Patch-Clamp Recording from Neuronal Dendrites

The dendrites of pyramidal neurons have fascinated neuroscientists for decades, yet until recently, they have remained off-limits to routine electrophysiological recording. Investigations prior to the mid-1990s used blind intracellular recordings with sharp electrodes (e.g., Benardo et al., 1982; Poolos and Kocsis, 1990). The limitations of this technique included the nonspecific leak induced by sharp electrode impalements (Spruston and Johnston, 1992) and the inability to record simultaneously from two or more positions on the same neuron. The advent of differential interference contrast microscopy using infrared imaging (IR-DIC) enabled visualization of living neurons in the brain slice without staining (Stuart et al., 1993). This technique allows the investigator to accurately identify neurons of interest and place patch pipets with high spatial precision on differing parts of the neuron, including the apical dendrites of pyramidal neurons. Because the neuron is visible almost in its entirety, it is now routinely possible to record simultaneously from different areas of the same neuron, such as the soma and dendrite. This advance allowed the first proof that dendritic action potentials are initiated at the soma and are propagated into the dendrites in a retrograde manner (Stuart and Sakmann, 1994). Dendritic recording techniques are now in wide use, providing new opportunities for gaining insight into the excitability properties of dendrites (e.g., Hoffman et al., 1997; Hausser et al., 2000; Poolos et al., 2002). The techniques of visualized patch-clamp recording from dendrites in the brain slice preparation are in most respects similar to those used for recording from somata (*UNIT 6.7*). A key difference, however, is the higher level of attention required for the tissue slicing technique, which is necessary to obtain viable neurons with intact dendritic processes.

This unit explains how to obtain dendritic patch-clamp recordings by themselves (see Basic Protocol 1) or in combination with somatic recordings (see Basic Protocol 2). In addition, the special techniques that allow preparation of healthy slices suitable for dendritic patch recordings are described (see Support Protocol).

DENDRITIC RECORDING

BASIC PROTOCOL 1

This technique enables patch-clamp recording from dendrites of pyramidal neurons in a brain slice preparation. It requires an upright microscope with IR-DIC optics, as well as a high-quality micromanipulator capable of movement with submicron resolution. Best results are obtained when brain slices are prepared using the stringent technique described in the Support Protocol. The basic patch-clamp technique can be used for cell-attached, whole-cell, or excised-patch recordings.

Materials

Brain slice (see Support Protocol)
Extracellular artificial cerebrospinal fluid (aCSF; see recipe), oxygenated by bubbling with carbogen (95% O_2/5% CO_2)
Internal pipet solution—e.g., for whole-cell or cell-attached recordings (see recipes)
Experimental chamber (see Fig. 6.19.1B)
34-G platinum wire semicircle held with nylon threads (see Fig. 6.19.1B)
Upright microscope with IR-DIC optics (e.g., Zeiss Axioskop 2 FSplus, Olympus BX-51), IR-sensitive video or CCD camera (e.g., Dage Instruments Newvicon,

