

Modular Electrode Array for Multi-site Extracellular Recordings from Brains of Freely Moving Rodents

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Multi-site extracellular recordings from awake, freely moving rodents are an insightful technique that allows deduction of the dynamics of neural activity within a network of brain regions. Multiple advances in the design and materials of recording setups are available in the literature. However, most of these designs require several skill sets to assemble the electrodes and are expensive. Here, we explain in detail a custom design to build a multi-site (16 sites) electrode array (EA) and record extracellular electrical signals (local field potential and multi-unit spiking activity) at variable depths in freely behaving rodents. This EA weighs ~3.0 g and costs less than \$30. It provides mesoscopic neural activity maps (at millimeter scale) at low spatial resolution, thus enabling the experimenting group to further target specific regions with more expensive high-density probes at the resolution of an individual neuron. The article outlines the processes of building and implanting the array and recording neural activity during a behavior task. We also highlight the limitations of our design and the necessary steps to troubleshoot common issues faced during the initial implementation of the protocols. Finally, we explain the specific data one would obtain while using the probes during social interactions between rodents. © 2022 Wiley Periodicals LLC.

Basic Protocol 1: Preparation of the electrode array

Basic Protocol 2: Surgical implantation of the electrode array

Basic Protocol 3: Recording of neural activity from the electrode array while a mouse performs social investigation of a novel conspecific

Basic Protocol 4: Histology and electrode registration

Keywords: electrode array • extracellular • local field potential • multi-site • network

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INTRODUCTION

Social behavior is associated with neural activity in numerous brain regions encoding multiple sensorimotor cues to make specific behavioral decisions (Chen & Hong, 2018; Wu et al., 2021). Multi-site extracellular recordings in freely moving rodents have been extensively used to study neural activity during behavior (Binns et al., 2005; see *Current Protocols* article Harris, Golder, & Likhtik, 2017; Hultman et al., 2018; Mague et al., 2020; Tatsukawa et al., 2019). This method enables researchers to simultaneously record

Basic Protocol 1: The electrode array (EA)	
Planning the EA design (step 1)	Day 1 3 hours
Preparing the EA (steps 1-22)	
Cut the electrodes (step 23)	
Measure impedance (step 24)	
Coat Dil on electrodes (steps 25-26)	
Attach Omnetics to Mill-Max adapter (step 27)	
Basic Protocol 2: Surgical implantation of EA	
	Day 2 3 hours
Pre-surgery steps (steps 1-4)	30 minutes
Anesthetize the mouse (steps 5-6)	1.5 hours
Preparing for craniotomy (steps 7-11)	
Attaching screws and craniotomy (steps 12-15)	
Insertion of electrodes (steps 16-18)	1 hour
Apply dental cement (steps 19-20)	
Basic Protocol 2: Post-surgery recovery	
Post-surgery recovery (steps 21-23)	Days 2-5
Basic Protocol 3: Electrophysiology and behavior recording	
Attaching headstage to the EA (steps 1-2)	Day 6-10* Each day(1 session) 1 hour * One session each day for 3 days of recording. Users can modify these to suit their experiments.
Habituation to the recording arena (step 3)	
Recording neural and behavior activity (steps 4-5)	
Finish recording (steps 6-7)	
Return the subject to home cage (step 8)	
Post-processing of the acquired data (step 9 and Understanding Results)	Day 14, 2 hours
Basic Protocol 4: Histology and registration of the electrodes	
Perfusion and removal of brain tissue (steps 1-7)	Day 10, 2 hours
Fixation of brain tissue (step 8)	Days 10-12
Sectioning and slide preparation (steps 9-10)	Day 13, 5 hours
Imaging sections in with fluorescent microscope (step 11)	
Locating electrodes using atlas (step 11)	Day 14, 1 hour

Figure 1 Flowchart detailing the order of the basic protocols and the time required for each. The chart divides the basic protocols into constituent steps (mentioned in parentheses) and the respective time required to finish those steps.

extracellular potentials and multi-unit spikes from multiple brain regions in awake behaving rodents. Here, we describe in detail a modular design and workflow to build an electrode array (EA) to target different combinations of brain areas and the process of surgical implantation of the EA. The workflow enables one to record local field potentials (LFPs) and multi-unit activity (MUA) during behavioral tasks, thus allowing the study of the subsequent neural network corresponding to the behavior. Commercially made and custom-made multiple high-density EAs (Lei et al., 2011; Meister, Pine, & Baylor, 1994) allow simultaneous recording from a few brain areas only when the targeted brain regions are adjacent. Thus, these probes do not allow recordings from multiple remote brain regions. The EA presented in this article allows recording from multiple sites located in any part of the brain, can be built by researchers not trained in EAs, and is less fragile in contrast to commercial probes (such as silicon probes). The field of neurophysiology is rapidly moving to multi-site high-dimensional data acquisition, which is predominantly complex in design and requires a high degree of skill and large amounts of funding. In this article, we present a relatively modular, inexpensive, and highly transferable method to record multi-regional network dynamics in awake behaving rodents. This EA may be

used as an intermediate step in screening for possible brain regions involved in specific behavior by recording low-resolution mesoscopic neural activity (LFPs). Subsequently, specific brain regions may be explored by employing high-density probes that resolve spiking at the single-unit level.

A previous protocol by Harris et al. (2017) highlights a methodology for recording multi-site neural activity from awake, freely moving rodents. Our method differs from this protocol in designing the EA to target any brain region and incorporating the steps in a time- and cost-efficient manner. The EA and recording devices were planned to reuse cost-intensive parts (such as Omnetics connectors) for multiple experiments, making the protocols effectively usable in many labs.

We provide a flowchart highlighting the whole process to aid the user in planning their experiments (Fig. 1). The process begins with a detailed explanation of designing a modular EA (Basic Protocol 1) to record from up to 16 brain regions simultaneously. Then, the steps to perform chronic implantation of the EA in a mouse brain, targeting brain regions accurately by using stereotaxic coordinates and successful recovery of the mouse, are outlined (Basic Protocol 2). Next, we explain how to run a multi-site neural recording while video-recording the freely moving animals during social encounters (Basic Protocol 3) and subsequently how to verify the brain regions from which the neural recordings were acquired (Basic Protocol 4). Finally, we discuss the causes of and troubleshooting steps for common issues in each of the basic protocols, considerations when planning such experiments, and the expected results from analysis of the collected data.

PREPARATION OF THE ELECTRODE ARRAY

This protocol describes preparing an EA that can simultaneously record extracellular neural activity from multiple brain areas in awake behaving mice (Figs. 2 to 4).

Materials

- Dental cement (and solvent solution; Unifast Trad)
- 0.9% (w/v) saline (from sodium chloride, Sigma, #S9888)
- DiI dye solution: 50 mg/ml DiI
(1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, Sigma-Aldrich, #42364) in 70% (v/v) ethyl alcohol (from 100%, Sigma, #65347-M)
- Custom-designed PCB boards [Fig. 3A, left; manufactured according to Gerber files "PCB design_3D probe 6-5-19" (see Supporting Information, File 2) by Brander PCB based on our customized design]
- Cutting wire (gold molybdenum cutting wire, 0.08-mm diameter; Mobitools; Fig. 3A, right)
- Microcutter (Hakko, #CHP-170)
- Pin socket (Interconnect Machined Pin Socket, Mill-Max, #853-43-100-10-001000)
- Pointed serrated pliers (Hakko, #CHP PN-5001)
- Scissors (tungsten carbide; AS Medizintechnik, #12-436-11)
- Dissection microscope (5× objective)
- Coated silver wires (76 µm, 300 Ω; A-M Systems, #785500)
- Scalpel (Fine Science Tools, #10003-12)
- Surgical blades (size 11; Swann-Morton, #0203)
- PCB holding device (see Supporting Information, File 1, Fig. 1E), including:
 - Part 1: Linear slide rail (MGN7H, 7 cm long; Venstpow)
 - Part 2: CNC carriage (3.4 × 2.6; MGN7H)
 - Part 3: 2-way rail slider (Fotomate)
 - Part 4: Tripod ball head Arca Swiss plate (Ulnazi)

BASIC PROTOCOL 1

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Part 5: 3D-printed PCB holders (see Supporting Information, File 3; built with BCN3D Sigmax R19 using PLA material)

Part 6: M3 screws (drill threads for M3 screws on CNC carriage)

Solder wire (recommended: 0.8 mm, 63% SN; Bryz Tech)

Soldering station iron (110 to 220 V; Hakko, #936)

Metal rod (aluminium or stainless steel, 5 to 10 cm long, 1-mm diameter)

Tungsten microwire (tungsten 99.95% CS, 50 μ m, 150 k Ω ; California Fine Wire Co., #100211)

5-cm fine forceps (Dumont AA - Epoxy Coated Forceps, Fine Science Tools, #11210-10)

Toothpicks

Alligator clip and stand

LCR meter (Tonghui, #TH2822)

1.5-ml Eppendorf tube

Omnetics connector (adapter; male Mill-Max connector soldered to 18-pin wire; Omnetics, #A79012-001; see Supporting Information, File 1, Figs. 1A to 1D)

1. Choose all brain regions to record from using the appropriate brain atlas (Franklin & Paxinos, 2019) and plan arrangement of microwire electrodes by choosing specific grooves on the custom-designed PCB boards.

