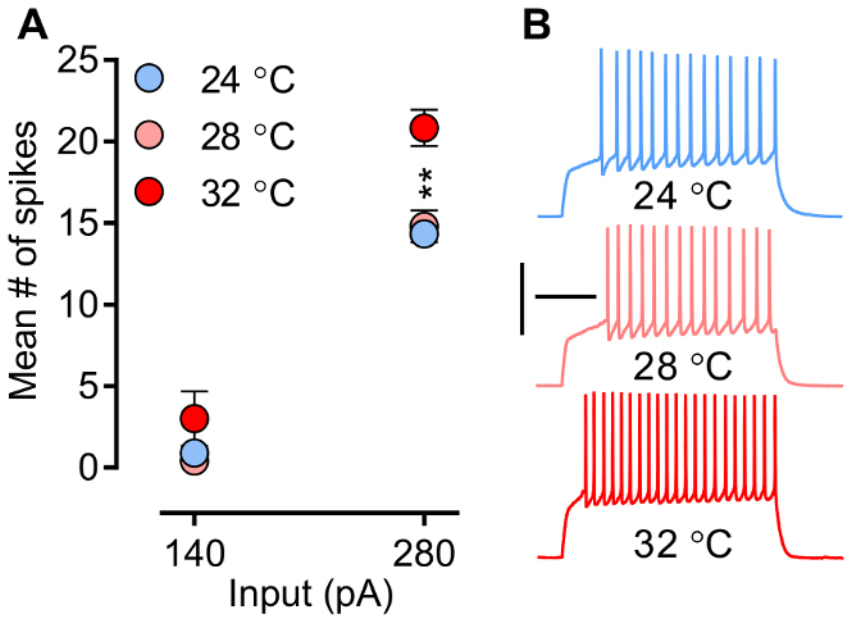

Figure 3. Neuronal Firing (Intrinsic Excitability) is Assessed in Current-clamp Mode. Here, a pre-defined and incremental series of current steps is given in order to elicit changes in membrane voltage, and thereby trigger action potentials. **A)** Mean spike number increases with temperature. **B)** Sample traces at 280 pA from NAc medial shell MSNs at three different temperature settings (24 °C, n = 9; 28 °C, n = 5; and 32 °C, n = 6). The temperature in the recording chamber directly affects spike frequency. However, note that although spike frequency does not seem to be altered at subphysiological temperature, it significantly increases when temperature reaches 32 °C, a physiologically relevant temperature. Neurons are held at -80 mV. Two-way ANOVA: interaction, $p < 0.0001$; temperature effect, $p = 0.0041$; post hoc tests: 24 °C and 28 °C are both significantly different from 32 °C, $^{**}p < 0.01$. Data are represented as mean ± SEM. Calibration: 200 msec, 50 mV. Please click here to view a larger version of this figure.