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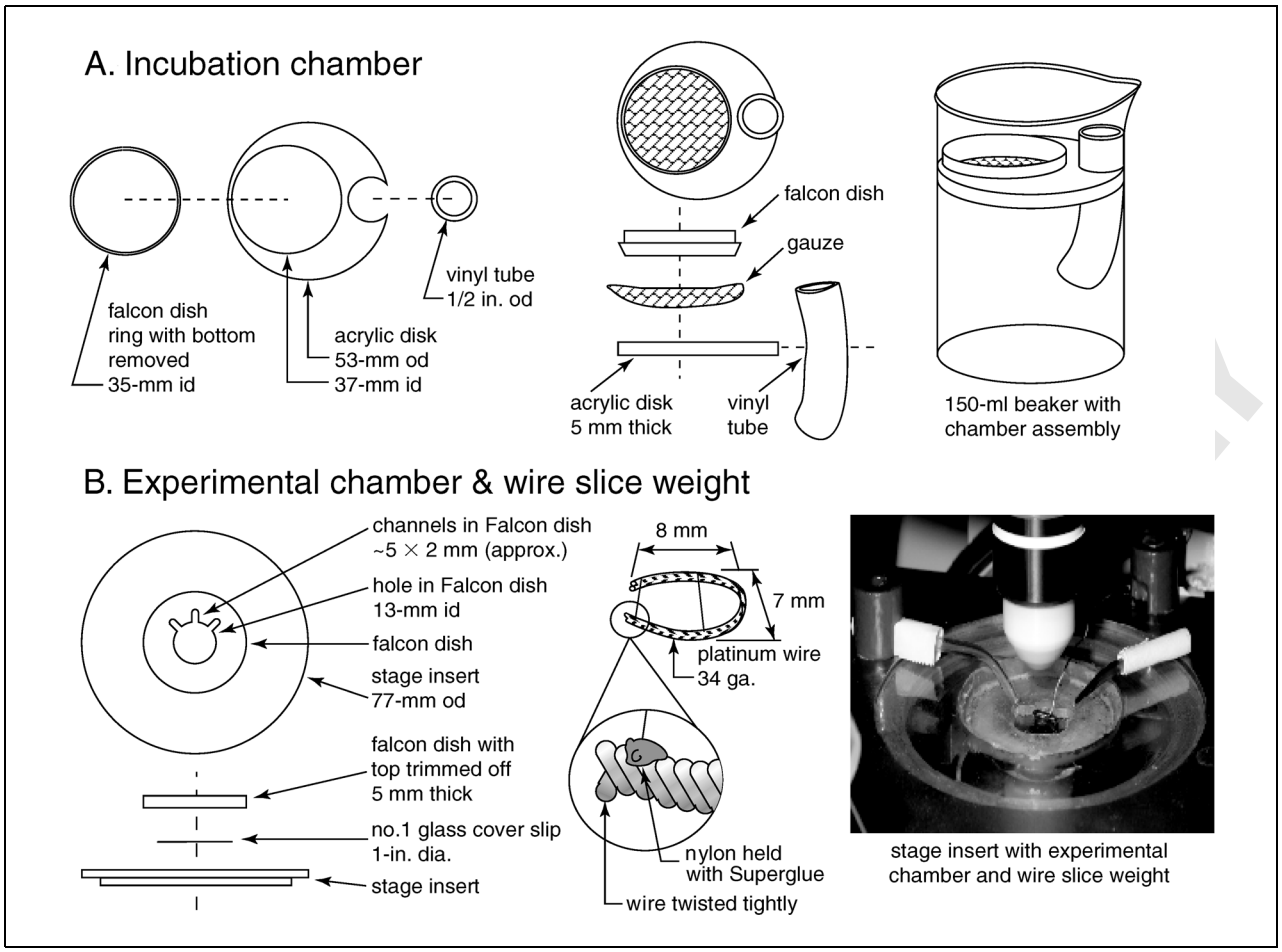

Figure 6.19.1 Schematics for apparatus construction. (**A**) Incubation chamber for warming slices. The incubation chamber can consist of a 150-ml beaker fitted with an acrylic ring that accommodates a 35-mm plastic petri dish (e.g., Falcon, Becton Dickinson). The bottom of the petri dish is removed (e.g., with a Dremel tool), giving a plastic rim that fits within the acrylic ring. A 2 × 2–in. piece of gauze is secured between the plastic rim and the acrylic ring to form a submerged surface to support the incubating slices. (**B**) Experimental chamber and platinum wire weight used to hold slice in place during recordings. The photo of the complete experimental assembly shows bath inlet and outlet tubes, as well as the ground electrode, arranged in the appropriate petri dish channels.

Hamamatsu C7500-50), and high-contrast monitor (e.g., Dage Instruments HR120)

Patch pipets (*UNIT 6.3*), preferably coated with Sylgard and fire-polished with a microforge (e.g., Narishige MF 830) just before use

5-ml syringe with tubing appropriate for pipet

Electrophysiology setup (*UNITS 6.1 & 6.6*) with high-resolution electromechanical, hydraulic, or piezoelectric micromanipulator (e.g., Sutter MP-225)

Pressure gauge (e.g., Dwyer Magnelic 2050)

Additional reagents and equipment for patch-pipet recording in brain slices using the DIC technique (*UNIT 6.7*)

Identify dendrite

1. Secure a brain slice in an experimental chamber with the "good side" up (see Support Protocol).
2. If necessary, weigh down the slice using a 34-G platinum wire formed into a semicircle encompassing the slice, with one or two nylon threads spanning the wire that will cross the slice and hold it down (Fig. 6.19.1B).

