A living guide to the theory, methods, and practical details of slice electrophysiology. Contributions welcome!

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Patch clamp recording in brain slices allows unparalleled access to neuronal membrane signals in a system that approximates the in-vivo neural substrate, while affording greater control of experimental conditions. In this document we discuss the theory, methodology, and practical considerations of such experiments including the initial setup, techniques for preparing and handling viable brain slices, and patching and recording signals. A number of practical and technical issues faced by electrophysiologists are also considered, including maintaining slice viability, visualizing and identifying healthy cells, acquiring reliable patch seals, amplifier compensation features, hardware configuration, sources of electrical noise and table vibration, as well as basic data analysis issues and some troubleshooting tips.

Contents:
Neurons use electrical signals to process and transmit information. These electrical signals are part of the “currency” of neural processing, and appear in multiple forms that can be recorded in a variety of ways. The simplest recordings are measurements of extracellular potentials, which are generated by current flow between different parts of individual neurons. These recordings can reflect either the spiking of neurons (as in single-unit or multi-unit recordings), or can be field potentials that are composed of a mixture of time-locked synaptic currents and cell spiking in a population of cells near the recording electrode. Neither of these types of recordings require access to the interior of the cells.

The electrical signals recorded outside of single neurons are generally very small, in the range of microvolts (\( \mu \)V) to a few millivolts (mV). While these are useful for understanding the coding of information in spike trains, or the spatial and temporal organization of synaptic inputs in laminar structures, they do not readily reveal the underlying mechanisms of synaptic integration or spike generation. A modern electrophysiological method called patch clamp, permits the measurement of transmembrane voltage and current with high resolution and low noise. What sets patch clamp apart from other methods, such as sharp-electrode intracellular recording, is its use of a tight seal between the recording electrode and the cell membrane. This seal essentially blocks external currents associated with the electrical activity of surrounding cells, allowing the experimenter to record even small subthreshold events in a single neuron. The development of these techniques to create high-resistance seals with the micrometer-sized pipette tips needed to record from small vertebrate neurons (Neher et al., 1978; Hamill et al., 1981) revolutionized electrophysiology.

There are several variants of the patch clamp method. The most commonly used method is best described as whole-cell recording. Of the other variants on the patch clamp method, the next most commonly used configurations are the cell-attached patch, followed by outside-out and inside-out patch recording. In this chapter, we will focus on the technical aspects involved in making whole-cell patch clamp recordings, and only briefly touch on cell-attached and outside-out patches.

When studying electrical signaling in neurons, there are two widely-used recording methods. Current clamp refers to recording the voltage across the membrane of individual cells. Voltage clamp refers to measuring the current that is associated with conductance changes in the membrane in response to voltage changes. Current clamp and whole-cell voltage clamp require electrical access to the interior of the cell. Both current clamp and voltage clamp can be very informative about the mechanisms by which cells fire particular patterns of action potentials or by which synaptic inputs change with time and are integrated. These recording methods also permit a variety of manipulations that can yield insight into the cellular physiology and biology of specific neurons, revealing mechanisms underlying processes such as learning, memory, decision making, hormonal regulation, the construction of motor activity patterns, and perception.

In this chapter, we focus on the use of brain slices when making patch clamp recordings. Brain slices are an in vitro preparation, created by sectioning fresh brain tissue (Yamamoto and McIlwain, 1966). Slices offer a number of advantages for analysis of cellular mechanisms and small networks, including excellent optical access to even small cellular elements such as dendritic spines, mechanical stability, and the ability to control the extracellular environment for ionic or pharmacological manipulations. Slices have an advantage over dispersed neuronal cultures in that they can retain much of the in vivo network structure and connection specificity, as well as the normal complement of cells. However they have disadvantages as well. Brain slices can only be used for a few hours after preparation, and they lack
many of the normal activity patterns that can be observed in vivo. Regardless, over the past 30 years, slices have been instrumental in deepening our understanding of cellular and synaptic physiology, and have significantly contributed to understanding local neuronal networks.
The patch clamp technique achieves superior recording fidelity by creating a high-resistance seal between the pipette glass and the cell membrane, then rupturing the membrane within the lumen of the pipette to allow measurement of electrical signals internal to the cell. In the methods we describe, a thin slice of brain tissue is held in a small chamber perfused with warm, oxygenated fluid, which approximates cerebrospinal fluid. This arrangement keeps the brain slice alive for several hours after dissection, during which the experimenter will attempt to patch neurons within the slice. The recording chamber is mounted on a microscope stage to allow the experimenter to visually identify cells within the slice. Glass patch pipettes, filled with an electrode solution approximating the composition of cytosol, are then positioned using precise micromanipulators to form a seal with the cell membrane.

By applying suction to the patch pipette, the membrane is ruptured, granting electrical access to the cell interior. However, this access is imperfect due to the electrical characteristics of the pipette. The goal of patch clamp, in the most general sense, is to measure and manipulate the voltage $V_m$ and resistance $R_m$ of the patched neuron (figure 1). This goal is confounded by the combination of electrical resistance at the tip of the pipette $R_s$ and capacitance across the cell membrane $C_m$ and pipette walls $C_p$. These confounds are addressed through a variety of techniques that are discussed throughout the chapter.

Fig. 2.1: Figure 1. Schematic of glass electrode patched onto cell with equivalent circuit diagram. : Voltage inside pipette; this is the voltage controlled or measured by the amplifier, less the electrochemical junction potential. : Pipette capacitance; typically a few picofarads. : Series (or access) resistance; this is the resistance separating the pipette from the cell body and is due mainly to the narrow pipette tip and organelles that may be blocking it. : Seal resistance; the resistance of the region of contact between the pipette and the membrane. To make quality recordings, this must be $> 1 \text{ G}\Omega$. : Membrane voltage; the voltage of the interior of the neuron relative to the bath. : Cell membrane capacitance. : Cell membrane resistance; also called input resistance. : Bath voltage, as measured by the ground electrode.
CHAPTER 3

Equipment

A typical patch clamp recording setup is shown in figure 2, and minimally consists of an amplifier, a microscope, a data acquisition system, a solution delivery system, and a chamber that holds the brain slice.

Fig. 3.1: Figure 2. A minimal patch electrophysiology rig. Left to right: Oxygenated ACSF is siphoned through a fluid heater and into the recording chamber where it continuously washes over the brain slice. Fluid is then aspirated out of the recording chamber and into a waste flask. A patch clamp amplifier headstage is mounted to a micromanipulator and holds the patch pipette, which currently impales the brain slice (detailed in figure 3). The headstage output is amplified, digitized, and finally recorded on a computer.

Fig. 3.2: Figure 3. Patch recording equipment. A) 63x ceramic, water-immersion objective. B) Silver chloride wire connected to headstage ground output. C) Heated aluminum holder for recording chamber. D) Plastic recording chamber with glass coverslip on the bottom. E) Brain slice bathed in warm, oxygenated ACSF. F) Glass patch pipette filled with electrode solution. G) Electrode holder. H) AgCl electrode wire. This wire fits inside the patch pipette and makes electrical contact with the electrode solution as well as the I) gold pin which conducts electrode potential into the amplifier headstage. J) Pressure control tube. This allows the experimenter to increase or decrease the pressure inside the patch pipette. K) Amplifier headstage.

3.1 Amplifiers

Currents in neurons span a wide range, from picoamperes (pA) for single channels and miniature synaptic currents, to tens of nA for currents through some voltage-gated ion channels. Similarly, voltages in neurons range from tens of μV for synaptic potentials to about 100 mV for action potentials. Consequently, specialized equipment is required to
amplify the signals before they are recorded. The amplifiers used in electrophysiology are uniquely suited to recording such signals while introducing as little noise as possible.

Whereas early patch clammers had to build their amplifiers, a variety of commercial amplifiers are now available that have capabilities and specifications that would be hard for an individual laboratory to duplicate. The choice of an amplifier should be guided by the goals of the experiments, as not all amplifiers have the same merit figures for noise, or bandwidths, and some are better suited for particular experiments. The amplifiers consist of two main parts. The headstage is usually placed close to the recording site, and may hold the electrode directly. The headstage is connected to the main part of the amplifier, which provides the controls, input and output signals, and usually interfaces with a computer. The headstage also provides a high-quality ground output that is used to ground the fluid in the recording chamber and provide a clean reference for measuring potentials.

If the goal is to make current clamp recordings, a voltage-follower headstage is recommended. This type of headstage has a very high input impedance, low input capacitance, and a fairly wide bandwidth. Usually these headstages are also equipped with a current injection circuit, and circuitry that allows compensation of the electrode capacitance. As a result, they can faithfully report the voltage that appears at the input terminal, or at the wire that is inserted into the electrode. With properly prepared electrodes, the measured voltage can be nearly identical to the voltage at the tip, although it will be affected by electrode capacitance, which creates a low-pass filter that will attenuate rapid changes in voltage, as well as the voltage drop introduced by any current flowing through the electrode.

When the goal is to make voltage clamp recordings, the headstage is usually configured as a current-to-voltage converter. Because of the bandwidth limitations that arise when high gain is needed (for example, pA to V), the amplifiers include additional internal circuitry that helps to extend the bandwidth of the amplifier. Such headstages can also be operated in a mode that allows voltage measurement (as in current clamp), but limitations of the circuit can result in distortions of the voltage waveform (Magistretti et al., 1988).

Some modern amplifiers have dual-function headstages that provide very good performance in both voltage and current clamp, making it possible to collect data in both modes from individual cells, and even to switch between modes in the middle of a sweep. For example, this can be useful if you wish to elicit an action potential or a train of action potentials, and then measure the currents associated with the ensuing afterhyperpolarization.

Many modern amplifiers offer computer control, while older amplifiers were designed in an era when computers were used only to collect data from the amplifier. Having digital control of the amplifier and some internal signal processing, such as filtering, can be advantageous since it allows the experimenter greater flexibility and can simplify the execution of particular experimental manipulations. On the other hand the use of digital circuits, including digital signal processing, in the amplifier can introduce additional noise sources that can be difficult to eliminate and sometimes are hard to detect unless a wide-band oscilloscope is available. High frequency noise that is above the Nyquist frequency sampling limit imposed by the rate selected for the analog-to-digital (A/D) conversion process becomes folded down into lower frequencies, and adds to the apparent low frequency noise. (The Nyquist limit is one-half of the A/D conversion frequency, per channel. For example if you sample the voltage channel every 100 μsec, then the sample frequency is 10 kHz and the Nyquist limit is 5 kHz. All signals coming into the A/D converter should be low-pass filtered below 5 kHz. In practice, for 5 kHz filtering, the sampling rate should be at least 20 kHz.) We have observed high frequency noise on the output (following internal filtering) of a digitally controlled amplifier, and suggest that for recordings under conditions where low noise is demanded by the experimental measurements, analog amplifiers without internal digital processing may be preferable, or additional external filtering following the amplifier may be required.