Note that the distance between neighboring grooves on the PCB board is 1 mm along both the x-axis and the y-axis. This is because in our hands, if the electrodes are placed at a distance of 500 μ m, they tend to bend significantly, to a state where their locations in the brain are indistinguishable. We chose three electrodes to target brain regions at a depth of 2.8 mm (Fig. 2, pink dots), which are 0.3 mm lateral to the midline. Another 13 electrodes (Fig. 2, red dots) were selected for targeting brain regions at a depth of 4.7 mm. There are more electrode grooves (60) than the number of channels (16) used for recordings in the EA design. For a specific experiment, users need to design their arrangement of electrodes based on the regions they wish to record. We have added our design and arrangement of electrodes (16 electrodes) for brain regions targeted to the example (Fig. 2).

The PCB boards have electrode grooves on the “back” (60 grooves; Fig. 3A, left, yellow arrow; 200 μ m in diameter), Mill-Max connector sockets (18 grooves in the blue outlined area, purple arrow), and grooves for holding the EA with a metal rod (blue arrows).

2. Take two PCB boards to prepare EA and clean any debris from electrode grooves using cutting wire (Fig. 3A, right).
3. Using a microcutter, break pin socket into 9×2 -pin pieces (Fig. 3B).
4. Remove and save first pin from the top row of connectors (for the reference wire) and last pin from the bottom row of connectors (for the ground wire) using pointed serrated pliers (Fig. 3C).
5. Using scissors and a dissection microscope, cut two 2.5-cm-long coated silver wires (0.003-in. diameter) and remove insulation from ~ 0.5 cm at both ends using a scalpel with a surgical blade (Fig. 3D).
6. Place one of the modified PCB boards from step 2 in one slot of the PCB holding device (see Supporting Information, File 1, Fig. 1E) and the second modified PCB board in the other slot of the PCB holding device (Fig. 3E).

We cut two parts of the PCB board on one side so that the edges do not cover the eyes of a CD1 mouse while implanting the EA (Basic Protocol 2). The modified PCBs can be observed in Figure 3F (left panel shows a back view and right panel shows a front view).

7. Place pins removed for reference and ground (see step 4; Fig. 3C) inside the respective grooves on the second PCB board (Fig. 3F, left, back view).

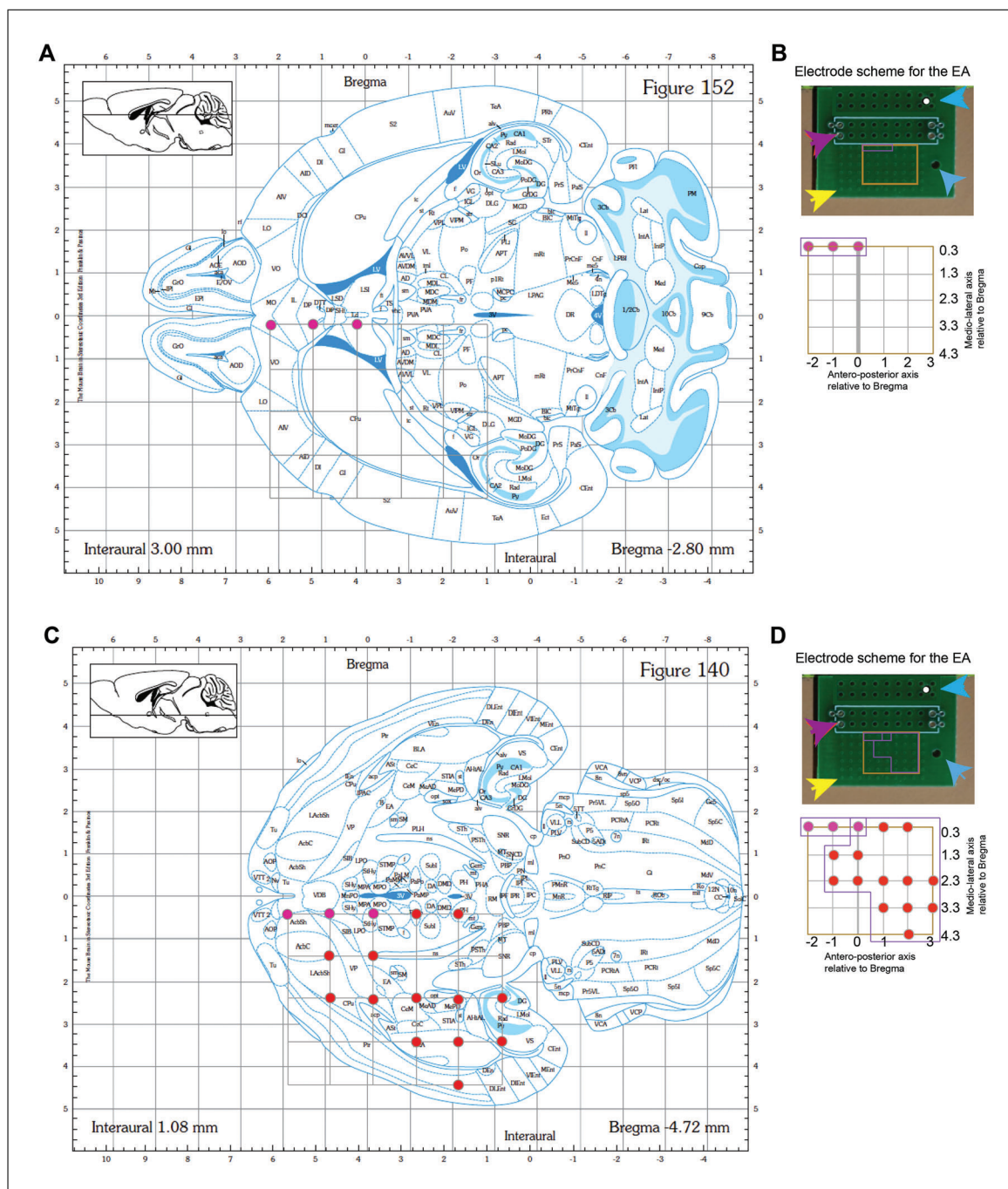


Figure 2 Targeting specific brain regions with the EA. (A) Image of a horizontal section at 2.8 mm deep from the brain surface (Fig. 152 in the atlas) with an overlaid grid depicting a 5 × 6 array design (gray-colored grid on left hemisphere). The pink dots depict the planned regions to target [these electrodes would be cut to a length of 4.8 mm (= 2 + 2.8 mm); see Basic Protocol 1, step 23]. (B) Scheme of the grooves in the EA where the three electrodes would be inserted for targeting the brain regions shown by pink dots in Figure 2A. Top: The yellow arrow shows the grooves for the electrodes, whereas the purple arrow is where the Mill-Max connectors would go. The two blue arrows are the grooves for attaching a metal rod to hold the EA. We used the second groove, marked with a white dot adjacent to a blue arrow, to insert the metal rod. Bottom: The EA is designed to target three regions of interest. (C) Similar to (A), a horizontal section at a depth of 4.7 mm with 16 electrodes overlaid in targeted brain regions is shown. The pink dots are the electrodes that were part of the EA design in (A) (with a shorter length of 4.8 mm), whereas the red dots are the electrodes for the other 13 brain regions that are found at this depth in the brain of the mouse (final length of each of the 13 electrodes = 6.7 mm). (D) Similar to (B), the EA design has 13 (red) long electrodes and 3 (pink) short electrodes. The arrows illustrate the same parts as in (B).