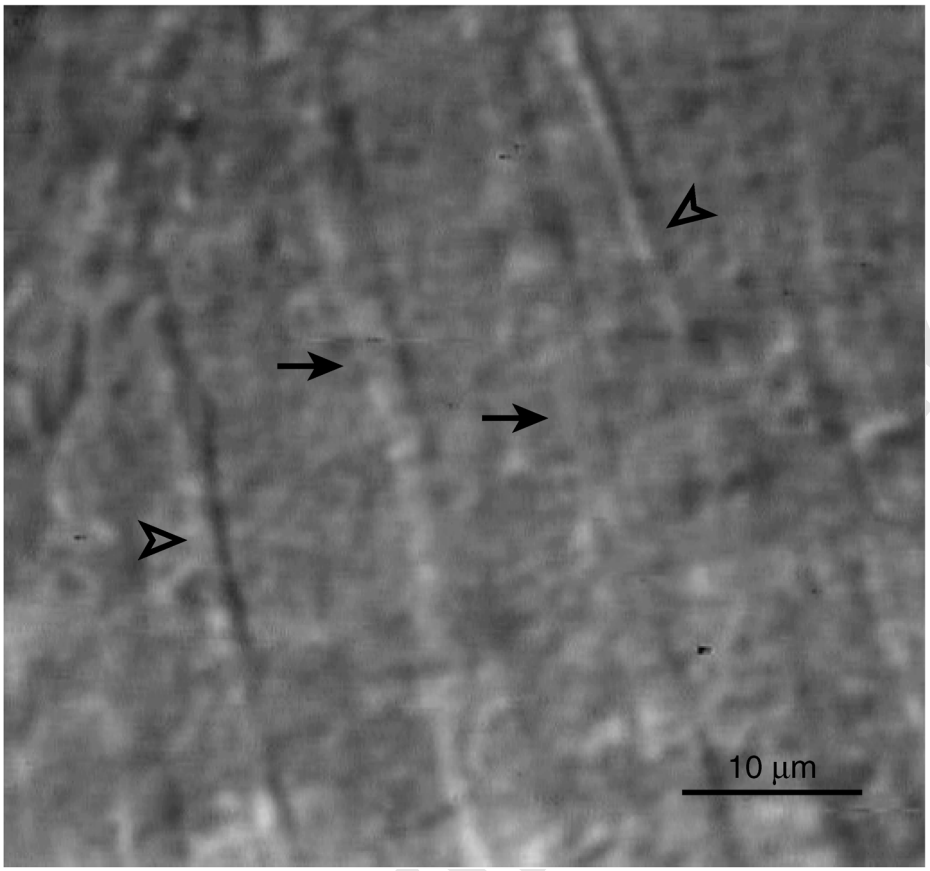

Figure 6.19.2 Video image of IR-DIC-visualized proximal CA1 hippocampal pyramidal dendrites. Dendrites which are suitable for patch recording (arrows) are noted for their comparatively large diameter, smooth surface, and low contrast appearance. Dendrites which are in poor health and not amenable to patch recording (open arrowheads) generally have a shriveled, high-contrast appearance. Scale bar, 10 μm.

3. Visualize slice on an upright microscope with IR-DIC optics, IR-sensitive video or CCD camera, and high-contrast monitor, and identify a viable dendrite of interest (*UNIT 6.7*).

   Viability is assessed by looking for processes that are plump, low-contrast, and smooth, and that have a "ground-glass" appearance (see Fig. 6.19.2). Dead or dying neurons will have a shriveled, high-contrast, and irregular surface. An exception to this rule is that unhealthy neurons can also appear swollen and translucent, and have no depth as though they are "ghosts" of previously healthy neurons.

   Developing a trained eye for assessing cell viability under DIC visualization is essential for dendrite recording. Many of the high-contrast processes that initially leap out on visual inspection are in fact unsuitable for patching, as they have undergone excessive damage during slicing and will be stiff and unyielding when a gigaohm seal is being formed. The most viable dendrites will have a more subtle, low-contrast appearance.

   The pipet tip size limits the diameter of the process that can be patched; however, because the dendrite is flattened to some extent during the patching process, with practice it is possible to patch processes not much bigger than the diameter of the pipet tip itself (1 to 2 μm).

4. Once a viable dendrite is chosen, ensure that it belongs to the neuron type of interest.

   For example, for CA1 hippocampal pyramidal dendrites, follow the dendrite both distally and proximally, observing whether it possesses the expected morphology and is continuous with a soma of expected location and morphology.

Prepare pipet

5. Select a patch pipet, preferably one coated with Sylgard and fire-polished just before use.

   Choosing the best pipet tip involves a tradeoff between the minimum size of the dendrite to be patched and the quality of the resulting recording. A small tip makes it possible to patch smaller processes (down to ~1 μm). On the other hand, a larger tip maximizes the current amplitudes that can be recorded in cell-attached voltage clamp, and reduces series resistance in whole-cell recordings. In general, unless isolating single channels, use the largest pipet tip practical for the dendrite to be patched. For example, for all but the most distal CA1 pyramidal dendrites, this may be a tip size of 1.5 to 2 μm, with a resistance of 5 to 10 MΩ.

6. Back-fill the pipet with appropriate internal pipet solution by immersing the tip in the solution and applying suction through a 5-ml syringe connected to the pipet end with tubing.

   As in any patch-clamp recording, the choice of internal pipet solution depends on the purpose of the experiment. For whole-cell recording, an intracellular solution is used; for cell-attached recording, an extracellular solution is used. Composition also depends on whether particular ion currents are to be isolated, e.g., by adding tetrodotoxin to isolate K^+ currents.

7. Attach pipet to the micromanipulator so that pipet is angled at 30° to 45° from the horizontal plane. Apply steady positive pressure (10 in. H_2O or 18 mmHg) to side port using tubing connected to a pressure gauge.

Position pipet

8. Advance pipet into the fluid meniscus formed under the microscope objective and locate the pipet tip in the center of the video screen. Monitor pipet resistance in voltage-clamp mode by applying repetitive 5-mV commands.