### 3.2 Electrode holders

Properly functioning and clean electrode holders are critical to the success of patch clamp recording. The holders are typically made from a polycarbonate shell, and are designed to provide mechanical stability for the electrode, low capacitance, electrical insulation, and a low-noise connection to the input of the headstage (figure 3G). Holders should be cleaned regularly following the manufacturer’s instructions, and they should always be cleaned whenever the electrode solution gets into the holder.
The wire in the holder is usually made from silver and is coated with a thin chloride layer. The wire should be cleaned by carefully polishing with 600 grit polishing paper and washed with ethanol to remove any residue and oils. At this point, it is recommended to handle the wire with either tweezers or gloves. The chloride layer can be created by placing a cleaned wire into diluted bleach for a day or two. The last 1-2 mm of the wire should be cleaned to bare silver so that it can make good contact with the gold pin of the electrode holder (figure 3I). In some experiments, solutions are used which have a large electrochemical potential against silver-chloride, so in this case it is important to provide a bridge, usually made of agar, and 3M KCl to connect to the silver wire. Several published protocols can be found in the literature (Shao and Feldman, 2007; Snyder et al., 1999). Chlorided silver wires may also be used as the headstage ground wire, in lieu of commercially available ground wires with AgCl pellets attached.

Patching requires the application of negative and positive air pressure to the back of the pipette, so the holder will have a single port. We use a 1-2 cm length of silastic tubing to provide a flexible joint, and then ~20 cm of polyethylene tubing to reach the table or the armrest on the isolation table (figure 3J). There are several methods of delivering air pressure. Some prefer to use a mouth-pipette, which provides excellent control over the pressure. We often use a 1cc tuberculin syringe (without the needle), and an adapter to connect to the tubing. With practice, the syringe can be used to make small changes in pressure that are either slow, for making seals, or fast, for rupturing the cell membrane.

When tightening the holder onto the headstage, it is often important to be careful to not make the holder too tight, as relaxation of the teflon coupler over time can introduce slow movement of the electrode. Similarly, the rubber retaining ring in most holders should not have much pressure on it when the cap is tightened, so that it does not relax and twist the electrode. A gentle, but firm finger tightening is sufficient.

Also, there is usually a gasket that seals access to the AgCl electrode at the back of the holder. This gasket is important to provide a pneumatic seal, and to keep fluid out of the connection to the headstage.

3.3 Microscope

Patch clamp recordings in brain slices are most successful when the cells to be recorded can be directly visualized, although “blind” patching is a technique that can be used under some circumstances. Direct visualization is usually achieved by using a fixed-stage, upright microscope with electrically insulating (for example, ceramic) water immersion objectives. The fixed microscope stage is often replaced with a fixed platform or a set of gantry towers that are fixed to the vibration isolation table, and the microscope placed on a translatable platform. An alternate approach is to fix the microscope to the vibration isolation table, and to have a translatable stage to hold the preparation and manipulators. This latter approach is commonly used when introducing laser light into the microscope, for example for 2-photon microscopy, since the optical platform must remain well aligned with the light source. It is usually not necessary to have more than 2 objectives on these microscopes. A low magnification (2.5-5X) long-working distance objective can be used to select the region of the slice and find the electrode for coarse positioning, while a high magnification (40X or 63X) 2-3 mm working distance water immersion objective is needed for visualizing and patching cells. It is best if the objectives can be exchanged and returned to the same focal position without requiring refocusing the microscope, and this is achieved with a sliding objective positioner. However, it is also possible to use a rotary objective turret, although this will require refocusing for each change of objective.

For young tissue or thin preparations, the use of Nomarski differential interference contrast optics (DIC) can help with the visualization of cell membranes and fine processes such as dendrites. In addition, the use of infrared (IR) or long-wavelength illumination reduces light scattering, and can be used to gain better visualization into deeper regions of the tissue. However, these long wavelengths also require a camera with good infrared sensitivity (usually a CCD camera) to actually visualize the preparation. Some CCD cameras have IR filters in front of the detector that reduce their natural IR sensitivity, and these have to be removed and replaced with an appropriate IR-transmitting filter.

For older, thicker or more heavily myelinated tissue, the use of DIC optics has little advantage, since the light polarization is partially randomized by the tissue. In this case, asymmetric or gradient illumination, followed by appropriate adjustments in image contrast on the monitor, works nearly as well. With modern cameras that have dynamic ranges of 12 or 16 bits, the contrast can be greatly increased around a mean level, allowing visualization of details that would be otherwise lost. The asymmetric or gradient illumination also helps increase the contrast. In the simplest case, such illumination can be obtained by adjusting the condenser off center from the light path, or by using a high-power IR.
light-emitting diode placed below the preparation and off the optical axis. We have used simple asymmetric illumination and image contrast adjustments to perform visualized patch recordings from neurons in 300 μm thick slices of adult mouse (> 80 days old) brainstem nuclei that have heavy myelination. In some cases, having software or hardware adjustments that allow the displayed image contrast to be enhanced, while subtracting background light levels, can also help visualize cells.

Blind patching is a technique whereby the patching is done without direct visualization (Blanton et al., 1989). In this case, the only feedback available is the electrical signal from the electrode. Blind patching can be done in vivo, or in thick tissues where no visualization is possible. However, the success rate is lower than for visualized patching.

Several approaches that fundamentally consist of optical workbenches with objectives and reconfigurable mechanical arrangements are now available. These may be preferable in some situations as they allow the rig to be changed to meet the demands of specific experiments or new optical configurations much more easily than if a dedicated microscope with enclosed optics is used.

### 3.4 Manipulators

Positioning the electrode requires the use of manipulators that allow smooth motion in 3 axes at the sub-micrometer level. Typically, this is achieved with mechanical, hydraulic, piezoelectric, or stepping motor manipulators. Each type has some advantages and disadvantages, but the current trend is towards piezoelectric and stepping-motor driven manipulators that have remote control units so that moving the electrode does not require touching the manipulator itself. The manipulators should be mounted securely on the same platform as the recording chamber. These manipulators will also often have a mechanical arrangement that allows the headstage and electrode holder to be easily brought out from under the objective to change electrodes.

### 3.5 Vibration Isolation

An important component of any patch clamp setup is reduction of building vibration. Most buildings have vibration that arises from air handling systems and nearby roadways (or railroad tracks), as well as foot traffic in the hall. Vibration that is transmitted to the electrode can make patching difficult or impossible. For patch clamp recording, tables can vary in size, although we typically use 30x48” tables with 4” deep tops to allow sufficient room for the microscope, light sources and ancillary equipment that is on the table. Smaller tables can be used as well, if they are located in an area with less vibration. Larger tables are only needed if there will be additional optics, such as lasers, on the table. The tables are “floated” using nitrogen supplied through a regulator. House air systems can be used if they have sufficient pressure, but it is recommended to provide an air filter and a water trap in the system to avoid mishaps that could damage the table.

### 3.6 Other hardware

Stimulators: One of the most common ways to activate pathways in a brain slice is to electrically stimulate the tissue using a bipolar or concentric electrode, usually no more than 250 μm in diameter. Simple stimulating electrodes can be made by twisting small gauge (22-30 ga) teflon-coated platinum wire together, cutting the ends flush with a sharp razor, attaching it to a twisted pair of wires that go to the stimulator, and inserting the platinum end through a fire-polished Pasteur pipette until the ends stick out of the pipette. A small drop of glue at the end of the pipette will help hold the wires in place. Commercial electrodes are also available in a variety of sizes and configurations from several vendors. The basic requirement for the stimulator is that the current (or voltage) and pulse duration be controlled. Typical pulse durations are 0.05 to 0.2 msec per stimulus. Voltages range from <1 to ~100 V, or if using constant current pulses, from 10’s of μA up to about 1 mA. Stimulus parameters are highly dependent upon the tissue type as well as the electrode configuration. The stimulator hardware consists of the pulse generator (this can be a computer or a stand-alone unit), along with an isolation unit that drives the electrode through a circuit that is electrically isolated.
from the rest of the setup. This isolation occurs either through an optical coupler, or a transformer. The output of the isolation unit should not be grounded.

Pipette puller: The preparation of the patch pipettes requires a puller suited to the purpose. Modern pullers are microprocessor-based devices that can create a pair of patch pipettes from glass blanks by heating the glass with either a filament or a laser, and cooling the glass with a jet of air. The choice of puller is not critical, as long as it is easy to modify the pulling pattern of heating and cooling and force. With some pullers it may also be necessary to have a microforge to fire-polish the tips of the electrodes. We have not found this necessary with a laser puller.

Slicers: The preparation of brain slices requires a slicer. To minimize damage to the tissue, slicers that use a vibrating blade that can be advanced through the tissue with a controlled rate, oscillation speed and distance, and angle, seem to work best. The slicer should be dedicated to brain slice preparation, as contamination with fixative or chemicals that might be encountered during histological processing is not conducive to the preparation of healthy, living brain slices.

Water filtration system: It is extremely important to have high quality water when preparing solutions for brain slices and patch clamp recordings. Contamination of the water used to make solutions by water treatment chemicals, bacteria, or various ions and salts that may be accumulated along the way, can lead to unexpected results and complications. The type of filtration system that is needed depends on the quality of water that is available to feed the system. For example, if your building provides reverse-osmosis treated water to each lab, then the system can be limited to the filter components needed to polish the water to a high quality. However, if you only have utility supplied water, you may need a reverse osmosis unit to generate a local supply that can be used to feed the polishing system. In our opinion, simple steam distillation of water is not sufficient. In addition, the water should be filtered with a 0.22 μm tissue-culture grade filter at the last step prior to use. It is also possible to purchase water, although this would be an expensive option.
Experimental Procedure

4.1 Recording Solutions

Slice dissection and recording takes place in artificial cerebrospinal fluid (ACSF) solutions (See Table 1). These solutions consist of salts, pH buffers, energy sources, and divalent ions. Experimenters often adjust their solutions for different purposes. The replacement of sodium with NMDG or sucrose during slice preparation can improve the survival of cells in the slice (Tanaka et al., 2008), both in the brainstem and cortex. Replacing sodium ions prevents cells from spiking, reducing excitotoxic damage, and reduces the activity of Na+/K+ pumps, reducing metabolic demand. Sodium pyruvate and myoinositol provide alternate entry points into cellular metabolism, and their addition seems to improve cell survival. Ascorbic acid acts as a free radical scavenger and may help cell survival. Ascorbic acid may be required in experiments that use drugs sensitive to free radicals.

While the divalent ion concentrations listed in Table 1 are common in brain slice experiments, it is important to recognize that these are significantly higher than those occurring in vivo. Recent experiments in the medial nucleus of the trapezoid body at the calyx of Held have revealed how the use of these high divalent concentrations can lead to conclusions from in vitro studies that may not apply in vivo (Lorteije et al., 2009). The use of high divalents dates from the early days of slice recording, where it was found that elevated calcium seemed to help with forming seals between cell membranes and pipette glass. The elevated calcium concentration also increases release probability at synapses, which makes synaptic responses larger and more reliable. However, solutions with a more “physiological” calcium and magnesium, such as 0.8 mM CaCl2 and 1.3 mM MgSO4, can ease the interpretation with respect to in vivo conditions. Alternatively, it is important in some experiments to provide both high release probability and low polysynaptic transmission, which can be achieved by using even higher calcium and magnesium (4 mM). Such concentrations are often used in photostimulation experiments where spatial maps of connectivity are the primary goal and time constraints prevent repeating maps many times to measure connections with low release probability.

