

Multi-electrode Recording of Neural Activity in Awake Behaving Animals

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Introduction

Perhaps one of the most important questions we seek to understand when studying the brain is how neural circuits process information. One of the key ways of addressing this question is to record the activity of individual neurons during well-controlled behaviors and/or presented stimuli. By measuring the spiking activity of the neurons during sensation or action, we can build models of the output of a given neural circuit. Then, by building a hierarchy of models, we can understand the broader flow of information.

Traditionally, experimentalists have acquired data and built these models based on data obtained one neuron at a time. In these types of experiments, activity of multiple neurons is assembled over many sessions during which stimulus parameters and/or animal behavior is carefully monitored and/or controlled. Isolating the signal of a single neuron requires precisely positioning the tip of electrode in the proximity of the soma, as described in Chapter 3. Then, a successful experiment requires maintaining the electrode stably in that position long enough to acquire whatever data are needed. For some brain regions this can be a painstaking process in which a large fraction of an experimental session is spent locating the neurons to be studied. In other cases, the challenge is that the action potentials of multiple neurons are detected by the electrode, requiring ultraprecise adjustment to isolate the activity of one from the rest. The impedance of the recording electrode, which is largely determined by the surface area of the recording region (Cogan 2008), largely determines where a given experiment lies along the continuum from zero-or-one neuron (high-impedance electrode) to one-neuron-from-many (lower impedance).

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4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 77

Simultaneous recording of neural activity using multiple electrodes has the obvious potential advantage of reducing the amount of time required to acquire data. In practice, the challenges of precise positioning mean that one cannot simply scale electrode count by sequential placement of individual impermanent electrodes. By the time the n^{th} electrode is targeted to a neuron, the signal on the first electrode has likely been degraded. Instead, many systems neuroscience experiments employ chronically implanted multichannel electrode arrays in which permanent electrodes are placed simultaneously in parallel. The multi-electrode approach enables the activity of many neurons to be recorded simultaneously, and chronic implantation can provide stable signals across multiple days (Chestek et al. 2011, Fraser and Schwartz 2012, Kentros et al. 1998, Tolias et al. 2007). As we will discuss later, chronic implantation typically entails the use of lower-impedance electrodes, requiring signal processing-based isolation of individual neurons by the shape of their action potentials (“spike sorting”). However, when electrode sites are close enough that the signals of individual neurons are detectable on multiple channels, confidence in the quality of unit isolation can actually be higher than in all but the highest-quality single-channel electrode recordings (Ecker et al. 2010, Gray et al. 1995, Harris et al. 2000, Henze et al. 2000).

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In addition to enabling faster “single-electrode” science, simultaneously recording the activity of multiple neurons enables the study of important aspects of neural circuits that cannot be observed a single neuron at a time. To understand this, we must consider how the activity of an individual neuron varies. When repeated stimuli are presented, the exact pattern of action potentials generated by a neuron is variable. In the simplest model, we might describe this variation as arising from two sources, “signal” and “noise.” The “signal” component of variation reflects how changes in the stimulus or the animal’s action modulates brain activity (e.g., a visual receptive field or a preferred direction of motion). The “noise” component reflects variability that arises from the randomness in synaptic transmission, membrane fluctuations, and so forth (Averbeck et al. 2006, Harris and Thiele 2011). Thus, to distinguish signal from noise, we need exquisitely precise control of experimental and behavioral parameters as well as repeated trials. However, even when these conditions are met, variations in the internal state of the brain (e.g., attention, alertness, or motivation) can modulate neural activity in uncontrolled ways (Averbeck and Lee 2004, Chalk et al. 2010, Goard and Dan 2009, Harris and Mrsic-Flogel 2013; Harris and Thiele 2011, Harris 2005, Karlsson et al. 2012, Lee and Dan 2012a, Niell and Stryker 2010, Ponce-Alvarez et al. 2012). When multiple neurons are recorded simultaneously, it begins to be possible to use statistical models to disambiguate brainwide variation from the fluctuations of individual neurons (Briggman et al. 2006, Chen et al. 2011, Churchland et al. 2007, Kass et al. 2005). In addition, by recording the activity of multiple neurons in an anatomically connected circuit, we can begin to understand exactly how neural ensembles transform patterns of information. Thus, multi-electrode recording has emerged as a key tool in expanding our understanding both of how neurons represent the world and how they generate these representations (Buzsáki 2004, Stevenson and Kording 2011).

Multi-electrode Recording Arrays

Several multi-electrode recording technologies are employed for chronic neural recording. We can divide them into two broad categories: commercially manufactured electrode arrays and customized “microdrives.” To form a complete system, these multi-electrode devices are coupled with multichannel neural recording systems.

Commercially manufactured rigid electrode arrays come in three general categories: microwire arrays (Nicoletis et al. 2003), micromachined electrode arrays (Normann et al. 1991), and multisite silicon probes (Drake et al. 1988, Normann et al. 1991, Vetter et al. 2004). As shown in Figure 4.1, microwire arrays (Microprobes for Life Sciences, Inc, Gaithersburg, MD; Tucker-Davis Technologies, Alachua, FL) and micromachined electrode arrays (Blackrock Microsystems, Salt Lake City, UT) are typically structured as a “bed of nails,” where long, narrow electrodes are insulated except for the very tips. Were these electrodes to be rigidly attached to the skull, micromovements of the brain (e.g., arising when animals vigorously interact with their environments) would cause rapid degradation in the quality of recorded signals. Consequently, for stable chronic recordings, a “floating” architecture is often used, in which a flexible tether connects the implanted electrode array to the connector used to record signals (see Fig. 4.1c). Typical

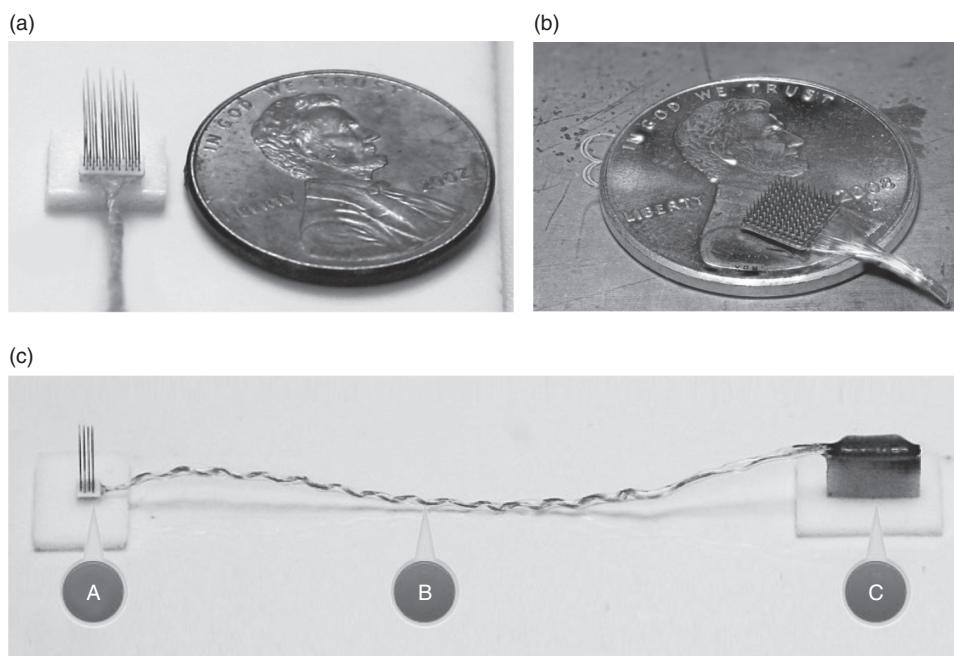


FIGURE 4.1 Two variants of a chronically-deployed electrode array, both structured as a “bed of nails.” a: Floating microwire array (Microprobes for Life Sciences, Inc.). b: Silicon micromachined electrode arrays (Blackrock Microsystems, Inc., photo by Bryan William Jones, used with permission). c: A flexible tether (B) connects the implanted electrode array (A) to the connector used to record signals (C). Photos (a) and (c) by Martin Bak, MicroProbes for Life Science, Inc., used with permission.

4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 79

interelectrode distances are 100–500 µm, meaning that signals from individual neurons are present on at most one electrode. By specifying the depth of the electrodes, these topologies can be customized for recordings targeting a particular layer of neocortex (these arrays are typically not useful for subcortical structures).

In contrast to targeting a particular plane of neurons, silicon probes have multiple recording sites along the depth axis of the probe (Fig. 4.2). This architecture has enabled local field potential recordings along the dendritic arbors of neurons, which are critical to understanding the architecture of vertically organized structures in the hippocampus and neocortex (Bragin et al. 1995, Buzsáki et al. 1992, Ylinen et al. 1995). Newer electrode topologies that place recording sites in an array at the tip of the electrodes have proved more successful in chronically recording the activity of single units (Mizuseki et al. 2009, 2012, 2014)—the key innovation that enabled this success is the ability to move the electrode array (Vandecasteele et al. 2012).

