Video Article

Large-scale Recording of Neurons by Movable Silicon Probes in Behaving Rodents

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Abstract

A major challenge in neuroscience is linking behavior to the collective activity of neural assemblies. Understanding of input-output relationships of neurons and circuits requires methods with the spatial selectivity and temporal resolution appropriate for mechanistic analysis of neural ensembles in the behaving animal, i.e. recording of representatively large samples of isolated single neurons. Ensemble monitoring of neuronal activity has progressed remarkably in the past decade in both small and large-brained animals, including human subjects[1-11]. Multiple-site recording with silicon-based devices are particularly effective because of their scalability, small volume and geometric design.

Here, we describe methods for recording multiple single neurons and local field potential in behaving rodents, using commercially available micro-machined silicon probes with custom-made accessory components. There are two basic options for interfacing silicon probes to preamplifiers: printed circuit boards and flexible cables. Probe supplying companies (http://www.neuronexustech.com/; http://www.sbmicrosystems.com/; http://www.acreo.se/) usually provide the bonding service and deliver probes bonded to printed circuit boards or flexible cables. Here, we describe the implantation of a 4-shank, 32-site probe attached to flexible polyimide cable, and mounted on a movable microdrive. Each step of the probe preparation, microdrive construction and surgery is illustrated so that the end user can easily replicate the process.

Video Link

The video component of this article can be found at http://www.jove.com/video/3568/

Protocol

1. Construction of the microdrive

All drives are made from the same basic elements: a moving part, which carries the electrode and a fixed part, which is anchored to the skull. An ideal microdrive allows smooth but long enough travel of the electrode in multiple small steps, is sturdy enough to prevent accidental movement of the electrode, easy to manipulate by the experimenter without interfering with the animal's behavior, small in size and light in weight. As a result of these competing requirements, different drives suite different applications.

Only 4 parts are needed to build our basic drive: a brass flat head screw, a matching nut, a plastic bridge prepared from a single row pin header and two custom-cut brass plates.

1. Break a 3-pin piece from the header
2. Gently pull out the middle pin.
3. Enlarge the hole by drilling through it with a drill bit size #55.
4. Cut a thread using the 00-90 tap.
5. Cut two pieces out of the brass plate.
6. File the edges of the plates with a Dremmel.
7. Drill a hole in the middle of both pieces using a drill bit size #65.

8. Assemble the drive pieces so that the brass plates are touching the pins. To accomplish this, insert the brass screw through, successively, the brass plate, the threaded pin header hole, the second brass plate, and the nut. Tighten the screw gently so that the assembly becomes stable.
9. Solder the pin ends to the brass plates.
10. File the protruding end of the screw.
11. Solder the nut to the screw. Be careful not to solder the nut to the brass plate.
12. Test the movement of the drive: turn the screw clock-wise to elevate the plastic bridge.

2. Preparing the silicon probe

Before fixing the probe to the drive, add extra insulation to the bonding area of the probe to prevent cerebrospinal fluid (CSF) or humidity from producing short-circuits:

1. Weigh and mix the Sylgard Elastomer components in a 10:1 ratio.
2. Using a sharpened cotton applicator, apply the Sylgard to the top end of the probe.
3. Let it dry in an oven preheated at 60°C for 2 hours.

To ensure that the recording sites are devoid of any debris, the probe tips need to be cleaned:

4. Prepare a 4 % dilution of Contrad detergent.
5. Let the probe soak in the detergent at 63°C for at least 2 hours.
6. Rinse the detergent off by dipping the probe repeatedly in distilled water.

Before fixing the probe to the drive, the impedance of each recording site should be checked:

7. Dip the probe in 0.9% saline, and connect it to an impedance-meter. If too many recording sites have incorrect impedance, repeat steps 2.4-2.6 or consider using a different probe. Here we use an Impedance Conditioning Module from Frederick Haer, Co. (FHC), combined with a homemade channel selector. Alternatively, a niPOD by NeuroNexus, Inc. or NanoZ by Neuralynx, Inc. allows the monitoring of the impedance of all the probe channels simultaneously.

3. Affixing the probe to the microdrive

1. Using a razor blade, cut multiple grooves into the bridge to create a rugged surface.
2. Appose the probe to the bridge of the drive. This procedure is best done under an operating microscope, by holding the drive with a clamp and adjusting the probe by a micromanipulator so that the shanks are perfectly parallel with the drive screw. This ensures that during advancement, the probe shanks move into brain tissue without 'cutting' through it. The exact depth of the probe tips relative to the base of the drive should be determined at this stage, taking into consideration the depth of the target structure from the surface of the skull.
3. The probe is then fixed to the bridge with grip cement.
4. Optional: for visualizing the probe track in the brain, DiI solution (1-2% diluted in ethanol) can be applied to the back of the probe at this stage.