   The tip size produced by fire polishing (1 to 2 μm) should yield resistance of 5 to 10 MΩ.

9. Lower pipet near surface of slice. Confirm location of dendrite and position pipet so that it is displaced in the *x* axis (perpendicular to long axis of dendrite) a small distance away from the process.

10. Apply a stronger positive pressure to the pipet, ~30 to 40 in. H_2O (or 55 to 75 mmHg), and lower pipet into slice.

    A pressure wave of internal solution flowing from the pipet tip should be observed.

Approach dendrite and form seal

11. Slowly advance pipet tip toward the dendrite in both *z* (perpendicular to surface of slice) and *x* axes. If necessary, maneuver the pipet around other large dendrites or somata. Position pipet tip so that it is just above (*z* axis) the dendrite and centered on it in the *x* axis.

12. Lower pipet onto dendrite slowly, depressing surface of cell.

    If the dendrite is healthy, it should not blow away under the pressure of internal solution flowing from the pipet, nor should it provide stiff resistance to being depressed (finally springing away with further pressure), but should yield a bit. A dimple should be seen under the pipet tip, resulting from the pressure of internal solution flow. It may be

necessary to move the tip in small increments in the x axis to keep it centered on the process and prevent it from moving away under the flow of solution.

13. Once the dimple is seen, release positive pressure on the pipet and apply a small amount of negative pressure while monitoring pipet resistance.

    As the seal forms, resistance will gradually increase to >1 GΩ.

14. If the seal does not form adequately, apply stronger negative pressure to pull more membrane into the pipet tip. If a gigaohm seal doesn't form within several minutes, abandon the recording and attempt with another dendrite.

    Ideally, a gigaohm seal forms within seconds of applying negative pressure; however, sometimes it can take several minutes. Cell viability probably influences this. Conversely, a high gigaohm seal which forms almost instantly after release of positive pressure can occur with nonviable "ghost" processes.

Adjust capacitance and series resistance

15. After the seal has formed, reposition the pipet by withdrawing it slightly in the *x*-axis direction, as removing the flow of internal solution from the pipet often causes the surrounding tissue to recoil somewhat.

For recording in cell-attached voltage-clamp mode

16a. Adjust capacitive transients using the amplifier and begin data collection.

For recording in whole-cell mode

16b. Rupture the patch by applying strong negative pressure.

    Setting the voltage command to –60 mV will prevent depolarization of the cell to near 0 mV upon break-in.

17b. Switch to current-clamp mode and apply small repetitive current steps to monitor series resistance.

18b. Gently apply several puffs of positive pressure to dislodge membrane fragments back into the cell.

    Series resistance should decline to 10 to 20 MΩ. Minimizing series resistance is essential for a high-quality whole-cell recording. The positive pressure "blow-out" of intracellular contents from the pipet is a delicate but important task. Too little positive pressure will only clog the tip; too much will ruin the gigaohm seal. A successful blow-out will produce a brief, small pressure wave visible in the cell.

19b. Adjust series resistance and capacitance compensation on the amplifier and begin data collection, periodically monitoring series resistance.

SIMULTANEOUS DENDRITIC AND SOMATIC RECORDING

BASIC PROTOCOL 2

This method is the same as Basic Protocol 1, except that two micromanipulators are necessary, along with the means to record two channels of electrophysiological data. The necessity of making two recordings in serial fashion and completing data acquisition before either recording deteriorates provides many opportunities for these experiments to fail. Therefore, it is recommended that the more difficult recording (usually the dendritic recording) be attempted first.

The materials are the same as for Basic Protocol 1, except that two manipulators, two amplifiers, and so forth are necessary. The two amplifiers will share the ground lead to the bath, which typically results in some increased electrical noise. The manipulators will have to be arranged so they do not interfere with each other; usually this means the two pipets will approach the cell from opposite sides.

1. Using the same criteria described above (see Basic Protocol 1, steps 1 to 4), identify a viable neuron of correct morphology with a clearly visible soma and apical dendrite.

   Because it is disproportionately difficult to patch somata that are more than one or two cell layers deep in a stratum (e.g., stratum pyramidale in hippocampus), it may be preferable to block the tissue prior to slicing such that the dendrites are oriented "down" into the slice, with the somata closer to the surface (see Support Protocol). For more dispersed neurons such as neocortical pyramidal neurons, this may not be an issue.

2. Establish dendritic recording using the same methods described in the remaining steps of Basic Protocol 1.

   Since the dendritic recording is more difficult to achieve than the somatic recording, this step should be performed first. If there is a choice between micromanipulators used for the two recording sites, use the better instrument for the dendritic site. It may be preferable to insert the somatic recording pipet into the bath and position it near the soma with only a small amount of positive pressure before approaching the dendritic site. This may lessen the chances of jostling the dendritic pipet while positioning the somatic pipet in the bath.