In contrast, in most chronic recording experiments rigid electrode arrays are initially implanted and then not moved. Unfortunately, precise targeting of recording sites to individual neurons is impossible during implantation. Thus, the most common approach for obtaining high-quality multi-electrode recording of many single neurons

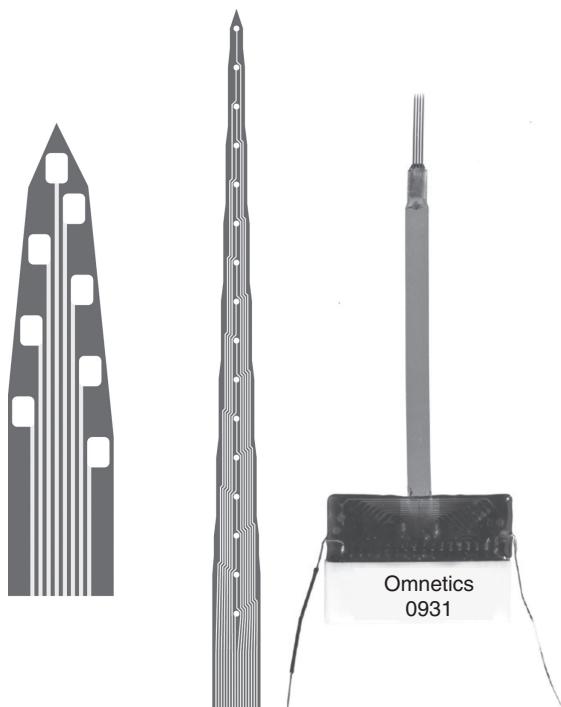


FIGURE 4.2 Silicon probes have multiple recording sites along the depth axis of the probe. **Left:** Actual device. **Right:** Sample recording site layouts. In general, the electrode pattern chosen depends on the neurons being targeted and the experimental paradigm. Drawings by Neuronexus, Inc., used with permission.

80 | BASIC ELECTROPHYSIOLOGICAL METHODS

uses microdrives (Fig. 4.3) (Gray et al. 1995, Jog et al. 2002, McNaughton et al. 1983, Wilson & McNaughton 1993). While there is limited commercial manufacturing of microdrives (Neuralynx, Inc, Bozeman, MT), including recently introduced drives that allow the depth of rigid electrode arrays to be adjusted after implantation (NeuroNexus, Inc.), most are still manufactured within individual laboratories. Microdrives are typically used with two- or four-channel electrodes (“stereotrodes” or “tetrodes”). Remarkably, the procedure for manufacturing these electrodes—twisting nichrome or platinum–iridium wires together, softening the insulation so that they will adhere, and cutting and electroplating the ends—largely follows the same procedure developed more than 30 years ago (McNaughton et al. 1983). A typical microdrive will have between 4 and 40 tetrodes, with smaller microdrives used for recordings in mice (where there is an implant weight limit of ~5 g) (Voigts et al. 2013) and larger microdrives used for recordings in rats (limited to ~40 g) (Kloosterman et al. 2009) or even monkeys (Tolias et al. 2007). The canonical design presented here for a 16-tetrode microdrive has a mass of ~35 g and can be expected to record the activity of >100 neurons in dorsal hippocampus (see the end of the chapter for a detailed, step-by-step protocol in the manufacture of a 16-tetrode microdrive).

Surgical implantation of chronic recording electrode arrays in rodents is beyond the scope of this chapter (see Vandecasteele et al. 2012). However, several factors are discussed below that merit special attention: (1) the ground and/or reference screw, (2) minimizing damage to cortex to minimize inflammation, and (3) ensuring good adhesion to the skull for stable recording.

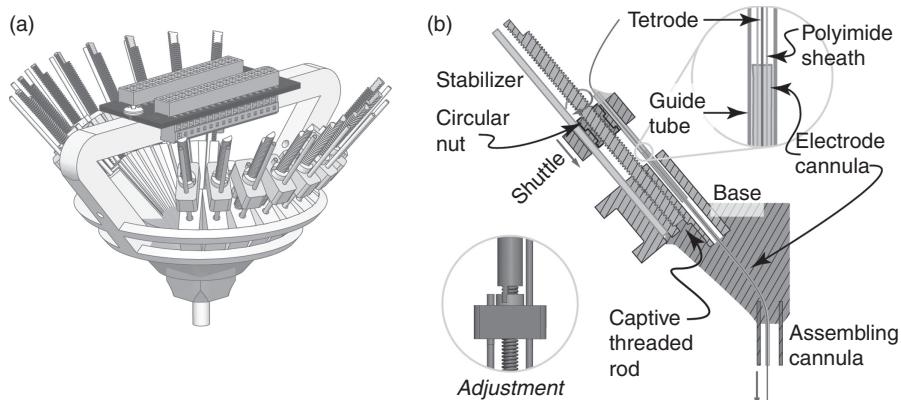


FIGURE 4.3 Microdrive arrays are typically hand-assembled. By using a very small threaded rod for each individual electrode, precise positioning can be achieved. a: Sketch of completed device showing 16 independently movable microdrives intended for tetrodes. b: Drawing of one microdrive assembly. In this design, shuttles are made by hand and coupled with 0–80 threaded rod, a custom 3D-printed base, and individual stainless-steel cannulas to form a complete drive.

Multi-electrode Data Acquisition Systems

A block diagram for a traditional multi-electrode recording system is shown in Figure 4.4. Neural activity recorded from each electrode is amplified by a small circuit board plugged into the electrode connector (the “headstage”), transmitted along a tether, further amplified, digitized, and then **further** processed by custom software on a personal computer. In the most recently available neural data acquisition systems (e.g., Neuralynx, Inc.; Tucker-Davis Technologies, Inc.; Plexon, Inc.; Blackrock Microsystems, Inc.; Ripple, LLC), the complete amplification and digitization process occurs in the headstage (Harrison 2008), reducing the number of wires in the tether from one per channel to a dozen or less.

Practice of Multi-electrode Recording

Multichannel Neural Activity

Software designed for recording multichannel neural activity will typically include the ability to monitor both local field potentials (LFPs) and spiking activity. The LFP is the lower-frequency (typically less than ~400 Hz) component of the acquired signal (see also Chapter 7). The low-pass filtering removes much of the signal energy that arises from the action potentials of nearby neurons and leaves a signal thought to be primarily driven by synaptic currents. Depth-dependent changes in LFP patterns are often extremely useful for fine positioning of recording electrodes. LFP is typically visualized as a series of scrolling records, as shown in Figure 4.5.

Action potentials are typically detected using an amplitude threshold. The recording software enables the threshold level to be optimized for each channel of the system. Neural “snippets”—windows of electrode signal around the times of threshold crossing (typically ~0.25 ms before and ~1 ms following)—are displayed for each electrode channel. For tetrode recordings (and other multichannel electrodes where more than one channel is associated with a micro-scale brain location), action potentials from a single neuron can often be detected on multiple channels. Thus in these cases, a threshold crossing on one channel triggers snippet acquisition for all associated channels. Furthermore, for tetrode recordings, the snippet display typically also includes a “cloud” view where the peak values on each channel are plotted against each other. This perspective allows for rapid estimation of the quality of signal isolation for individual neurons, which appear as isolated clouds. Both snippet and cloud views display not only the most recent threshold crossing detected but also some history (see Fig. 4.5). Proper selection of the threshold is different during recording and during adjustment and is discussed below.

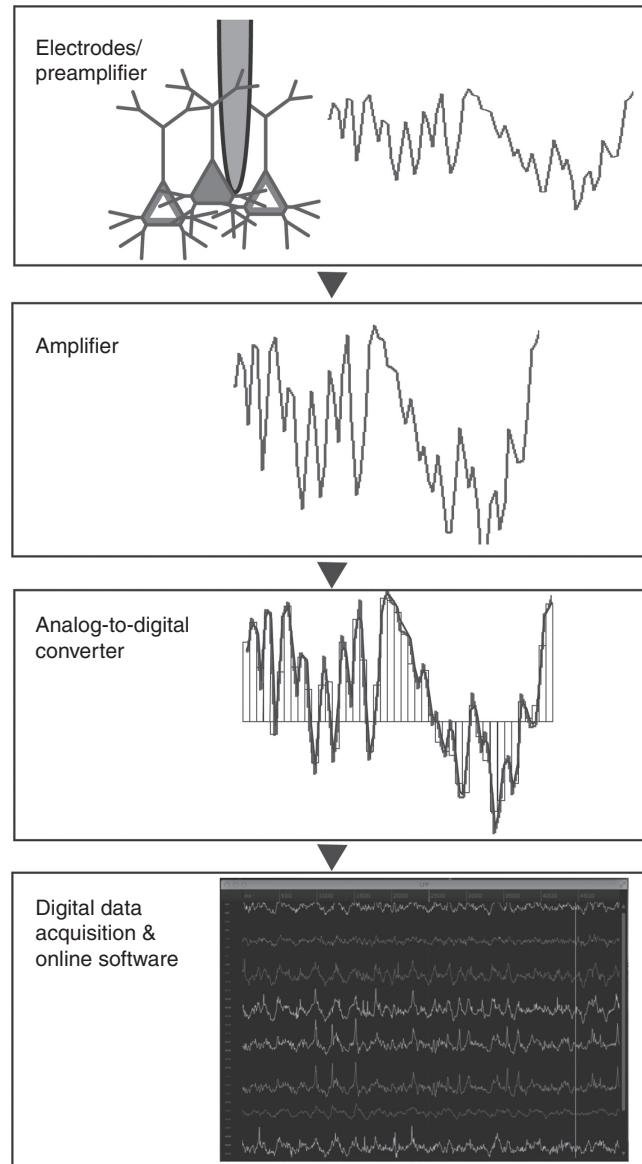


FIGURE 4.4 In a multi-electrode recording system, data acquisition comprises several steps. Top: The signal is acquired from implanted electrodes, (middle top) amplified from microvolt levels to a range appropriate for analog-to-digital conversion, (middle bottom) digitized, and (bottom) stored and displayed on one or more computers. In most traditional multi-electrode recording systems, only the first step occurs at the animal's head, where a preamplifier drives the signals up a long multiwire tether to the amplifiers. Custom integrated circuits have enabled an emerging trend in which the amplification and digitization take place on the animal's head, resulting in a significant reduction in the number of wires in the tether.