When making ACSF, the components are added in the order given in Table 1, and are weighed out on a balance (with 0.1 mg resolution) as accurately as possible. Between uses, salts are stored in a dessicator to minimize water absorption. The divalent ions are added last, just before use, to prevent precipitation. This solution is either warmed to 34 °C in temperature controlled water baths, or chilled in a freezer for 30-45 minutes before use. The solution should be oxygenated and pH equilibrated by gassing with 95% O2 - 5% CO2. The pH should be between 7.35 and 7.40. Inadequate gassing can lead to a more basic pH, and can be a cause of poor slices. We do not recommend making “stocks” of the incubation and recording solutions for three reasons. First, without equilibration with 95% O2 - 5% CO2, the solutions will become basic over time, which causes the divalent ions to precipitate out of solution. Second, with the sugars in the solution, it does not take long to get bacterial growth, and bacterial endotoxins are not conducive to good slice health. Third, if a stock is incorrectly prepared, several days’ (or even months’) worth of experiments may be disrupted. With practice, it only takes about 15 minutes to prepare 1-2 L of solution, and this is readily done on the morning of each experiment.
4.2 Electrode Solutions

Pipettes are filled with an electrolytic solution whose primary function is to conduct current between the electrode and the interior of the cell (or exterior membrane surface). Because patch electrodes also facilitate the exchange of soluble molecules, pipette solutions used in intracellular recordings are designed to mimic the contents of the cytosol (or CSF, in the case of cell-attached patch) to preserve the natural function of the cell. Pipette solutions vary according to the goals of the experiment, and this is an aspect of the experimental design that requires some consideration. Standard recipes for potassium gluconate and cesium solutions are given in Table 2. Potassium gluconate is generally used for current clamp experiments, when normal cell activity is desirable. For voltage clamp, cesium based solutions are often used because Cs+ ions block K+ channels, which increases the length constant of the cell (see Section 4.7.6). QX-314, a Na+ channel blocker, is often added to cesium-based solutions to block action potentials.

Electrode solutions are usually used in small quantities, and so are prepared in batches, and stored in single use aliquots of 100 or 200 $\mu$l at -80 °C. The usable lifetime of an electrode solution is ~3 months, although this may vary with the content. When retrieving the solutions from the freezer, it is important to vortex each solution, since during freezing components may come out of solution or a gradient in osmolarity may appear. The solution should then be centrifuged briefly to pull down any debris. Alternatively, or in addition, the solutions can be passed through a 0.22 $\mu$m filter. The solution is then stored capped in an ice bath during use, and is discarded at the end of the day. The pipettes are filled as needed immediately prior to use. We have found that filling pipettes in batches that sit all day prior to use usually leads to low success rates.

The filling solutions used for patch work are often slightly hypoosmotic with respect to the bath. There are two reasons for this choice. First, as a seal is made onto a cell, the higher osmolarity in the the cell relative to the pipette helps push the cell membrane closer against the pipette tip, aiding in forming a seal with less hydrostatic pressure. Second, a portion of the cell’s osmolarity is made up of elements with low diffusibility, so using a lower osmolarity in the pipette itself is less disruptive and less likely to lead to cell swelling over time.

Many electrode solutions used in patch clamp recording have unusual combinations of ions, particularly anions, that introduce electrochemical potentials both at the electrode tip and between the solution and the wire that connects to the headstage. These potentials interfere with the accurate measurement of the cell membrane potential. While the potential between the wire and the electrode solution can be eliminated by adjusting the offset controls of the amplifier easily enough, the tip potential can be problematic and it is necessary to know its value in order to know the proper membrane potential of a cell in either current or voltage clamp. The tip potential is different when the cell is in the bath, and when it has access to the cell, since the electrochemical potential in the two conditions is different. Typically the tip potential has to be measured. One way to do this is to place a filled electrode into a bath containing the electrode solution, and using an amplifier in current clamp, measure the potential. Next, while keeping a very slight amount of positive pressure on the electrode to minimize mixing with the bath solution, exchange the bath for a normal extracellular solution, and measure the potential again. The difference is the tip potential that would exist when the electrode is in the bath, but which (mostly) dissipates when the electrode is in the cell. Typically, for a 140 mM K-gluconate based electrode solution with low (4-8 mM) KCl, this potential will be about -12 mV. This means that, if the electrode potential is set to zero outside the cell, and measured at -50 mV (or clamped to -50 mV) when the electrode accesses the cell, the actual resting (or holding) potential is -62 mV.

4.3 Patch Electrodes

Standard patch clamp electrodes are made from borosilicate glass pipettes which are heated and stretched to form a pipette with a blunt tip ~1-2 $\mu$m in diameter (figure 4). Since patch electrodes can only be used once and have a very limited lifespan, they are most often made on-site using programmable pipette pullers. These pullers work by heating and melting the center of a glass pipette and pulling the two halves away from each other to form two identical tapered electrodes. The exact shape of the electrode is determined by the timing and power of heating as well as pulling force. Two competing factors must be considered and balanced when pulling pipettes. First, the shape of the pipette determines its electrical resistance and capacitance, both of which should be minimized to improve recording fidelity.
Typical pipettes have a resistance of 2-10 MOhm and capacitance of only a few pF. Second, pipette tips that are too large (>2.5 um) or too small (<1 um) may be difficult to patch with or unable to maintain long-term access to the cell.

![Fig. 4.1: Ideal patch pipette shape. The pipette is pulled in multiple stages. The first stage is a long, narrow pull which thins the tip to help it fit under the objective. The following stages produce a rapid taper (about 15°) to reduce resistance and end with a 1.5 μm tip.](figures/rendered/04_pipette.png)

The resistance of a patch pipette is inversely proportional to both the angle and diameter of the tip (approximated as a conical conductor). Thus to reduce resistance, both values should be maximized; however, tip diameters greater than ~2 μm may be difficult to patch with. With the tip pulled to ~15°, it should be possible to produce pipettes with low resistances around 2-5 MΩ. Such low resistance is crucial for voltage-clamp experiments measuring currents greater than a few hundred pA, such as evoked synaptic currents or currents through ion channels. For current clamp recordings, higher resistances up to 20 MΩ may be acceptable as long as the access resistance to the cell is stable for the duration of the recording.

Another factor to consider is that the pipette must fit comfortably between the objective and the recording chamber (see figure 3). For objectives with a short working-distance, it may be necessary to reshape the pipette tip. We shape tips by pulling in multiple stages: the first stage produces a long, narrow pull and subsequent stages taper the tip more quickly (figure 4). The long first stage provides more room under the objective but does not significantly increase the resistance because the last 100 μm of the tip accounts for roughly 95% of the total resistance.

The choice of glass can be important in some experiments, and so familiarity with the different compositions made by different manufacturers can be helpful. Typical pipette glass is borosilicate-based, and will have an outside diameter of 1.0-2.0 mm, and an inside diameter of 0.5-1.6 mm. The thickness of the glass wall is generally maintained in proportion to the diameter of the tip as the glass is pulled, and for patch-clamp recording, thicker walled glass is generally preferable to reduce pipette capacitance. Patch pipette glass often includes a small interior filament that acts as a wick to draw electrode solution into the tip. Some pipettes are produced with the raw ends fire-polished. This is necessary to prevent the otherwise sharp glass from scratching the thin AgCl layer on the silver wire. Pipettes can be easily polished by holding the back end over a small flame for a few seconds, until the glass glows orange.

Producing clean, correctly-shaped pipettes often requires much trial and error. Once pipettes are pulled, they may be individually inspected under 10X and 40X objectives. This screening process is used both to guide adjustments to the puller to obtain the desired diameters and tip shapes, and to discard pipettes that are broken, fouled or which fall outside the desired tip diameter. It is of the utmost importance that the tips of patch electrodes be clean. Thus, pipette blanks should only be handled by the ends to avoid placing skin oils in the region that will be heated. Pulled pipettes are only used on the day they are made, because of increased chances of tip fouling and potential hydration of the fine tip glass that could affect the dielectric properties of the glass and introduce recording noise.

An optional final step in preparing pipettes is to coat the tip to reduce capacitance to the bath and improve the dielectric properties. The effect of the coating is to reduce recording noise, and to improve the ability to fully and properly compensate the electrode capacitance for voltage-clamp recordings. When performing voltage-clamp studies, we consider the application of a coating essential. In experiments in which only current-clamp recordings are done, this step can be skipped, although the reduction of capacitance reduces the amount of compensation needed, which in turn reduces the overall noise level of the recording. There are two approaches that are commonly used. The first is to use a conformal coating, such as Sylgard (Dow Corning 184). This is a two-part mixture that can be painted to within 50 μm of the tip using a fine needle while viewing the tapered region of the pipette with a dissection microscope. The mixture cures in several seconds by applying heat (we use a paint stripper on its low setting). The Sylgard can also be stored uncured in the freezer (-20 °C) for about 2 weeks, and we find that preparing the mixture about 24 hours prior to first use is also helpful. A second approach is to wrap the tip of the pipette with a 2-3 mm wide strip of Parafilm, which

### 4.3. Patch Electrodes

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can be melted with gentle heat, or to dip the tip of the pipette in molten parafilm, while keeping positive pressure on
the pipette to maintain a clear tip.

Filling the pipette with electrode solution is also an important step and one where problems can occur. While there
are various commercial filling needles, these tend to be expensive and hard to keep clean, often leading to clogged
electrodes. A different approach is to use 100 \( \mu l \) pipette (those made with a harder plastic seem to work best), tips
pulled over a flame to create a very fine tube (figure 5AB). With care and practice, these make filling tips that are only a
few hundred \( \mu m \) in diameter. The fillers can be inserted into a 1 ml syringe and backfilled from the electrode solution
stock (figure 5C) or capped over a pre-filled syringe and filter (figure 5D). The major advantages of these fillers are that
they are disposable if they become dirty, are economical, and are easily remade. For making fillers and fire polishing
pipettes, we have created a small burner from an 18-gauge blunt needle that provides a flame size similar to a match.

Fig. 5: Making patch pipette fillers from disposable pipette tips. A) Heating a 100 \( \mu m \) disposable pipette
tip over a small flame. The tip should be rotated to produce even heating and care should be taken to avoid burning
the plastic. B) As soon as the tip has melted through, remove it from the heat and pull into a thin tube (this takes some
practice). Cut the tube with a sharp blade to avoid crushing it. C) Filler made from tip of pipette inserted into 1 ml
syringe. D) Filler made from base of pipette attached to 1 ml syringe and a low-volume, 0.2 \( \mu m \)-pore syringe filter.

### 4.4 Dissection and Slicing

The preparation of brain slices containing healthy cells is critical to the success of patch recording. The goal is to
extract a section of the brain such that the cells of interest are close to the surface of the slice and any other required
network connections are intact elsewhere in the slice. Furthermore, we need to make sure that the cells/tissue are
still sufficiently alive and undamaged and that they can be visualized well enough to facilitate patching. Producing
viable brain slices can be very difficult and proven methods often vary widely between brain regions. The main
factors affecting slice viability are: 1) Prevention of ischemic damage by dissecting and slicing quickly, often in well-
oxigenated, ice-cold ACSF (the ACSF may actually be partially frozen). 2) Prevention of excitotoxic damage through
use of specialized ACSF solutions. 3) Prevention of mechanical damage by avoiding compression/stretching of brain
tissue and by using well-tuned slicers with appropriate blades.