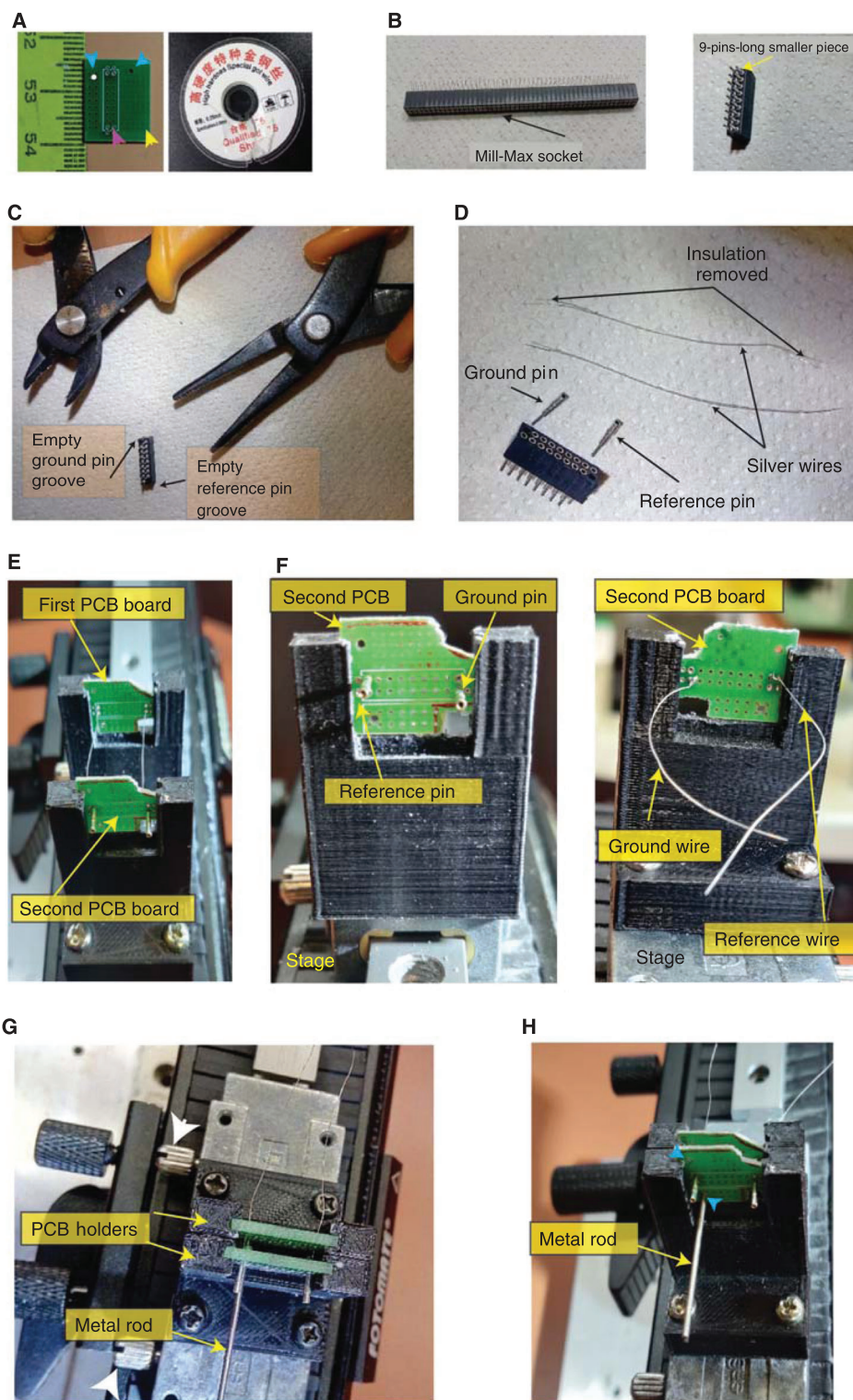


Figure 3 The EA, part 1. (A) Left: The PCB with grooves for electrodes (yellow arrow), Mill-Max connector (purple arrow, the whole area outlined in blue with 18 grooves), and a metal rod to hold the EA (blue arrows; users can choose one of these). We used the second groove, marked with a white dot adjacent to a blue arrow, to insert the metal rod. Right: Wire for cleaning dust from the grooves for electrodes. (B) The Mill-Max connector socket (left) is cut with nine pins (right). (C) The pins were removed from the connector for the reference and ground wires using pointed serrated pliers. (D) Insulation from silver wires was removed for soldering them to the reference

(legend continues on next page)

and ground pins. **(E)** The first PCB and the second PCB, with the soldered pins and silver wires, were placed into the PCB holders on the stage. **(F)** The second PCB in the holder on the stage and the reference and ground wires (right; front face, which will be closer to the back face of the first PCB) are soldered to Mill-Max pins (left; back face, where the electrodes would be bent into grooves and the Mill-Max connector would be inserted) through the PCB. **(G)** The second PCB is aligned to the first PCB, with the soldered wires inserted into the respective reference and ground grooves of the first PCB. **(H)** Inserted metal rod to hold the EA after preparing it. The user can insert the rod in one of the grooves shown by the blue arrows.

8. Solder silver wires to the reference and ground pins (Fig. 3F, right, front view) using solder wire and a soldering station iron.
9. Align 3D-printed PCB holders in the PCB holding device such that one can thread the soldered ground and reference wires through the first PCB board's respective grooves (Fig. 3G).
10. Tighten PCB holders' M3 screws (Fig. 3G, white arrows) onto the stage and angle so that one can visualize electrode grooves on the PCBs under the microscope.

Extra lamps (e.g., 3W LED clip-on table lamps) directed over the stage help to efficiently locate the grooves on the PCB and the electrodes.

11. Insert a metal rod (1-mm diameter) through second PCB's large side groove (Fig. 3H, in one of the grooves indicated by blue arrows, as in Fig. 2).

This metal rod should be aligned perpendicularly to the array, which is essential to target the brain regions with higher depth. Note that we provide two grooves for inserting a metal rod to hold the EA (Fig. 3H, blue arrows), which are useful for further steps such as applying dental cement, performing impedance measurements, coating electrodes with dye, and implanting the EA into mouse brains. The user can use either of the grooves; however, in our setup, we found that the groove corresponding to the blue arrow (white dot) provides better visualization of the surgical area (left hemisphere of the mouse brain) during the implantation step.

12. Using scissors, cut tungsten microwire (50- μ m thick) into pieces \sim 5 cm long for electrodes.

Users can cut multiple electrodes (at least 20) for later usage and keep them in a small box (10 \times 10 \times 5 cm). We used 16 electrodes for each EA but kept about 32 cut electrodes for building the EAs.

13. Under the microscope, use 5-cm fine forceps to place each electrode (total of 16 electrodes; Fig. 4A, yellow arrow) inside first PCB board and then slide it into respectively aligned groove of the second PCB board.
14. Using the same fine forceps, move electrode wires behind the respective groove of the second PCB board (to \sim 1 cm in length).
15. Repeat steps 13 and 14 for all other electrode wires according to the layout chosen in step 1 (Fig. 4B).
16. Using toothpicks, place dental cement between the two PCB boards and over the electrodes to keep them sturdy and immovable (Fig. 4C).

Take extreme caution not to get dental cement on the holders or stage, which will prevent removal of the boards when you are finished designing. Additionally, do not disturb the exposed ends of the electrodes or the grooves of the Mill-Max connectors.

17. Use a scalpel with a surgical blade to scratch/remove insulation from the surface of the electrode wires that are behind the second PCB board.
18. Hold each electrode's de-insulated end with fine forceps and put it inside an empty groove of the socket from step 4 (Fig. 4D) using toothpicks.

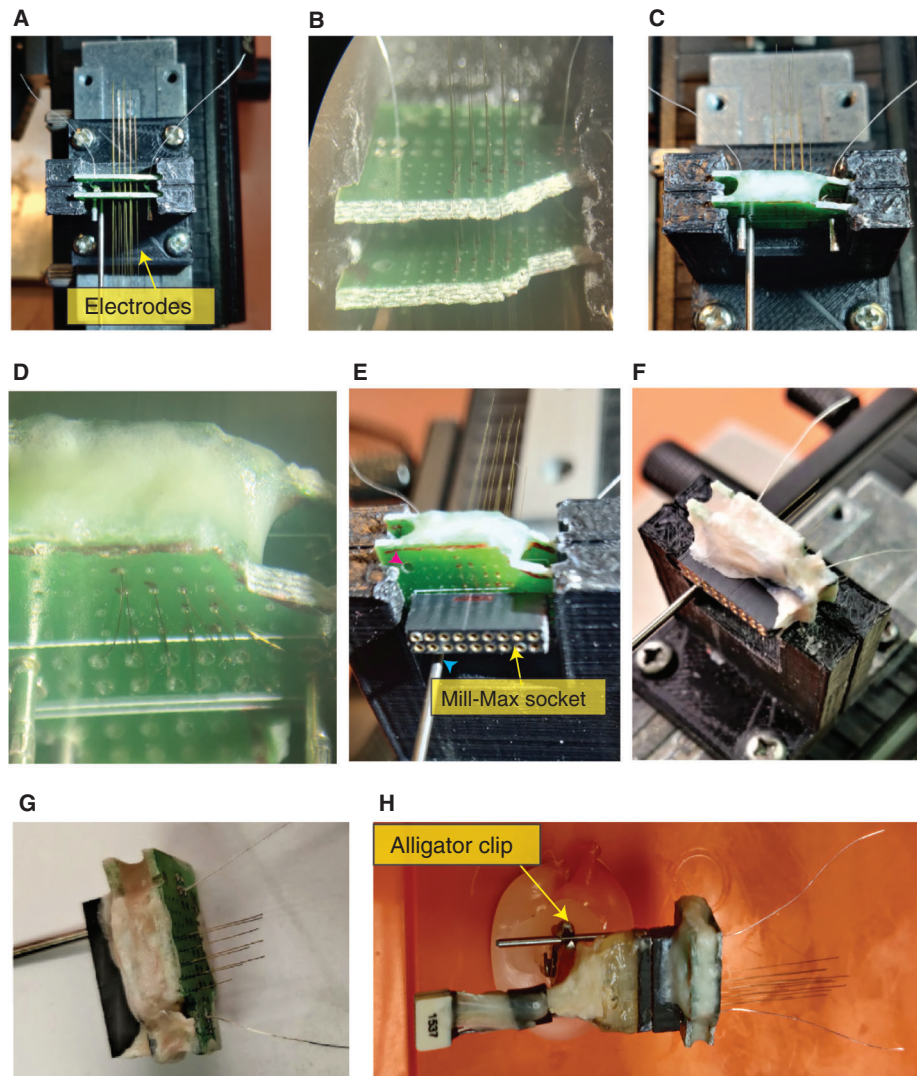


Figure 4 The EA, part 2. (A) The electrodes (yellow arrow) are inserted into the grooves of the first PCB (as per the EA design in Fig. 2) and then into the aligned groove of the second PCB. (B) Zoomed-in view of the electrodes in both PCBs. (C) The electrodes were glued between the PCBs with dental cement. (D) Zoomed-in view of the second PCB with electrodes bent and placed inside the grooves for the Mill-Max connector. (E) The Mill-Max connector is placed into specific grooves over the second PCB. The red and blue arrows indicate grooves (similar to Fig. 3H); one can decide to insert the metal rod into either one of these grooves. (F) The EA after adding excess dental cement to cover the open ends of the electrodes between the two PCBs. (G) The EA after the dental cement has dried. Electrodes were cut to their final length as described in Basic Protocol 1, step 23. (H) The EA with the Mill-Max connector is attached to an alligator clip for storage until surgery.

