Protocol

Efficient Method for Whole-Cell Recording in Freely Moving Rodents Using Ultraviolet-Cured Collar-Based Pipette Stabilization

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Whole-cell recording is a key technique for investigating synaptic and cellular mechanisms underlying various brain functions. However, because of its high sensitivity to mechanical disturbances, applying the whole-cell recording method to freely moving animals has been challenging. Here, we describe a technique for obtaining such recordings in freely moving, drug-free animals with a high success rate. This technique involves three major steps: obtaining a whole-cell recording from awake head-fixed animals, reliable and efficient stabilization of the pipette with respect to the animal's head using an ultraviolet (UV)-transparent collar and UV-cured adhesive, and rapid release of the animal from head fixation without loss of the recording. This technique has been successfully applied to obtain intracellular recordings from the hippocampus of freely moving rats and mice exploring a spatial environment, and should be generally applicable to other brain areas in animals engaged in a variety of natural behaviors.

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

Acrylic paint, black (e.g., Crafter's acrylic [DecoArt])
Adhesive, UV-cured (Norland NOA63)
Agarose (2% [w/v] in physiological saline; Sigma-Aldrich A9539)
Agarose, darkened (4% black acrylic paint in 2% agarose)
Analgesic (e.g., Buprenex [Reckitt Benckiser Pharmaceuticals])
Bupivacaine hydrochloride monohydrate (Sigma-Aldrich B5274)
Dental acrylic (e.g., Jet Denture Repair Package [Lang Dental 1234PNK])
Dental adhesive, light-cured (e.g., OptiBond FL [Kerr Dental])
Dental composite, light-cured (e.g., Charisma [Heraeus Kulzer])
Experimental animals

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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)
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

The procedure for whole-cell recording in freely moving rodents requires several custom-designed parts that have been described previously (Lee et al. 2014). Contact the authors for further details.

Acquisition software (e.g., Patchmaster [HEKA Elektronik])
Acquisition system, analog-to-digital (e.g., InstruTECH [HEKA Elektronik])
Acrylic paint marker, black (e.g., DecoColor)
Air tubing and tubing connectors (World Precision Instruments)

Attach a three-way tubing connector, a pipette electrode wire, and an air tube as shown in Figure 1D.

Amplifier, with miniature headstage (e.g., npi electronic ELC-03XS; see Fig. 1B)
Anesthesia system (e.g., VetEquip 901806)
Audio monitor (e.g., Grass Technologies AM10)
Behavioral arena, electrically shielded and grounded
Cable counterweighting system

The weight of the headstage/light-emitting diode (LED) assembly is relieved by counterweights attached to a few locations on the recording wires coming from the headstage via pulleys. Carefully adjust the weights such that the head-mounted device floats at the level of animal's head when not attached to the animal.

Camera, charge-coupled device (CCD) (optional; see Step 25.i)
Collar, synthetic sapphire, 3 mm tall, with a 1.55-mm diam (± 0.005 mm) hole at the center (Rayotek Scientific; see Fig. 1A, inset)

The collar must be custom-designed and fabricated precisely, as the hole size determines the amount of UV adhesive required around the pipette.

Curing unit (e.g., Translux Power Blue [Heraeus Kulzer])
Eye cover, hinged, custom-made (see Fig. 1E, top)

The hinged eye cover—made from a piece of lightweight black paper placed in front of the headplate holder—is designed to protect the animal's eyes from the lights and the experimenter's movements. Because of its low weight, the animal can easily lift the cover with its nose when the animal's head is released for free behavior.

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
Gripper jaws, custom-made (Fig. 1C)
Headplate and rapidly releasable headplate holder, custom-designed (Fig. 1A,D)

It is essential to make the headplate holder quickly releasable. Unscrewing by a half-turn allows the headplate clamp to rotate because the spring beneath it lifts it, thus allowing the headplate to be released by pulling it up (by the wires on the headstage).