Blades: The choice of cutting blade can be critical to successful slice preparation, especially in older tissue. The most
commonly used blades are commercially available double-edged stainless-steel razor blades. These vary in quality
however, and different types should be tried to determine which ones work best for a specific brain region. “Platinum
plus” blades have worked well in the brainstem and cortex, while other types of blades have been found to yield
very poor cutting. Reusable blades made of sapphire or ceramic are also excellent choices, especially if they can be
resharpened. Blades should be cleaned prior to use, and stainless-steel blades should only be used for one cutting
session. Cleaning is necessary to remove oils and other protective chemicals used to retard oxidation and corrosion of
the blades. We clean by first briefly washing the blade in acetone with a cotton swab, followed by a 70% ethanol rinse,
and finally a distilled water rinse. The blade is then dried, and placed in the chuck of the slicer.

We discuss preparation of slices from two different brain regions below, to illustrate two different approaches to
creating viable brain slices. The first method, for neocortex, follows a conventional approach, while the second
method, which we use for cochlear nucleus, demonstrates how variations on the procedure may be best applied for
different brain regions. Prior to removing the brain, all solutions need to be at an appropriate temperature and properly
oxygenated, surgical tools should be located and clean, and the cutting blade should be in place. The goal is to make
the time between decapitation and the incubation of the slices in the holding chamber as short as possible. At the same
time, it is critical to be careful with the tissue, and to handle it gently. In each approach, the animals are first deeply
anesthetized, according to an approved protocol, and decapitated.

Cortex: The skull is exposed, cut down the midline with fine-tipped scissors, and peeled back with rongeurs (in adult
animals where the skull is thick) or with fine-tipped scissors (in younger animals when the skull is thin). Care should
be taken not to touch the brain itself when removing the skull. The brain is removed after carefully cutting major
cranial nerves that may enter or leave near the tissue of interest. The brain is then “rolled” out using a small spatula,
into an ice-cold dissection solution (see below for composition). The tissue is trimmed, using fine scissors and scalpel
blades. The key elements in trimming are to obtain a flat surface that is parallel to the desired plane of section that
can be used to glue the brain block to the stage, and to remove any excess tissue that does not contribute to stabilizing
the brain block during cutting. It is helpful to have a specific sequence in which the trimming is performed, as with
practice this can greatly speed the preparation.

The next step is to place the tissue block on the chuck that will go into the slicer. We usually prepare the mounting
position by laying down a small platform made of 4% agar (made in 150 mM NaCl) to support the tissue, and place
an agar wall behind the platform. In some cases, these agar supports are cut with an angled surface to help orient the
brain when it is glued down. A drop of cyanoacrylate glue is placed on the platform just in front of the wall. The
tissue is then picked up by sliding it onto a small piece of ashless #50 filter paper (this paper is “hard” and can hold
small blocks of tissue even when wet), such that the part of the tissue that will be against the wall is against the tissue
paper. The chuck is placed at an angle such that the wall can support the tissue by gravity. The tissue block is then
transferred onto the cutting chuck by sliding it against the wall until it comes in contact with the glue, at which point
the filter paper is slid out from under the tissue. It is important in this step that the glue not come in contact with
the filter paper. We next mount 4% agar support blocks (usually ~2x2x6 mm posts) that are glued to the stage and
gently abut the tissue, to minimize movement of the tissue during cutting. Cutting takes place in a cold solution in a
previously frozen cutting chamber surrounded by an ice slurry.

Brainstem (cochlear nucleus): The squamous portion of the occipital bone over the cerebellum is removed with
rongeurs (fine scissors are sufficient in mice), exposing the cerebellum and brainstem. The brainstem is briefly washed
with warmed oxygenated ACSF. The temporal bone is carefully retracted laterally, the floccular and parafloccular
lobes of the cerebellum are gently lifted, and the exposed eighth nerve (both the auditory and vestibular branches) is
then sectioned with the tip of a #11 scalpel blade. Care is taken to minimize stretch of the nerve while cutting. The
brainstem is transected rostral to the inferior colliculus with a spatula, and again caudal to the obex, removed from the
skull, and rinsed again in ACSF. A small tissue block containing the cochlear nucleus of the left side is then isolated
from the brainstem. The brainstem is bisected at the midline longitudinally with a scalpel, and trimmed rostral and
caudal to the cochlear nuclei with scissors. The choroid plexus lying above the cochlear nucleus is gently teased away
with #5 forceps. Most of the cerebellum is cut away at the cerebellar peduncles with scissors. The rostral and caudal
ends of the block are trimmed at an angle approximately parallel to the long axis of the cochlear nucleus. A final cut
is made parallel to the desired cutting plane; this may be along the midline or across the ventral surface. This block is
transferred using a strip of hardened #50 ashless filter paper, blotted to remove excess fluid, and mounted on the chuck
of the tissue slicer with cyanoacrylate glue. The tissue is supported with agar blocks from behind and on the sides.
The chuck and tissue are immersed in a warmed carbonated cutting solution in the bath.

For either tissue region, slices are cut by carefully advancing the blade into the tissue under visual control. Often, a
few 500 μm thick slices are quickly taken until the desired region is reached, and then the cutting thickness is adjusted
to 250-350 μm, and a series of slices collected. As each slice is taken, it is checked under a dissecting microscope to be
sure that it is from the appropriate region and is not damaged, and then is transferred to the incubation chamber using
the blunt end of a Pasteur pipette whose tip has been broken off and fire polished so a pipette bulb can be attached.
We prefer this method over using small paint brushes, as there is less mechanical stress to the tissue slice during the
transfer.

### 4.5 Slice Incubation

Slices are commonly allowed to incubate for 30-60 minutes at 32-34 °C after slicing to allow them time to recover and
re-equilibrate to the ACSF environment. After this the slices are usually incubated at room temperature. The slices
are held in an incubation chamber that may be in a water bath, or on the bench for room temperature incubation. We primarily use a simple chamber that consists of a 100 ml glass beaker. A sintered-glass gas dispersion tube is inserted into the chamber and about 60 ml of ACSF is added, and well gassed with 95% O2-5% CO2. The slices rest on a permeable nylon mesh that forms the bottom of multi-well tray. This tray is hung in the chamber so that the ACSF covers the mesh, but is 1-2 mm from the top of the tray walls. The top of the chamber itself is loosely covered with parafilm to minimize evaporation over time. Other incubation chambers have been used as well. The key requirements are that bathing solution is exchanged around the slices by a stirring action (usually provided by the gas dispersion system), that the solution is well gassed, that evaporation is controlled, and that the chamber and gas dispersion system does not leach chemicals into the incubation solution.

4.6 Recording chamber / perfusion / harps

After incubation, slices are held in a small volume (~0.3 ml) recording chamber mounted on the stage of an upright microscope. The slice rests either on a coverglass or on a small section of netting, and is held in place by another net stretched across a stainless steel harp. Warm, oxygenated ACSF is perfused over the slice at 2-8 ml/min by siphoning from a flask. The incoming perfusate is warmed to 38 °C just prior to entering the chamber with a feedback controlled heater, resulting in a solution temperature at the slice of 33±1 °C. While some experimental procedures, such as analysis of network organization using photostimulation, can be done at room temperature, it is nearly always preferable to record at elevated temperatures to more closely approximate the normal kinetics of ion channels, the release properties of synapses, and the engagement of intracellular signaling cascades.

The fluid level in the recording chamber is regulated by positioning an aspirator above the surface of the water. While a properly configured aspirator should maintain the bath at a constant level, it is nevertheless important to monitor the chamber to avoid overflows which may damage equipment or underflows which may damage the slice.

To prevent electrical noise, it is very important that the fluid in the recording chamber be electrically isolated from everything except the patch and ground electrodes. If the chamber overflows, it is possible this will create a new electrical path to ground or other parts of the setup, introducing a new potential noise source.

4.7 Patching

4.7.1 Finding viable cells

You are finally ready to patch a cell. The first task, then, is to find a cell that appears to be healthy and is in the correct location of the slice. The appearance of healthy cells will vary somewhat between brain regions, but typically these will appear to have a smooth, translucent interior and a smooth cell membrane (figure 6, white arrows). Unhealthy cells often appear either shriveled or bloated, have a rough or abnormally transparent interior, or have a visible nucleus and nucleolus (figure 6, black arrows). However, these features are not always diagnostic of cell health and it is possible to bias the cell selection by avoiding healthy cells with an abnormal appearance. Ultimately, trial and error may be the best way to determine which cells can be patched successfully, and which appearances are associated with healthy cells. In a few cases, the situation may call for alternative methods such as shadow patching (using fluorescent electrode solution and looking for dark regions indicating a healthy cell that is impermeable to fluorophore) or blind patching (using electrical signals rather than visualization to determine when the pipette has contacted a cell) instead of direct visualization.

It is preferable to avoid cells very close to the slice surface, as they are likely to have been severely damaged during slicing. Generally, one should attempt to patch cells as deep as possible given the limitation of visibility in the slice. For relatively transparent tissue such as neocortex from young animals, it may be possible to visualize cells up to 50-100 μm deep. For older or heavily myelinated tissue, visibility may be limited to 20 μm or less. In this situation, having properly adjusted illumination and a good camera is crucial. It may also be necessary to use software that allows large contrast adjustments or background subtraction.
Once a candidate cell has been selected, center the cell within the camera’s visible range and switch back to a low-power objective in preparation for positioning the patch pipette.

### 4.7.2 Filling patch pipettes

For each cell you wish to record, a new patch pipette must be prepared and filled with electrode solution. It is recommended to make all patch pipettes at the beginning of the day (about 6-12 should suffice, depending on the experiment) but do not fill the pipettes with electrode solution until immediately before each is to be used. The day’s aliquot of electrode solution should be thawed, vortexed, centrifuged, and kept chilled on ice. Note that vortexing is critical because just-thawed electrode solutions may have a large osmolarity gradient from the top of the tube to the bottom.

To fill the pipette, either 1) attach a plastic filler (see Patch Electrodes and figure 5) to a 1 ml syringe and draw a small amount of electrode solution into the syringe, or 2) draw all of the electrode solution into the syringe and cap with a 1 ml aliquot filter and a plastic filler (figure 5C,D). Insert the filler as far as possible into the pipette and inject enough solution to fill approximately 2/3 of the pipette. The exact amount needed will be just enough such that the electrode solution makes contact with the AgCl wire in the pipette holder. If the pipette is overfilled, electrode solution may seep into the pipette holder and this can add noise to the recording. Air bubbles between the AgCl wire and the tip of the pipette may increase the resistance of the electrode. These can be removed by sharply tapping the pipette against the counter.

Many modern pipette blanks have a glass filament that is fused to the inside of the tubing. The filament creates a capillary flow of fluid that helps bring the electrode solution to the pipette tip. It can also act as an electrical conductor. These pipettes can be filled from the back and the tip will fill over several seconds to a minute, with no bubbles in the tip. In the absence of such a filament, pipettes can be also filled from the tip by applying suction to the back while the tip is dipped into the recording solution. This will bring a tiny amount of the solution into the tip, then the pipette must be filled the rest of the way from the back.

When the pipette is filled, place it over the AgCl wire and secure it snugly into the pipette holder. Do not over-tighten the retaining cap, as this can put torque on the rubber sealing gasket and cause the pipette tip to drift slowly as the rubber relaxes.

### 4.7.3 Approach

The most important thing to remember while patching is that clean glass is very sticky. Whatever touches the tip of your pipette first will adhere to it permanently. We keep positive pressure inside the pipette so that electrode solution is constantly flowing through the tip, ensuring that nothing touches it until we are ready. Most electrophysiologists use a syringe to provide this pressure, while some prefer to use their mouth. It may also be helpful to use a pressure gauge.