19. Make a note of each electrode wire and the respective groove of the socket (Fig. 4D).

The electrode wires, which are now behind the second PCB board (after step 16; the part of the electrode wires marked as electrodes in Fig. 4A) as they were inserted through the electrode grooves (Fig. 3A, left, yellow arrow) are scratched to remove insulation and inserted into the Mill-Max grooves of the second PCB board (Fig. 3A, left, purple arrow). Notice that the reference and ground pins are already placed inside the respective Mill-Max grooves, and therefore, only 16 grooves are available to receive the de-insulated electrode wires.

Write down the corresponding channel numbers (follow the channel arrangement of the Omnetics connector in the Supporting Information, File 1, Fig. 1B) for the connectors

according to the electrodes arranged in the array. While post-processing the recorded files (see Basic Protocol 3, step 9), users will need to correlate each channel to the corresponding electrode and brain region from the sections (see Basic Protocol 4, step 11).

20. Align socket above the second PCB's grooves. Press connectors inside while ensuring that each lead of the pins connects with the electrode surface (Fig. 4E).
21. Apply dental cement to stick the two PCB boards and the silver wires together (Fig. 4F).
22. Hold probe by clipping the metal rod to an alligator clip attached to a stand and apply additional dental cement to stick the two PCB boards and the silver wire and metal rod holding the two PCBs together.

It takes ~2 hr for the cement to completely dry and harden (Fig. 4G).

23. Cut electrodes 2 mm longer than the dorsoventral coordinates of the targeted brain regions using scissors under the 20× objective of the microscope (Fig. 4G).

This step requires handling the scissors precisely to cut the electrodes. Preparing a list of the final length of each electrode (the dorsoventral coordinates for each brain region + 2 mm for keeping the EA above the skull) aids in achieving better results in this step.

24. Use an LCR meter to check impedance of each electrode (50- to 150-k Ω range) and silver wires (150 to 300 Ω) by immersing the wires in 0.9% saline and measuring the relative changes from respective Mill-Max connectors.

To confirm that there is no crosstalk between two electrodes, we test the impedance between all pairs of electrodes. A pair of electrodes with no crosstalk would have a high impedance (>10 M Ω). In contrast, a pair of electrodes with crosstalk will show low impedance, on the order of ≤ 100 k Ω . These crosstalking electrodes should not be considered as viable channels for recordings from specific brain regions.

25. Dip electrodes in a 1.5-ml Eppendorf tube filled with DiI dye solution twice for 5 s each.
26. Remove electrodes from the solution and let them air dry for 5 s.

The cell membranes of the brain tissue near the electrodes absorb this lipophilic dye, thus aiding in locating the respective electrodes in the brain (see details in Basic Protocol 4 for registration and histology).

27. Attach Omnetics connector (see Supporting Information, File 1, Fig. 1D) to the EA and hold EA with an alligator clip in a box to avoid damage (Fig. 4H).

SURGICAL IMPLANTATION OF THE ELECTRODE ARRAY

This protocol describes stepwise procedures for pre-surgical preparation of the mouse, surgical implantation of the EA (Fig. 5), and post-surgical care. In our lab, a similar procedure was done with rats while implanting 6-16 electrodes.

Materials

70% (v/v) ethyl alcohol (from 100%, Sigma, #65347-M)
C57BL6 or CD1 male mouse (10 weeks old, ~30 g; Invigo)
Isoflurane (Piramal)
Duratears (Alcon)
3% (w/v) hydrogen peroxide (Millipore, #88597- 100ML-F)
Sterile 0.9% (w/v) saline (from sodium chloride, Sigma, #S9888), 4°C
Dental cement (and solvent solution; Unifast Trad)
Histoacryl (Braun, #2021/1265)
Baytril (5%; Bayer)

**BASIC
PROTOCOL 2**

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Norocarp (5%, carprofen; Norbrook Lab)

Antibiotics and anti-inflammatory drugs

Surgical tools:

Narrow pattern forceps (Fine Science Tools, #11002-20)

Spring scissors (Fine Science Tools, #15009-8)

Drill bits (RA41, SBT)

Graefe forceps (Fine Science Tools, #11051-10)

Dry sterilizer (dry beads, Merck, #Z380075)

Surgical area:

Dissection microscope (5× objective)

Stereotaxic frame (Kopf, Model 1900)

Tabletop lamp (3W LED clip-on table lamp)

Manipulator (Sutter motorized manipulator, MPC-385 system)

Heating pad (custom made for heating animals to 37.0°C; any other commercially available device can be used)

Low-flow anesthesia system (SomnoSuite®, Kent Scientific)

20G and 22G needles

Sharpie marker (black; Sharpie, #30051)

Drill (dental driller, Marathon, Shiyang)

Watch screws (Fine Science Tools, #19010-11)

Phillips screwdriver (size #0; Tekton, #26653), sterilized

Kimwipes (Kimberly-Clark, #34120)

EA (see Basic Protocol 1)

Toothpicks

1-ml syringes and 31G needles

NOTE: The following procedures were approved by the Institutional Animal Care and Use Committee. Users must also acquire approval from an equivalent committee before performing the following steps in any animal.

NOTE: We used CD1 mice for the experiments shown in Figure 7. However, in our hands, both strains of mice can carry the EA, but CD1 has a higher survival rate post-surgery than C57 (see Critical Parameters).

1. Sterilize all surgical tools using a dry sterilizer and afterward dip tools in 70% ethyl alcohol and let them dry.
2. Wipe surgical area, including the dissection microscope, stereotaxic frame, tabletop lamp, manipulator, and heating pad, with 70% ethyl alcohol (Fig. 5A).
3. Set stereotaxic frame to default coordinates and bring manipulator to the center.
4. Ensure that microscope objective is kept at the medium level of its dynamic zoom range.

Check that the microscope and all other surgical devices are functioning correctly before proceeding with the actual surgery.

5. Mildly anesthetize a C57BL6 or CD1 male mouse using 5% isoflurane flowing into the smaller box of the low-flow anesthesia system and observe breathing rate of the mouse.

CAUTION: *The isoflurane must be drawn into a 10-ml syringe for the anesthesia system (SomnoSuite®) in a fume hood as it is a potent anesthetic gas. An air-suction or negative-airflow channel should also be attached to the stereotaxic frame (see step 6) to reduce exposure to isoflurane.*