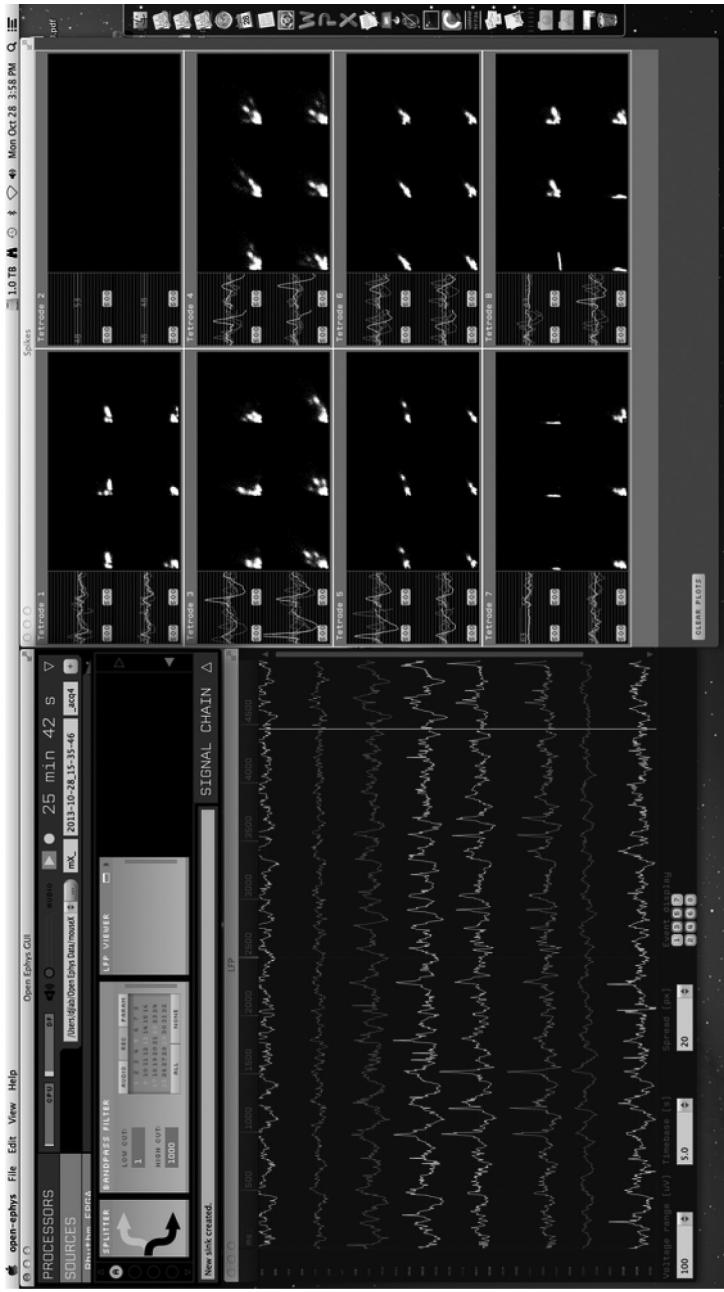


FIGURE 4.5 A screenshot of the Open Ephys GUI shows two open panels. Left: LFPs are visualized as a series of scrolling records, with a different color for each tetrode. Right: Waveform snippets acquired from each tetrode are plotted, and the peak amplitudes of each snippet are juxtaposed pairwise to form six two-dimensional clouds of points. By visualizing the pairwise signals, an experimenter can quickly assess whether action potentials of one or more neurons can be isolated. (Courtesy of Dan Johnston and Joshua Siegle, used with permission.)

Ground and Reference

During neural recording experiments, the configuration of the data acquisition system can have significant effects. All voltage measurements are differential, requiring a return path for the signal, and neural signals are no exception. The electrode against which the voltage of the recording electrode is measured is called the return electrode. In many cases, the return electrode is also connected to the ground for the neural recording electronics. A good return electrode is essential for high signal quality. A common choice is a conductive, biocompatible (stainless steel or titanium) screw implanted over the cerebellum. Care should be taken to ensure not only proper placement, with the bottom of the screw in contact with cerebrospinal fluid, but also a quality conductive solder joint between the screw and the connecting wire to the headstage (Fig. 4.6). For stainless-steel screws, an appropriate flux solution (i.e., N-3, LA-CO Industries, Inc., Elk Grove Village, IL) is required for the solder bond to form. The postimplantation quality can be tested by measuring the DC resistance between the animal's ear pinched in a saline-soaked gauze pad and the ground/reference connection on the headstage. Values greater than $\sim 20\text{ K}\Omega$ suggest that some portion of the return path is defective.

After the signal on each channel is amplified and digitized, a second type of referencing can be applied. This is simply accomplished by differencing the signals between a signal electrode and one designated as a reference. Reference electrodes are typically chosen based on their positioning far from neural sources—fiber tracts such as the corpus callosum are common choices. This digital referencing can enable action potential recording despite persistent external sources of noise that are common to multiple electrodes. As shown in Figure 4.7, large-amplitude noise will saturate the analog-to-digital conversion, resulting in maximal digital values, and thus digital referencing will not

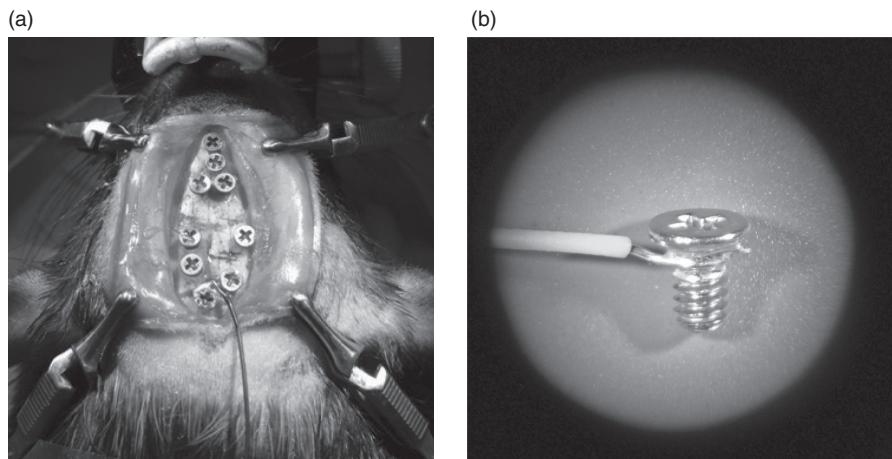


FIGURE 4.6 a: During surgery, a stainless-steel ground screw (arrow) is implanted into the skull over the cerebellum at the same time as implant fixation screws. b: A simple way to create a conductive connection to stainless steel is to wrap a stainless wire around the top threads of a fixation screw, and then apply solder using an appropriate flux.

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4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 85

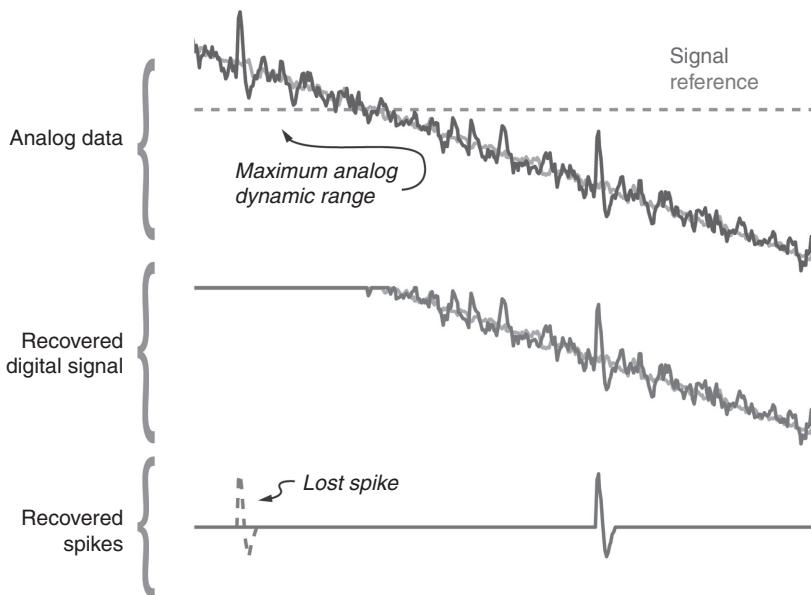


FIGURE 4.7 Digital referencing of the signal from neural data electrodes to another electrode(s) in the brain can sometimes mitigate the effects of large noise sources. In this example, a large noise event has perturbed both the signal and reference electrode channels in the analog data. Even though the level of nonsignal noise is much higher than the amplitude of the action potentials, the second spike can be recovered after subtracting the local reference. However, in the case of the first spike, both signal levels have exceeded the maximum input to the ADC, and thus small variations are erased.

be able to recover small signals. LFP signals are often present on electrodes used for digital referencing. Thus, care must be taken when recording/analyzing LFPs that might include signals from both recording and reference electrodes.

System Setup

Before plugging in the animal for the first time:

- Visually check the tether and headstage. Examine headstage(s) and cable for damage. Ensure that long tethers are mechanically supported (typically with elastic or counterweight systems) in such a way that animals will be able to move freely within the experimental environment. Many investigators additionally use a commutator to allow for animal rotation.
- Use a multimeter to measure the DC voltage between the pins of each headstage that will connect to electrodes and the pin that will connect to the ground screw. A non-zero voltage reflects a hazardous condition and is commonly caused by incorrect cable connections or damage to the headstage(s).
- Verify signal acquisition. Turn on data acquisition software. Plug a signal generator into the headstage(s). Verify that there is minimal noise and that 10 to 100 μ V, 40 to 1,000-Hz sinusoidal signals can be properly detected. Most neural data acquisition

86 | BASIC ELECTROPHYSIOLOGICAL METHODS

systems enable real-time audio monitoring of received neural data. This is an invaluable tool for both setup and recording. Under most circumstances, environmental sources of noise can be detected at this stage of setup.