Headstage, miniaturized, equipped with custom LED assembly (npi electronic; see Fig. 1B)
Heating plate, equipped with magnetic stirrer
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)

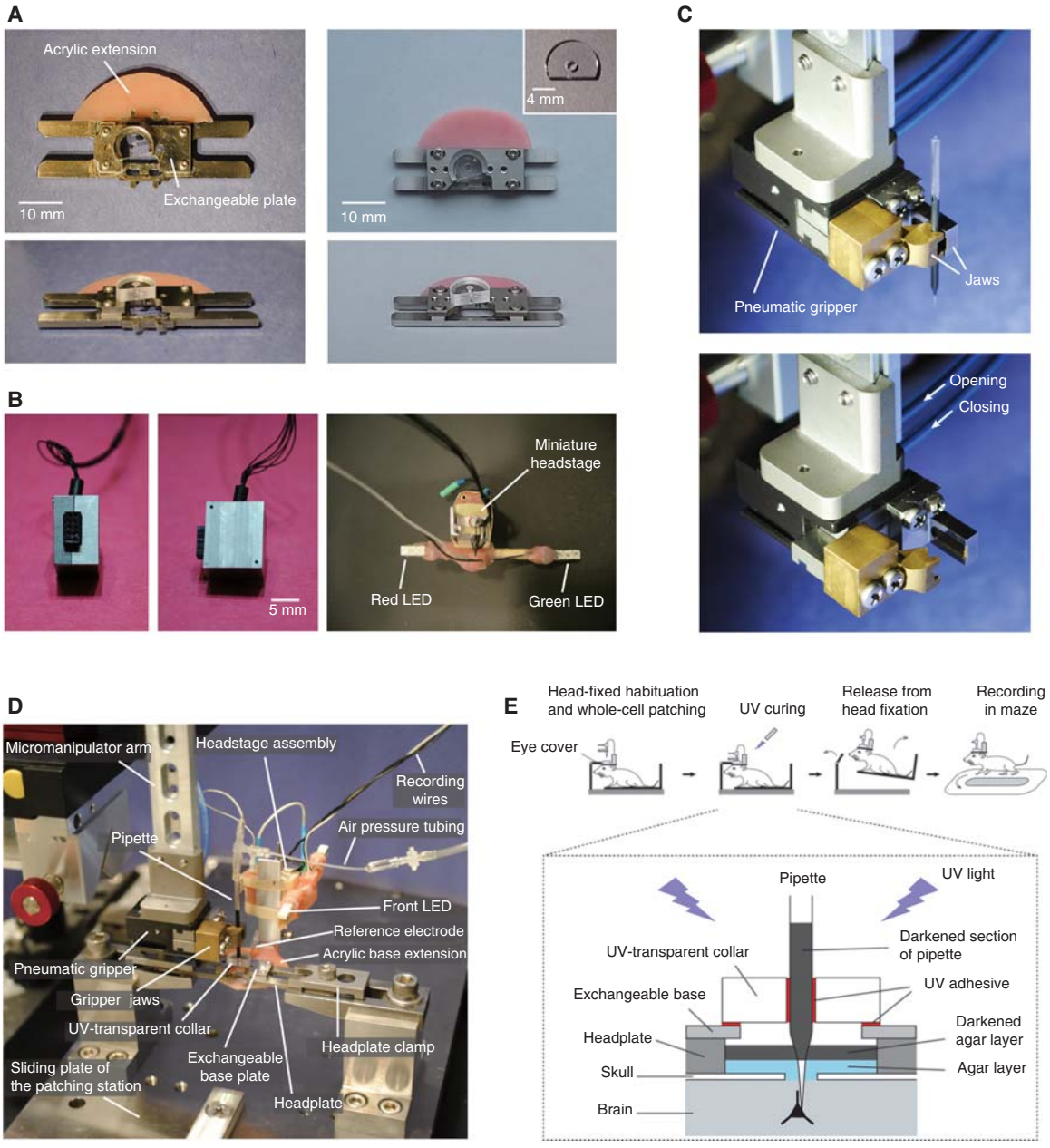

FIGURE 1. Awake-patched whole-cell recordings in freely moving animals. (*A*) Headplate for rats (*left*) and mice (*right*) with acrylic extension and an exchangeable base with a UV-transparent collar placed on it. A close-up of the collar (*inset*). (*B*) Miniaturized headstage (*left* and *center*) and a top view of a headstage assembled with LEDs (*right*). (*C*) Pneumatic pipette gripper. Custom-made jaws hold (*top*) and release (*bottom*) a pipette. (*D*) Arrangement of head-mounted equipment and head-fixed patching station with a rapidly releasable headplate holder. (*E*) Procedure for awake-patched whole-cell recordings in freely moving animals (*top*). Note that the animal is sitting inside the transfer box until it is released for recording in the maze. Arrangement of UV-transparent collar/UV-cured adhesive-based pipette stabilization (*bottom*). (Modified, with permission, from Lee et al. 2014.)

Patching station

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

Photodiode power sensor (Thorlabs S120VC)

Pipette electrode wire, Ag/AgCl

Solder a 7- to 8-mm-long silver wire to an insulated connecting wire with a gold pin at the other end. Chloride the silver wire from the tip to near the soldered end.

Pipette puller (Sutter Instrument P-97)
Pneumatic grippers, 2-jaw (Zimmer Group MGP801N or MGP803N)
Position tracking system (e.g., Neuralynx)
Power meter console (Thorlabs PM100D)
Reference electrode, Ag/AgCl

Prepare a 30-mm-long silver wire and a 3-mm-long piece of thin tubing (with an OD slightly larger than 1/32 in.). Insert the wire into the tubing. Glue the tube at the middle of the wire. Chloride the wire to one side of the tube. Solder a gold pin to the other side. Insert the wire into a 6- to 7-mm-long piece of plastic (e.g., Tygon) tubing (1/32-in. ID) such that the piece of thin tubing glued at the middle of the wire wedges into the longer tube.

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.

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

Clean and sterilize instruments thoroughly before use.