To begin, you should be looking at the slice through a low-power objective that is still centered on your target cell. Using the micromanipulator, position the tip of the pipette in the center of the field of view above the fluid surface.
Make sure there is positive pressure on the pipette, then lower it into the fluid but do not yet touch the brain slice. Note that sometimes salts may crystalize or debris may appear on the surface of the bathing solution; these should be aspirated from the surface, or the cause identified and eliminated, because they can foul the tip of the pipette as it enters the solution, making patching difficult or impossible.

At this point, adjust the amplifier’s pipette offset (this is described in the manual for your amplifier). Configure your amplifier to output 20 ms, -10 mV pulses (or for current clamp, use 1 nA) and calculate the resistance of your pipette from the recorded response (Fig. 7). Most electrophysiology software will have built-in features for measuring pipette resistance. Monitor the pipette resistance continuously until the cell is patched. If the resistance increases unexpectedly, discard the pipette (it has probably clogged). Record the pipette resistance for every patched cell as this information can be very useful when analyzing data.

Fig. 4.4: Figure 7. Voltage-clamp recording from cell-attached pipette before (dashed line) and after (solid line) adjusting the pipette capacitance compensation. The seal resistance has increased to 1.6 GΩ. A) Photo of patch pipette in bath. B) Voltage clamp command and current recording from patch pipette in bath. The voltage clamp requires 2.4 nA of current to effect a 10 mV pulse, indicating a pipette resistance of 4.2 MΩ.

4.7.4 Into tissue

With the pipette tip still above the surface of the slice, switch to a high-power objective. If the pipette tip is no longer visible, you may need to move the tip a small distance until it appears in the view. Be cautious—if you cannot see the pipette, it is easy to accidentally drive it into the slice or the objective. Once the tip is visible, proceed slowly down toward the surface of the slice, continuously refocusing to keep the tip in view.

For most situations, it is acceptable to simply center the pipette tip over the position of the target cell and descend directly downward to the cell. For tougher, myelinated tissue or for deeper cells, it may be advantageous to push the pipette in diagonally along its axis. This will avoid some amount of tissue compression that may result from going straight downward. Most micromanipulators can be configured for this purpose.

As the pipette tip enters the surface of the slice, you should immediately see the tissue gently spread away due to the pressure in the pipette. If you do not see this, then it is likely the tip is already clogged or there is insufficient pressure (and this pipette should be immediately discarded). Too much spread however is not a good sign, as it means that you are flooding the slice with the electrode solution. Depending on the solution, this can depolarize nearby cells, and the mechanical action of the flow may also disrupt the tissue. If this occurs, reduce the pressure.

As you proceed closer to the cell, you may encounter obstacles such as fibers or other cells. The positive pressure will push some obstacles out of your way, while other obstacles will need to be avoided. Some trial and error may be necessary at first. Remember, the goal is to arrive at the target cell with a clean pipette tip. Positive pressure makes this possible, but some situations will require finesse as well.

4.7.5 Near cell

When the pipette tip is within 10 μm or so of the target cell, correct the amplifier’s pipette offset again. Press the tip slowly into the center of the cell to form a visible dimple (figures 8A, 9). This dimple is your indication that there is indeed nothing else between the pipette and your cell. Release the pressure on the pipette and wait while monitoring
Fig. 4.5: **Figure 8.** Patch procedure. A) Approach the cell with positive pressure in the pipette. The surface of the cell should form a visible dimple. B) Release pressure on pipette, then apply gentle suction to seal the membrane against the pipette. This is the cell-attached configuration. C) Apply sharp suction to the pipette to rupture the membrane, granting electrical access to the cell interior. This is the whole-cell configuration. D) From whole-cell, pull the pipette very gently away from the cell until E) the membrane separates and re-closes. This is the outside-out configuration.

Fig. 4.6: **Figure 9.** Left: A ‘dimpled’ cell immediately before being patched. Right: voltage-clamp recording shortly after releasing pipette pressure. The resistance at the pipette tip has increased to 66MΩ.

the resistance of the electrode. The cell membrane should immediately come into contact with the electrode tip and begin to form a seal.

The membrane that has adhered to the pipette tip may spontaneously rupture, so it is important to prepare for this. After the seal resistance has increased past about 100 MΩ, voltage-clamp the pipette near the estimated resting potential of the cell, less the junction potential (for example, if a typical cell rests at -75mV and the junction potential is -12 mV, voltage clamp the pipette at -63 mV with brief steps to -73 mV). The resistance should continue to increase over a few seconds to a minute, going from a few MΩ to over 1 GΩ (**figure 10**). If resistance is not increasing quickly enough, gentle suction on the pipette can encourage a seal to form. If your software allows, it can be very helpful to watch the pipette resistance plotted over time.

Fig. 4.7: **Figure 10.** Voltage-clamp recording from cell-attached pipette before (dashed line) and after (solid line) adjusting the pipette capacitance compensation. The seal resistance has increased to 1.6 GΩ.

After forming a gigaohm seal, the pipette is considered “cell attached” (**figure 8B**). In this mode, it is possible to cleanly record action potentials from the patched cell, but little else should be visible. If your amplifier has built-in pipette capacitance compensation, now is the perfect time to adjust those settings (your amplifier manual should discuss this in detail). This will minimize the transients at the beginning and end of the voltage command step (**figure 10**).

### 4.7.6 Break-in

Once a gigaohm seal has been formed, access to the cell can be obtained. Apply brief pulses of suction to break the membrane within the lumen of the pipette (**figure 8C**). There are several ways to do this. In our lab, we typically use
a 1 cc tuberculin syringe to create the suction, using small, quick, pulls on the plunger (0.01-0.03 cc displacements).
The negative pressure needed to break into the cell varies with cell type, the preparation, and the pipette tip diameter
and taper. Under the best conditions, a displacement of less than 0.1 cc is sufficient to provide a clean break in. Once
the break in is achieved, the negative pressure is immediately released. A traditional way to apply suction is to use a
mouth-pipette tube. This also gives good control of the pressure. Another way is to use a controlled negative pressure
generating system, such as a column of water, along with a valve. However, the complexity of such a system may not
be worth the effort to maintain it compared to using the simpler methods. Many amplifiers also offer “zap” and current
pulse controls that can be used to try to break the luminal membrane by voltage-breakdown. However, we have not
found these to be very effective in the cochlear nucleus or auditory cortex, and when access to the cell is achieved it
has high resistance and is not stable.

When whole-cell access is obtained, the membrane current trace will consist of a fast transient current that decays back
towards the baseline (figure 11). The amplitude of this transient is inversely proportional to the series resistance (lower
resistances generate larger transients), while the time constant of the transient decay is approximately the product of
the series resistance and the effective cell capacitance seen by the electrode. The access resistance should be low.
Using -10 mV steps, a -1 nA peak current would correspond to 10 MΩ access and 2 nA to 5 MΩ (as follows from
Ohm’s law). The time constant corresponds to the speed of the uncompensated voltage clamp and the fast component,
corresponding to charging of the soma, usually should be well under 1 ms.

![figures/rendered/11_patch_examples4.png](figures/rendered/11_patch_examples4.png)

Fig. 4.8: Figure 11. A) Whole-cell patched neuron filled with fluorescent dye. B) Voltage-clamp recording from
the same neuron. The steady-state current is about 80 pA, indicating an input resistance of 125 MΩ. The peak of the
charging transient is 900 pA past the steady-state, indicating an access resistance of 11 MΩ.

### 4.7.7 Compensating series resistance and whole cell capacitance

In an ideal voltage clamp, the membrane potential at the patched cell is exactly equal to the requested command
voltage. In practice, several factors prevent perfect control of the membrane potential. The small tip of the electrode,
combined with cell debris inside the tip, creates a series resistance between the interior of the electrode and the interior
of the cell. When current is passed through this series resistance, it results in a voltage difference, so that the resulting
membrane potential is no longer equal to the command voltage, but is shifted in a direction that depends on the sign
of the current flowing through the electrode at any instant in time. When currents are large, the resistance of the bath
electrode may also contribute to this error. Series resistance, when combined with the capacitance of the pipette and
of the cell, also introduces a complex low-pass filter that affects how rapidly the voltage at the cell can be changed,
and how rapidly the amplifier can detect changes in the cell voltage.

Voltage clamp amplifiers include compensation circuitry that attempts to correct these effects by including a feedback
circuit that takes into account the series resistance and cell capacitance, and injects current through the electrode in
an attempt to faithfully follow the command voltage. This compensation is essential for experiments that require
precise control of the membrane potential and accurate recordings of fast or large currents. It also effectively increases
the bandwidth of the clamp, resulting in tighter control of membrane potential during rapid changes in membrane
conductance.

The drawbacks to series resistance compensation are that it introduces additional high-frequency noise to the recording,
and it is prone to producing oscillations that may damage or destroy the cell if configured incorrectly. For recordings
that require very low noise, where the currents are slow, and where a voltage error can be tolerated or is demonstrably
small (e.g., measuring small currents where the voltage error is also small), it may be preferable to disable series
resistance compensation.
The accuracy of compensation is limited by the extent to which the user is able to adjust the settings to closely reflect the electrical circuit of the pipette and cell. The details for configuring series resistance compensation are found in the manuals of the amplifiers, and because the compensation circuitry varies between amplifiers, those recommendations should be followed. A few important points are in order however. First, any cell with an extended dendritic tree will have a capacitive transient that has multiple time constants. However, the amplifiers are all designed to compensate a single time constant, e.g., a spherical cell body with no processes. Thus, care in adjustment must be used to focus on the correct (somatic) time constant. Second, stable recording conditions need to be attained. Any change in access resistance, or even the bath fluid level, can affect the conditions needed for optimal compensation, and will result at best in incorrect compensation, and at the worst in the system going into oscillation and destroying the cell.

At this point, we wish to raise an important limitation of voltage clamp that is all too often ignored in the literature. Only the point of the cell immediately adjacent to the electrode is properly “voltage-clamped”. There is a large and local spatial gradient over which clamp fidelity decreases, usually on the order of 100 μm or so (see Fig. 12). This can be partially corrected by using the amplifier’s compensation circuitry and by using electrode solution that blocks ion channels to increase the electrotonic space constant of the cell. This issue has been discussed by a number of authors over the years (Spruston et al., 1993; Williams and Mitchell, 2008). In most central neurons, even under the best conditions, only the cell body and proximal ~50 μm of the dendrite is under good control of the voltage clamp. Thus, it may be advisable to record in current clamp to minimize the potential for voltage clamp problems, or if appropriate, patch recordings directly from dendrites might be considered.

Even though the entire neuron cannot be clamped, recording in a voltage clamp mode has several advantages for examining synaptic responses. The clamp keeps the membrane potential relatively constant and below spike threshold, so that synaptic inputs are not likely to drive spikes unexpectedly. In addition, voltage clamp can largely remove the effect of membrane capacitance from conductance changes generated near the recording electrode, which can improve the signal-to-noise ratio for detection and measurements of synaptic inputs, especially when measuring single quantal events such as miniature excitatory or inhibitory postsynaptic potentials. Finally, even though dendritic synaptic events are not fully clamped, relative changes can be measured under different conditions in individual cells, while holding the membrane potential (and synaptic driving force) constant. However, this requires careful consideration of the potential influence of any manipulations on the quality of the clamp and on non-uniform changes in driving force across the dendritic tree.