- Eliminate noise. Common sources of noise are “ground loops” and nearfield radiation. We commonly think of neural data being transmitted along one wire, but just as in our discussion of referencing, all signal paths must be a circuit. Ground loops arise when multiple high-impedance return paths for signals are shared with other equipment. In this case, it is very common to detect 50/60-Hz noise coupled from power supplies into the neural data. Ground loops can be alleviated by (1) ensuring there is only one conductive ground path from the animal to the recording system (avoiding, e.g., a grounded, conductive behavioral environment) and (2) using only DC-powered equipment and ensuring that DC power is “low ripple.” Radiative sources of noise are more insidious than ground loops. These arise because neural recording electrodes act like antennas. Thus, local sources of radiative energy can induce noise. Note that this noise need not be within the frequency band of neural activity (i.e., <20–30 kHz); enough power will overcome the anti-aliasing filters and be modulated into the neural activity band. Common sources of radiative noise include switching power supplies (commonly found in DC power adaptors), fluorescent lights, and other scientific equipment. These noise sources can be alleviated by electrically shielding either the noise sources or the neural recording environment with grounded conductive material such as copper mesh or aluminum foil.
- Verify experiment. Check for proper functioning of any behavioral control or measurement apparatus (e.g., operant conditioning, video monitoring). Verify that these data are properly synchronized with neural data.

Electrode Positioning

Disruption or removal of the membranes that cover the brain surface (thick dura and thinner dura and arachnoid) inevitably result in an inflammatory response (Polikov et al. 2005). One of the consequences of this inflammation is expansion of the cortical sheet that decreases in the days (up to two weeks) following surgery. Thus, for electrodes lowered during or immediately following implantation, the depth of recording sites relative to target neurons will change over time. If this movement causes the electrodes to move through and beyond the target recording depth (i.e., as reduction of inflammation shrinks the tissue above), resulting damage can drastically reduce the quality of final recordings (Fig. 4.8a). Consequently, the final positioning of recording electrodes to target neurons should be postponed until after inflammation has subsided.

Electrode movement can also be irritating to tissue, causing further inflammation and even seizures in severe cases. Thus, care should be taken to move electrodes slowly. Specifically, during daily adjustments, the electrode should be lowered or raised slowly, in a smooth manner, at a rate of ~10 $\mu\text{m}/\text{s}$. Even when electrodes are moved slowly and carefully, some tissue displacement will take place (see Fig. 4.8b), resulting in drift of the

4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 87

final location overnight of up to 25% additional displacement. This can be ameliorated by retracting the electrode a small amount after advancing it, but care should always be taken that this overnight drift will not move the electrode beyond the desired location. To reduce the effects of inflammation and tissue displacement, the distances electrodes are advanced should follow an exponentially decreasing pattern.

When advancing electrodes, care should be taken to securely restrain the movement of the implant without adversely restraining the animal. The implant should be held close to the base to prevent abrupt movements from applying a torque that could cause deimplantation. ~~The use during implantations~~ of special adhesives and bone-preparation agents such as Metabond (Parkell, Inc.) applied between skull and methylmethacrylate implant material can reduce these risks. The adjustment tool should also be held carefully to prevent sudden relative movements that could damage electrodes. It is easiest to make depth adjustments in sleeping animals. However, a trained investigator will hold the implant firmly with one hand and the adjustment tool with the other, while still allowing the animal to move slowly and securely. When animals are being adjusted, it is helpful that they be contained in a low-static environment. Either a raised platform or a ~~three~~-sided box with high walls and no top is an appropriate choice (exploring animals will typically try to climb out of low-walled environments). Allowing the animal

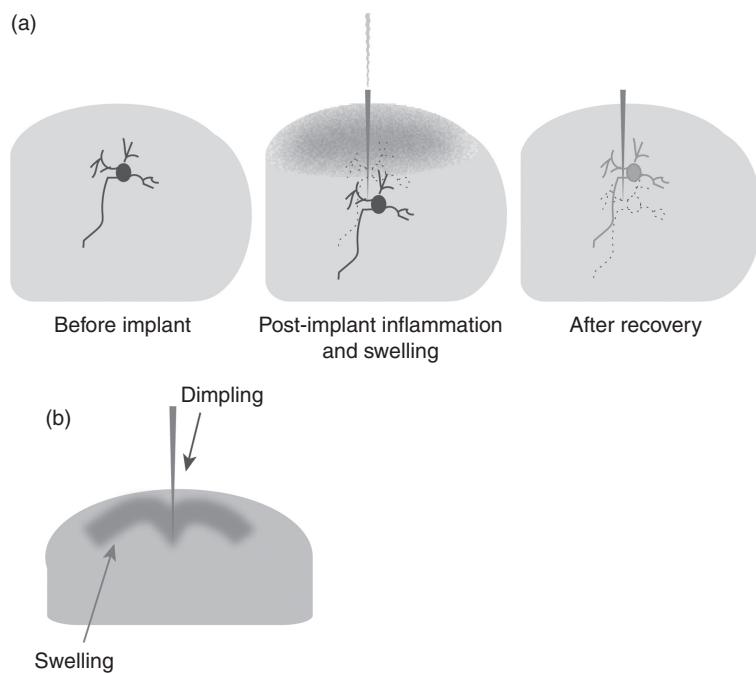


FIGURE 4.8 a: Implantation can cause swelling, which shifts the relative position of target neurons. If the electrode is lowered to the target too early, deswelling can result in the electrode passing through and damaging or destroying the target area. b: Even when electrodes are moved slowly and carefully, some tissue displacement in the direction of movement and potentially slight damage will take place. Thus, electrodes should always be moved slowly and gradually.

88 | BASIC ELECTROPHYSIOLOGICAL METHODS

to explore the adjustment environment thoroughly before surgery (or prior to electrode adjustment) can reduce novelty-induced movements.

The process of electrode advancement and positioning requires experience. Thus, for experiments with very specific targets, new investigators should carefully note various patterns of neural firing and LFP and depths during advancement, comparing measured depths with an atlas. After final location is verified in postexperiment histology, these notes will facilitate subsequent accurate positioning. While patterns of neural activity are quite depth-dependent, the investigator must keep careful track of the depth of each electrode, ideally with at least 50- μ m precision.

Electrode positioning in the days following surgery typically takes the following pattern:

Day 0: Advance electrodes into brain. Use audio monitor to listen to signal from each electrode during movement. Pause periodically to assess LFP and audio signals. If possible, record depth at which the brain surface is reached (often a subtle “pop”). Continue to advance until first action potentials are detected.

Day 1: Advance reference electrode to a quiet location (e.g., white matter). Assess signal on all other electrodes, then advance ~100–200 μ m to find large action potentials. Note the quality of each channel of each electrode for a baseline against subsequent changes (which might reflect damage).

Days 2–5: Advance all electrodes to ~500–1,000 μ m above final target depths. Move no more than 1–2 mm per session, and move furthest on earlier days. Observe LFP and spiking phenomena specific to different lamina (e.g., thalamocortical spindles in deeper cortex, decreased background activity in corpus callosum, sharp wave ripple complexes in hippocampus). These can serve as a check for measured depths, and conflicts between expected and observed depths of these features can reflect the degree of tissue swelling.

Days 6–9: Observe LFP and spiking on each electrode to assess the stability of the recording depth. Begin moving toward target area. Keep in mind that because of tissue stretching, electrodes will travel an additional 10–20% overnight. One strategy to minimize this uncertainty is to advance and then partially retract. The goal for final adjustment is to move ~50–75% of the remaining distance each day; as movements of less than ~25 μ m are impossible, this will place the electrode at the target in 3–5 days. With tetrode recordings, extracellular action potential waveforms with peaks of 200–400 μ m are expected, and occasionally values >1 mV can be stably recorded.

Days 10+: Observe LFP and spiking on each electrode. Stable implants should not exhibit laminar differences in patterns—changes in depth may reflect skull changes that will result in deimplantation. Recording quality is typically stable for 5–10 days but eventually will begin to decrease, reflecting local foreign-body responses. Advance electrodes as desired to find new neurons.

4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 89

The following is an example case of the process for lowering electrodes to the pyramidal cell layer of the dorsal aspect of hippocampal area CA1. Note that cortical swelling typically depresses the dorsal hippocampus ~300–400 µm. Thus, we expect target depths initially to be deeper and then to become shallower. Depending on the degree of inflammation, this can take 5–12 days.

Day 0: Advance electrodes into brain. Continue to advance until first action potentials are detected. Depending on exact coordinates, these neurons will often have motor, somatosensory, or visual receptive fields that can be activated by movement, stroking the animal, or flashing a light.

Day 1: Advance reference electrode to corpus callosum (expected at a depth of 2–2.5 mm). Assess signal on all other electrodes, then advance to top of corpus callosum (expected at similar depths).

Day 2: Advance all electrodes beyond bottom of corpus callosum. Very quiet background activity will begin to change as hippocampal sharp wave ripples (present during sleep and immobility) and ensemble activity become more apparent. If single excitatory neurons or dying cells are detected, stop and quickly retract electrode 100–200 µm. Move electrodes forward until ripples are clearly audible, then retract 300 µm. Figure 4.9 depicts signals expected at different hippocampal laminae.

Day 3–6: Observe LFP and spiking on each electrode to assess swelling and stability. Again move electrodes forward until ripples are clearly audible, then retract 300 µm. After a week, or once deswelling (stronger sharp wave ripples than expected) is observed, begin final adjustment.

Day 7: Advance electrodes until ripples are apparent and 40- to 60-µV peak multi-unit activity is present. Pull back 75 µm. Note that most ripples will be accompanied by downward-going sharp waves above stratum pyramidale.

Day 8: Advance electrodes until multi-unit activity grows and single units can begin to be distinguished (100- to 200-µV peaks). Pull back 25–50 µm.

Day 9: Advance electrodes 25 µm. Note that for this small of an adjustment, sometimes no immediate effect is noticeable.

