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Protocol

In Vivo Patch-Clamp Recording in Awake Head-Fixed Rodents

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Whole-cell recording has been used to measure and manipulate a neuron's spiking and subthreshold membrane potential, allowing assessment of the cell's inputs and outputs as well as its intrinsic membrane properties. This technique has also been combined with pharmacology and optogenetics as well as morphological reconstruction to address critical questions concerning neuronal integration, plasticity, and connectivity. This protocol describes a technique for obtaining whole-cell recordings in awake head-fixed animals, allowing such questions to be investigated within the context of an intact network and natural behavioral states. First, animals are habituated to sit quietly with their heads fixed in place. Then, a whole-cell recording is obtained using an efficient, blind patching protocol. We have successfully applied this technique to rats and mice.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Agarose (2% [w/v] in physiological saline; Sigma-Aldrich A9539)
Analgesic (e.g., Buprenex [Reckitt Benckiser Pharmaceuticals])
Bupivacaine hydrochloride monohydrate (Sigma-Aldrich B5274)
Dental acrylic (e.g., Jet Denture Repair Package [Lang Dental])
Dental adhesive, light-cured (e.g., OptiBond FL [Kerr Dental])
Dental composite, light-cured (e.g., Charisma [Heraeus Kulzer])
Experimental animals

This protocol has been successfully used with juvenile rats (age of 4–6 wk) and adult mice (age of ∼3 mo).

Intracellular pipette solution <R>
Isoflurane (e.g., IsoSol [Vedco])
Ketamine hydrochloride/xylazine hydrochloride (Sigma-Aldrich K4138)
Paraformaldehyde (4%)
Phosphate-buffered saline (PBS) (0.1 M)

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Physiological saline (i.e., 0.9% NaCl)
Silicone elastomer (e.g., Kwik-Cast Sealant [World Precision Instruments])
Veterinary ophthalmic ointment (e.g., Puralube [Dechra Veterinary Products])

Equipment

Acquisition software (e.g., Patchmaster [HEKA Elektronik])
Acquisition system, analog-to-digital (e.g., InstruTECH [HEKA Elektronik])
Ag/AgCl pipette with reference electrode wire
Amplifier (e.g., npi electronic ELC-03XS)
Anesthesia system (e.g., VetEquip 901806)
Audio monitor (e.g., Grass Technologies AM10)
Curing unit (e.g., Translux Power Blue [Heraeus Kulzer])
Faraday cage
Glass capillaries, borosilicate, with filament, 1.5 mm OD, 0.87 mm ID (Hilgenberg)
Glass capillary, reference electrode-holding (~15 mm long, 1 mm OD)
Headplate, custom-designed (Fig. 1A)
Light source, halogen (Schott KL1500 HAL)
Manometer (Sigmann Elektronik)
Microdrill (Foredom)
Microdrill bit (0.45 mm)
Micromanipulator, motorized, 3- or 4-axis (e.g., Luigs & Neumann)
Micropipette storage jar (e.g., World Precision Instruments)
Patching station

This custom-designed unit consists of a base plate to which a headplate holder (Fig. 1B) is attached.

Pipette puller (e.g., Narishige PC-10 or Sutter Instrument P-97)
Reference electrode-holder cap

An ~5-mm-long piece of Tygon tubing (1/32 in. ID) with one end blocked is suitable for this purpose.

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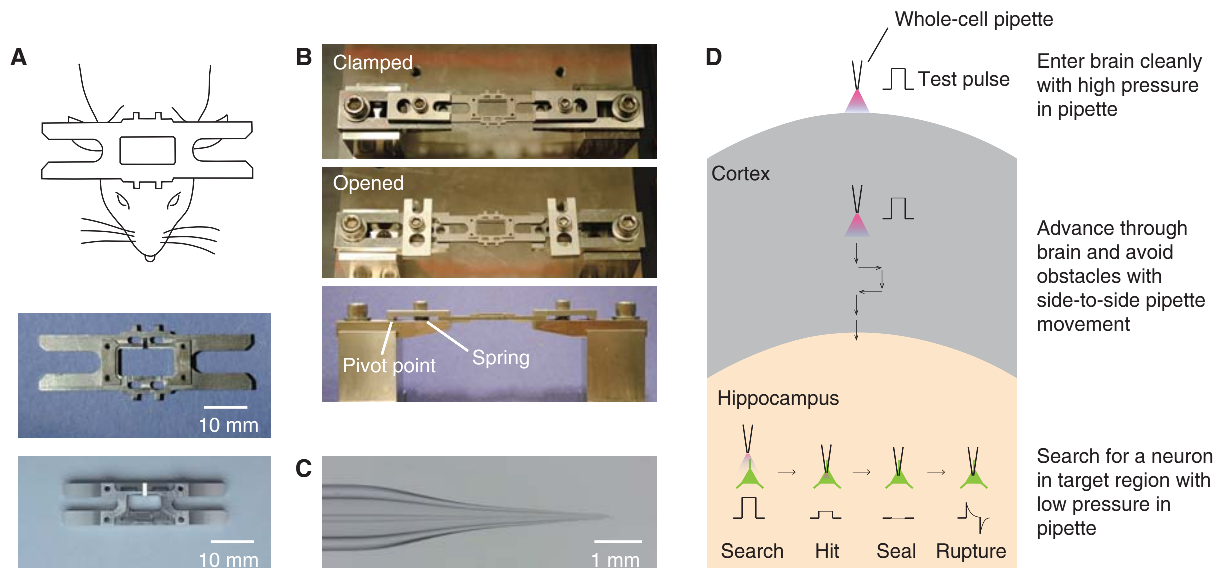