### 4.7.8 Inside-out, outside-out

Outside out patches (figure 8D, E) are very useful in evaluating the voltage-dependence of ion channels, and the kinetics of neurotransmitter receptors. These patches have a very low capacitance, and can be well controlled under voltage clamp. They can be pulled from cells in slices, including from fine dendrites. A major advantage of using isolated membrane patches is that the space clamp problem is eliminated. A second advantage is that the site where the channels with particular currents are located can be determined. Disadvantages include the fact that the channel function may be disturbed by wash-out of essential proteins or intracellular ions, or even by introducing an unusual curvature to the patch membrane.

To pull an outside-out patch, first obtain a whole-cell recording (figure 8C). We find that the best patches are pulled within about 10 minutes of accessing the whole-cell configuration, and this provides time to perhaps fill the cell with a dye and to obtain a characterization of the intrinsic physiology. Next, switch the amplifier to voltage-clamp, holding the cell at -60 mV, and provide 10-50 msec long voltage pulses going to -70 mV. It is best not to compensate the amplifier at this point, as visualization of the access resistance and clamp time constant is better obtained by watching the uncompensated currents on an oscilloscope. Begin forming the patch by slowly drawing the pipette away from the cell, at a rate of a few μm per 10 seconds, stopping frequently, in a direction normal to the cell surface at the point of contact (figure 8D). Watching the oscilloscope, you should see an increase in access resistance, as indicated by a decrease in the peak current at the beginning and end of the step, once the pipette is more than about 5 μm from the cell, and a thin bridge of membrane may be visible connecting the pipette to the cell. Continue pulling slowly until the capacitive charging transient at the beginning and end of the voltage pulse becomes very small, and the input resistance of the patch increases as indicated by a decrease in the small steady-state current during the step. The holding current should be less than a few 10’s of pA. You should be able to continue pulling the pipette away and up so that the tip is in the bath above the slice (figure 8E). At this point you have an outside out patch. Applying voltage steps may reveal...
small currents (10-200 pA), especially with depolarization. Loss of the patch is indicated by a large increase in the holding current and noisy traces.

Note that this procedure can also result in a resealing of the membrane of the cell that the patch was pulled from, and it is often possible to image the cell after recordings from the patch are complete.

### 4.7.9 Running the experiment

When the cell is patched, you are ready to run your experiment. Patched cells can be temperamental, so it is important to monitor the health of the cell for the duration of the experiment. The major indicators of a failing cell are decreased or increased resting membrane potential (10-15 mV above or below the typical resting potential) and decreased input resistance. These can be monitored by periodically recording the response to current or voltage pulses similar to the procedure used during patching. Alternatively, the cell can be continuously monitored by generating an audible signal from the amplifier.

Additionally, access resistance may increase during the experiment. Although some increase in access is normal, it may cause problems if it continues to increase past 15-20 MΩ in current clamp, or by more than 8-10% in voltage-clamp. Applying very brief, gentle pulses of pressure to the patch pipette may help lower the access resistance, but can also rupture the patch seal.

In some experiments, it is desirable to voltage clamp cells at a membrane potential that is well away from the normal resting potential. For example, to measure currents through NMDA receptors, it is common to clamp the cells at a positive potential, such as +40 mV. Even with Cs-based electrodes, we find that many types of cells do not tolerate being held at positive potentials for more than about 10 seconds at a time. It is usually best to step the cell between a normal holding potential and the positive potential, and apply stimuli during the positive step. Subtraction of traces with and without stimulation may be needed when using such a protocol, as Cs+ does not completely block all potassium currents, and some time-dependent current may remain.

### 4.7.10 Data analysis

Patch-clamp experiments most commonly generate time-series analog signal recordings (for example, a 1-dimensional array of membrane voltage or current values). These signals are analyzed using a variety of general signal processing techniques as well as less common techniques devised specifically for analysis of neuronal signals. Given a model of the system we are studying (be it a channel, membrane, neuron, or circuit), the objective of any analysis is to measure one or more parameters of the model from signals in the recording. However, the presence of noise and other interfering signals can make this challenging.

Prior to analysis for signals of interest, it is common to digitally filter the recording to remove unwanted noise and offsets. To remove a baseline offset, it is usually sufficient to subtract the mean or median value derived from a quiescent period of the recording. Bessel or Butterworth filters are frequently used to remove both high-frequency noise and low-frequency baseline fluctuations. Any filtering must be applied with caution to avoid altering those aspects of the signal that are to be measured. For example, many filters introduce frequency-dependent phase delays that can affect the measurements of event timing in the signal. Filtering can also generate ringing artifacts in response to rapid changes in the incoming signal or noise spikes. They can also alter the apparent kinetics of rapidly activating currents measured under voltage clamp, such as occurs with voltage-gated sodium and calcium conductances, and some fast synaptic conductances. In general, it is wise to always check that the chosen filtering (or any automated analysis, for that matter) produces the expected results for a set of known inputs, by always checking filtered signals and the subsequent analysis results manually.

Signals of interest in patch clamp recordings can be divided roughly into two categories: evoked events, and spontaneous events. Evoked events are somewhat easier to analyze because their timing usually follows a predictable delay with respect to the stimulus (electric shock, photostimulation, etc.). Evoked events include excitatory and inhibitory postsynaptic currents, action potentials, and direct perturbations of the membrane potential or holding current. Such events are analyzed to characterize their shape in some way. For example, sudden shifts in membrane potential may be fit to exponential decay curves to determine their time constant; action potentials are measured for their amplitude,
width, afterhyperpolarization depth, rising and falling slopes, and other criteria; and postsynaptic conductances are analyzed for amplitude, latency, rising and falling kinetics, or total charge transfer.

Analysis of spontaneous (or otherwise poorly timed) events requires extra effort because the timing of events must be determined before they can be measured. In some cases, it may be difficult to unambiguously distinguish events from background noise, or to separate overlapping events. Numerous techniques for event detection have been developed. Most of these work by filtering the signal such that each event is reduced to a single, sharp spike that can be clearly distinguished from the background noise. The timing of these spikes is then detected by searching for regions of the signal that exceed a predefined threshold. A commonly used and more sophisticated analysis uses a template matching algorithm (Clements and Bekkers, 1997). In this approach, a short template with the expected event shape is slid across the trace in time, and the error in the fit (with the baseline and peak amplitude as the adjustable parameters) is returned at each point in time. The regions with the best fits that exceed a statistical criterion are then identified for subsequent analysis. An alternate treatment that can be used in current clamp recordings uses deconvolution to estimate the time course of a current from the voltage traces (Richardson and Silberberg, 2008). This method can be useful for isolating and measuring the amplitudes of overlapping events.
5.1 Slice viability

Difficulty keeping sliced brain tissue alive is one of the most common problems in slice electrophysiology. The reasons for this are not well understood, and the remedies are often highly specific to each brain region. However, many of the rules for producing viable slices are common across all brain regions:

- **Reduce ischemia:**
  - Become faster at dissection and slicing
  - Make sure you are using a well-oxygenated dissection buffer.
  - Try doing the preparation at different temperatures (cooling or warming the tissue during dissection and slicing).

- **Reduce excitotoxicity:**
  - Use NMDG / sucrose solutions - reducing Na+ may reduce action potential firing, and minimize synaptic release. It may also reduce energy demands.
  - Cut in high Mg2+ / low Ca2+ solutions.
  - Try cooling to reduce activity.
  - Check the pH and osmolarity of all solutions:
    - pH should be 7.2-7.4 for all solutions. If needed, supplement buffers with HEPES to help control pH.
    - Reduce bicarbonate to 20 mM in cases where CO2 content of gas tank may be low.
    - Osmolarity should be about 290mOsm for electrode solution and 310 for ACSF. Reduce evaporation, or add sucrose to make osmolarity appropriate.

- **Reduce mechanical trauma:**
  - Try different blades for slicing.
  - Cut slower if the tissue is not cutting (sticking to blade, rolling, or compressing).
  - Cut faster if possible.
  - Check blade angle—the blade should be pointed roughly 10 degrees downward, such that the bottom tapered edge of the blade is horizontal.
  - Try a slice orientation that severs fewer or only smaller processes. For example, some neurons are mostly planar, and survive better when the slice is parallel to the main plane of the dendritic tree.
Use more care during dissection. Do not touch the region you wish to study, avoid compressing or shocking the tissue. Do not expose the tissue to air any longer than absolutely necessary.

Cut any cranial nerves before removing the brain from the skull, as the tension from stretching these may damage some areas of the brain.

Some slicers impart a small amount of vertical vibration to the blade and may need to be tuned to avoid this.

- Use younger animals; these cells tend to be much more resilient.
- Look in the literature for proven protocols (for your brain region), and talk to people using those regions.
- Be systematic: start with something that works, and change one variable at a time.
- Look deeper into the slice (may require illumination adjustment).
- Wait at least 1hr after cutting before starting any recordings.

Because slice preparation and patch clamp have a steep learning curve, we suggest starting with a simple experiment to be sure that you have a handle on how to make everything work together. Start out with younger animals (P10-P14 for rats or mice), and just try to perform current clamp experiments on cells. Once you can regularly get cells with good spike heights and resting potentials, then it is time to advance to your project. Be persistent and expect to spend weeks to months becoming proficient with these techniques.

5.2 Electrical noise

Noise is common in electrophysiology equipment, and the noise both contaminates the recordings and in some cases can mask signals. There are five primary sources of noise. 1. Line noise. This appears as 50 or 60Hz, often with harmonics (integer multiples of 50/60Hz), and can come from several sources including unshielded power cords, overhead lights, and incorrect grounding practices. 2. Power supply noise. This is typically also at harmonics of 50 or 60 Hz, and may result from power supplies that are not working correctly, poorly designed or incorrectly grounded. For example, switching power supplies are convenient because they are small and light, and do not require heavy transformers or large filter capacitors. However, they can generate wideband noise anywhere from 15 kHz to many MHz and this noise may become aliased into the recorded signals. 3. Electrochemical junction noise. This appears as an unstable, fluctuating noise on a time scale of milliseconds to seconds. It may be caused by salt solution that is spilled, for example near the chamber, that creates a battery between dissimilar metals, or that bridges different ground connections. 4. Digital equipment noise. Computers, some modern computer-controlled amplifiers, microprocessor-based devices, and digital and analog cameras have high frequency clocks or oscillators that are often not properly electrically isolated from ground circuits, and the signals can get into the ground system. Because these are high frequency (sometimes in the MHz range), they can be hard to troubleshoot, and may require particular attention. We have found that even top-of-the-line electrophysiology amplifiers can “leak” such signals into the rig and cause problems. 5. Ground loop noise. These are noises caused by currents that circulate in the ground system of the rig, through cables and the various grounds associated with the equipment.