Day 10: Advance electrodes in 25-µm steps as required. When electrodes are within stratum pyramidale while ripples are prominent, sharp waves are not present.

Daily Practice of Neural Recording Experiments

In most awake behaving rodent experiments, the first steps of a typical experimental day will involve retrieving the animal from the vivarium, placing it into the container used for electrode positioning, plugging the animal in, and checking and setting parameters for the neural recording software. Ideally, the software should be set to

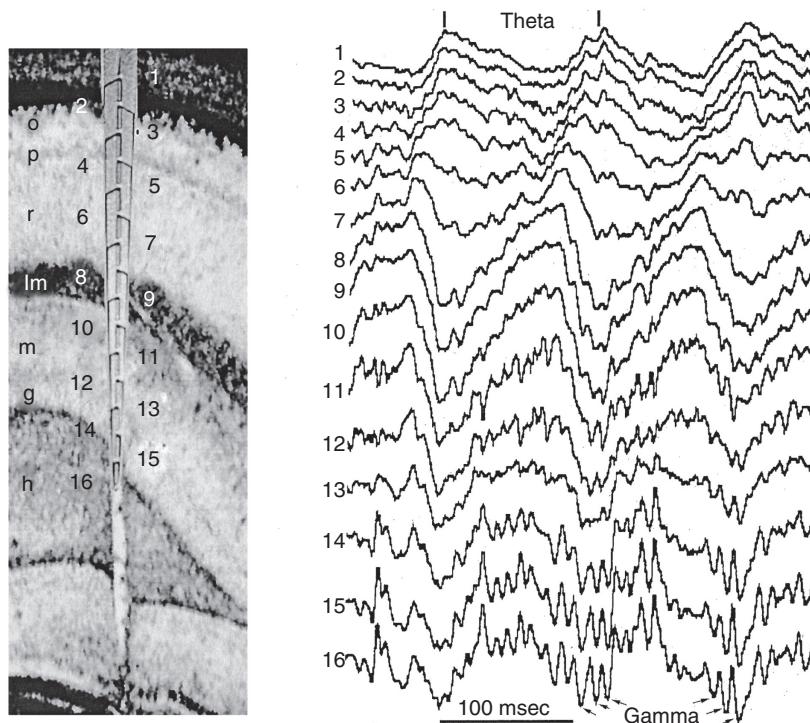


FIGURE 4.9 LFP patterns expected at different hippocampal laminae. (Reproduced from (György Buzsáki, 2002) with permission.)

record broadband data from all electrodes. This enables all LFPs and spikes to be extracted after the experiment. However, the data requirements (e.g., 14 GB/h for 64 channels sampled at 30 kHz) can sometimes be excessive. Hence sometimes investigators simply choose to save LFP—filtered and decimated to, for instance, 1,500 Hz—and thresholded snippets. If this is the case, care must be taken to select the proper thresholds for spike detection. As shown in Figure 4.10, if thresholds are set too high, the activity of potentially isolatable single neurons will be lost. If thresholds are set too low, the refractory nature of spike sampling can result in only the very beginning of large action potentials being detected. For most recordings, a safe range for threshold values is 45–75 µV.

The experimenter should follow the following steps:

1. Check integrity of headstages, tethers, and so forth. If anything has been reassembled, use a multimeter to measure voltage between recording channels and ground (a non-zero voltage is a hazardous sign of a misconnection).
2. Check that behavioral apparatus and behavioral recording system (e.g., video) are functional.
3. Observe animal for signs of pain or distress.

4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 91

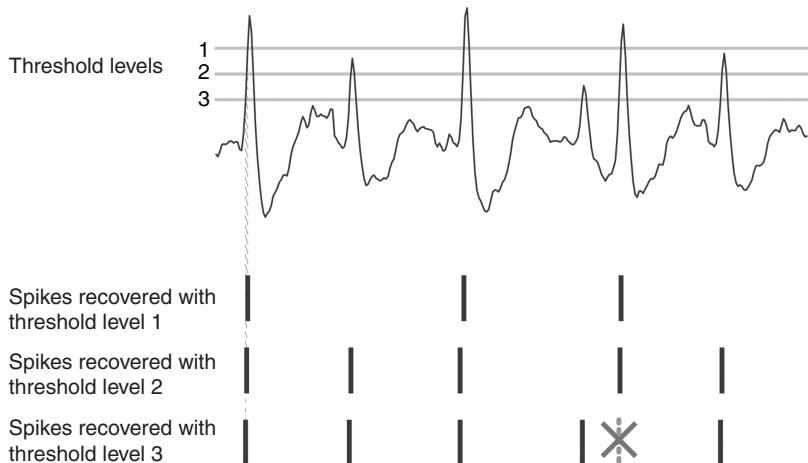


FIGURE 4.10 When selecting the threshold for spike detection, care must be taken to select a proper level. If the threshold level is too high, potentially isolatable neurons with smaller action potential waveforms will be missed. The negative effects of a threshold level that is too low are more subtle. The biggest problem comes from the fact that sampling a spike snippet blocks another spike from being detected for some amount of time. Thus, if the threshold level is too low, nonisolated neural activity can trigger acquisition that prevents immediately subsequent spikes that follow from being detected.

4. Start neural data acquisition software and ensure functionality.
5. Ensure there is enough disk space for neural and behavioral data.
6. Plug in animal.
7. Observe LFP and audio signal of reference electrode. Ensure no action potentials are apparent.
8. Observe and record signal quality on all channels. Especially watch for any day-to-day changes.
9. Set thresholds appropriately for each electrode.
10. Initiate saving and run experiment.
11. (*following experiment*) Close data files
12. Observe and record signal quality on all channels. Make small adjustments on electrodes—remember that slow drift will make the effect of these adjustments not apparent until 12–24 hours later.
13. Unplug animal and return to cage.

End-of-Experiment Procedures

Electrolytic Lesions

Following the conclusion of neural data acquisition, it is often important to assess the anatomical location of the recording electrodes. Preapplied dye can be used to deposit a record of the track of the electrode in the tissue (DiCarlo et al. 1996), but the best technique to discover the location of the electrical recording site is an electrolytic lesion.

92 | BASIC ELECTROPHYSIOLOGICAL METHODS

These lesions are achieved by passing current through the recording electrodes, creating a small scar that can be seen later in histological sections.

To create an electrolytic lesion with tetrodes, an investigator needs a current generator (World Precision Instruments A365 or similar), a headstage connector breakout cable, and a timer. The following protocol can be followed:

1. Anesthetize the animal using an approved method.
2. Set the current level of the current generator to 30 μ A. Set the current generator to “unipolar” current mode.
3. When areflexia is reached, connect the negative terminal of the current generator to the implant headstage connector position corresponding to the ground screw. Connect positive terminal to the first electrode for which a lesion is to be created. All channels of a tetrode can be connected or a single channel can be used. The experimenter should select channels that produced high signal quality, avoiding, for instance, broken or shorted electrode channels.
4. Activate the “On” setting of the current generator for 3–4 seconds. When current is flowing, the audio feedback tone will change pitch.
5. Move on to the next electrode.
6. Allow the animal to waken from anesthesia and return to cage. Animal can be euthanized immediately, but more robust lesions will be present if this is delayed for 24 hours.

Histology

Following electrolytic lesions, animals should be euthanized. For best results, follow euthanasia with transcardial perfusion of 4% paraformaldehyde. Electrodes may be retracted following perfusion or the entire microdrive and brain can be extracted and postfix fixed for 24–48 hours. This later procedure often results in preservation of detectable electrode tracks, which can facilitate post hoc reconstruction of recording history in conjunction with electrolytic lesions. Following full fixation, the electrodes should be extracted and the brain prepared for sectioning. Typically 30 to 50 μ m sections are used to locate electrolytic lesions in tissue stained for anatomical visualization either in bright field (e.g., cresyl violet) or in fluorescence (e.g., NeuroTrace, Life Technologies). An example section is shown in Figure 4.11.

Spike Sorting

Extracellular neural recordings nearly always record the activity from more than one neuron. Most of these neurons will have small indistinguishable action potential waveforms, but some will have unique signatures that can be consistently distinguished from those of the multi-unit mass and other single neurons. In the case of tetrode recordings, action potential waveforms need only be distinguishable in one of the four channels of the electrode. Consequently, in areas such as hippocampal area CA1 with densely packed neurons, >10 neurons can often be detected simultaneously with one tetrode.

4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 93

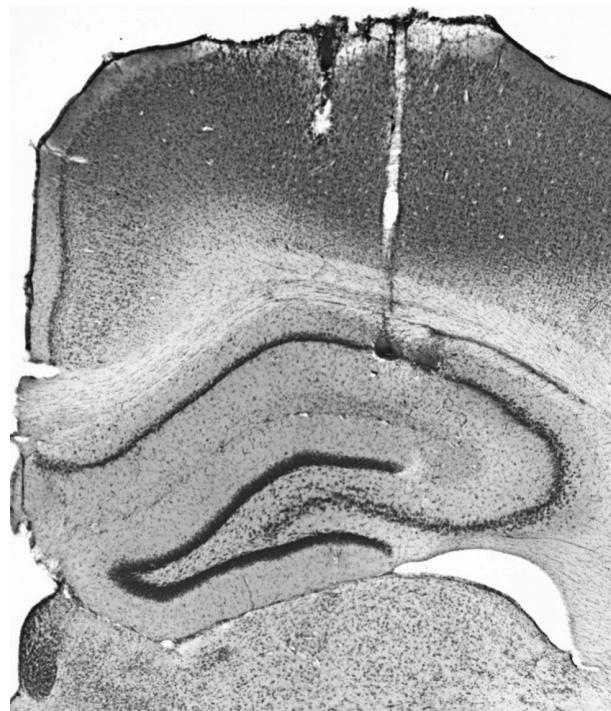


FIGURE 4.11 Sample histological section prepared using cresyl violet and imaged in brightfield illumination, showing electrode tracks and electrolytic lesions. (Photomicrograph by Shantanu Jadhav, used with permission.)