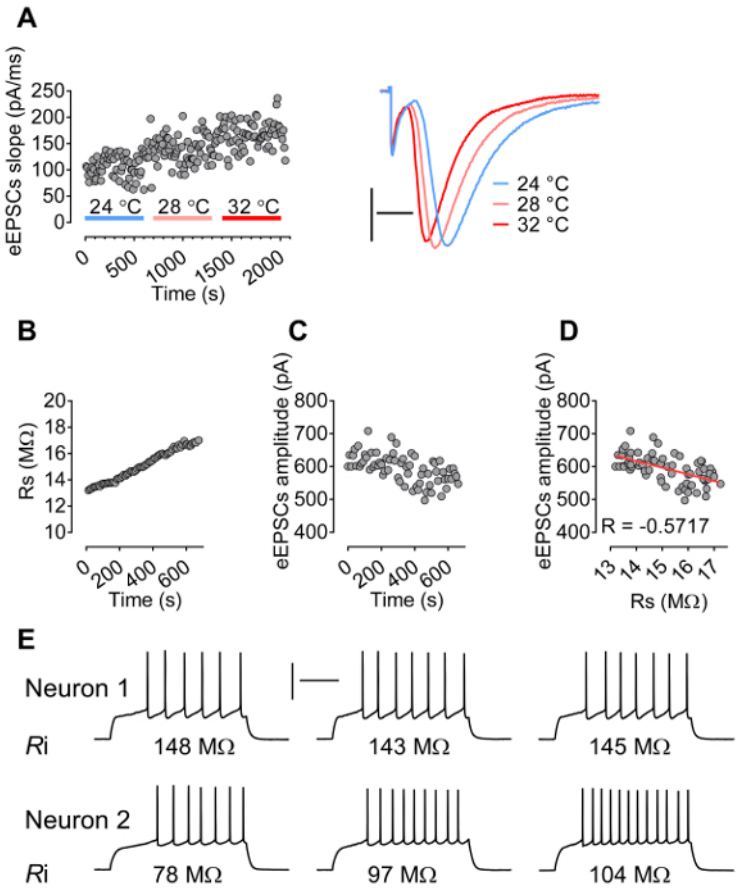

Figure 4. Effect of Temperature, R_s, and R_i on the Waveform of the Electrical Signal Under Study. A) Example of eEPSC amplitude from a single NAc shell MSN. Increasing the temperature from 24 to 28 °C and to 32 °C increases the slope of eEPSCs. Note that temperature-induced changes in eEPSCs slope occur rapidly. Here, eEPSCs slope is assessed in voltage-clamp mode. Calibration: 5 msec, 100 pA. **B-D)** Example of eEPSCs slope from a single NAc shell MSN. When R_s increases (**B**), the slope of eEPSCs decreases (**C**). **D)** Correlation analysis of eEPSC slope as a function of R_s. Pearson's R = -0.5717, p<0.0001. Neurons are voltage-clamped at -80 mV. **E)** Example of traces from two neurons showing the effect of R_i on the capability of the neuron to generate spikes. Neurons are current-clamped, and held at -80 mV. Calibration: 200 msec, 50 mV. Please click here to view a larger version of this figure.

Discussion

This protocol describes the basic procedure for performing whole-cell patch-clamp experiments on neurons in brain slices. However, the complexity, potential and sensitivity of this technique cannot be fully described in this article. Here, we have tried to delineate the most basic steps and underscore important parameters that must be controlled for achieving successful and rigorous whole-cell recordings. For further theoretical learning, many books and articles have been published on both whole-cell patch-clamp recording in brain slices[3,21-24] and on methods that can refine the solutions used[25-27] in order to enhance cell viability. In order to routinely perform proper recordings, improvement of technical skills through intensive practice is required. Nonetheless, with proper application of the steps mentioned, cells can be patched hours post-mortem, providing important information about changes in synaptic functions and intrinsic excitability.

In general, besides the importance of carefully preparing both ACSF and internal micropipette solutions, each step from the brain dissection, slicing, achieving successful whole-cell configuration, and obtaining rigorous and unbiased data requires intensive practice. Primarily, it is critical to generate healthy brain slices. Briefly, rapid dissection of the brain (ideally <45 sec), maintenance of a low temperature (0 - 2 °C) while slicing, and appropriate slicing solutions all play an important role in ensuring cell health. It is noteworthy to mention that slicing solutions may differ between laboratories and according to the cell type and/or brain region that will be investigated. When slicing the NAc or dorsal striatum, our laboratory and others use kynurenic acid for the slicing solution to minimize excitotoxic processes[28-33], however, other methods can also be used, such as sucrose-based solutions[34], high Mg^{2+}/low Ca^{2+} solutions[35], *etc*. These are only few examples and can be adjusted according to the sensitivity of the brain or brain region to excitotoxic processes (*e.g.,* due to age). For further information on solutions and cell viability, please see [25-27]. Ultimately, the concentration of anions, cations, and other drugs (*e.g.*, ascorbate, glutamate receptor antagonists) that compose slicing solutions is determined so that it mimics cerebrospinal fluid and minimizes as much as possible excitotoxic processes that occur during slicing. The protocol presented in this article describes standard solutions that were routinely used in authors' previous studies [28-31] when recording from MSNs in the NAc or the dorsal striatum in brain slices. Furthermore, proper adjustment of the osmolarity for both ACSF and internal micropipette solutions are critical for successful seal formation and maintenance of whole-cell configuration. To create a concentration gradient from extracellular solution to intra-pipette solution, ACSF osmolarity should be higher than for internal micropipette solutions. Ideally, the difference can range from 10 to 30 mOsm.