FIGURE 1. Whole-cell recordings in awake head-fixed animals. (*A*) Drawing showing position of headplate attached to a rat's head (*top*). Headplate designs for rats (*middle*) and mice (*bottom*). (*B*) Rapidly releasable headplate holder. (*C*) An example of a recording pipette. (*D*) Whole-cell patching procedure for awake head-fixed animals. (*B*, reprinted, with permission, from Lee et al. 2014.)

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Stereoscope (e.g., Olympus SZ61)
Stereotaxic apparatus (e.g., Narishige SR-6)
Surgical instruments (Fine Science Tools)

Clean and sterilize instruments thoroughly before use.

Temperature control system (e.g., FHC)
Vibration isolation table (Newport)

METHOD

Headplate Implantation Surgery

Use a temperature control system to maintain the body temperature of the experimental animal at ~37°C during surgery.

1. Using an anesthesia system, sedate the experimental animal using 1.5%–2.0% isoflurane.
2. Affix the animal's head in the stereotaxic apparatus.
3. Apply ophthalmic ointment to the subject's eyes. Cover with small pieces of black paper.

   This protects the subject's eyes during surgery.
4. Subcutaneously inject bupivacaine (2 mg/kg body weight) into the subject's scalp.
5. Make an incision along the scalp midline. Open the skin to expose the skull. Scrape away the connective tissue covering the bone.
6. Mark the coordinates overlying the region of the brain of interest.

   For example, if the dorsal part of the rat hippocampal subregion CA1 is to be studied, mark a point 3.5 mm posterior and 2.5 mm lateral to the bregma.
7. Taking care to avoid the target site, apply three to four drops of dental adhesive to the skull. Light-cure the adhesive.
8. Apply a 0.5- to 1-mm thick layer of dental composite on top of the cured adhesive. Light-cure the composite.
9. Affix the headplate to the cured composite on the subject's skull with a layer of dental acrylic. Cover the inner wall of the headplate completely with dental acrylic such that the area inside the headplate is isolated electrically from the rest of the recording station.

   Make sure there is no gap between the skull and the headplate.
10. Using dental acrylic, attach a reference electrode-holding capillary to the headplate.
11. After surgery, proceed as follows:
    1. Rehydrate the experimental subject by injecting it subcutaneously with ~2 mL warmed saline/100 g body weight.
    2. Administer an analgesic (e.g., 0.05 mg Buprenex/kg body weight) immediately after surgery and for the next 2 d.
    3. House experimental animals individually until they are fully recovered (at least 2 d).

Habituation to Head Fixation

12. Affix the headplate attached to the experimental subject's head to the patching station's headplate holder. Leave the animal undisturbed for ~5 min on the first day. Over the course of 4 d, gradually increase the time of habituation to ~1 h.
13. Once the animal is habituated to head fixation, expose it to experiment-related noises during each training session.

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14. Continue fixation training daily with 1 h-sessions until the animal is sufficiently accustomed to being head-fixed and can sit quietly for several minutes at a time without movement.

    Obtaining a giga-seal is difficult if an animal moves during patching. Proceed to Step 15 only with animals that are sufficiently habituated to head fixation. In our hands, >50% of animals proceed to patching.

Craniotomy and Target Depth Determination

15. On the first day of patching, anesthetize the animal with 1.5%–2.0% isoflurane. Fix its head into the patching station.
16. Drill a 1–2 mm diameter hole in the skull at the previously marked target coordinates.
17. Optionally (required for rats; for mice this step can be omitted), use a ≥26-gauge needle and/or microscissors to cut a small opening in the dura (~0.5 mm diameter).