The process of disambiguating the spikes from multiple neurons is known as spike sorting. The problem of fully automated spike sorting has occupied engineers for some time (Carlson et al. 2014, Einevoll et al. 2012, Lewicki 1998, Wild et al. 2012). However, despite slow progress, most experimentalists will still use a manual or semiautomated approach for spike sorting. When spike snippets have been extracted during recording, spike sorting can begin immediately. Alternatively, following broadband recording, the acquired signal must first be filtered (typically 600 Hz to 6 kHz band-pass filter) and then snippets isolated. Some open-source packages such as KlustaSuite (<http://klusta-team.github.io/>) accomplish both the spike extraction and spike sorting tasks, whereas others such as MClust (<http://redishlab.neuroscience.umn.edu/MClust/MClust.html>) require spike snippets to be extracted first.

The waveforms of well-isolated single neurons will have features that are completely distinct from those of other neurons and stable across the time frame of a recording session. Some quantitative measures for the quality of isolation have been developed (Hill et al. 2011, Joshua et al. 2007, Schmitzer-Torbert et al. 2005). In cases where neurons are not well isolated, neural activity is often described as “multi-unit” and isolated clusters recorded on the same electrode are referred to as “units” rather than “neurons.” In many cases, the shape of action potentials within a cluster of poorly isolated multi-unit

94 | BASIC ELECTROPHYSIOLOGICAL METHODS

activity may still provide information for further decoding or other analyses (Chestek et al. 2011, Ventura 2008, Wood & Black 2008, Zhiming et al. 2013).

Conclusion

Chronic recording from large ensembles of neurons has revolutionized systems neuroscience. Large ensemble recordings with excellent signal quality in rats and mice can be achieved using lab-constructed multitetrode microdrives. Many investigators find aspects of the process—construction of microdrives, elimination of noise in a recording environment, data processing, and so forth—initially quite challenging. With practice, however, trained experimentalists can initiate and conduct recording experiments and analyze the resulting data very rapidly. By carrying out these three aspects of an experiment in parallel in a staggered fashion, a complete experiment involving four to eight subjects can often be conducted in 9–12 months.

While we have described the steps in acquiring the signals of individual neurons in the context of chronic recordings in freely behaving animals, the systems and procedures have many similarities with those used in experiments involving head-fixed animals. These preparations often entail additional concerns about noise and, in the case of anesthetized subjects, maintenance of temperature and anesthetic plane. For acute experiments, where the electrodes are inserted during the recording session and subsequently removed, the microdrive can be replaced with larger, stereotactically positioned actuators. Furthermore, the inflammatory responses that can impair initial signal quality in chronic recording contexts typically do not present immediately, meaning that investigators can often record dozens of neurons daily using, for instance, multisite electrodes (Niell and Stryker 2010).

Careful design of behavioral paradigms coupled with high-quality recording using the techniques described above can yield experimental data that are so rich that subsequent analyses often produce new and important scientific understanding over many years (e.g., Mizuseki et al. 2014). Thus, while the experimental process can seem more tedious than other approaches in neuroscience that yield more immediate data, the profound understandings gained are more than worth the effort.

Protocol: 16-Tetrode Microdrive Construction

1. Overview

For chronic tetrode recording in behaving rats, an in-house-built microdrive array has a number of advantages over commercial ones—particularly its low cost (<\$100) and customizability. The mechanism presented in this chapter was modified from designs used previously. Figure 4.12 shows a drawing of a “standard” 16-tetrode microdrive as well as versions we have built for specialized contexts such as simultaneous recording in multiple regions and simultaneous recording and stimulation.

4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 95

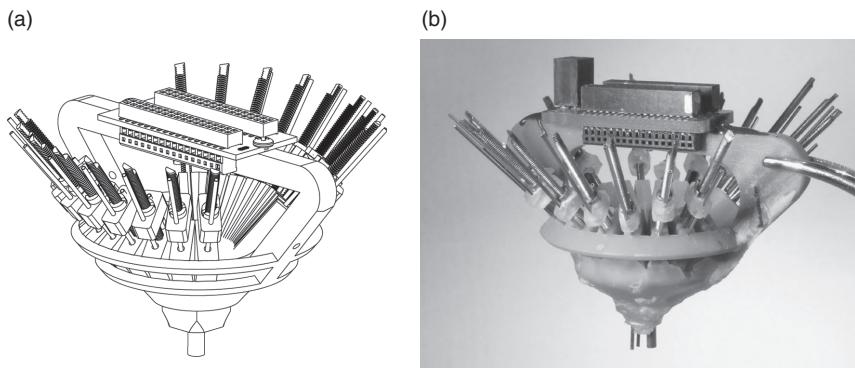


FIGURE 4.12 Drawing (a) and photograph (b) of a “standard” 16-tetrode microdrive.

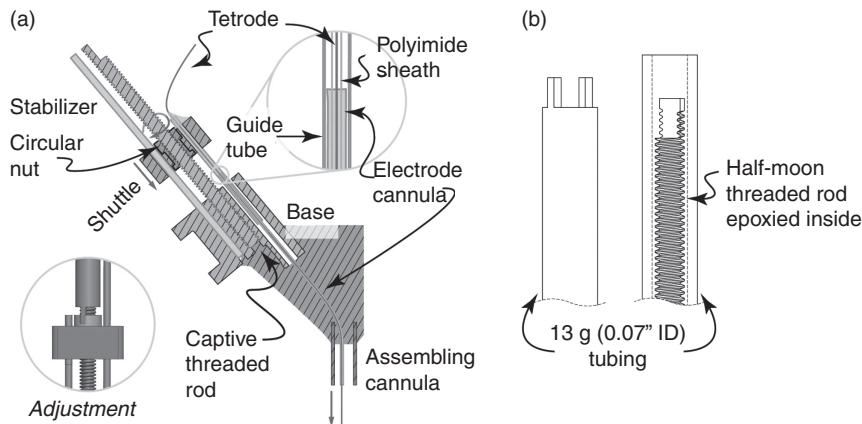
The design described below has evolved from one initially developed for a NASA mission (Knierim et al. 2000, Knierim and McNaughton 2001), with important contributions from Loren Frank and Mattias Karlsson (Frank et al. 2004, Karlsson et al. 2012, see also Lansink et al. 2007). Figure 4.13a shows the working design principle for the microdrive array. A portion of threaded rod is fixed in the 3D-printed (i.e., using a stereolithography process) body of the drive array. Each microdrive shuttle contains a circular nut that can turn freely but is captive (i.e., cannot translate within the shuttle). Using a toothed adjustment tool to turn the captive nut results in linear motion. With standard right-handed threaded rod, a right-handed rotation moves the shuttle downward. Using off-the-shelf 0–80 threaded rod, one turn corresponds to 318 µm of travel. A tetrode is glued to the shuttle within a polyimide sheath that itself is contained within a guide tube for mechanical stability. The polyimide sheath protects the tetrode at the entrance of the stainless-steel cannula that directs the tetrode to the bottom of the drive and from there to the brain. One or more assembling cannulas collect the tetrode cannulas into a bundle over the target regions of the brain, and further align them vertically so that the tetrodes exit the device parallel to each other and perpendicular to the skull.

Preparing a microdrive array for implantation is a straightforward process. The main steps are making the shuttles, assembling and affixing the tetrode and gathering cannulas within the 3D-printed body, and making and loading tetrodes into the final assembly. Following an experiment, the tetrodes can be replaced, and the microdrive array can be sterilized (using ethylene oxide or other low-temperature means) and reused on a subsequent subject.

2. Needed supplies

- a. Stainless-steel tubing, 23 g, 30 g, and 14 g (Component Supply, Inc.), cut to length using a rotary tool. Small tubing should be cut using a diamond wheel.
- b. 5.5 or 6 mil wire (Component Supply, Inc.), cut to length with wire cutters or a rotary tool with a diamond wheel

96 | BASIC ELECTROPHYSIOLOGICAL METHODS

**FIGURE 4.13** Schematic of a working design of a microdrive array.

- c. Threaded rod 0–80 (Component Supply, Inc.), cut to length and half-moon-carved using a rotary tool
- d. Polyimide tubing (Micro-Lumen, Inc.), cut to length with scissors.
- e. Methylmethacrylate powder and resin (Patterson Dental)
- f. Cyanoacrylate glue (normal or thick)
- g. Teflon lubricant
- h. Petroleum jelly mold release
- i. Custom-machined parts—slotted brass nuts, mold base; see designs online
- j. 3D-printed drive body, 3D-printed cannula mold (see designs online at <http://github.com/kemerelab/MicrodriveDesigns>)
- k. Electrode interface board (see designs online at <http://github.com/kemerelab/MicrodriveDesigns>), connectors
- l. Tetrode wire
- m. Tetrode heatshrink tubing, conductive paint
- 3. Needed tools
 - a. Tetrode assembly station (Neuralynx, Inc.)
 - b. NanoZ impedance measuring system (with adaptor) (Neuralynx, Inc.)
 - c. 0–80 tap
 - d. Cautery pen (Bovie)
 - e. Acrylic mixing dish
 - f. Belt sander
 - g. Rotary tool with abrasive and diamond cutting wheel
 - h. Slotted nut turn tool (make from 13 g tubing, see Fig. 4.13b)
 - i. 0–80 turn tool (make from 13 g tubing and half-moon-cut threaded rod, see Fig. 4.13b)

4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 97

4. Making the shuttles

- Begin by cutting needed tubing to length. This is a good time to additionally cut the 30 g tetrode cannulas and the assembling cannula that will be needed in future steps. The items needed for each of the 16 microdrives are shown in Figure 4.14. Note that microdrives with more complex targeting plans will require multiple assembling cannulas whose sizes are chosen appropriately.
- The shuttle mold is a square piece of brass (typically ~3 mm thick) with a centered 0–80 tapped hole flanked by holes drilled for 23-g tubing (0.75 mm separation).
- Install a pair of 23-g tubing, a 0–80 threaded rod, and a slotted nut onto the mold (Fig. 4.15a). This holds the slotted nut in the right orientation for acrylic to be added.
- Use a disposable bulb pipette to apply a drop of Teflon lubricant into the groove area of the slotted nut and onto the surface of the mold.
- Mix and apply methylmethacrylate cement. Apply a small quantity while cement is less viscous to ensure that no voids form between slotted nut and stabilizing rods (see Fig. 4.15b). As cement becomes more viscous, add more cement to form the appropriate ~3 mm height for the shuttle (see Fig. 4.15c).