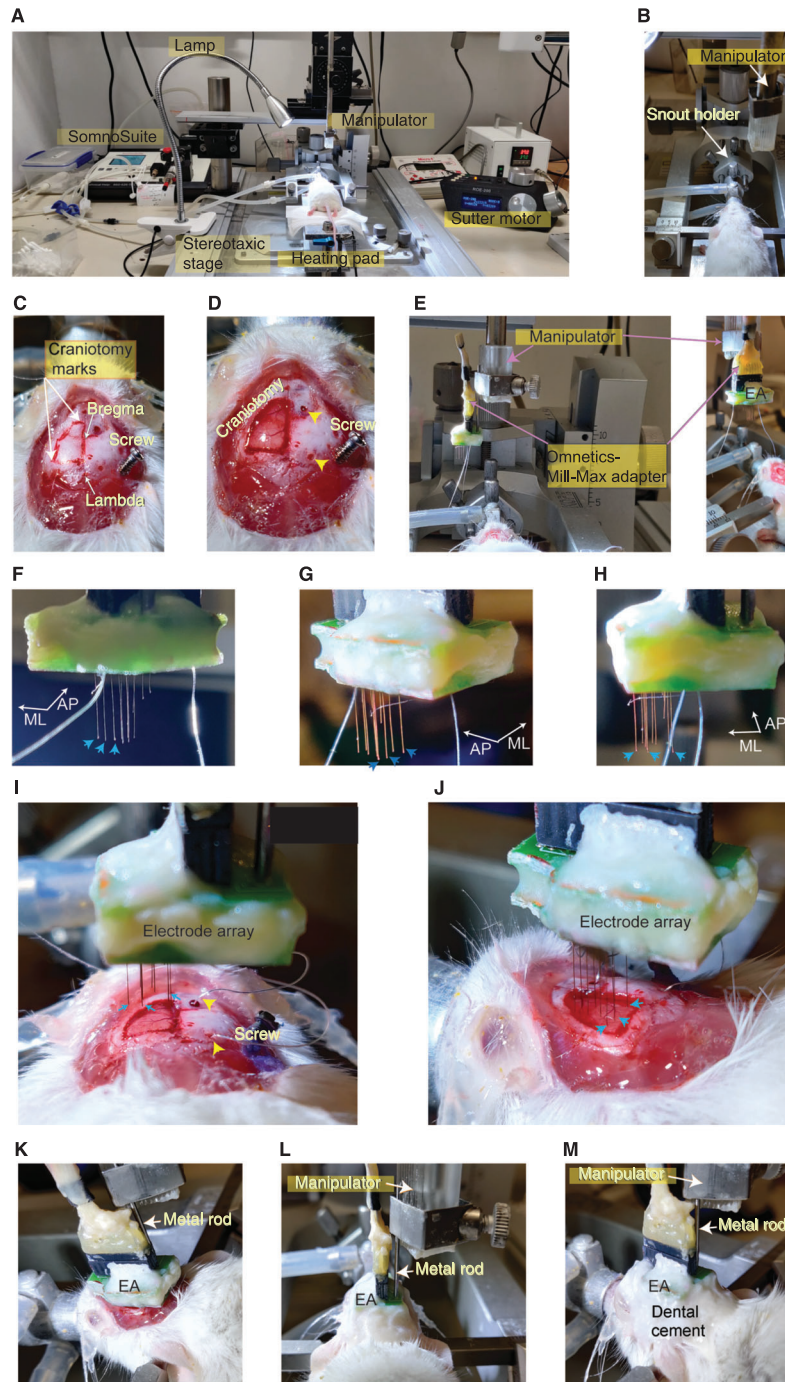


Figure 5 Surgical implantation of the EA. **(A)** The layout of the surgery area, with an isoflurane pump, tabletop lamp, stereotaxic stage, mouse on a heating pad, and manipulator and Sutter motor controls (left to right). **(B)** The zoomed-in image depicts the arrangement of the mouse snout and head in the stereotaxic apparatus and the manipulator above the mouse head. **(C)** Specific markings denoting the bregma and lambda points, the burr holes, the screw, and the area of the skull in the left hemisphere to be removed during craniotomy. **(D)** Brain surface after craniotomy, with the burr holes for the reference and ground wires (yellow arrows) and the screw. **(E)** Left: The EA was attached to the manipulator with the metal rod and moved over the mouse head. Right: The attached EA and the mouse head from the lateral side. **(F)** A zoomed-in view of the EA from the lateral side, with the most extended electrodes marked with blue arrows. **(G)** A zoomed-in image of the EA from the lateral side (opposite image in Fig. 5F), with the longest electrodes marked with the blue arrows. **(H)** A zoomed-in image of the EA under the microscope, with all three most extended (legend continues on next page)

electrodes (blue arrows) in a perpendicular line from the mid-sagittal sinus. (I) The reference and ground wires are placed into respective burr holes (yellow arrows), and the EA is lowered closer to the brain surface. (J) Lateral view of the craniotomy, with the most extended electrodes (blue arrows) touching the brain surface of the mouse. (K) Lateral view of the EA, with electrodes at their final depth (dorsoventral coordinates of the most extended electrodes), inside the left hemisphere of the mouse brain. (L) Dental cement was applied over the EA, the screw, and the exposed skull while holding the EA to the manipulator. (M) Lateral view of the dried dental cement over the mouse head with the EA.

6. Once the mouse has a delayed or slow breathing rate, fix mouse in the stereotaxic frame (Fig. 5B) and set anesthesia flow rate to ~0.5% isoflurane (200 ml/min by air) at the snout mask.
7. Apply Duratears over mouse's eyes to keep them hydrated throughout the procedure.
8. Expose skull by removing the skin from the area extending from between the ears to the eyes using narrow pattern forceps and spring scissors.
9. Apply a few drops of 3% hydrogen peroxide over exposed skull to clear connective tissues.
10. Note coordinates using a 22G needle attached to the manipulator for bregma and lambda sutures intersecting with the sagittal suture (Fig. 5C). Adjust plane of the mouse skull to be aligned with both the medio-lateral and anterior-posterior axes.

For adjusting the plane of the mouse skull, the snout holder is adjusted by moving the Kopf stereotaxis frame in specific directions and taking measurements of the bregma and lambda coordinates. We estimate the difference in depth (coordinates of the dorso-ventral axis) at bregma and lambda to be $<10\ \mu\text{m}$, whereas there is no change in the medio-lateral coordinates (i.e., bregma and lambda are on the same line as the mid-sagittal suture).

11. Using a Sharpie marker, mark areas covering the most anterior, posterior, and lateral coordinates of the targeted areas (Fig. 5C).

These markings are the extent to which the skull would be drilled and removed for placing the EA (Fig. 5C, red lines).

12. Using the drill and a drill bit, make two burr holes for placing reference and ground wires (Fig. 5D, yellow arrows).

These burr holes could be in the right hemisphere if the EA is implanted into the left hemisphere $\geq 2\ \text{mm}$ away from the craniotomy.

13. Make a bigger burr hole at ~2.5 mm lateral to the craniotomy for the watch screw. Hold watch screw with Graefe forceps and sterilize with 70% ethyl alcohol. Place sterilized watch screw into the burr hole and wind it into skull using a sterilized Phillips screwdriver (Fig. 5D).

The watch screw supports the EA and keeps the whole EA adherent to the skull. While placing the screw, avoid placing it too deep into the skull, thereby avoiding any lesions over the cortical tissue.

14. After placing the screw, gently drill over outlines of the Sharpie markings made in step 11 (Fig. 5C), making the skull thinner and more fragile. Repeat this process until edges of the drilled skull break.

The drilling over the skull may cause rupture of the underlying blood vessels. To reduce bleeding, apply cold saline and gentle pressure from the drill bit on areas with dense vasculature (for instance, the mid-sagittal sinus).

15. Bend a 20G needle tip to an ~90° angle using long forceps and use this needle to remove drilled skull piece. Wipe excess bleeding using Kimwipes and clean exposed brain with cold sterile 0.9% saline (Fig. 5D).

16. Attach EA (with the Omnetics connector) to the manipulator using the metal rod and align to exposed brain (Fig. 5E).

Make sure that the EA is oriented correctly. The deepest area targeted will have the most extended electrode, and this will be the first to hit the brain surface while lowering the EA. There are three electrodes (marked with blue arrows in Figs. 5F, 5G, and 5H) based on our EA design that give an estimate of the depth at which the whole EA is inside the brain tissue when these electrodes reach the brain surface (measure of the dorso-ventral coordinates). This procedure helps the user target the EA accurately to multiple brain regions.

17. Before lowering the EA, place reference and ground electrodes into their respective burr grooves (Fig. 5D, yellow arrows).
18. Gently lower EA into the brain while noting the dorsoventral coordinates (Fig. 5I, electrodes at the brain's surface, blue arrows represent the deepest electrodes; Figs. 5J and 5K, electrodes positioned at their final depth inside the brain).
19. Using toothpicks, apply a mix of dental cement and Histoacryl over exposed skull and EA while avoiding the brain tissue and the electrodes (Figs. 5L and 5M).

Make sure to apply dental cement over the space between the EA and the screw and further over the exposed skull without letting the cement flow into the craniotomy. Apply a less viscous layer of the mixture of cement and Histoacryl around the edges of the exposed skull and over the screw. Another layer of more viscous mixture can then be applied over the EA, skull, and screw.

20. After the dental cement dries, remove manipulator holding the EA with the metal rod.
21. Move mouse to a cage with the heating pad, set at 37°C. Administer Baytril (0.03 ml/10 g bodyweight) and Norocarp (0.03 ml/10 g bodyweight) subcutaneously using a 1-ml syringe and 31G needle. Let mouse recover for a few hours before moving it back to its home cage (step 22).

The heating pad temperature must be set at 37°C. The mouse can be placed back in its home cage (step 22) once it starts moving and shows signs of wakefulness.

22. Keep mouse in a new home cage in isolation to limit disturbance to the EA.
23. Administer antibiotics and anti-inflammatory drugs every day for a couple of days for recovery from surgery.

RECORDING OF NEURAL ACTIVITY FROM THE ELECTRODE ARRAY WHILE A MOUSE PERFORMS SOCIAL INVESTIGATION OF A NOVEL CONSPECIFIC

**BASIC
PROTOCOL 3**

The following steps guide the reader in setting up an arena for recording neural activities during social interaction between two mice. We have used Intan acquisition setups for recording extracellular activity. However, any other commercially available extracellular recording setups (for example, Plexon or Neuralynx) could be used similarly. The user acquires raw files of electrophysiological recordings from all channels of the EA along with a video file of the behaving subject from the recording session. The user can analyze these files based on the acquisition board file formats and preferred toolboxes for electrophysiological recordings (see annotation to step 7).