Achieving a successful whole-cell configuration is another important step for conducting efficient recordings. First, pipette capacitance can be adjusted once the pipette is placed in the bath. Although automatic settings are usually properly set, it is advisable to use fast and slow adjustments of cell capacitance with caution as these can damage the cell when not appropriately performed. Second, brief membrane suction that is necessary to rupture the membrane will lead to a significant opening of the membrane, and thereby allow a good communication between intracellular and intra-micropipette milieu. This will ensure that R_s will remain relatively stable throughout the recording. If using Cs-based micropipette solution, the membrane resting potential should be assessed immediately upon establishment of the whole-cell configuration (see step 5.8). Indeed, the diffusion of Cs^+ inside the cell causes the loss of membrane resting potential. To determine the proper resting potential, the liquid junction potential must be assessed [20]. However, the experimenter may report the resting potential that is observed after breaking the membrane (after step 5.8) and choose not to adjust for the liquid junction potential. In all cases, it must be mentioned in the article's method section. Upon establishment of the whole-cell configuration, C_p can also be obtained and can be used as an indirect parameter to assess cell health and/or cell type. Third, when recordings began, other parameters must be rigorously monitored. Critical factors that must be controlled when assessing neuronal excitability are temperature, R_s, and R_i.

As mentioned above, R_i and C_p can be indicative of cell health and/or cell type. For example, the plasma membrane, acting as an insulator, separates charge (resulting from the different composition of the intracellular and extracellular solutions), which together constitute the membrane capacitance. The larger the membrane surface (neuronal-specific), the higher the capacitance. It is then not surprising that specific neuronal types exhibit C_p and R_i (mathematically related to C_p) that are within the same range. R_s is directly related to the size of the pipette tip, and therefore is usually indicative of the quality or the size of membrane opening. Briefly, upon establishing whole-cell configuration, the cytoplasm becomes electrically continuous with the solution in the micropipette and completely isolated from the external medium. R_s (or R_a) originates from the resistance for the current to flow from pipette to cytoplasm. For some recording conditions (*e.g.*, current-clamp mode or voltage-clamp recording of voltage-gated ion currents), R_s must be compensated properly (refer to Ref.[3,21-24] or amplifier manual guide for proper R_s compensation).

As described in **Figure 4**, R_s is particularly important as it can dramatically affect the electrical signal waveform, *e.g.*, EPSC amplitude. Nonetheless, R_s must be carefully monitored for off-line interpretations of any observed effects. In case the membrane has not been ruptured properly, micropipette tip clogging or re-closure of the membrane may occur, in which case R_s increases and bias the waveform of the electrical signal under study (**Figure 4B-D**). In summary, numerous problems can be encountered while recording, and those usually fall under three categories: i) tissue-related, *e.g.*, increased cell mortality due to poor dissection, maladjustment of ACSF osmolarity, and hypoxia; ii) equipment-related, *e.g.*, noise and grounding problems, temperature control, slice and micropipette positioning, *etc.*; and iii) data interpretation, *e.g.*, observed changes can be the result of undesired experimental artifacts biasing the data like changes in electrical waveform-altering parameters (R_i, R_s, temperature, see **Figure 3 & 4**) rather than the result of experimental manipulations.

Although whole-cell recording in brain slices is a powerful technique for assessing experience-dependent plasticity, this approach limits the interpretation of data. In particular, three important limitations of whole-cell recording technique are that: (i) changes in function and expression levels of specific proteins (*e.g.*, ion channels) cannot be distinguished; (ii) because this technique assesses current flow through the whole membrane (or substantial part), it does not provide accurate sub-cellular localization of the ionic currents or changes that are observed; and (iii) invasiveness of whole-cell configuration leads to the dialysis of the cell content, and thereby to the disruption of intracellular molecular machinery necessary for some phenomena to develop or to be expressed. One way to avoid dialysis is to use sharp electrode recordings or the perforated patch technique[3,21,23]. Regarding the latter, pore-forming antibiotic molecules such as nystatin can be added to the pipette solution. Formation of these pores will allow the recording of currents without disrupting the second messenger mechanisms within the cell. Nonetheless, recent advancements in nanotechnology and the development of nanoelectrodes[36] provide powerful tools for improving neuronal recordings. Such technological advancement in neuroscience are still under development and are now putting in our reach the possibility to perform patch-clamp and intracellular recordings with minimal invasiveness, *i.e.*, keeping the intracellular milieu intact, and investigating the functions of ion channels within sub-cellular compartments that were so far not accessible with classical patch-clamp electrodes[37].

Disclosures

None of the authors have competing interests or conflicting interests.

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