3. Form the somatic recording using the same methods described in Basic Protocol 1.

   Mechanical forces from positioning the somatic pipet on the cell surface should not appreciably disturb the dendritic pipet. In whole-cell mode, however, significant depolarization may be observed at the dendritic site when the cell is approached by a somatic pipet ejecting K^+-based internal solution under pressure.

SUPPORT PROTOCOL

PREPARATION OF BRAIN SLICES

This method produces brain slices of high quality with viable dendritic processes. The central difference from more conventional methods involves intracardiac perfusion of a low Na^+ and Ca^{2+} solution to clear red blood cells from the brain, and to achieve partial replacement of the brain extracellular milieu with a solution that minimizes Na^+ and Ca^{2+} loading into cells. Also described is a process for blocking the brain to produce brain slices with the optimal orientation of the apical dendrites from CA1 hippocampal pyramidal neurons. The blocking procedure is illustrated in Figure 6.19.3.

Materials

Anesthetic: e.g., ketamine/xylazine/acepromazine
Experimental animal (e.g., rat)
Perfusion solution (see recipe), ice cold and bubbled with carbogen (95% O_2/5% CO_2)
Vetbond (cyanoacrylate glue, 3M)
Extracellular solution (see recipe), bubbled with carbogen
No. 10 scalpel
Large dissecting forceps, toothed
Surgical scissors
Large hemostat
18- and 25-G hypodermic needles
Intravenous (i.v.) perfusion set and delivery system (e.g., 60-ml syringe reservoir with appropriate tubing)
Pyrex dish, round, 3-in. diameter with 1-in. sides
Iridectomy scissors, carbide-edged
Bone rongeurs
Weighing minispatula with a 30° bend in the blade
Filter paper disk, 2-in. diameter
Petri dish, chilled

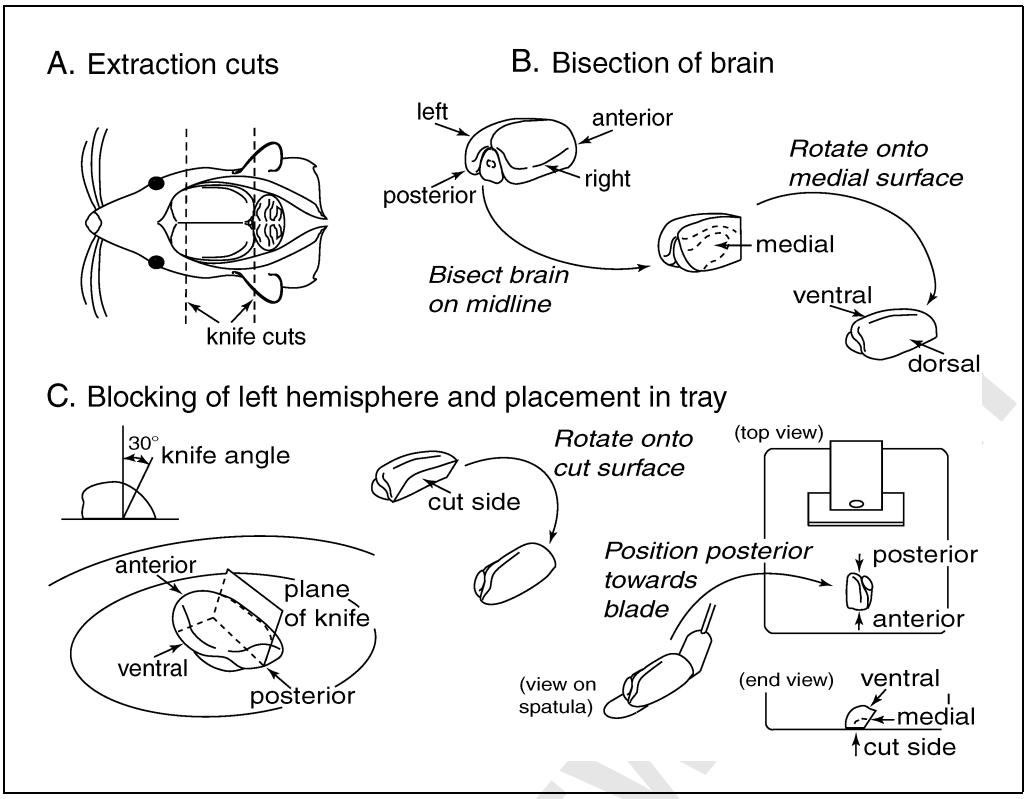

Figure 6.19.3 Procedure for blocking brain to produce optimal orientation of CA1 hippocampal pyramidal neuron apical dendrites. (**A**) Knife cuts used to extract brain. (**B**) Separation of cerebral hemispheres. (**C**) Blocking left hemisphere and placement in tissue slicer.