Materials

Mouse with implanted EA (see Basic Protocol 2)
Isoflurane
Stimulus mouse

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Glass jar (17 cm high \times 13 cm in diameter)
 Cotton swabs
 Intan headstage (RHD 16ch; Intan Technologies, #C3334) connected to Intan RHD USB interface board (Intan Technologies, #C31000) with SPI cable (12-pin RHD SPI cable connector, Intan Technologies, #C3216) or similar
 Plexiglas arena (37 \times 22 \times 35 cm) in custom-made recording box, covered with aluminium plates serving as faraday cage, grounded to electrophysiology system to remove external noise
 Camera recording software (FlyCap SDK, Teledyne FLIR)
 Desktop computer (RAM = 16 GB, memory = 500 GB, \geq 4 USB3 inputs)
 Monochromatic camera (Flea3 USB3, Teledyne FLIR)

NOTE: The recording box is lined with stripes of red LEDs for monochromatic dark recordings using a camera positioned at the top. The Plexiglas arena for the mouse to behave inside is aligned with the best window for video-recording the mouse behavior.

1. Move mouse with implanted EA from its home cage to a glass jar containing a cotton swab soaked with isoflurane until the mouse begins shallow breathing.
2. Attach EA to the Intan headstage connected to the Intan RHD USB interface board with an SPI cable or similar.
3. Place mouse in the Plexiglas arena in a custom-made recording box and let it recover and habituate to arena for \sim 10 min.
4. Begin recording neural activity and video using trigger mode in camera recording software on a desktop computer and TTL strobes relaying from the monochromatic camera to the interface board (see Supporting Information, File 1, Figs. 1F and 1G).
5. Record a pre-encounter period of 5 min and then introduce stimulus mouse.
6. Stop recording after 5 min of interaction between the stimulus and the subject.
7. Save all acquired files from recording and remove stimulus.

The user acquires files corresponding to the video of the mouse behavior and a set of .rhd files (in the case of an Intan acquisition board, as in our case), which can be further extracted as raw electrical signals in the form of voltage changes for the whole recording period. One must refer to the user manual and software provided by the acquisition setup suppliers (Intan in our case; however, Plexon, Neuralynx, or others can be used similarly). The raw .rhd files can be converted to .mat (Matlab format) files using openly available conversion codes provided by Intan. Further, the electrophysiological parameters (like LFP power, coherence, and firing rate) can be analyzed using custom-written MATLAB codes. The user can employ extensively documented, openly available toolboxes for extracellular neurophysiology like NeuroExplorer (provided by Plexon) and Chronux (Chronux.org) to analyze their recorded data. Further, the results expected from such recordings, specifically in a social interaction test, are described below in the Understanding Results section (Fig. 7).

8. Move subject out of the arena and hold its head while removing headstage. Return subject to the home cage.

Subjects can undergo multiple recording sessions every day. We recorded two sessions every day for each subject (50 min total: 10 min habituation + 10 min Session 1 + 10 min habituation + 10 min Session 2). We have observed fewer social interactions in mice when the recording sessions are longer than the above scheme.

9. Post-process acquired files from step 7 after completing all recording sessions and registration of electrodes (locating the ends of each electrode in the brain tissue; see Basic Protocol 4, step 11).

HISTOLOGY AND ELECTRODE REGISTRATION

The following protocol explains the process of visualizing and further registering the location of each electrode in the implanted mouse brain (Fig. 6).

BASIC PROTOCOL 4

Materials

Mouse with implanted EA (see Basic Protocols 2 and 3)
Ketamine (1 g/10 ml; Vetoquinol)
0.9% (w/v) saline (from sodium chloride, Sigma, #S9888)
4% (w/v) formaldehyde (FA; see recipe)
Mounting medium (Sigma, #F4680)

1-ml syringes and 31G needles
Small scissors (Fine Science Tools, #14090-09)
Wooden board (15 cm long \times 5 cm wide \times 15 cm high)
Absorbent napkins (60 \times 60-cm absorbent pads for dogs)
Rubber bands
Toothed forceps
26G needle
Saline pump (10 W, AB25, Goso)

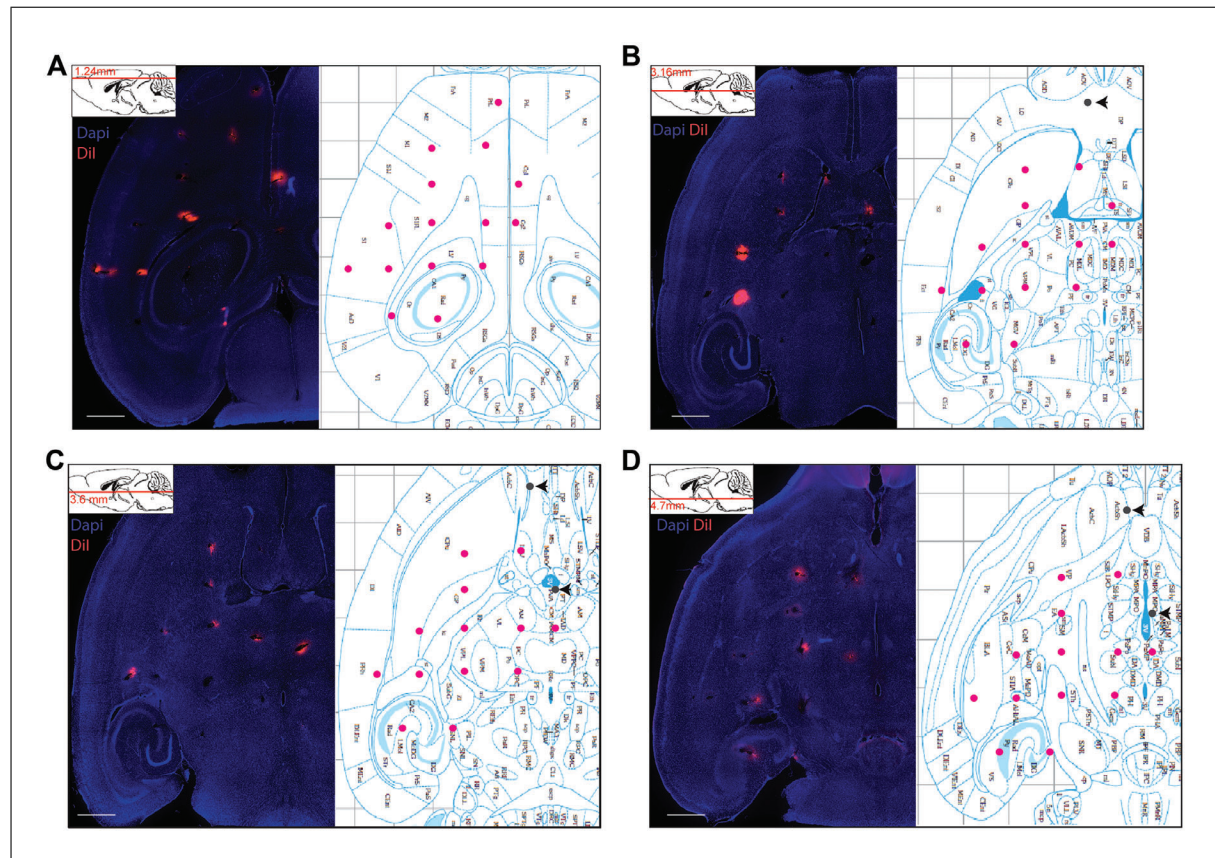


Figure 6 Locating the electrode ends in serial sections of a brain previously implanted with an EA. (A) Left: Horizontal brain section image at 1.24 mm deep from the brain surface (top-left cartoon), with electrode marks (Dil in red and gross anatomy based on DAPI staining in blue). Right: Corresponding section from the brain atlas, with red dots depicting the electrodes found in the section in the left figure. Scale = 1 mm. (B) Similar to (A), horizontal section at a depth of 3.16 mm below the brain surface. Gray dots represent the electrode that is missing in this section. Thus, sections above this depth may locate the region targeted with this electrode. (C) Similar to (B), the horizontal section corresponds to a depth of 3.6 mm from the brain surface. (D) Similar to (B), horizontal section matching the atlas at a depth of 4.72 mm from the brain surface.

Blunt forceps
50-ml Falcon tubes
Vibratome (VT1000s, Leica)
Superfrost Plus glass slides (Menzel-Glaser, Thermo Scientific)
Coverslips
Wide-field fluorescence microscope (Nikon Eclipse Ti)

NOTE: We followed the procedure explained in detail in other protocol articles (DiCarlo, Lane, Hsiao, & Johnson, 1996; see Current Protocols article Paletzki & Gerfen, 2019).

1. Deeply anesthetize mouse with an implanted EA using 0.3 ml (100 mg/kg body-weight) ketamine via intraperitoneal injection using a 1-ml syringe and 31G needle.
2. Once the mouse is anesthetized, grasp its head to prevent movement while removing EA and pull EA out to ensure that the dental cement is not adherent to the skull. Cut any adherent dental cement using small scissors.

