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A Novel System for Recording from Single Neurons in Unrestrained Animals

Helen Sherk and Elizabeth J. Wilkinson

Dept. of Biological Structure University of Washington

Abstract

To observe neural activity in animals engaged in natural behavior, it is often desirable to minimize or eliminate restraint of the animal. We have developed a simple system for recording from single units in unrestrained cats. An implant with multiple guide tubes and a tiny microdrive is placed inside the recording chamber. An indwelling Pt-Ir microelectrode is advanced incrementally during recording sessions that occur over a period of weeks or months. Electrodes can be easily replaced. We obtain excellent recording stability, and also have been able to sample extensively from a region of cortex or brain stem in a single animal. The essential electronics have been miniaturized and sewn into a light-weight walking jacket, so that we can collect data from a cat who is not connected to any fixed equipment.

Introduction

Many methods have been developed for recording from single units in behaving animals (Hubel, 1959; Evarts, 1968; see Discussion for other citations). The less restraint imposed on the animal, however, the more challenging the problem becomes. Difficulties include instability of recording, limitations on positioning of electrodes, problems with electrode replacement, dural thickening or overgrowth, and data storage. We wished to record from completely unrestrained cats with a system that was easy to use, that yielded stable recording, and that resulted in large samples of units per cat. In our experiments, cats walk through tunnels and down a long test alley, and so can not be attached by a cable to any fixed electronic device. Cats move around freely between alley runs, and often jump into the start box by themselves, so we needed a system that provides robust recording stability. Finally, we wanted to sample an extensive stretch of cortex in a single electrode penetration, so that it was essential to be able to advance the electrode incrementally over 6–10 mm. This paper describes the system that we have developed to meet these needs.

Corresponding author: Helen Sherk Address: Dept. of Biological Structure, Box 35-7420 University of Washington Seattle, WA 98195
Phone: 206-685-1733 Fax: 206-543-1524 E-mail: has@u.washington.edu.
Elizabeth J. Wilkinson Dept. of Biological Structure, Box 35-7420 University of Washington Seattle, WA 98195 Phone: 206-685-1733
Fax: 206-543-1524 E-mail: wilkie@u.washington.edu

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Materials and Methods

Cats

Three male cats, obtained from a commercial breeder, were used. All procedures were approved by the University of Washington Animal Care Committee, and conformed to the N.I.H. "Principles of laboratory animal care".

Chamber

A chamber is attached to the skull in an anesthetized cat; this is done in a surgical suite using sterile procedures. The chamber is a piece of titanium tubing (OD 22 mm, ID 19 mm; TitaniumSports.com), with one end shaped to fit the skull. The chamber must be very securely attached, since in future procedures we clamp the chamber to immobilize the anesthetized cat's head; moreover, awake cats sometimes knock their chambers against solid objects (cats don't react to such impacts, indicating that they are not aversive). We place 7-8 titanium skull screws in a ring around the chamber, and bond the chamber to the screws with dental acrylic. To ensure good growth of bone into the screw threads, we grit-blast the screws, producing a microscopic roughening of the titanium (Svehla et al., 2000; Moroni et al., 2002). To get good adhesion of acrylic to the chamber, we grit-blast it, and also place 3 or 4 2-56 stainless steel set screws near its lower end that protrude outward like tiny spokes.

For electrical grounding, we place a gold-plated pin that extends through the skull, and is attached via stainless steel or platinum wire to a small screw in the chamber wall. We make a craniotomy smaller than the chamber diameter, and cover the chamber with a tall cap (height = 38 mm) which is held on with 3 2-56 cap screws.

Guide tube implant

The central feature of our recording system is an implant holding several guide tubes and a microdrive (see Fig. 1A). The implant base is made of plastic such as Delrin, cut from a sheet 3/16" thick. It holds 3-5 guide tubes, made of 25 g thin-wall hypodermic tubing (Small Parts), each sharpened at one end. After drilling holes for the guide tubes in the plastic, we machine conical depressions in the surface of the plastic, each extending to about half its thickness and centered on one hole. A guide tube is inserted so that its top end is flush with the bottom of the conical depression. Guide tubes are fixed in place with epoxy ("Plastic Steel Epoxy", Devcon; all references to epoxy are to this variety). Each conical depression is coated with the epoxy to produce a smooth surface ending at the guide tube lumen, thus forming a funnel to steer the electrode tip into the guide tube without damage.