4. Preparing the skull

Prior to surgery, the reference and ground electrodes, and the parts of the on-head Faraday cage are prepared:

1. Cut two 2"-long pieces of copper wire, and solder one end of each insulated copper wire for about 1 mm.
2. Using a needle, scrape the head of a 00-90, 1/8" stainless steel screw and solder one piece of copper wire to it. Soldering such stainless steel ground screw-electrodes requires an appropriate flux (e.g., N-3 All purpose flux from La-Co) and high soldering tip temperatures. Carefully prevent any solder from flowing into the groove of the screw. This will be used as the ground electrode. Repeat with another screw and copper wire to prepare the reference electrode.
3. Cut trapeziodal pieces from copper mesh. These pieces will be assembled to protect the headstage.

Surgical instruments and preparation are the same as used in many small animal surgeries. The entire surgery is done under deep isoflurane anesthesia, using aseptic conditions, according to NIH approved guidelines. Please note that the (mock) surgery shown in this video is for demonstration purposes only. For appropriate visibility and filming purposes, several preparatory steps, surgical precautions and postoperative procedures are not shown/visible or discussed.

Prior to surgery, all components and supplies should be sterilized, following appropriate procedures (see Guidelines for Survival Rodent Surgery; http://oacu.od.nih.gov/ARAC/surguide.pdf). During surgery, a sterile field on the skull is prepared and isolated by sterile drapes. At the end the surgery, a broad spectrum antibiotics is applied locally and a long-acting pain killer is given intramuscularly (e.g., buprenorphine, [Buprenex] 0.05 mg/kg). In addition, painkiller (e.g., Ibuprofen) is provided in the drinking water at approximately 60 mg/kg/24 hrs for 5 days. For proper surgical and anesthesia procedures, consult appropriate sources[12].

4. Install the animal in the stereotaxic apparatus, shave and clean the scalp[13].
5. Cut the skin along the midline and push aside the scalp. Remove the periosteum, clean and dry the skull.
6. Measure the location and distance between bregma and lambda, and determine the x and y coordinates of the probe implantation site accordingly using a stereotaxic atlas[14]. Mark the site by scraping a cross on the skull with a scalpel.
7. Drill the skull using a round head drill bit (size ¼) and drive support screws (stainless-steel, 000-120, 1/16") halfway into the bone, on different bone plates on the top and on the side of the skull. The screws will provide anchors to securely bond the headgear to the skull.

8. Drill holes above the cerebellum and insert the ground and reference electrodes prepared in step 4.2. For recording local field potentials (LFP), the choice of the reference site is critical. This site is chosen because cerebellar LFP is the smallest of all cortical regions and muscle artifacts are minimal at this midline location.
9. Apply dentin activator (Metabond kit) using a tiny brush over the entire surface of the skull. Rinse it with 0.9% saline.
10. Apply dental cement (Metabond kit; follow manufacturer's instructions for mixing) on the skull, carefully covering anchor screws and ground and reference electrodes, but leaving the probe implantation site clear.
11. Secure the four copper mesh flaps (prepared in step 4.3) to the skull. For this, cement the narrow base of each of them to the anterior, left, right, and posterior sides of the skull. The copper should never be in direct contact with the bone but always separated by a layer of cement.

5. Preparing the brain surface

1. Using a round head drill bit, drill around the implantation site in multiple stages, while frequently irrigating the bone with saline.
2. Carefully remove the bone flap and irrigate the brain surface.
3. For inserting a multiple-shank probe, a large strip of dura is removed. Two tools are needed to remove the dura: a scalpel and a hook prepared from an insect needle (alternatively, a standard tungsten microelectrode). Bend the tip of the needle by pushing against a hard surface (e.g. glass microscope slide), and attach it to a handle (here, a piece of wooden Q-tip; alternatively a microdissecting needle holder).
4. Lift the dura with the hook, and cut it with a scalpel. Special care is taken to avoid damaging the pia, vessels and the surface of the neocortex. Small bleeding can be resolved by saline irrigation. If major bleeding occurs or the neocortex is compromised in any way, one should consider terminating the surgery and preparing another animal.

6. Implanting the probe

At this stage, the density and orientation of cortical surface vessels are carefully evaluated. Stereotaxic coordinates should be adjusted, because the probe has to penetrate the brain in an area free from larger vessels.

For implantation, the drive assembly can be held with an alligator clip attached to the stereotaxic holder. Uninterrupted visibility of the brain surface and the tips of the probe are critical for successful penetration.