To minimize noise, one must first start by stripping the rig down to just the amplifier, the microscope, and the computer. This means disconnecting all cables at both ends, and turning off (and unplugging) all other equipment. Remove anything from the vicinity of the microscope that is not being used. Unconnected or dangling cables should be stowed. The microscope body should be grounded to the table, and the table grounded to the Faraday cage, with at least 1/4” wide stranded wire strapping of as short a length as practical. All connections should be made using screws and toothed washers, and all surfaces that are to be bonded should have exposed metal at the point of contact (sand off any paint or oxidation). Place a small amount of saline in the recording chamber, and a filled pipette on the headstage. Monitor the output of the amplifier with an oscilloscope. We also find that using a spectrum analyzer (or spectrum calculated from digitized data) is extremely helpful in identifying noise sources and eliminating them. The amplifier headstage should only be connected to the electrode, and the high quality signal ground (usually located on the amplifier headstage) should be connected only to the reference electrode in the bath. In some cases, it is worthwhile to either leave the electrode floating in the air above the recording bath, or to patch a ball of ‘Sylgard® in the recording
chamber (essentially, making a high-impedance seal; under these conditions, small currents are more easily detected). The amplifier itself may have a separate ground connection (on the back) and this can be used as the reference (ground) for the Faraday cage, table and microscope (and the equipment rack if one is used). With the amplifier filters open (>50 kHz bandwidth), re-examine the noise levels and try to identify and correct noise from any additional sources.

Items that are connected to the microscope can also cause problems, and should be addressed next. Any ungrounded conductive objects in proximity to the recording chamber may act as an antenna, which picks up electromagnetic radiation, and couple it capacitively into the recording area. This may include micromanipulators, parts (or all) of the microscope, and the experimenter’s body. While some microscopes have a specific ground point that can be used, many parts of the microscope are not bonded electrically to this point, and are therefore ungrounded. This often occurs because items are painted or anodized, or because they are separated by a thin layer of grease. Items attached through couplers, such as cameras, should not be considered to be grounded, and may need a separate grounding strap. Anything on the stage that is anodized (or is on an anodized stage) will not be well grounded, and may need a separate connection.

After eliminating sources of capacitively coupled noise, begin adding the other equipment to the setup one piece at a time, evaluating the noise at each stage, both with the equipment connected and off, and with it turned on. Often this will identify an offending item, which might need to be moved, or might need additional attention for grounding.

Ground loop noise can be difficult to eliminate. Ground loops occur when there is more than one path for current to flow between two points in a system. This can occur through shielded cables that connect two pieces of equipment which otherwise share a ground connection with each other. There are also common-mode currents that may flow on a shared signal or ground path that may contribute to interference. There are several treatises on this problem in the professional audio literature (for example, Waldron, Web Resource). There are two points with regards to small rigs. First, maintain as best as possible a “star” ground configuration for all equipment. In a star configuration, there is one central reference point, and all common connections go to that point. While this topology minimizes the chances of creating ground loops between different pieces of equipment, it is not always practical. Second, keep the headstage and its reference input completely separate from the rest of the system grounds (remember also that the interior of the recording chamber and any connecting fluid compartments must be completely electrically insulated from the rest of the system). Third, consider the signal paths associated with connecting cables between equipment items. In some cases, it may be useful to isolate the ground side of the connection in a signal cable, but this is not always recommended. Not all manufacturers follow the same rules for signal grounding in their equipment, and this can cause interesting problems. Sometimes, even short “ground” leads can pick up radiated signals and introduce additional noise. It should never be necessary to disconnect (“lift”) the safety electrical ground in a piece of modern equipment if the manufacturer has arranged this correctly (e.g., connected to the equipment case, and separate from the signal ground paths). In some cases, where signals >100 kHz are problematic, the use of toroidal cores or ferrite chokes around the connecting cables may be helpful. The size and permeability of the core or choke should be commensurate with the frequency of the signal to be blocked. Sometimes signals from nearby AM or FM radio stations, hospital dispatchers, or even a cell phone or tablet computer in the vicinity of the rig, can introduce unwanted energy onto the cables around a rig and add noise. Remember that some of these devices have a wireless connection that operates in the 2-5 gigahertz range, where wavelengths are short, and even a short ground strap or a stray wire can operate as a receptive antenna at these frequencies.

A day spent disassembling and reassembling a rig while monitoring the noise levels can be very helpful both in terms of understanding how the rig is configured, and in terms of understanding the various sources of noise in and around the rig. One must take a very systematic approach and try to keep the rig as “clean” both physically and electrically as possible, only then will you be rewarded with a low-noise setup whose data traces will make you proud. Regular maintenance, including cleaning the rig and checking the noise level, and maintaining a log of noise measurements under a fixed set of conditions, is also advisable.

As mentioned earlier, another source of noise that sometimes appears is caused by salt spills (even evaporated spills with just salts in a humid environment). If the salt is in a location that can add currents through a ground loop, or create a loop, it can act like an unstable battery. An example is salt bridges between the recording chamber and the metal platform that holds the chamber. Here, the salt creates an unstable resistance possibly with an electrochemical potential between the high-quality ground used in the recording bath (connected to the headstage) and a general ground used for reduction of capacitive noise pickup. For this reason, amongst others, it is important to clean up all spills.
immediately. It is also important to take apart and clean any items that may get salt inside them (e.g., microscope, substage condensers, translation stages, manipulators) as soon as possible after a spill. Spills should be carefully cleaned up with water, followed by 70% alcohol, and wiped dry. If spills happen frequently, some items may need to be treated with a thin layer of grease or a rust preventative.

When troubleshooting noise, remember also that the tubing used to bring solutions to the preparation and to remove the solution contain a conductive solution that can also be capacitively coupled to other noise sources. Sometimes shielding the tubing, or changing its placement, can help. Peristaltic pumps can also introduce noise through the fluid delivery system, and should be avoided when possible. However, peristaltic pumps are sometimes needed when using expensive or limited chemicals in a recirculating bath.

5.3 Vibration

Vibration isolation tables are designed to dampen vibrations that commonly occur in buildings, usually in a low-frequency range that depends on the size of the table. If the electrode is vibrating under the microscope, then there may be a mechanical connection that is essentially short-circuiting the isolation table. Anything that goes on or off the table can contribute to this. Whenever possible, use cables that are flexible to bring signals to and from devices on the table, and clamp (or tape) the cables to the edge of the table where they leave. Allow the cables to hang (do not make them tight) so that vibration from other non-isolated instruments and racks is less-well coupled to the table. Devices with fans, such as some high-performance CCD cameras, can also contribute to vibration, especially if they are mounted at the top of the microscope. In extreme cases it may be necessary to replace the fans, manually balance them, or find a way to mechanically uncouple the camera from the microscope.
Frequently Asked Questions

Q: I don’t see any living cells in my slice.
A: This is one of the most common problems in slice electrophysiology (See Troubleshooting: Slice Viability). The first thing to remember is that the ideal dissection, slicing, and incubation procedures vary considerably between brain regions, so find out what has already worked for other researchers slicing the same region. If you believe you are doing everything correctly, try cutting slices from younger animals, which are typically much easier to work with. Think critically about your procedure and remember the major causes of cell death in brain slices: pH, ischemic damage, excitotoxic damage, and mechanical damage. Consider also that a dead slice should have many cells that appear to be dead, whereas a slice with no cells may simply indicate an illumination or imaging problem. Finally: be persistent.

Q: I am not able to form a gigaohm seal
A: The most common cause is that the tip of the pipette is fouled. This can have several causes: 1. fingerprints / dust on pipette glass before pulling, 2. pipettes are too old (more than a day) or left uncovered too long, 3. the pipette contacted crystallized salt on the surface of the recording chamber water, 4. the pipette tip contacted brain tissue before it reached the cell due to insufficient pressure inside the pipette. 5. The bathing solution contains serum or bovine serum albumin. If you have to use a solution that contains these, make the seal first in a solution that does not contain proteins, then switch solutions.

Other reasons: 1. A dimple was not visible on the cell before releasing pressure (this often means that something else was compressed between the pipette and the target cell) 2. The cell is dead.

Q: I get a gigaohm seal very quickly, but the cell seems to be gone immediately after breaking in (indicated by very low input resistance or very high resting membrane potential).
A: The cell was probably dead before you patched it. Cells that look similar to this one are also likely to be dead; try changing your cell selection criteria.

Q: I can patch a cell, it looks healthy, but I lose it 10 minutes later.
A: This is often caused by a drifting pipette. Check to see that it has not moved more than about 10 μm from its location at the time of patching. If the pipette has moved, see the following question.

Another possibility is that vibrations transmitted to the pipette tip caused it to detach from the cell. This can be caused by vibrating equipment (unbalanced camera fans are a common culprit), poor isolation from ground vibrations, or the experimenter touching the setup. It is recommended to monitor the cell’s health frequently (or use an audible indicator of electrode potential) to increase the probability that you will discover the cause of a lost cell.

If this happens consistently, it is possible that your internal solution is poisoning the cell. If the cell becomes swollen or shriveled after patching, it is possible that the osmolarity of your internal solution is too low or too high. Try using a different aliquot of internal solution, or borrowing an aliquot of a different batch of internal solution from another experimenter to see if that helps the problem.

Q: My pipette tip is drifting! What do I do?
A: This is most commonly caused by over-tightening or under-tightening the electrode holder cap, which compresses and strains the O-ring holding the electrode. The O-ring relaxes slowly over time, causing the electrode to drift. Applying a small amount of grease to the O-ring can help release this strain before it becomes a problem. Also be sure that nothing is touching the pipette such as the edge of the recording chamber or the objective and that the pressure tube and headstage cable are properly secured to prevent transmission of any strain to the electrode holder. Another source of drift can be temperature changes in the vicinity of the headstage, or a malfunctioning manipulator.

Q: My patch pipettes keep clogging.

A: Clogged pipettes are a common but easily solvable problem. These are most commonly caused by either particulates suspended in the electrode solution or a dirty pipette filler. Electrode solutions should either be centrifuged at the beginning of the day or filtered immediately before filling the pipette, or both. We recommend against using most commercial pipette fillers, as they are difficult to clean. Instead make new fillers daily from plastic pipettes (see Section 4.3).

Q: My ACSF solution looks cloudy or has a precipitate.

A: This is often a sign that the pH of the solution is not in the right range (7.2-7.4). Check the pH. If the solution is being gassed with carbogen, check to be sure that the tank really contains 95% O2 and 5% CO2. One of the authors had an experience where the supplied tank did not have any CO2 in it, and this rapidly led to the death of the slices.

Q: My series resistance (or bridge balance) starts out OK, but increases over the course of the experiment.

A: Series resistance should be kept to a minimum (less than 15-20 MOhm) when possible. Increased series resistance is usually a sign that the tip of the pipette is clogging or the membrane is resealing. Sometimes a little pressure or suction will reopen the tip and allow access. It may also be a sign that the pipette is drifting away from the cell. In this case, visually check the pipette position and check the “My pipette tip is drifting!” FAQ. Increasing the diameter of your pipette tips can help avoid this problem.

Q: I can’t seem to compensate the amplifier in voltage clamp.

A: First, be sure that you understand the compensation procedure in the manufacturer’s manual, and practice the procedure on a model cell. Second, make sure that the electrodes are properly coated to reduce capacitance, and that the electrode series resistance is low (less than 5-10 MOhm). Make sure that the holding potential and the voltage step are in a linear range for the cell you are working with. The activation and deactivation of voltage-dependent channels can happen on the same time scale as the transients that you are trying to eliminate, making it difficult to properly adjust the compensation. Finally, remember that neurons with extensive dendrites do not appear to the clamp amplifier as a simple RC circuit with a single time constant, but have a large number of time constants. However, the amplifiers only are designed to provide proper compensation for a cell with a single time constant. In voltage clamp, you should be attempting to compensate the fastest time constant, and will not be able to correct for the slow components.