Syringe, equipped with blunt-ended 18-gauge needle

This is used for applying agarose.

Temperature control system (e.g., FHC)
Transfer box with urine absorber, custom-made

Cover the floor of the box with laboratory tissue and then a sintered aluminum plate such that urine will be absorbed through it, allowing the animal to remain more comfortable.

Ultraviolet curing system with bifurcated light guide (e.g., Hypercure 200 [Hologenix])
Vibration isolation table (Newport)

METHOD

All the training and patching procedures are performed while animals are head-fixed and sitting inside the transfer box. Once a whole-cell recording has been obtained and stabilized on the head, the animal can be released from head fixation and transferred to the behavioral arena. Wear gloves and a face shield to protect exposed skin from the UV light.

Headplate Implantation Surgery

1. Prepare the headplate with dental acrylic such that it extends horizontally to form a flat, semicircular acrylic "base" (see Fig. 1A).

   This base will serve as the site of attachment for the headstage/LED assembly (see Step 11).

2. Attach the headplate to the skull of the experimental subject as described in Steps 1–11 of Protocol: **In Vivo Patch-Clamp Recording in Awake Head-Fixed Rodents** (Lee and Lee 2016).

Habituation to Head Fixation and Release

3. Place the experimental animal in the transfer box. Fix the animal's head into the custom-designed rapidly releasable headplate holders (see Fig. 1E, top left).
4. Close the hinged eye cover to protect the animal's eyes from the lights and experimenter movements (see Fig. 1E, top left).
5. Habituate the animal to head fixation as described in Steps 12–14 of Protocol: **In Vivo Patch-Clamp Recording in Awake Head-Fixed Rodents** (Lee and Lee 2016).
6. Habituate the animal to the release process (i.e., being rapidly released from head fixation and having its head lifted while remaining in the transfer box):

   i. Using the sliding plate, move the animal away from the gripper and light guides.

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ii. While pressing down firmly on the headplate with a metal bar, unscrew the plate from the holder.

iii. Remove the bar. Simultaneously, swiftly and gently pull up the recording wires coming from the headstage/LED assembly to lift the animal's head.