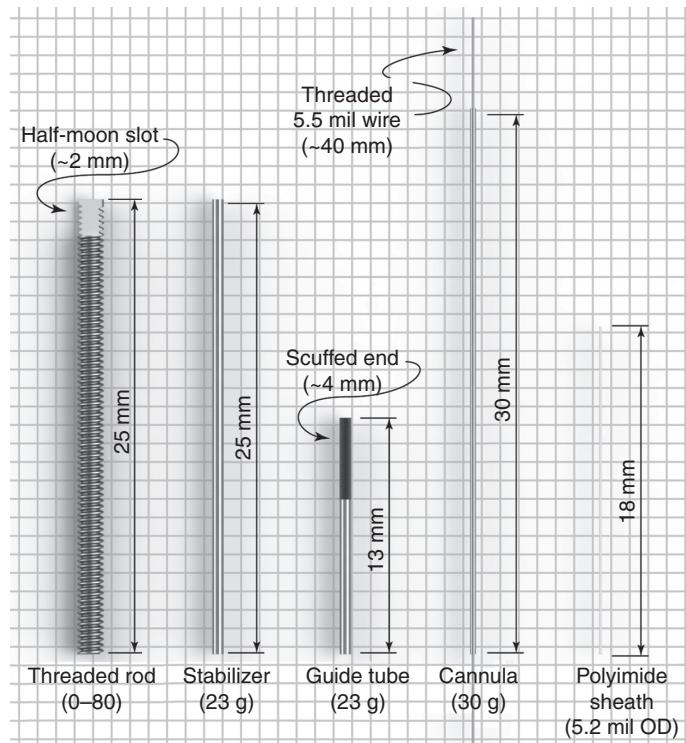


FIGURE 4.14 Items necessary to construct each microdrive.

98 | BASIC ELECTROPHYSIOLOGICAL METHODS

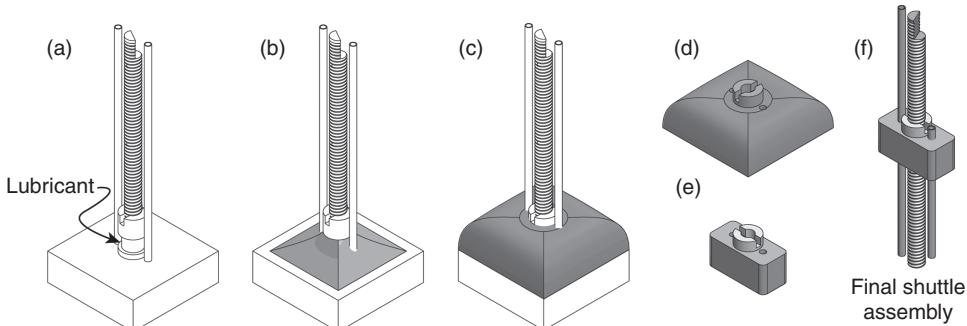


FIGURE 4.15 Construction of a microdrive shuttle. a: The shuttle mold with 23-g tubing, a 0–80 threaded rod, and a slotted nut. b, c: Application of methylmethacrylate cement to approximately 3 mm in height. d: Removal of unshaped shuttle from mold. e: Final shuttle after sanding. f: A completed shuttle.

- f. Remove tubing and threaded rod and use pliers to snap unshaped shuttle from mold (see Fig. 4.15d). Shape length and width of final shuttle on belt sander or rotary tool (see Fig. 4.15e). Test slotted nut for free rotation and discard if there is more than minimal friction or any wobble (friction or axial/lateral shifting will adversely affect final recording quality).
- g. Scuff the upper portion of 23 g guide tube using a file or rough sandpaper and glue into place in shuttles using cyanoacrylate (the glue will adhere more robustly to scuffed stainless steel). Take care not to let glue into interior of guide tube.
- h. Use a 23 g needle to ream the opposite hole in shuttle such that 23 g stabilizer translates freely. A completed shuttle is shown in Figure 4.15f.
5. Assembling the drive body
 - a. Use a 23 g needle to ream the channels for the 23 g guide tubes in the 3D-printed microdrive array base. Use a 0–80 tap to tap the holes for the threaded rod.
 - b. Insert 5.5 mil wires into 30 g tetrode cannulas. This will prevent the cannulas from collapsing when being bent during assembly.
 - c. Prepare the assembling cannula, scuffing the top ~2 mm of the outside.
 - d. Insert all 16 stuffed 30 g cannulas into the assembling cannula. In the case of a more complex layout with multiple assembling cannulas, insert the proper number of 30 g cannulas into each.
 - e. Mount this assembling cannula(s) into targeting block, and, as shown in Figure 4.16a use a clamp to support 3D-printed drive base above it.
 - f. As shown in Figure 4.16b, carefully bend each 30 g cannula into an inner slot of the drive base and mount a shuttle over it (by inserting 23 g guide tube over the 30 g cannula, inserting 23 g stabilizer, and screwing threaded rod into microdrive array base). This will capture the 30 g cannula in place. Take care that the 30 g cannulas are not tangled as they leave the assembling cannula(s).
 - g. Lower drive base to final height (see Fig. 4.16c). This will further bend the tubing, so be careful to avoid kinks.

4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 99

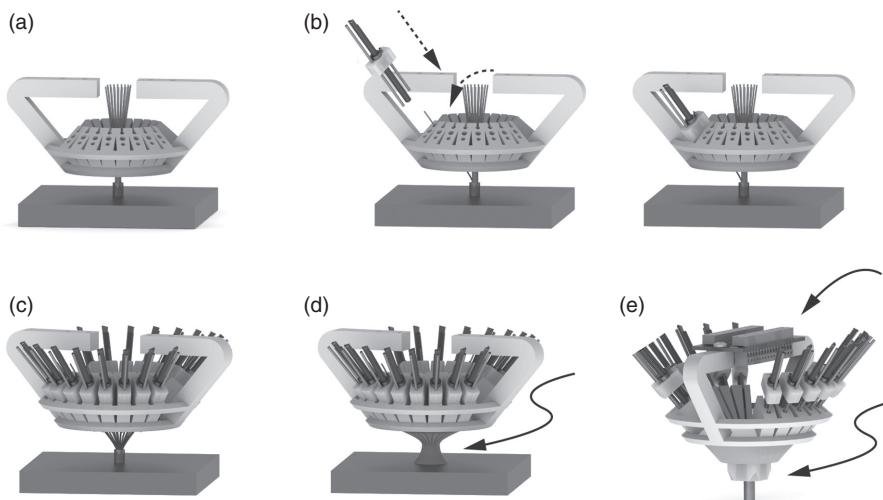


FIGURE 4.16 Assembling the drive body. a: The assembled cannula is mounted into the targeting block. b: Each 30-g cannula is carefully bent into an inner slot of the drive base and a shuttle is mounted over it. c: The drive base is lowered to its final height. d: Methylmethacrylate is applied over the web of 30-g cannula and interface. e: A rectangular bottom facilitates holding the drive during tetrode insertion.

- h. Apply petroleum jelly to inner 23 g/30 g interface to prevent cement from entering and to targeting block surface to facilitate later removal.
- i. Ensure that shuttles are lowered fully onto microdrive array base. As shown in Figure 4.16d, carefully apply methylmethacrylate cement over web of 30 g cannula and interface with assembling cannula and microdrive. Note that the (lowered) placement of each guide tube will now constrain the maximum depth to which each shuttle can be lowered.
- j. After cement has hardened (typically ~20 minutes, although full strength is not reached for up to 24 hours), remove assembly from targeting block. Shape applied cement with a belt sander or rotary tool to minimize weight. A rectangular bottom facilitates holding the drive during tetrode insertion (see Fig. 4.16e).
- k. Fill in interstitial spaces in bottom of assembly cannula by deeply inserting stainless-steel wire with pliers. This ensures that fluid cannot enter and remain after implants.
- l. Carefully cut the assembly cannula from the bottom, together with the 30 g cannulas and any added wire fill, using a rotary tool with a diamond cutting wheel. It is critical that the 30 g cannulas still be stuffed with 5.5 mil wire during cutting to prevent permanent stoppages.
- m. Cut the top of the 30 g tubing to final length and clean the inner portion of the opening using the tip of a needle. Ensure that 5.5 mil wire still moves smoothly through each 30 g cannula.
- n. Record the pattern of cannulas at the bottom of the assembling cannula. Note which microdrive corresponds to which bottom position. In a tightly packed

assembling cannula, adjacent tetrodes will protrude with about 125 µm of separation. Having a record of the pattern will facilitate understanding where each tetrode is within the brain.

- o. Mount an electrode interface board (EIB) to the top of the microdrive array using small screws and/or epoxy. In the latter case, be careful not to allow contamination of the electrical contacts.
6. Loading tetrodes

Below is a summary of one process for loading tetrodes, which involves attaching each channel of the tetrode to the male pin of a connector. Other approaches are also popular, such as the use of a small gold pin or solder to affix each wire into a hole in the EIB. Most of the process can be performed using magnifying goggles, but the use of a stereomicroscope is strongly recommended.