The EA should be removed by pulling it out only perpendicularly to the brain surface to avoid disrupting the brain tissue. The screw attached to the skull usually comes out with the dental cement. If the screw is still attached to the surface, it can be removed later with the skull bones as it does not disturb the brain tissue.

3. Place anesthetized mouse in the supine position on a wooden board covered with absorbent napkins and use rubber bands to hold paws so one has easy access to the chest cavity. Expose heart by removing the rib cage with small scissors and toothed forceps and placing a 26G needle attached to a saline pump into left ventricle. Make a small cut over right atrium to decrease the pressure and allow cleared fluids to be absorbed by the napkins.
4. After ~30 ml of 0.9% saline is perfused through the mouse vascular system, replace saline with 4% FA.
5. After perfusing with 30 ml FA, stop flow and remove needle from the ventricle.
6. Cut skull along the mid-sagittal suture while avoiding the tissue below the skull.
7. Use blunt forceps to break skull and remove brain gently by cutting the cranial nerves adherent to the ventral regions and pia tissue attached to the brain.
8. Keep brain immersed in a 50-ml Falcon tube containing 4% FA for 3 days at 4°C.
9. Take 50- μ m sections of brain using a vibratome along the horizontal axis to visualize the EA marks (DiI fluorescence).
10. Mount sections serially on Superfrost Plus glass slides and add mounting medium to hard-set a coverslip.
11. Image serial whole sections in the DAPI and TRITC channels using a wide-field fluorescence microscope. Use an appropriate brain atlas (Franklin & Paxinos, 2019) for electrode registration.

This imaging aids in identifying gross anatomical landmarks (see mouse brain atlas; Franklin & Paxinos, 2019) and specific electrode marks (Fig. 6). Electrode tracks can be seen as blobs of red dye (DiI stain) in subsequent horizontal sections (Fig. 6, red dots represent electrodes in the same arrangement as in the EA design step in Basic Protocol 1, step 1, and gray dots/black arrows are electrodes that are no longer present at the respective depth of the section). With the help of the atlas, the user can locate the electrodes and the brain regions to match the EA design and thereby the respective channel of the recorded electrophysiological signals.

REAGENTS AND SOLUTIONS

Formaldehyde (FA), 4%

500 ml 8% (w/v) formaldehyde stock solution (see recipe) (4% final)

500 ml double-distilled H₂O

9 g sodium chloride (Sigma, #S9888) (0.9% final)

Adjust pH to 7.4 with 1 N HCl or 1 N NaOH

Prepare immediately before use and keep at 4°C

CAUTION: Formaldehyde solution for fixation purposes should be prepared immediately before use from stock solution.

Formaldehyde stock solution, 8%

Heat 800 ml double-distilled water to 65°C and add 80 g paraformaldehyde (Sigma-Aldrich, #P6148) in a fume hood. Stir solution for 2 min and then slowly add 2 to 3 ml 5 N NaOH (until a clear solution appears). After the solution cools down to room temperature, filter solution using Whatman no. 1 filter paper and add water to a final volume of 1 L. Store ≤ 2 weeks at 4°C.

CAUTION: Formaldehyde is toxic, and preparation requires heating the solution, releasing a considerable amount of fumes. Therefore, it is essential to follow safety procedures to prepare the FA solution using a fume hood.

COMMENTARY

Background Information

Extracellular recordings from behaving rodents have been used in electrophysiology studies (O'Keefe & Dostrovsky, 1971; Ranck, 1973) for a few decades. With advancements in recording technologies, multi-site brain recordings from freely moving rodents have become the norm (Hultman et al., 2018; Mague et al., 2020). EA designs for multi-site extracellular recordings in freely moving mice have classically required skilled personnel to fabricate the EA, and fabrication is a time- and labor-intensive process. Our modular design and its reuse of expensive parts allow such studies to be performed by a more diverse range of groups. Though we use a social behavior paradigm in Basic Protocol 3, other behavioral tests, such as open-field, elevated plus-maze, and operant tasks, can be performed with similar EA designs targeting any set of relevant brain regions. Mesoscale electrophysiological studies can equip the experimenter with network-level dynamical datasets and respective inferences. As large-scale neural dynamics and individual computational hypotheses of network dynamics are evolving in systems neuroscience (Pesaran et al., 2018; Stevenson & Kording, 2011; Urai, Doiron, Leifer, & Churchland, 2022), we hope that our modular EA design will aid experimenters in gaining such insights.

Critical Parameters

The EA design presented here weighs ~ 3.0 g without the tethered cable. Thus, it is

ideal for neural recordings from adult mice. In our hands, we observed more successful surgeries in CD1 mice than in C57BL/6J mice. While fabricating the EA (Basic Protocol 1), proper contact of each electrode wire with the respective pins of the Mill-Max connector is essential for good neural recordings. Additionally, the reference and ground wires must accurately touch the brain tissue to reference voltage differences in the recordings. Furthermore, the EA design is limited to recordings from brain regions ≥ 1 mm apart along the anteroposterior and mediolateral axes. In our hands, if the electrodes are placed at a distance of 500 μ m, they tend to bend significantly, to a state where their locations in the brain are indistinguishable.

There may be multiple sources of noise introduced during recording in electrical devices. To avoid this noise adding to the recording in Basic Protocol 3, ground wires from the main electrical power supplies of any devices around the recording setup should be connected to the ground of the recording device. Further, shielding the arena using a faraday cage (1-mm aluminium plates) and grounding this cage is essential for reducing electrical noise from external devices that could corrupt the recorded brain activity.

Another essential aspect that aids in better recordings is the state of the experimental animal in Basic Protocol 3. Careful handling of the experimental animal (Gouveia & Hurst, 2019) and habituating the experimental animal to the recording setup can drastically reduce

Table 1 Troubleshooting Guide for Using a Modular Electrode Array for Multi-site Extracellular Recordings in Rodents

Problem	Cause	Solution
The Mill-Max grooves are closed due to an overflow of dental cement	An excessive amount or low viscosity of dental cement was applied over the electrodes between the two PCBs	Apply small amounts of the dental cement mixture and repeat the process multiple times to cover all electrodes
Crosstalk between two electrodes	The ends of electrode wires that were bent and inserted into specific grooves of the second PCB are in contact	While folding the electrode wires and placing them inside the Mill-Max grooves of PCB, cut excess wire to accommodate the space between the two PCBs
Electrode impedance too high ($> 1 \text{ M}\Omega$)	The electrode coating was not removed correctly, so there is no possible connection between the Mill-Max pins and the electrode wire	Remove the coating from the electrode at precisely the site where it folded to enter the Mill-Max grooves in the PCB
Excessive bleeding while removing the drilled skull fragment	Superficial vessels were torn during the removal of the drilled skull	Absorb the excess blood using Kimwipes and apply a few drops of cold saline
Implanted mice are weak 1 or 2 days after surgery	The mouse is avoiding food consumption, which indicates that the healing from surgery is incomplete	Administer saline to avoid dehydration due to low consumption of water
	The analgesic dose was not enough to relieve the pain	Administer two doses (12 hr apart) of analgesics on the third day post-surgery
The EA is loose and falling off the head of the mouse	The mouse was removed prematurely following EA implantation, before the dental cement dried	Check that the dental cement is completely dry by gently pushing with a toothpick
	The skull screw was loose when attached to the skull, failing to hold the EA and skull together with dental cement	After attaching the screw to the burr hole on the skull, make sure it does not move when pushed gently with a toothpick. If needed, make a smaller burr hole and attach the screw into this hole. Cover the previous burr hole with bone wax or Vaseline to avoid infection.
	The dental cement was applied to the wet skull surface	Make sure to wipe the skull with Kimwipes to dry the surface and further scratch the surface with a bent 22G needle tip for better attachment of dental cement
The RHD recording software shows noise from all channels even after connecting the EA to the headstage	The attached headstage is loose and feeding a suboptimal signal to the RHD interface board	Take the mouse out of the arena and re-attach the headstage
The DiI marks are not visible in the fluorescent images	The electrodes were not appropriately coated with the dye	For future experiments, try to increase the electrodes' coating with dye (five dips for 5 s each)