Guide tube lengths are determined by the target brain structure. For recording from cortex, the guide tube should be long enough to penetrate the dura, but should extend minimally into the cortex itself. For subcortical recording, guide tubes extend deeper into cortex, but never into thalamus or brainstem, to avoid damage to small subcortical structures. An L-shaped stylus made of 31 g hypodermic tubing is placed in each guide tube to occlude the lumen when not occupied by an electrode, and thus exclude fluid or debris. The long leg of each stylus has a length matched to its guide tube, so that the tip is about flush with the end of the guide tube.

A tiny microdrive (3-NRMD-A1, Crist Instruments) is attached to the implant via stainless steel pins and epoxy. The part of the microdrive that is intended to hold the electrode is modified so that a piece of thin-wall 19 hypodermic tubing, about 3 mm long, can be secured to it with epoxy (see Fig. 1A). This tubing will be used to hold the electrode.

Another sterile procedure is carried out in order to place the implant. We anesthetize the cat and record from a suitable structure (for example, LGN) in order to determine the appropriate

location for the implant. The electrode is held in a conventional microdrive mounted on a stereotaxic frame. After removing the electrode, we lower the implant into place, with the sharpened guide tubes penetrating through the dura, and the plastic part of the implant resting solidly on the skull. Exposed dura is covered with gel foam, and dental acrylic is used to fix the implant in place, covering the exposed skull and bonding to the inner wall of the chamber. Finally, we remove one stylus and replace it with an electrode. The electrode is point-loaded into the guide tube, with the smooth conical opening minimizing or preventing electrode damage. Electrode preparation and attachment are described below.

Implants can also be used to inject muscimol (for inactivating small regions of brain) or other substances (see Wilkinson et al., 2007).

Microelectrodes

We use parylene-coated platinum-iridium microelectrodes (40 mm long, made from 135 μ m diameter wire, impedance 2-2.5 M Ω ; Nano Bio Sensors LTD). A microelectrode is prepared by cutting it to the desired length and stripping the insulation from the last 1 mm with a razor blade. The electrode is jacketed with a piece of polyimide tubing (0.0071" ID; A-M Systems), leaving about 8 mm of electrode protruding at the tip end. (The polyimide provides stiffness and a close fit to the inside of the guide tube, preventing leakage of CSF.) A lead is attached to the electrode; it is made of 32 g wire with "ultra flexible" insulation (Cooner Wire), 25 mm long, soldered to a gold-plated male miniature connector pin (A-M Systems), which is cut to a length of 7 mm (see Fig. 1B). Insulation is stripped from about 1 mm at the free end of the lead; this bare length is placed side by side with the stripped end of the electrode, and they are bound tightly together using a short piece of fine wire (about 40 g). The join is embedded in epoxy. The last step is to attach an "arm" to the electrode with epoxy; this is an L-shaped piece of 22 g hypodermic tubing, with one leg about 3 mm long which will fit into the microdrive, and the second leg of a length determined by the distance of the target guide tube from the microdrive.

When the microelectrode is put into a guide tube, the "arm" is slipped into the 19 g tubing segment on the microdrive. We use viscous UV cured adhesive to fix the microelectrode "arm" solidly to the microdrive ("Structural tack & seal", Dymax). We make several successive applications of adhesive to this join, curing the adhesive after each one with a UV-conductive fiber optic cable. To ensure that the electrode records well, we connect it to a headstage via its lead and record in the anesthetized cat. The microdrive is advanced by turning the top screw (340 microns/revolution). When we encounter neural activity, we disconnect the electrode, leaving the lead dangling freely inside the chamber, and replace the protective cap on the chamber.

Changing electrodes

As described below, we recorded neural activity daily or every other day until spikes were lost, presumably because the electrode had passed out of gray matter. This process generally required at least a few weeks. We then put a new electrode in a different guide tube. Although it is possible to carry out this operation in an awake cat, we usually anesthetize the cat so as to reliably immobilize his head. The head is held with a clamp which securely grips the titanium chamber. With a tiny scalpel we cut away the UV-cured adhesive that holds the electrode onto the microdrive. After removing the electrode we place a sterile stylus in its guide tube, and go through the procedure outlined above to place a new electrode.

Electronic devices

Because we needed electronics for amplifying and filtering the neural signal that could be easily carried by the cat, as well as a method of starting and stopping data collection remotely, we

designed 3 small devices, each built on its own printed circuit board. These were a headstage, a main board with a filter and amplifier, and a control board. The control board is used to turn on and turn off data collection when the cat passes under dim incandescent lights at the entrance and exit of a long test alley; it will not be described further.