Vibrating tissue slicer (e.g., Vibratome 1500; Electron Microscopy Sciences OTS 4000)

Incubation chamber: 150-ml Pyrex beaker fitted with an acrylic ring and 35-mm-i.d. plastic petri dish to support gauze platform (see Fig. 6.19.1A)

35°C water bath

Cutting blade, preferrably glass (Pelco knife maker, Ted Pella) or artificial sapphire (Delaware Diamond Knives)

Dumont tweezers

Additional reagents and equipment for anesthetizing animals (*APPENDIX 4B*)

NOTE: All protocols using live animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) and must follow officially approved procedures for the care and use of laboratory animals.

Perfuse animal

1. Anesthetize the experimental animal (*APPENDIX 4B*).

   A mixture containing 21 mg/kg ketamine, 3.2 mg/kg xylazine, and 0.7 mg/kg acepromazine given i.p. is effective, but other agents (e.g., pentobarbital, volatile anesthetics) work as well.

2. Position the animal in a supine position, securing the upper limbs with laboratory tape so the abdomen is well exposed. Confirm that anesthesia is sufficiently deep by testing for the lack of lower limb retraction from a foot pinch.

3. Make a midline incision with a no. 10 scalpel through the skin from the upper thorax to the mid-abdomen.

4. Grasp the abdominal wall with large, toothed dissecting forceps, and use surgical scissors to make a cut below the sternum through the abdominal wall and rib cage. Extend cuts in a U-shaped pattern to create a flap of the chest wall which can be retracted with a large hemostat.

   This will completely expose the heart.

5. Insert an 18-G hypodermic needle connected to an i.v. perfusion set into the apex of the heart, advancing the tip just enough to enter the left ventricle.

6. As soon as the needle is inserted into the heart, use scissors to make a cut into the right atrium, which will allow exsanguination during perfusion.

7. Perfuse with ice-cold oxygenated perfusion solution at a rate of 10 to 15 ml/min via an appropriate delivery system.

   This can be accomplished using a gravity-fed delivery system such as a 60-ml syringe reservoir placed at a height of ~24 in. above the animal to feed the i.v. perfusion set. The flow rate is controlled using the thumbwheel of the perfusion set.

   It is critical that the perfusion solution be as cold as possible when it enters the heart. To keep the solution as close to 0°C as possible, the perfusion tubing can be run through an ice water bath.

8. Continue perfusion until the fluid leaving the right atrium no longer resembles blood, but is pinkish or mostly clear.

   This process should take no more than 90 to 120 sec.

Remove and block brain

9. Remove the head and plunge it into ice-cold perfusion solution in a 3-in.-diameter Pyrex dish with 1-in. sides.

10. Make a midline scalpel incision through the scalp.

11. Using carbide-edged iridectomy scissors, cut through the midline of the skull, starting at the posterior aspect. Remove the skull flaps with bone rongeurs to expose the cerebral hemispheres.

12. Make anterior and posterior cuts through the brain in the coronal plane (Fig. 6.19.3A). Using a weighing minispatula with a 30° bend in the blade, sever the brainstem and dislodge the brain from the skull.

13. Let the freed brain rest in cold perfusion solution for a minute, and then make a mid-sagittal incision to separate the brain into two hemispheres (Fig. 6.19.3B).

14. Remove the left hemisphere (leaving the right hemisphere in the cold solution) and place medial-side-down on a 2-in.-diameter filter paper disk in a chilled petri dish. Orient the hemisphere so the anterior end faces forward and the posterior end faces toward the dissector.

15. Make a scalpel incision through the dorsal aspect of the hemisphere, angled rightward at 30° from the vertical (Fig. 6.19.3C).

16. Transfer hemisphere to the vibratome stage using a weighing spatula so that the 30° cut surface becomes the base on which the brain rests and the posterior end faces forward (Fig. 6.19.3C). Affix the cut surface to the stage with Vetbond. Fill slicing chamber with ice-cold perfusion solution.

   The ventral aspect of the brain should now be face up and tilted to the right.

   The blocking procedure optimizes the angle of the apical dendrites within the tissue slice for CA1 hippocampal pyramidal neurons. The desired result is for the dendrites to course

towards the surface of the slice ("up") along their length. This increases the likelihood of making distal apical recordings since the dendrite will be closer to the surface of the slice rather than disappearing into the middle of the slice. A different orientation may be preferred for dual somatic/dendritic recordings (see Basic Protocol 2). Also, because the hippocampus is a curved structure, not all slices cut using this procedure will have the apical dendrites in the correct orientation. Finally, a different blocking procedure is required for preparing neocortical slices, and will depend on the cortical region being studied.

17. To slice the right hemisphere, repeat the blocking process, but angle the scalpel incision through the hemisphere leftward at 30°.

    The authors recommend slicing one hemisphere at a time, leaving the unsliced hemisphere in cold perfusion solution until ready for use. Some investigators slice both hemispheres simultaneously.

Slice brain

18. Before slicing, fill the incubation chamber (Fig. 6.19.1A) with extracellular solution and place in a 35°C water bath. Bubble the solution with carbogen via a 25-G hypodermic needle connected to tubing from the carbogen source. Set the carbogen flow at a level that will not agitate the slices.