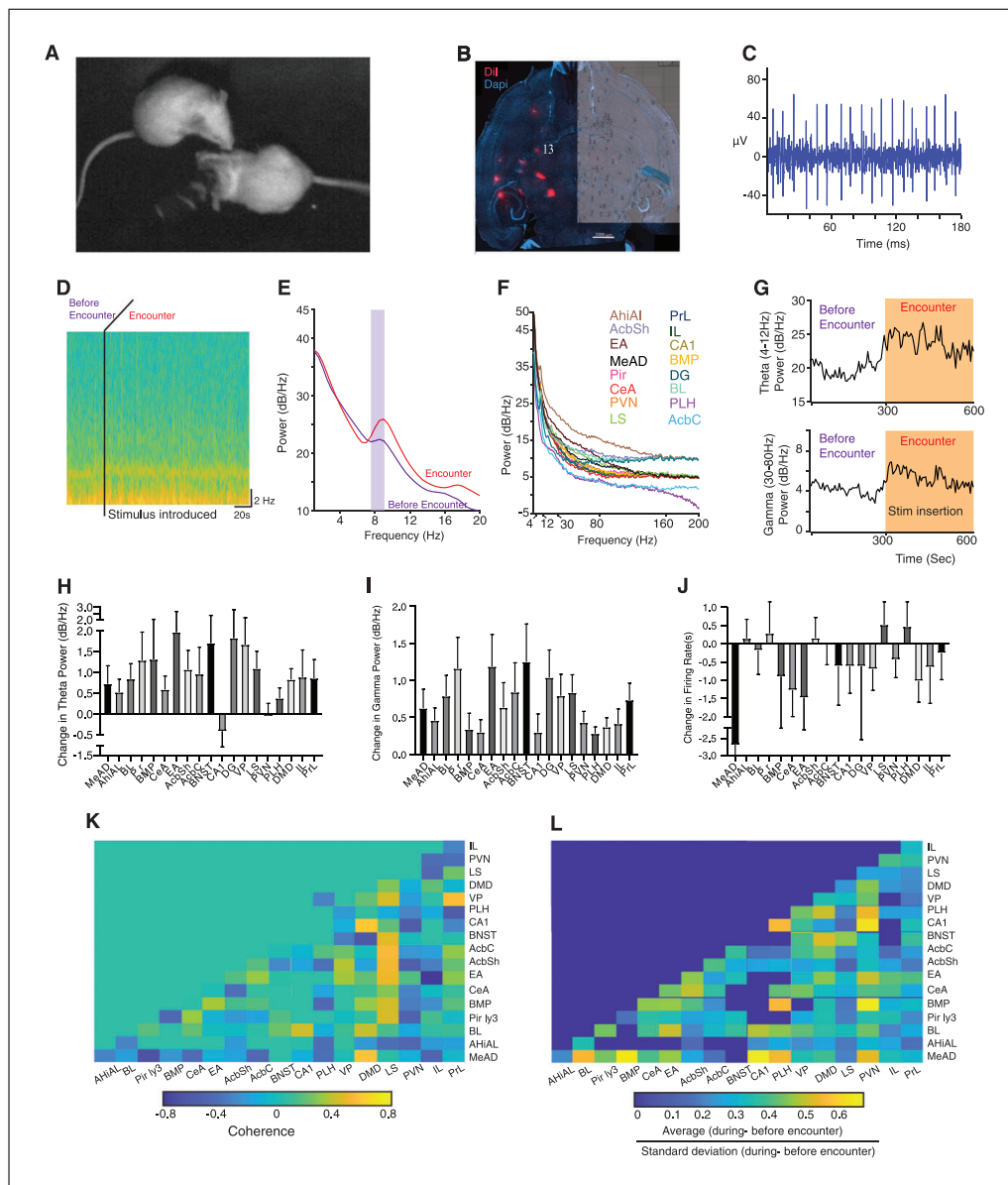


Figure 7 Neural signals from male mice during social interactions. **(A)** Social interaction between an EA-implanted ICR male mouse and a male stimulus (top view, zoomed-in picture). **(B)** Electrode marks (red) in the brain of an implanted mouse; horizontal plane (-4.7 mm deep from the bregma). Scale = 1 mm. **(C)** Multi-unit spiking activity from the globus pallidum (electrode 13 in Fig. 7B). **(D)** Spectrogram of the LFP signal from the accumbens core (AcbC) during the encounter with the male stimulus. **(E)** LFP power spectral density plots from the AcbC before and during the encounter period. **(F)** LFP power spectral density plots from 16 brain regions targeted in the ICR male mouse during the encounter with the stimulus. **(G)** Theta power (up) and gamma power (down) traces were recorded from the AcbC during the entire recording session. **(H)** Change in theta power during the encounter compared to before the encounter, from 44 sessions of social encounter for 19 mice. The bar graphs represent the mean \pm standard error of the mean theta power of each brain region. **(I)** Same as in (H) for gamma power. **(J)** Same as in (H) for the change in the firing rate of multi-unit spiking activities. **(K)** Theta coherence between brain areas before encountering the social stimulus. **(L)** Change in theta coherence between brain areas during the encounter with respect to the stimulus. The pair of brain regions in at least five sessions from all the recordings were included in the coherence analysis. AhiAl: amygdalohippocampal area, anterolateral part; AcbSh: accumbens nucleus, shell; EA: extended amygdala; MeAD: medial amygdala, anterodorsal; Pir: Piriform cortex, ventral; CeA: central amygdala; PVN: paraventricular hypothalamic nucleus; LS: lateral septum; PrL: prelimbic cortex, IL: infralimbic cortex; CA1: field CA1 of the hippocampus, BMP: basomedial amygdala, posterior part; DG: dentate gyrus, ventral; BL: basolateral amygdala; PLH: peduncular part of lateral hypothalamus; AcbC: accumbens core.

the animal's stress levels. These practices help prevent the animal from being stressed due to novel handling experiences immediately before actual behavior tasks. Moreover, experiments must be carried out after the mouse shows signs of complete recovery (moving freely and consuming food; Basic Protocol 2).

Troubleshooting

Please refer to Table 1 for a troubleshooting guide.

Understanding Results

In the example shown in Figure 7, we used an EA to record from multiple (up to 16) brain areas of CD1 mice during social interactions with a same-sex, age-matched conspecific (Fig. 7A). Electrode tip locations were identified post-mortem using the DiI marks in horizontal brain slices (Figs. 6 and 7B). The EA design allowed us to record extracellular signals, which depend on multiple parameters, such as the electrode's impedance and the sampling rate. Specifically, rhythmic LFP signals in the range of 1 to 300 Hz can be separated from MUA using a bandpass filter (Butterworth) (Fig. 7D; bandpass filter between 300 and 5000 Hz; see electrode 13 recordings from the globus pallidum in Fig. 7C).

Rhythmic LFP potential signals may be inspected in time using a spectrogram, enabling visualization of changes in the power of different frequencies during the session (Fig. 7D). The frequency-power relationship may also be quantitatively analyzed for specific periods of the behavior using power spectral density (PSD) analysis. For example, Figure 7E demonstrates a marked increase in the amplitude of 6 to 9 Hz (theta rhythm, purple shade) in the nucleus accumbens core between the periods before and during social interaction, suggesting increased theta rhythmicity during social interactions. Separate PSD profiles may be calculated for each brain region of a given animal (Fig. 7F). The power in given bands, for example, the theta (4 to 12 Hz) and gamma (30 to 80 Hz) bands, as well as the MUA rate, may be separately plotted and analyzed in time to demonstrate temporal changes (Fig. 7G).

We specifically analyzed the theta and gamma rhythms from 1- to 300-Hz filtered signals (Figs. 7H, 7I, and 7J), as this frequency range is the most studied and has been shown to encode arousal in general and specifically during social interactions (Geschwind & Levitt, 2007; Tendler & Wagner, 2015; Uhlhaas & Singer, 2006; Wass, 2011). Theta rhythmicity has been shown to coordinate

neural ensembles between regions that are anatomically distantly located (Buzsáki & Draguhn, 2004; Fries, 2005), whereas gamma rhythmicity has been reported to originate from inhibitory local circuits (Buzsáki & Wang, 2012). Moreover, these rhythms were reported to be affected in rodent models of human pathological conditions associated with impaired social behavior, such as autism spectrum disorder (Lazaro et al., 2019). The motivation to analyze a specific frequency band is guided by the specific experimental aim and the brain regions being targeted by the user.

Another parameter extracted from these recordings for each band of rhythmic LFP signals is coherence, a measure of synchronous activity between pairs of brain regions (Fries, 2005, 2015). In the example heat maps shown in Figure 7, we plotted the coherence level before the social interaction (Fig. 7K) and the change in theta coherence during the social interaction (Fig. 7L). The coherence between two brain regions before and after social interaction increased in some brain regions while decreasing in others, suggesting remodeling of functional connectivity in the entire network during social interaction.

Time Considerations

Basic Protocol 1: Preparing EA: ~3 hr.

Basic Protocol 2: Surgical implantation of EA: ~3 hr for surgery, 3 days for post-surgery recovery.

Basic Protocol 3: Recording neural activity and video of behaving mouse: 50 min for each session of social behavior test.

Basic Protocol 4: Histology and electrode registration: ~1 hr for perfusion, 72 hr for post-perfusion in 4% FA, ~3 hr for preparing sections, ~2 hr for fluorescent imaging.

Analysis: 3 to 4 hr for analysis of video and neural recording data.

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Author Contributions

Alok Mohapatra: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review and editing; **Shai Netser:** Conceptualization, Methodology, Project administration, Resources, Software,

Supervision, Validation, Visualization, Writing – review and editing; **Shlomo Wagner**: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review and editing.

Conflict of Interest

The authors have no conflict of interest to disclose.

Data Availability Statement

The data, codes, and material that support the protocols are available from the corresponding author upon reasonable request.

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