We have designed and built both single and dual channel versions of the electronics used for data collection; for simplicity, only the single channel version will be described. The headstage circuit, shown in Fig. 2A, is a non-inverting amplifier that “rolls off” low frequencies using a 10 uF capacitor, thus acting as an AC amplifier (Horowitz & Hill, 1989). Headstage gain = 16.7. The signal is sent to the main circuit board (Fig. 2B), starting with a 4-pole high-pass Butterworth filter (−3dB point = 580 Hz), and then going to a 4-pole low-pass Butterworth filter (−3dB point = 5,300 Hz). Finally, the signal passes through a variable gain amplifier (gain = 4–10). The experimenter can adjust the gain using a small potentiometer with a plastic knob (Vishay/Spectrol; Mouser Electronics). Maximum gain of the system, including the filter stages, is 1,107. Power is supplied by a pair of flat 3 V batteries (Ultralife Batteries).

In order to make the electronics as compact as possible, all components are surface-mounted. We use the Eagle Layout Editor (CadSoft Computer, Inc.) to design PC boards and generate Gerber files; boards are fabricated by Advanced Circuits. The headstage board is 9×19 mm; the main board is 50×25 mm. The two-channel versions are only slightly larger (the main board, for example, is 65×30 mm) because we used a smaller package size for the passive components.

Because our cats are actively moving around, the electronics must be rugged. Surface-mount construction has proven quite satisfactory. Connectors linking the different electronic devices are a greater challenge; they should be sturdy, small, and light-weight. We use plastic, positive latch, 0.100” pitch connectors (Molex; Mouser Electronics). For cabling, we use extra flexible multiconductor cable (Cooner Wire).

Mounting of electronic devices

The headstage is attached to a stainless steel recording cylinder that fits over the chamber, replacing the cap worn between recording sessions; it is held with 2-56 cap screws (see Fig. 3). A shield, cut from 0.25 mm thick brass, is screwed onto the outer wall of the cylinder, pressing the headstage tightly in place. A gold-plated miniature female connector is soldered to the headstage, and extends into the interior of the cylinder; the microelectrode lead plugs into this connector. A metal lid caps the cylinder, and is secured with a 2-56 cap screw.

Note that the titanium chamber, the recording cylinder, and the brass shield are all grounded to the brain. It is important to keep this internal ground insulated from external grounds such as the cat's skin or the experimenter's hands, so the outside of the cylinder and shield are painted with epoxy (black on outside of cylinder in Fig. 3).

The main circuit board and the control board are sewn into a cat “walking jacket” (see Fig. 4). Each cat has his own jacket. The design is modeled after commercially available jackets sold in pet stores, with a collar that is fastened with a plastic buckle, and a velcro closure around the chest. The circuit boards are sewn onto the dorsal aspect of the jacket, and the batteries are in a side pocket. A cable from the headstage leads to the jacket. Output from the main circuit board goes to a tiny computer with an A to D converter (“Stingray” = RL2; Tucker Davis Technologies) which is held by velcro onto the ventral aspect of the jacket. The jacket together with electronic devices, cables, and batteries, but excluding the computer, weighs 61 g. The computer weighs 73.5 g.

Data collection

When recording from a stationary cat, data are downloaded via a cable from the main circuit board to a rack-mounted device (RP2; Tucker Davis Technologies) that is connected to a PC. The programs for data collection using the Stingray and the RP2 are equivalent; the chief difference is digitization rate of the signal (24 kHz vs 25 kHz). Whenever the absolute value of the signal exceeds a threshold set by the user (for example, $5 \times$ root mean square of the averaged signal), a segment of signal 0.92 msec long is captured; the captured segment is centered on the time point at which the signal crossed threshold. Each captured segment is time-stamped. During subsequent data analysis, we use spike-sorting software, written in the lab, to select spikes belonging to distinct units. The RP2 has 32 Mbytes of memory, which is sufficient to store about 60 minutes of data from a neuron with a high rate of spontaneous firing. The Stingray has 64 Mbytes of memory.

In order to synchronize the spike record with videotape records made during data collection, the RP2 or Stingray turns on an LED for 100 msec at the start of each second during spike capture (the LED risetime (150 microsec) is negligible). LEDs are mounted in view of both videocameras used when the cat is stationary. When the cat is walking, the videocameras see an LED attached to his jacket (see Fig. 5). During data analysis, the first frame in which the LED is on marks the start of each second of data collection.