19. Adjust the angle of the cutting blade to 20° to 25° from horizontal. Set the vibration amplitude high enough that slicing produces no evidence of shearing or stretching of tissue. Minimize the speed of the blade advance as much as practical while still allowing the overall sectioning process to occur in a reasonable period of time (e.g., 5 to 7 mm/min).

    Any of several tissue slicers may produce satisfactory results. Recommended manufacturers are Vibratome, Leica, and Electron Microscopy Sciences. The choice of slicing blade may also vary. In general, good results are achieved with glass blades (fabricated with a Ralph-type knife maker using 6-mm glass strips, Ted Pella) or with synthetic sapphire blades. Less satisfactory results have been obtained with steel razor blades, regardless of maker.

20. Make a first pass into the hemisphere, sectioning away unnecessary tissue until the desired tissue plane comes into view.

21. Using Dumont tweezers, peel away the meningeal membranes adhering to the cortical surface, as they tend to resist cutting and can deform and stretch the tissue during passage of the blade.

22. Cut subsequent tissue slices at 350- to 400-µm thickness, taking care to keep the bath solution at 4°C or less. Arrange cut slices at the bottom of the bath with the just-cut surface face-up.

    The slicing process tends to produce a "good" side and a "bad" side of the slice. The upper surface (i.e., the side of the slice that was exposed by the previous sectioning) tends to be more damaged than the underside of the newly cut slice. This may result from the greater contact of the upper surface with the vibrating blade, as opposed to the underside, which is more mobile during cutting. Paying attention to the orientation of the "good" side will save much time during the recording process.

23. After all slices have been cut but before removing them from the bath, block further as needed to remove unwanted tissue.

24. Using a dropper constructed from the blunt end of a Pasteur pipet, transfer the slices to the incubation chamber (step 18) and incubate 10 min with carbogen bubbling. Transfer the chamber to room temperature for 1 hr before recording, while still oxygenating.

High-temperature incubation improves slice quality, possibly by rapidly restoring the activity of homeostatic ion pumps. Some investigators use longer periods of incubation (up to 1 hr). The sudden exposure of slices to 35°C seems to work better than a gradual rewarming or no rewarming at all (Moyer and Brown, 1998).

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see ***APPENDIX 2A****; for suppliers, see* ***SUPPLIERS APPENDIX****.*

Extracellular solution

125 mM NaCl
2.5 mM KCl
1.25 mM NaH_2PO_4
25 mM $NaHCO_3$
2 mM $CaCl_2$
2 $MgCl_2$
10 mM dextrose
Store up to 1 week at 4°C

Internal pipet solution, cell-attached (for K^+ currents)

125 mM NaCl
10 mM HEPES
2.5 mM KCl
2 mM $CaCl_2$
1 mM $MgCl_2$
1 μM tetrodotoxin (TTX)
Adjust pH to 7.3 with 1 M NaOH
Store up to 1 month at 4°C

Internal pipet solution, whole-cell

120 mM $KMeSO_4$ or K-gluconate
20 mM KCl
10 mM HEPES
0.2 mM EGTA
2 mM $MgCl_2$
4 mM Na_2-ATP
0.3 mM Tris-GTP
Adjust pH to 7.3 with 1 M KOH
Store in ??-ml aliquots up to 6 months at −20°C

These storage conditions will preserve ATP/GTP activity.

$KMeSO_4$ produces less blockade of the K^+ currents mediating slow afterhyperpolarizing potentials than K-gluconate (Velumian et al., 1997).

Perfusion solution

110 mM choline chloride
2.5 mM KCl
1.25 mM NaH_2PO_4
25 mM $NaHCO_3$
0.5 mM $CaCl_2$
7 mM $MgCl_2$
7 mM dextrose

continued

1.3 mM ascorbic acid
3 mM pyruvic acid
Store up to 1 week at 4°C

Prior to use, chill the solution until a thin layer of ice has formed, then stir the ice into a thin slurry using a hand-held ice grinder (Braun).

Sucrose may be substituted for choline chloride in equi-osmolar amounts. (Total osmolarity should be verified with an osmometer to be ~300 mosm.) Ascorbate and pyruvate may improve tissue resistance to hypoxia during the slicing procedure (MacGregor et al., 1996; Lee et al., 2001).

COMMENTARY

Background Information

Visualization of brain slices using IR-DIC optics has revolutionized neuronal electrophysiology. The ability to identify individual neurons and their processes in the slice preparation allows more efficient and accurate patch-clamp recording than previous "blind" methods because neurons can be visually selected by type and health. More importantly, IR-DIC visualization has made recording from pyramidal neuron dendrites routine, again for reasons of efficiency, and because visualization allows simultaneous recording from two or more regions on the same neuron (e.g., soma and dendrite). Dual simultaneous recordings have answered many fundamental questions about dendritic electrophysiology, such as the sites of action potential initiation and propagation within dendrites. Because dendrites occupy >90% of the surface area of pyramidal neurons, many important questions regarding the electrical properties of neurons will require dendritic electrophysiological investigation.