Craniotomy and Target Depth Determination

7. Perform craniotomy and target depth determination as described in Steps 15–21 of Protocol: **In Vivo Patch-Clamp Recording in Awake Head-Fixed Rodents** (Lee and Lee 2016).

Prepare Painted Patch Pipettes

8. Pull patch pipettes (4–7 MΩ) before each patching session.

9. Paint the pipettes with a black acrylic paint marker to prevent a voltage offset caused by direct UV illumination of the internal solution.

Paint the pipettes under a stereoscope. If the paint layer is too thick, the pipette will get stuck in the hole of the UV-transparent collar. If it is too thin, UV illumination will cause a voltage offset. Painting two-thirds of the pipette length is generally enough to protect 5 µL of internal solution. Leave ~4 mm of length from the pipette tip unpainted to allow one to inspect the tip for trapped air bubbles (the unpainted area will be protected by a layer of agar containing black paint).

Awake Patching and Pipette Stabilization

10. Fix the animal's head in the patching station.

11. Attach the miniaturized headstage/LEDs assembly to the headplate (Fig. 1B,D).

This attachment should be reversible such that the assembly can be easily detached at the end of each patching session.

12. Insert an Ag/AgCl reference electrode through the reference electrode-holding capillary.

13. Apply UV-cured adhesive to the bottom of the UV-transparent collar, then place the collar on top of the exchangeable base plate (which is itself attached to the headplate with screws; Fig. 1A,D).

Giga-seal formation will not occur if the pipette tips are contaminated with adhesive. If the same collar is used for two or more pipettes, verify that the hole in the collar is not blocked by adhesive. If necessary, clean the hole with tissue before inserting a fresh pipette.

14. Fill a painted glass pipette with ~5 µL of the intracellular pipette solution.

15. Insert the prepared pipette into the air-pressure-controlled gripper attached to the micromanipulator.

16. Apply a small amount of UV adhesive around the pipette. Insert the pipette into the hole of the collar (Fig. 1E).

As the pipette is inserted, the adhesive fills the gap between the pipette and the inner surface of the hole.

17. Place UV light guides in front and back of the animal's head. Point them at the collar at ~45° with respect to vertical.

Perform the following calibration before the patching session. Use the photodiode power sensor and power meter console to determine the power at a given distance from the end of each light guide. The UV intensity from each light guide should be ~42 mW/cm^2 at the site of curing. Adjust the distance between the end of the light guide and the collar to control the intensity.

18. Obtain a whole-cell recording:

i. Use the micromanipulator to position the pipette tip a few hundred microns above the brain surface.

ii. 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.

It is critical to enter the brain surface through a clean spot. Especially avoid spots with clearly visible blood.

iii. 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.

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

v. Apply a layer of 2% agarose around the pipette and covering the craniotomy. Add a second layer of 2% darkened agarose on top of the first layer such that both the craniotomy and the unpainted tip of the pipette will be protected from UV illumination.

vi. Advance the pipette using 1-µm steps to search for a target neuron.

vii. When there is an increase in the pipette resistance (by ~20% or more) for four to five 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.

viii. 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.

19. Observe the quality and stability of the recording for 2–5 min.

Monitor both the resting membrane potential and the series resistance carefully; proceed to Step 20 only if they are stable. Otherwise, retract the pipette, remove the collar, remove the agar, wash the craniotomy surface with physiological saline, and repeat the process from Step 13 with a new pipette.

20. Cure the UV adhesive by illuminating the collar with UV light using two 5-sec pulses, separated by a 5-sec gap.

This step anchors the pipette rigidly to the collar and the collar rigidly to the exchangeable base plate.

See Troubleshooting.

Release and Transfer Animal to the Arena

21. Carefully cut the air pressure tube to the pipette.

22. Carefully open the air-pressure-controlled gripper that holds the recording pipette.

23. Release the animal from head fixation as described in Step 6.i–iii.

Make sure that the headplate does not hit the headplate holder below; otherwise, recordings can be easily lost.

24. Move the transfer box containing the animal adjacent to the behavioral arena. Allow the animal to walk out of the box into the arena.