Procedure

To simplify the recording procedure, we have 5 identical “recording kits” that are gas-sterilized before use. Each kit contains the essential items: recording cylinder with attached headstage, lid for the cylinder, jeweler's screwdriver, and tiny hemostats (for plugging the electrode lead into the headstage). A recording session begins with the cat placed in a cat bag with his forelegs free (e.g., Fig. 3). The protective cap is removed and the recording cylinder is placed on the chamber. We plug the electrode lead into the headstage, and connect the headstage to the main board on the cat's jacket, which is draped across the cat's back. We advance the microdrive with a jeweler's screwdriver while playing the neural signal on an audio monitor and displaying it on an oscilloscope. Sometimes the spike that we recorded in the previous day's session is still present, so that it is necessary to advance to find a new neuron. More commonly, we advance to find isolated and responsive spikes. When a satisfactory location has been found, we put the lid on the recording cylinder and commence collecting data via the RP2. With the cat in the cat cradle, we generally capture 3-8 chunks of data, each 4-6 minutes long. We then take the cat out of the cat bag, put on his jacket, and attach the Stingray to the jacket. We record neural activity in walking cats while letting them traverse a long test alley. Generally, we collect data from 6-12 alley trials and then download the data from the Stingray to a PC.

Electrical noise

We have not experienced any 60 Hz noise or other forms of electrical interference from devices such as fluorescent lights or computer monitors. However, static electricity discharges do show up on spike records and can destroy op amps in the electronic devices. Grounding the experimenters almost eliminates this problem, which we accomplish by removing our shoes.

Results

Implant durability

We have recorded from 3 cats fitted with 4 implants (2 successively in one cat). Recording commenced at least 2 days after placing each implant, and continued for up to 32 weeks. Recording continues in one cat; in a second cat we intend to make further penetrations via the current implant, as the implants apparently have unlimited life spans.

When the dental acrylic around the implant was well sealed to the inner wall of the chamber, the chamber remained completely dry. Leaks between the acrylic and chamber wall led to fluid build-up on top of the acrylic, though never to intracranial infection. Dampness inside the chamber often reduced spike size during a recording session, presumably due to a high-resistance short to ground. Leaks can be repaired without anesthetizing the cat.

Ease of recording

One of the attractive features of this recording system has been ease of use. In a typical recording session, the preparation time prior to the start of data collection, including putting the cat in the bag, replacing his protective cap with a recording cylinder, and finding a responsive, well-isolated cell, was about 15-20 min. An entire recording session usually took about 1.5 hr, and we generally recorded from a given cat every day or every other day. Our usual practice has been to collect data from a single cell in one session, but sometimes we have advanced the electrode during a session, thus acquiring data from a second or occasionally third cell. It is thus possible to accumulate substantial data samples in a moderate amount of time. In the 2 cats in which we have recorded from visual cortex, we have sample sizes of about 101 and 137 units. In the third cat, in which we recorded from the midbrain for a limited period of time before proceeding to injection experiments, we have a sample of 59 units.

Recording stability

We have recorded single unit activity in 3 settings: 1) in stationary, bagged cats (see Figs. 3 and 6); 2) in cats walking through a long test alley (see Fig. 5); 3) in cats roaming freely about the lab. In all 3 conditions isolation was good and movement artifacts were rare. Isolation would sometimes be lost if the cat shook his head vigorously; however, recording more often remained stable through the most enthusiastic swats at toys, head shifts, running, and pouncing (see Fig. 6). The most common occasion on which we lost units was at the start of testing in the cat alley, possibly relating to brain movement occurring when the cat got into the start box. We suspect that the location of the electrode tip in the cortex played a role in stability: stability is worst when the electrode has just entered the cortex, and is near the surface.

Electrode durability

While all electrodes recorded well when first placed in the brain, we found that tungsten electrodes deteriorated over the next 3-6 weeks, yielding reduced isolation. The platinum-iridium electrodes described in Methods, in contrast, have maintained their recording properties for much longer periods. The second set of spikes in Fig. 7 were recorded 11.5 weeks after electrode placement.

Problems encountered

The most common equipment problem that we have experienced concerned the attachment of the electrode to the microdrive. In one implant, the piece of 19 g tubing repeatedly separated from the microdrive, indicating that care must be taken to ensure a robust attachment. We also found that we needed to use a generous amount of UV-cured adhesive to attach the electrode "arm" to the 19 g tubing to ensure that the electrode did not come loose.