Critical Parameters and Troubleshooting

Visualization

Much of the technique of dendritic recording remains similar to patch recording from cell bodies. The major difference is the optimum use of the optics to ensure a high percentage of quality recordings. The adjustment of the IR-DIC optics to provide maximum contrast and penetration into tissue is essential (*UNIT 6.7*). The authors have found dichroic IR filters (e.g., Chroma D770/40) to be better than low-pass RG9 filters. Likewise, the Newvicon IR-sensitive video camera is superior to most CCD cameras, although the Hamamatsu C7500-50 comes close and is much smaller in size. An improved IR-DIC image pays dividends in electrophysiology results by allowing identification of healthy, relatively low-contrast processes which may lie deep in the slice.

Health of slices and dendrites

Beyond optics, the most important area of variability is slice health, particularly when mature animals are used. Slice health tends to decline significantly after about 3 weeks of age in rats, yet neuronal ion channel properties in pyramidal neurons have not reached their adult configuration until 5 to 6 weeks (Zhu, 2000). IR-DIC imaging has made it all too clear that brain slices of adult animals can have dismally unhealthy-appearing neurons, even though extracellularly recorded field responses (e.g., population spikes.) are present. Nonetheless, careful attention to the process of brain slicing will yield tissue with many healthy neurons and dendrites worthy of patch-clamping.

Many investigators have found that intracardiac perfusion is essential for healthy adult brain slices. Not only does perfusion clear the vasculature of red blood cells (which diminish the quality of IR-DIC), but slice health is improved by a partial replacement of the extracellular milieu with a low Na^+, low Ca^{2+} perfusion solution, diminishing the deleterious effects of hypoxia-induced excitotoxicity and Na^+ overload. Cooling the brain also mitigates excitotoxicity. Since the process of perfusion cools the brain less quickly than rapid decapitation and immersion into cold saline, it is important to chill the perfusate as much as possible and perfuse only as long as necessary to achieve wash-out of cerebral blood.

Learning to identify healthy dendrites requires trial and error. The most effortless recordings of the highest quality come from fat, smooth, low-contrast processes. In fact, the more easily visible and high-contrast a dendrite is, the less likely it belongs to a healthy neuron. This highlights the importance of good IR-DIC optics in allowing one to seek out the less obvious processes.

Tissue slicers

It is not clear that choice of vibratome is critical in achieving good slice quality. Good results have been achieved with several models. The choice of cutting blade may be more important, with anecdotal evidence suggesting that sapphire or glass blades are superior. The former have the advantage of requiring no fabrication but need to be replaced periodically, which can be expensive; the latter are single-use and exquisitely sharp but require some practice to fabricate.

Patching

The actual process of patching dendrites has much in common with the technique used for somatic patch-clamp recording. The added challenge is in trapping a small, sometimes mobile process with a pipet tip that may be of a comparable diameter. Again, healthy cells are the most permissive of patching. Unhealthy processes tend to roll or spring away from the pipet, likely due to increased stiffness of the shrunken dendritic membrane. It is also more difficult to form a gigaohm seal on an unhealthy cell, and voltage-gated currents may run down quickly once a seal is formed or may even be absent immediately after break-in. Other problems that may occur in slices that are of less than optimal quality are the inability to break in after a seal is formed in whole-cell mode, or poor resting potential after break-in. Troubleshooting of problems in patch-clamp recording in general is covered in Table 6.7.1.

Anticipated Results

These techniques should allow the routine recording of smalldiameter ($\geq$1 to 2 μm) pyramidal neuron dendrites, with at least one or two successful recordings per experimental preparation. In rat hippocampal CA1 pyramidal neurons, recordings can be made in apical dendrites up to 300 μm from the cell soma; in neocortical pyramidal neurons, recordings can be made up to the distal tuft ($>$700 μm in layer V neurons). Interneurons with pyramidal-like morphology tend to have even larger-diameter dendrites than principal neurons; recordings in these dendrites are possible virtually throughout the length of the apical dendrite.

Time Considerations

As with almost any kind of slice-based electrophysiology, an experiment occupies most of the working day. Preparing and freezing of solutions plus dissection setup takes an hour or so, the animal preparation and slicing another half-hour, and incubation of slices an hour. Once the tissue is ready for experimentation, it is best used within 4 to 5 hr, especially if experimentation is done at $\geq$30°C. Since the gradual decline in slice viability begins as soon as the preparation is made, experiments should be initiated as soon as the incubation period is complete to attain the highest quality recordings.

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Key References

Stuart et al., 1993. See above.

First application of IR-DIC optical techniques to patch-clamp recording in brain slices.

Moyer and Brown, 1998. See above.

Detailed examination of tissue slicing parameters that affect neuronal health when using mature animals.

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