Recording

25. Record the membrane potential in current-clamp mode:

i. If necessary, track the animal's position using a CCD camera that captures the LEDs attached to the headstage/LED assembly.

ii. Continue recording until the baseline membrane potential depolarizes above physiological levels (either suddenly or gradually) or the series resistance increases significantly.

If another recording is to be attempted in the same recording session, fix the animal's head into the patching station, hold the pipette with the gripper, detach the exchangeable base plate from the headplate, retract the pipette (with exchangeable plate and collar attached), remove the agar, wash the craniotomy surface with physiological saline, then repeat from Step 13 with a new pipette.

26. After each recording session, cover the brain surface with a layer of 2% agarose, followed by a layer of silicone elastomer. Remove the reference electrode. Cap the reference electrode-holding capillary.

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If the same animal is used for two or more recording sessions on the same day, provide a 1–2 h rest period in its home cage between sessions. We have obtained multiple recordings from hippocampal area CA1 in the same hemisphere and have not in any cases observed any noticeable impairment in the network activity.

Histology

27. Recover the morphology of the recorded cell as described in Steps 31–34 of Protocol: **In Vivo Patch-Clamp Recording in Awake Head-Fixed Rodents** (Lee and Lee 2016).

    See Troubleshooting.

TROUBLESHOOTING

Problem (Step 20): Pipette fails to anchor properly when cured using UV illumination.

Solution: Establish that the UV light intensity is sufficiently high; check the UV intensity regularly and adjust it to the recommended value. Apply enough UV adhesive such that it completely fills the gaps between the collar and the pipette and between the collar and the exchangeable base plate.

Problem (Step 20): Illumination with UV light changes the membrane potential permanently.

Solution: This can occur when the paint on the pipette is not thick enough and thus does not effectively prevent the UV light from illuminating the internal solution directly. Paint pipettes under a stereoscope and ensure there are no unpainted gaps. A higher-than-recommended UV intensity could also be a problem. Check UV intensity and adjust it if too high.

Problem (Step 27): The recorded cells cannot be observed histologically.

Solution: Because the pipette cannot be gently retracted while the animal is not head-fixed, recordings generally end as a result of the loss of the whole-cell configuration. This can lead to biocytin leaking from the cell interior. Therefore, if histological recovery is required, the brain should be perfused immediately after the end of recording.

DISCUSSION

Different approaches involving both whole cell (Lee et al. 2006, 2009, 2012, 2014; Epsztein et al. 2010, 2011) and sharp (Long et al. 2010; English et al. 2014; Hamaguchi et al. 2014; Schneider et al. 2014; Kosche et al. 2015; Vallentin and Long 2015) intracellular methods have been attempted to overcome the high sensitivity of intracellular recordings to mechanical instability so that the technique can be applied more readily to freely moving animals (Long and Lee 2012). Rigid fixation of the recording pipette to the animal's head can yield stable, long-lasting, low series resistance whole-cell recordings in freely moving animals (Lee et al. 2006, 2009, 2014). Success is attributed to a high rate of giga-seal formation facilitated by the use of a three-axis micromanipulator (see Protocol: **In Vivo Patch-Clamp Recording in Awake Head-Fixed Rodents** [Lee and Lee 2016]), together with a strong, air-pressure-controlled gripper, as well as virtually 100%-efficient UV-based pipette stabilization (Lee et al. 2014). In particular, the nearly instantaneous stabilization of the pipette using the UV-cured, collar-based method allows whole-cell recordings to be obtained and stabilized in awake, drug-free animals. The protocol described here has been used in juvenile rats and adult mice by multiple experimenters with success rates of 40% or higher, where the success rate is defined as the percentage of all whole-cell recordings in which one has attempted UV-based pipette stabilization that ultimately leads to a good-quality intracellular recording in the freely moving animal (irrespective of the duration of the recording). Using this protocol, we have obtained 19 successful recordings from 16 rats; after release from head fixation, recordings lasted from 11 sec to 8.4 min (mean = 2.1 min), during which the rats traveled 1163 ± 232 cm on a linear track.

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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