Q: There is a high frequency intermittent spiky noise on my recordings.

A: Turn off your cell phone and pager, or move them away from the rig. Make sure that the aspiration of solution from the chamber is not causing a charge separation (sometimes using a fine silver wire for the first 10-20 cm in the aspiration tube will help keep this from happening).
Equipment and Reagents

Slice and Patch specific equipment (Vendor lists are not exhaustive): * Vibration isolation table (Newport, Kinetic Systems, Technical Manufacturing Corp.) * Pipette Puller (Sutter, Narashige, Warner) * Upright fixed-stage microscope (Zeiss, Nikon, Olympus) * Hot air gun * Binocular (stereo) dissecting microscope * Tissue slicer and light source (Leica, Pella, Camden, FHC) * Recording amplifier (Axon/Molecular Devices, Heka, Dagan, NPI, ALemibic Instruments) * Micromanipulator - Sutter, Burleigh, Luigs and Neumann * CCD Camera * Oscilloscope (needed for troubleshooting; > 10 MHz bandwidth, 2 channels) * Slice chamber (Warner Instruments, Scientific Systems Design, Cell MicroControls) * Stage temperature controller (Warner Instruments) * Translation Stage (Sutter, Burleigh, custom designs) * Faraday cage (optional but recommended - can be purchased with vibration table or homemade) * Computer * Data acquisition system or cards (Molecular Devices Digidata for use with pClamp, National Instruments, Data Translation, Cambridge Electronic Design).

Standard Laboratory Equipment: * Balance * pH meter * Osmometer * Pipettors (10, 100, 200, 1000 μl) * Refrigerators, freezers (-20 non-defrosting and -80 °C) * Centrifuge (Eppendorf, tabletop). * Waterbath (electronic control, room temp - to 40 °C)

- General Glassware (flasks, beakers, graduated cylinders)
- Sintered glass gas dispersion tubes (Corning)
- Tygon and teflon tubing (We use Teflon tubing for all solution lines, and Tygon for gas distribution)
- Silastic tubing (for flexible joints between Teflon sections and glass, or for to the suction line to the electrode holder).
- Polyethylene tubing (pipette suction line)
- Teflon valves (Cole Parmer)
- Pipette Glass (Garner Glass Co. KG33 or N51, Sutter Instruments 1.2 mm pre-polished, WPI, Dagan, AM Systems).
- Holding chamber (small glass beaker with insert to hold slices)

Data acquisition software (pClamp, Cambridge Electronic Design, or custom, such as Acq4 (Campagnola et al., 2014))

Data analysis software (Igor Pro (Wavemetrics, Inc. Oswego, OR) with TaroTools (https://sites.google.com/site/tarotoolsregister/) or Neuromatic (http://www.neuromatic.thinkrandom.com), pClamp (Molecular Devices), AxoGraph (http://www.axograph.com), MATLAB (The Mathworks, Natick, MA)

Reagents: Salts should be purchased from a reliable vendor, and should be at least ACS grade or better. In particular, pay attention to the level of impurities in the salts. Storage should be according to the vendor’s recommendations. We have indicated our preferred storage below (D = dessicator, R = refrigerated at 4 °C, F = freezer at -20 °C, F80 = freezer at -80 °C). Dessication may not be a problem if you live in a dry area such as the Southwestern desert, but in the American South, it can be important.

- NaCl (D)
• KCl (D)
• – KH2PO4 (D)
• MgSO4 (D)
• CaCl2(D)
• Glucose (D)
• Sucrose (D)
• N-Methyl-D-Glucamine (B)
• Ascorbic Acid (R)
• Myo-inositol (R)
• Sodium pyruvate (R)
• – HEPES (D)
• EGTA (D)
• K-gluconate (D)
• Mg-ATP (F80)
• Na-GTP (F80)
• phosphocreatine (F80)
• Alexa-fluor 488 or similar dye, hydrazide salt (F)
• Lucifer Yellow (K+ salt). (F)

List of Definitions
* ACSF - Artificial Cerebrospinal fluid
* Internal solution - the solution that is used in the patch pipette, usually similar to the internal salt contents of a cell.
* Electrode solution - see Internal solution
* Dissection buffer - A variation of ACSF that is used during tissue dissection and cutting. May have substitutions of ions (e.g., NMDG for sodium chloride), and different pH buffer systems.
* Amplifier - An electronic unit that connects to a headstage, and provides filtering, current and voltage command adjustments, electrode resistance compensation. The amplifier may be computer controlled or may only provide analog signals to the computer.
* Headstage - the portion of the Amplifier that is placed close to the preparation and usually also holds the electrode.
* Patch Pipette - A small diameter glass capillary that has been pulled to have a rapid taper to a small tip (1-2 μm diameter) that is fire polished.
* Recording chamber - A polycarbonate or plexiglass chamber on the microscope stage, usually with a glass bottom. The chamber can be heated, has inlet and outlet connections for solution exchange, and provisions for positioning a reference electrode.

Further Reading

• The Axon Guide - This is a dated, but still very useful book. It can be found on the Molecular Devices website, and also on various laboratory websites.


References


Waldron, T. A practical Interference Free Audio System (parts 1 and 2). Web resource: http://www.nutwooduk.co.uk/archive/Old_Archive/020918.htm


Figure Legends

Figure 1. Schematic of glass electrode patched onto cell with equivalent circuit diagram. Vp: Voltage inside pipette; this is the voltage controlled or measured by the amplifier, less the electrochemical junction potential. Cp: Pipette capacitance; typically a few picofarads. Rs: Series (or access) resistance; this is the resistance separating the pipette from the cell body and is due mainly to the narrow pipette tip and organelles that may be blocking it. Rseal: Seal resistance; the resistance of the region of contact between the pipette and the membrane. To make quality recordings, this must be > 1 GΩ. Vm: Membrane voltage; the voltage of the interior of the neuron relative to the bath. Cm: Cell membrane capacitance. Rm: Cell membrane resistance; also called input resistance. Vb: Bath voltage, as measured by the ground electrode.

Figure 2. A minimal patch electrophysiology rig. Left to right: Oxygenated ACSF is siphoned through a fluid heater and into the recording chamber where it continuously washes over the brain slice. Fluid is then aspirated out of the recording chamber and into a waste flask. A patch clamp amplifier headstage is mounted to a micromanipulator and holds the patch pipette, which currently impales the brain slice (detailed in Figure 3). The headstage output is amplified, digitized, and finally recorded on a computer.
Dendrites. Note the action-potential like waveforms with no compensation. Even with 95% compensation the voltage (inset), and also increases the outward potassium current. E) Command voltage and actual voltage at the soma and corresponding to unclamped partial action potentials. Increasing compensation brings the currents under better control to depolarization from -60 to -20 mV. With no compensation, the sodium conductance leads to an oscillatory current, creasing compensation, and at best the voltage step is only about half of the command step. D) Currents in response to -60 to -70 mV. Compensation increases the amplitude of the capacitive charging transient is 900 pA past the steady-state, indicating an access resistance of 11 MΩ. B) Voltage clamp command and current recording from patch pipette in bath. The voltage clamp requires 2.4 nA of current to effect a 10 mV pulse, indicating a pipette resistance of 4.2 MΩ.

Patch procedure. A) Approach the cell with positive pressure in the pipette. The surface of the cell should form a visible dimple. B) Release pressure on pipette, then apply gentle suction to seal the membrane against the pipette. This is the cell-attached configuration. C) Apply sharp suction to the pipette to rupture the membrane, granting electrical access to the cell interior. This is the whole-cell configuration. D) From whole-cell, pull the pipette very gently away from the cell until E) the membrane separates and re-closes. This is the outside-out configuration.

Voltage-clamp recording from cell-attached pipette before (dashed line) and after (solid line) adjusting the pipette capacitance compensation. The seal resistance has increased to 1.6 GΩ. A) Photo of patch pipette in bath. B) Voltage clamp command and current recording from patch pipette in bath. The voltage clamp requires 2.4 nA of current to effect a 10 mV pulse, indicating a pipette resistance of 4.2 MΩ.

Figure 7. Voltage-clamp recording from cell-attached pipette before (dashed line) and after (solid line) adjusting the pipette capacitance compensation. The seal resistance has increased to 1.6 GΩ. A) Photo of patch pipette in bath. B) Voltage clamp command and current recording from patch pipette in bath. The voltage clamp requires 2.4 nA of current to effect a 10 mV pulse, indicating a pipette resistance of 4.2 MΩ.

Patch recording equipment. A) 63x ceramic, water-immersion objective. B) Silver chloride wire connected to headstage ground output. C) Heated aluminum holder for recording chamber. D) Plastic recording chamber with glass coverslip on the bottom. E) Brain slice bathed in warm, oxygenated ACSF. F) Glass patch pipette filled with electrode solution. G) Electrode holder. H) AgCl electrode wire. This wire fits inside the patch pipette and makes electrical contact with the electrode solution as well as the I) gold pin which conducts electrode potential into the amplifier headstage. J) Pressure control tube. This allows the experimenter to increase or decrease the pressure inside the patch pipette. K) Amplifier headstage.

Cut the tube with a sharp blade to avoid crushing it. C) Filler made from tip of pipette inserted into 1 ml syringe. D) Filler made from base of pipette attached to 1 ml syringe and a low-volume, 0.2 μm-pore syringe filter.

Neuron examples in a cortical brain slice under gradient illumination. Black arrows indicate unhealthy or dead cells, white arrows indicate healthy cells, and a grey arrow indicates a borderline cell. (This figure is a composite of multiple images from different regions of a slice.)

Figure 6. Neuron examples in a cortical brain slice under gradient illumination. Black arrows indicate unhealthy or dead cells, white arrows indicate healthy cells, and a grey arrow indicates a borderline cell. (This figure is a composite of multiple images from different regions of a slice.)

Whole-cell patched neuron filled with fluorescent dye. B) Voltage clamp command and current recording from patch pipette in bath. The voltage clamp requires 2.4 nA of current to effect a 10 mV pulse, indicating a pipette resistance of 4.2 MΩ.

Making patch pipette fillers from disposable pipette tips. A) Heating a 100 μm disposable pipette tip over a small flame. The tip should be rotated to produce even heating and care should be taken to avoid burning the plastic. B) As soon as the tip has melted through, remove it from the heat and pull into a thin tube (this takes some practice). C) Apply sharp suction to the pipette to rupture the membrane, granting electrical access to the cell interior. This is the whole-cell configuration. D) From whole-cell, pull the pipette very gently away from the cell until E) the membrane separates and re-closes. This is the outside-out configuration.
in the dendrite (long dashed lines) varies with time and does not reach the command level. B-E: Insets show the first 1 msec of the current traces (B,D) or voltage (C, E) for each compensation level. Model: “Type I” neuron (Rothman and Manis, 2003), with sodium, delayed rectifier, hyperpolarization-activated cation conductance, and leak conductance in soma. Dendrites have a delayed rectifier, Ih current and leak conductance, with Ri = 150 Ω-cm.


Waldron, T. A practical Interference Free Audio System (parts 1 and 2). Web resource: http://www.nutwooduk.co.uk/archive/Old_Archive/020918.htm

CHAPTER 9

Indices and tables

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