    Be careful not to make the dura opening larger than ~1 mm, as this can otherwise lead to tissue swelling. Be especially careful not to damage the brain tissue below when targeting cortical areas.

18. Insert an Ag/AgCl reference electrode through the reference electrode-holding capillary.
19. Insert an extracellular glass electrode (1–3 MΩ). Search for characteristic activity by listening to the audio monitor.

    This step might not be necessary for shallow cortical target areas but is essential for deeper targets such as the hippocampus (for which the characteristic activity of the CA1 pyramidal cell layer is intermittent bursting).

20. Once the target depth has been established, protect the brain surface by covering it with a layer of 2% agarose, then cover the agarose with a layer of silicone elastomer. Remove the reference electrode, and cap the reference electrode-holding capillary.
21. Return the animal to its home cage for at least 2 h of recovery before starting a whole-cell recording session.

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Awake Whole-Cell Recording

22. Head-fix the animal in the patching station.
23. Insert an Ag/AgCl reference electrode through the reference electrode-holding capillary.
24. Fill a glass pipette (prepared in advance; 4–7 MΩ) with ~5 µL of the intracellular pipette solution.

    See Fig. 1C for an example of a glass pipette. However, other pipette shapes have also been successfully used in our hands and those of others.

25. Using the micromanipulator onto which the headstage is mounted, position the pipette tip a few hundred microns above the brain surface. Apply high pressure to the inside of the pipette (~800 mbar for a deep target such as the hippocampus, ~300 mbar for a superficial target). Lower the pipette onto the brain surface (Fig. 1D).

    It is critical to enter the brain surface through a clean spot. Especially avoid spots with clearly visible blood. When the dura is not removed in mice, the electrode passes through the dura, but patching through dura does not otherwise require a different technique.

26. While maintaining high-positive pipette pressure, slowly lower the pipette in 5-µm increments into the brain to the search depth (e.g., in the case of the dorsal CA1 pyramidal cell layer, 100–150 µm above the estimated target depth).

    It is critical to keep the pipette tip clean until attempting giga-seal formation. Carefully monitor the pipette resistance (a good indicator of the cleanness of the pipette tip) while advancing the pipette.

    See Troubleshooting.

27. When the pipette reaches the search depth:
    i. Reduce the positive pressure applied to the pipette to 25–35 mbar (i.e., "search pressure").

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ii. Advance the pipette using 1-µm steps to search for a target neuron (Fig. 1D).

iii. When there is an increase in the pipette resistance (by ∼20% or more) for 4–5 consecutive steps, attempt to form a giga-seal by removing the positive pressure and applying gentle negative pressure (<10 mbar).

At this point, hyperpolarizing the pipette holding potential to −65 mV can facilitate giga-seal formation.

See Troubleshooting.

28. Rupture the membrane within the patch pipette by applying a brief and strong pulse of negative pressure (>100 mbar for ∼0.2 sec) to achieve the whole-cell configuration.

If no giga-seal is attained or the break-in to whole-cell configuration is unsuccessful, retract the pipette and repeat Steps 24–28 with a new pipette.

29. At the end of each recording, withdraw the recording pipette slowly to facilitate resealing of the membrane for histological recovery.

30. When a recording session is finished:

i. Cover the brain surface with a layer of 2% agarose, followed by a layer of silicone elastomer.

ii. Remove the reference electrode. Cap the reference electrode-holding capillary.

iii. Return the animal to its home cage.

If the same subject undergoes two or more recording sessions on the same day, permit the animal to recuperate in its home cage for 1–2 h between sessions. (We have obtained up to five whole-cell recordings from hippocampal area CA1 in the same hemisphere and have not, in this or any other cases, observed any noticeable impairment in the network activity.)

Histology

31. After the last recording session, deeply anesthetize the animal by administrating ketamine (200 mg/kg body weight)/xylazine (10 mg/kg body weight).

32. Perfuse the animal transcardially with 0.1 M PBS, then with 4% paraformaldehyde.

33. Extract the brain. Fix overnight in paraformaldehyde. Transfer to PBS.

34. Slice the brain into 150-µm thick sections. Visualize neuronal morphology using an avidin–biotin–peroxidase method (Horikawa and Armstrong 1988).

When multiple neurons are recorded from the same hemisphere, unambiguously identifying each neuron is usually difficult. Therefore, for unambiguous identification, filling only one neuron per hemisphere is recommended.

See Troubleshooting.

TROUBLESHOOTING

Problem (Step 26): Resistance increases when the pipette is inserted into the brain.