1. Slowly lower the probe down to approximately 1 mm above the intended target, while constantly irrigating the craniotomy with saline. For neocortical recording, the probe tips are lowered into the cortex approximately 0.5 mm and lifted back near the surface.
2. Seal the craniotomy by applying a warm melted mixture of wax and paraffin oil through a needle (10-20g of wax in 10 mL paraffin oil, heated at 65°C). Prior to application, cool the mix to 30°C and test the density. It should be soft enough to allow easy probe movement). To facilitate complete coverage, the mixture may be melted in situ by approaching the hardened wax with the tip of a micro-cauterizer.
3. Attach the bottom of the drive to the skull with grip cement, being careful to leave the nut free to turn. It is of utmost importance to avoid any accidental "bump" of the drive at this stage, otherwise the probe will damage the cortex. After the drive is secured to the skull, smooth movement of the probe should be verified.
4. Cement the connector part of the probe to the skull.

7. Building the on-head Faraday cage

1. Pull up and assemble the copper mesh flaps into a protective cylinder around the probe and drive. The cylinder also serves as an electrical shield against environmental noise and the slow wave artifacts produced by the charged whiskers in the behaving animal.
2. Adjust the cylinder height by cutting away excess material so the copper mesh is level with the top of the probe connector.
3. Solder the wires from the reference and ground screws to the appropriate pins of the connector. Also solder adjacent copper mesh flaps together to ensure their electrical continuity, and solder the ground wire to the copper mesh.
4. Apply a layer of grip cement on the copper mesh to reinforce it and to prevent any direct contact between the metal and the animal's skin. Optionally, apply a layer of epoxy resin to further reinforce the headgear.
5. Test the movement of the drive screw.
6. Cover the top of the headgear with a piece cut from a rubber glove.

8. Recording in the freely moving animal

1. After appropriate post-operative care, connect the animal to the recording system using a high impedance headstage and a lightweight, ultraflexible multi-strand cable. Counterbalance the weight of the headgear.
2. Test the quality of recording each day in the homecage. The position of the recording sites is judged by both unit firing patterns and the shape of the local field potentials. Lower the probe gradually by turning the screw by small increments (typically 1/8 to 1/4 turn per day, i.e. 35-70 micrometers) until the target structure is reached.

9. Representative Results

Electrophysiological signals (local field potential and unit activity) vary depending on the recorded structure and the current behavior of the animal. Figure 1 shows examples of 32-channel CA1 hippocampal recordings while the rat is exploring an open field. Note the prominent 8 Hz (theta band) oscillation of the local field potential during exploration with superimposed spiking on multiple shanks and sites (examples of spikes indicated by arrowheads). To analyze neuronal unit activity, spikes are detected and sorted into single units using cluster analysis of their waveforms[15-16].

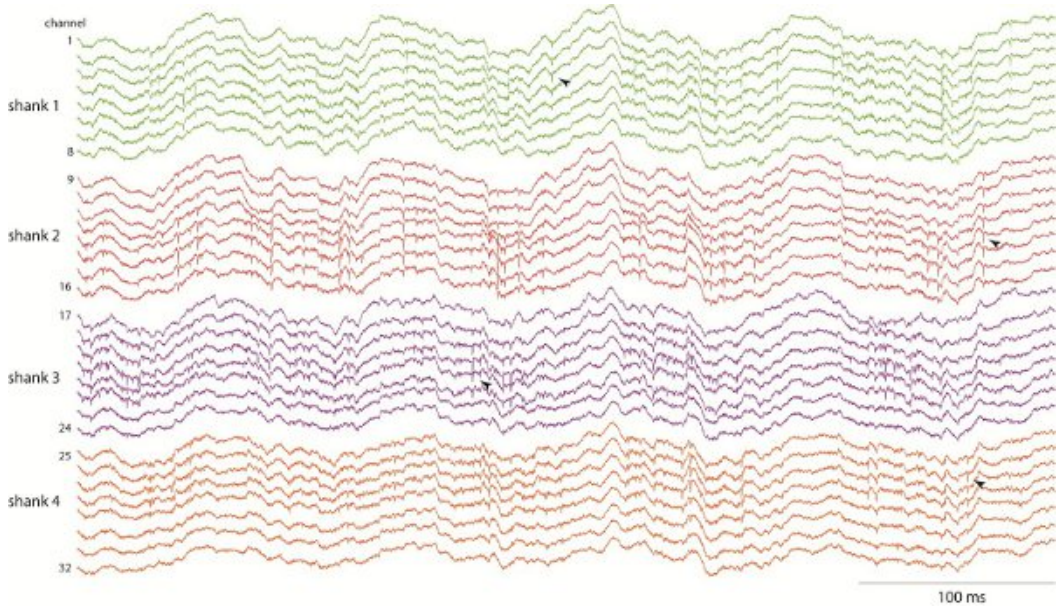

Figure 1. CA1 Hippocampal recordings in the behaving rat using a 4 shanks x 8 sites silicon probe. Recordings are wideband and sampled at 20 000 Hz, which allows to study both local field potential oscillations (e.g. "theta" band 8 Hz rhythm) and neuronal spiking activity.