Early in a penetration, electrodes sometimes got stuck in the guide tube. A tiny drop of hydrogen peroxide applied to the funnel that the electrode passed through generally freed the electrode.

Discussion

Two different approaches have been used for recording single units from awake, mobile animals, employing either rigid microelectrodes or "floating" microwires. The advantage of

microwires is their stability of recording (Palmer, 1978; Sasaki et al., 1983; Armstrong & Drew, 1984; Chang et al., 1988; Jaeger et al., 1990; Banks et al., 1993; Westby & Wang, 1997; Ludvig et al., 2001). However, the recording properties of individual microwires are rather unpredictable, their performance deteriorates over time, and they are often difficult to move. Other experimenters (Hubel, 1959; Beloozerova & Sirota, 1993; Drew, 1993; Grohrock et al., 1997) have used rigid electrodes, which can be more easily replaced or advanced in the brain, and more consistently isolate spikes. The method that we have developed uses rigid electrodes but also provides excellent stability. It maintains the advantages of rigid electrode procedures: electrodes can be replaced relatively easily, and the quality of recording is reliably good. Most importantly, the electrode can be advanced incrementally, making it possible to optimize spike isolation, and to sample over a long extent of gray matter.

In our procedure the electrode remains in the brain for weeks. Probably this contributes to stability, and it minimizes the preparation time for a recording session. Our method took as its starting point that developed by Beloozerova and Sirota (1993), who obtained consistently excellent recording using indwelling, movable electrodes in walking cats. With their approach, however, data collection is limited to about 1 month per cat because the dura is removed over a region of cortex. In our method, the long time in which the implant remains in place allows the dura and skull to regrow, presumably fixing the guidetubes in place and probably contributing to stability.

Prior recording methods in mobile cats and monkeys have involved a cable attached to the animal. We eliminated the cable in order to give our cats complete mobility. However, we regard our solution to this problem (the use of a tiny computer carried by the animal) as an interim one. Telemetry would eliminate the need for the computer. Grohrock et al. (1997) designed a telemetric system for recording from squirrel monkeys, and Nieder (2000) developed a telemetric system for recording from barn owls, but we lacked the expertise to replicate their transmitters. Suitable commercial telemetric systems presumably will become available in the future.

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Fig. 1.

A: An implant used in one cat (Douglas) for 5 months. A Crist microdrive is mounted on top of the plastic base. Visible on the surface of the base are the wide ends of the 5 “funnels” leading to the lumens of the 5 guide tubes. Note the piece of 19 g hypodermic tubing epoxied to the microdrive. B: Microelectrode prepared for recording, with attached lead terminating in a male gold miniature connector pin.

Fig. 2.
Circuit diagrams for the headstage (A) and the main board (B).

Fig. 3. Douglas lying in the cradle during a recording session, watching a moving target. Note the recording cylinder with the brass shield that encloses the headstage (the cylinder hides the chamber from view). A white cable connects the headstage to the main board sewn into the cat's black jacket, which is draped across his back.

Fig. 4. One cat (Elm) wearing his recording jacket. Visible are the knob of the potentiometer used to adjust the amplifier gain (lower arrow), and the light sensitive diode that turns on and off data collection when the cat walks through the test alley (upper arrow).

Fig 5.

One cat (Birch) walking through the test alley during a recording session. The LED worn on his cheststrap flashes briefly every second during recording in order to synchronize video frames with the spike record. Below are the first 8 spikes collected during this trial; the spike persisted throughout 6 runs through the alley. On the right side these spikes are shown superimposed (arrow). The duration of the baseline for each spike is 0.92 msec.

Fig. 6.

Frames from a video sequence taken while recording from Birch. At top are 3 non-sequential frames taken at the times indicated (in seconds) during data collection. The cat is watching a waving pipecleaner; in the second frame he strikes at it, and in the third frame he traps it with his paw. Below is a series of spikes, with time-stamps above each (light gray waveforms were excluded during spike-sorting). The spikes enclosed by the heavy line were fired during the period corresponding to the video frames above. (On the right, these 28 spikes are shown superimposed.) Note the absence of movement artifacts.

Fig. 7. Spikes recorded from a cortical neuron shortly after placement of a Pt-Ir electrode (Day 3), and from a different neuron 11.5 weeks later (Day 76). Each set of spikes is shown superimposed below.