Solution: If the dura is dissected (e.g., as for rats), pipette resistance should not change on entry. Repeat the dissection of the dura, or target the pipette to a clean spot on the dissected area. If inserting the pipette through an intact dura (e.g., as in mice), pipette resistance might increase temporarily, but it should return to the original value as soon as the pipette penetrates the dura. If the resistance does not recover, increase the positive pressure applied to the inside of the pipette.

Problem (Step 26): The pipette encounters an obstacle (e.g., a cell ahead of the target depth, a blood vessel, etc.) during its advance to the search depth.

Solution: Generally, an increase in resistance by 10% or more is indicative of an obstruction. Retract the pipette a few tens of microns until the resistance returns to its original value, then advance it again. In many cases, this will allow one to bypass the obstacle without any increase in resistance.

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If this remains unsuccessful after several attempts, retract the pipette until the resistance returns to its original value, reposition it to the side by up to 50 µm (usually <10 µm), then advance the pipette again in an attempt to bypass the obstacle (Fig. 1D). Repeat as necessary to avoid all obstacles on the way to the search depth.

Problem (Steps 26–27): Pipette resistance cannot be maintained until giga-seal formation is attempted.
Solution: Excessive movement while the subject is restrained can translate to large brain movements. Wait until the animal stops moving, extend the habituation period, or reject the subject in favor of a calmer animal. In practice, fewer than half of the animals need to be rejected for this reason.

Problem (Step 27): Gigaohm seal cannot be established.
Solution: Using a microscope, examine the pipette tip for clogging; in such cases, the positive pressure applied to the inside of the pipette cannot effectively push away thin dendrites or the extracellular matrix. If the same issue occurs repeatedly using different pipettes, try the following steps to eliminate the source of the clog: filter the internal solution; clean the inside of the glass capillaries before pulling pipettes; rechloride the recording wire. If no clog is evident, advance the pipette further into the membrane after the increase in resistance before removing positive pressure (e.g., instead of 4–5 steps of 1 µm, use 6–8 steps of 1 µm).

Problem (Step 27): The pipette does not hit the somata of neurons.
Solution: If the pipette hits dendrites, glia, or other structures in the brain, estimation of the target depth might be incorrect. Repeat Step 19 to find the correct depth of the target brain region. If the pipette hits dendrites, the search pressure might be too low. If the pipette does not hit any structures during the search for a neuron, the search pressure might be too high.

Problem (Step 34): The recorded cells cannot be observed histologically.
Solution: If a recording ends because of a loss of the whole-cell configuration, there is a greater chance that the biocytin will leak out, resulting in poor visualization of the recorded neuron. If histological recovery is required in such cases, perfuse the brain as soon as possible. If the whole-cell configuration is still intact at the end of a recording, slowly withdraw the recording pipette to allow the membrane to reseal, thus limiting biocytin leakage.

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DISCUSSION

Because of the high sensitivity of whole-cell recording to mechanical perturbations, in vivo whole-cell recording has been performed more often in anesthetized animals. Recently, an increasing number of studies have performed whole-cell recording in awake head-fixed animals (Margrie et al. 2002; Crochet and Petersen 2006; Harvey et al. 2009; Lee et al. 2014; Tan et al. 2014). Under optimal conditions, the success rate, recording duration, and recording quality rely largely on the extent of brain movement. Thus, habituating animals to sit quietly for long enough periods of time is important for successful recording. If an appropriately habituated (i.e., calm) animal is used, the patching method described here can usually achieve more than one recording per every three attempts (i.e., three different pipettes). Because it can be particularly challenging to obtain a giga-seal while the animal is moving, one should attempt giga-seal formation only while the animal is sitting quietly; once a tight seal is formed, recordings can tolerate gentle-to-moderate brain movement. In many experiments, active movement of the animal is required, such as for comparing processing between a quiet period and a period with locomotion (Bennett et al. 2013; Polack et al. 2013; Schiemann et al. 2015), or running on a treadmill to navigate virtual environments (Harvey et al. 2009; Domnisoru et al. 2013; Schmidt-Hieber and Häusser 2013; Bittner et al. 2015). In these cases, selecting animals that walk or run smoothly—which cause less-sudden brain motions—is critical for long-lasting recordings.

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RECIPE

Intracellular Pipette Solution

Reagent	Final concentration
Potassium gluconate	135 mM
HEPES	10 mM
Na_2-phosphocreatine	10 mM
KCl	4 mM
MgATP	4 mM
Na_3GTP	0.3 mM
Biocytin	~0.05% (w/v)

Prepare in distilled water. Adjust pH to 7.3 using KOH (target osmolarity is 295 mOsm). Store the solution for up to 1 yr at −20°C.

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