Material/Procedure	Possible replacement	Comment
Pin header	Adapt the choice of the header to your needs (length of the pins). A 2-row header can also be used. Build the drive as described, on the first row. This way the second row becomes part of the moving bridge, and a probe can be attached to the pins of the second row.	
Impedance conditioning module + homemade channel selector	Neuronexus niPOD-32 or Neuralynx NanoZ	Allows to test the impedances of all channels at once
Gas anesthesia	Injectable anesthetics suitable for survival surgery (e.g. Ketamine-Xylazine)	For surgeries lasting several hours, it is easier to monitor the state of the animal and continuously adjust the dose with volatile gas anesthesia, but careful use of injectables is possible.
Grip cement to affix the probe to the drive	Superglue (Loctite)	Works if the surface of the probe to attach is small (i.e., not directly connected to a printed circuit board).
DiI to visualize the probe track a posteriori	Other dyes	DiA has been successfully used
	Electrolytic lesions after the last recording session	Allows to know the depth of particular probe sites with better precision.
	Time	In long term chronic recordings (over a month), microgial activation at the contact of the probe (revealed by Nissl-staining) may be sufficient to visualize the track
Wax/oil mixture to close the craniotomy	Artificial dura	Not recommended, as it may stick to the probe
	Silicon mixture (Dow Corning)	Successful replacement of wax. Different qualities exist, a soft one is recommended.
Grip cement to reinforce the hat	Epoxy	Possible but with the risk of the hat being too flexible (depending on the hat shape, coppermesh strength -two layers can be used). A combination of both (a ring of cement at the bottom and top, and connecting stripes, epoxy on the remainder of the hat surface) is recommended.

Table 1. Alternatives to reagents and equipment used. Please click here to see a larger version of this figure.

Discussion

This movie illustrates the implantation procedure of silicon probes for chronic large-scale recordings in the behaving rat. Critical steps to ensure quality recordings of neuronal activity arise from the fragility of both biological (brain tissue) and technical (silicon probe) materials. Special care should be taken while handling the probe to avoid any contact of shanks with any remotely "hard" surface (for example, the shanks would break if one tried to implant them in the brain without removing the dura). Similarly, any injury to the brain tissue (while preparing the brain surface for implantation, or from bumping into the probe or drive once it is implanted) would result in damaging the cells and jeopardizing the recording of unit activity. In addition, the electrical path of the grounding should be checked, as any circuit interruption between the cerebrospinal fluid, the ground screw, the copper wire, the copper mesh flaps and the ground pin on the connector, would result in a large movement artifacts and/or line noise (50 Hz or 60 Hz). If the Faraday cage is not high enough, the protruding micro-drive may act as an antenna. The antenna effect can be prevented by grounding the drive as well (solder another copper wire between the drive and the copper-mesh). The reference signal path should be similarly checked.

We illustrated the implantation of a single silicon probe, but multiple site recordings using multiple probes and drives can be readily accomplished after some practice. In addition, we are using similar but smaller drives for implanting silicon probes in the mouse brain. The commercially available silicon probes and probe-flex cable-connector components, along with the small size of multichannel preamplifiers have drastically simplified the preparation process compared to previous techniques. Today, it is as easy to record from 64 to 128 sites simultaneously in a behaving rodent as from 2 sites with wire electrodes just a decade ago.

Silicon probe technology is undergoing rapid development and widespread use[17]. Preamplifiers can be integrated with probes[18], and smaller headstages, multiplexers or telemetric systems are being manufactured commercially, pushing the limits of physiological recordings to further limits.

Recent theoretical and experimental studies with silicon probes[17,19] indicate that with properly refined large-scale recording methods, combined with new mathematical insights and modeling studies, one will be able to record from a representatively large fraction or perhaps every neuron from the brain volume surveyed by a multiple shank silicon probe (thousands of cells in ~1 μm[3;5-17]). However, given the correlational nature of these measurements, the cause-effect relationship among neuronal activity patterns remains inevitably ambiguous. A thorough understanding of how coordinated ensemble activity emerges from its neuronal components requires at least two additional steps. The first one is the identification of the multiple neuronal cell types, each of which uniquely contributes to assembly behavior - literally like members of an orchestra. The second, and complementary step, is a principled manipulation of the spiking activity of identified cells or cell groups, in a manner engineers interrogate electronic circuits[20]. The recently developed molecular optogenetic tools can be used to manipulate specific cell populations by local light stimulation[20-22]. The efficient combination large scale recordings and optical methods with silicon probes[23] provides the means for both identifying and selectively driving specific cell populations, therefore allowing to address the causal relationships in brain networks.

Disclosures

No conflicts of interest declared.

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