- a. Make tetrodes. See Nguyen et al. (2009) for details.
- b. Cut the four wires on top of the tetrode to length (2–6 mm).
- c. Gently rub the hot tip of a cautery tool along the final ~2 mm of each wire. This will strip the insulation.
- d. Cut small pieces of 50-µm-diameter heatshrink tubing to ~2 mm in length. Mount a four-pin connector in a clamp in the focal zone of the microscope. Put a drop of conductive silver paint (Silver Print II, GC Electronics) onto a nonporous surface. Using tweezers, carefully dip the end of a tubing segment in the silver paint so that capillary action causes it to half-fill.
- e. Grasping the tetrode in your fingers, use tweezers to place a dipped tubing segment on each of the four wires at the end of the tetrode.
- f. Carefully use tweezers to place each tubing segment onto one of the pins of the connector. As shown in Figure 4.17a, the tubing segments should be oriented so that the junction of four tetrode wires is adjacent to the body of the connector. Ensure that the portion of the tetrode's wire with insulation removed is in contact with the connector pin inside each tubing segment. Using a heat gun, shrink the tubing segments, attaching the tetrodes to the four-pin connector.
- g. Load a polyimide sheath into a microdrive shuttle such that it protrudes ~1 mm (see Fig. 4.17b). This will require that the polyimide sheath also is contained within the 30 g cannula, as shown in Figure 4.13.
- h. Plug four-pin connector into the EIB, and use carbon-tipped forceps to carefully insert tetrode into the polyimide sheath/shuttle/drive (see Fig. 4.17c).
- i. Using the electroplating setup, test the tetrode for shorts and continuity. Remove and replace if any tetrode channels are shorted together or not connected. As this requires plugging into the EIB, it is sometimes easiest to test a group of tetrodes all at once.
- j. Using a small piece of scrap wire or other tool, apply cyanoacrylate glue to attach the tetrode, the polyimide sheath, the 23-g guide tube, and the shuttle. Be careful to avoid adhering the grooved nut to the body of the shuttle.

4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 101

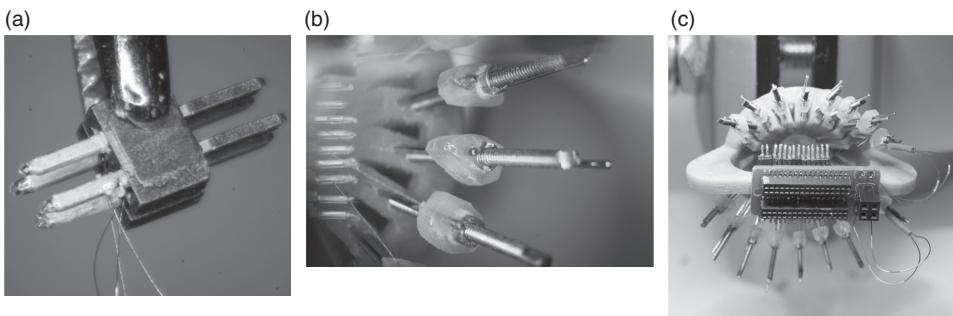


FIGURE 4.17 Loading tetrodes. a: The tubing segments should be oriented so that the junction of four tetrode wires is adjacent to the body of the connector. b: A polyimide sheath is loaded into a microdrive shuttle such that it protrudes ~1 mm. c: The tetrode is carefully inserted into the polyimide sheath/shuttle/drive.

7. Plating

- a. Carefully cut the protruding end of each tetrode to appropriate length. Typically, this corresponds to the full travel distance of the corresponding microdrive such that the tip of the tetrode retracts just within its cannula when it is fully retracted.
 - b. Measure preplating impedance in saline. Check again for short-circuited connections and nonconnected channels.
 - c. Electroplate tetrodes. Small plating currents (0.2–0.5 μ A flowing from plating solution to tetrode for ~0.5 s) will result in a slower but well-controlled process.
 - d. Rinse in distilled water and measure impedance in saline. Repeat plating, rinsing, and measuring until desired final impedance (typically 175–250 kOhms at 1 kHz) is reached and is stable after 1 hour.
 - e. Expect two to three rounds of plating. Higher currents and very low impedances will often correspond with short-circuited connections between the channels of the tetrode (although modifications of the plating solution can achieve lower impedances; Ferguson et al. 2009).
 - f. Retract tetrodes into drive for presurgical sterilization.
8. After the experiment
 - a. Remove old tetrodes and attached shuttles by unplugging from EIB. Using needle-nose pliers, grasp and twist each 23-g guide tube to remove from shuttles. Guide tubes can be soaked in acetone to dissolve cyanoacrylate glue and remove polyimide tubing, or discarded.
 - b. Carefully remove skull fragments and methylmethacrylate cement added to microdrive array during implantation to reveal bottom 3–4 mm of assembling cannula. Most of the cement should be removed using a rotary tool. Use flush cutter shears to carefully fracture and remove the remaining cement to reveal the undamaged assembling cannula. With care, the microdrive array can be returned to a preimplantation state and used many times.

Frequently Asked Questions

Q: How can I manually measure the impedance of tetrodes? How can I manually electroplate tetrodes?

A: Rather than using a multiplexing impedance tester/electroplatier, some investigators choose to measure the impedance of each channel of each electrode independently. Devices made for this purpose (e.g., IMP-2, Bak Electronics, Inc., Sanford, FL) emit a very-low-current sine wave and measure the resulting voltage, allowing for impedance measurement up to several megaohms. These devices can be used in conjunction with a current generator (e.g., A365, World Precision Instruments, Inc., Sarasota, FL) to electroplate a single electrode to a desired level of impedance.

Q: When starting data acquisition, what parameters should I use for the filter used for detecting spikes? What is a good beginning value for the spike detection threshold?

A: Most data acquisition systems use a passband filter on incoming neural activity to remove low-frequency LFP signals and high-frequency noise before performing spike detection. A good initial setting for this passband is 300–6,000 Hz. If there are many neurons with large action potentials, using a slightly narrower band (e.g., 600–6,000 Hz) may improve the separability. For electrodes with ~200-k Ω impedance, a good initial value for the spike threshold is 40 μ V. As described in the text, in the presence of large action potentials, it is often advisable to increase to 60 μ V or more.

Q: How do I pick a reference electrode? When should I move my reference electrode?

A: Investigators employ two common strategies for choosing a local reference electrode for postsampling noise reduction (see text). One strategy is to pick an electrode post-implantation that has poor signal characteristics (e.g., a bad channel). The advantage of this approach is the fact that maximal use is made of the microdrives in an array. Disadvantages include the fact that the chosen reference electrode will typically have impedance characteristics that allow it to sense action potentials, the horizontal targeting may not be ideally located for a target reference area, and it can sometimes be challenging to know which tetrode will be “bad” on the first few days of recording. One strategy is to implant a dedicated electrode with lower impedance than typically used for neural recording. The advantage of this strategy is that the signal properties are optimized for referencing. The disadvantage is that one or more of the microdrives in a microdrive array must be dedicated for the purposes of referencing. Thus, a dedicated reference is appropriate when a region with little neural activity (e.g., a white matter tract) is not present in the vicinity of the recording electrodes.

In general, the reference electrode should be placed somewhere with “noise” characteristics (e.g., artifacts due to jaw muscle movements) in common with the data electrodes and no “signal” (i.e., neural activity). The second criterion can be partially

4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 103

achieved by locating the reference electrode in a white matter tract such as the commissural fibers below cortex, as action potentials are rarely detected near axons. Note that lower-frequency LFP signal is still present in these regions, and thus any analysis must either be unreferenced or otherwise account for the location of the reference electrode. In general, the reference electrode should be moved so as to keep the reference signal free of action potentials. This sometimes requires multiple adjustments in the first few weeks of a recording experiment.

Q: What amplitudes should I expect of well-isolated action potentials? How can I tell if I'm recording from one or multiple neurons?

A: A well-isolated neuron typically displays action potentials in the range of several hundred μ V. Signals >1 mV are observed on occasion. In addition to amplitude, a well-isolated neuron will also display stability in action potential waveform shape, with variability comparable to the level of background noise. Careful electrode adjustment should result in neurons whose action potentials are stable over many hours or even multiple days. Any analysis should be predicated on the shape of the action potential waveform having been stable over the period analyzed. Instability can often be seen simply by plotting the peak amplitude of the action potential waveform as a function of time. Note that in bursting neurons, the situation is more complex, as the shape of action potentials in bursts *does* decrease in amplitude. In most scenarios with action potentials from multiple neurons detectable on the same electrode(s), the action potential shape or amplitude will be clearly different. A key test, however, is to evaluate whether the interspike interval of a putatively isolated unit shows violations of a biophysical refractory period (1–2 ms). Any violations strongly suggest the presence of multiple neurons in the sample.

Q: In what increment should I move the electrode to find better activity? How do I adjust microdrives in an awake animal?

A: The adjustment process described in the text addresses reaching the target region, using electrophysiological signs and the recorded depth of the electrode relative to an atlas or other experimentally derived depth map. Once the electrode is in the vicinity of the desired neurons, however, adjustment should proceed with minimal electrode steps. A minimal step will result in a small but noticeable change in the audio monitor signal, and sometimes even these minimal steps should be counterbalanced by retractions (i.e., 40–60 μ m forward followed by 20–30 μ m backward). High-quality isolations cannot be assessed until the following recording session as a result of slow relaxations of the brain tissue. Adjusting a microdrive in a moving animal is a challenging process. Addressing the needs of the animal—food, water, or safety in the form of a dark corner—may facilitate adjustment. Carrying out the process during the rest phase of the animal's diurnal cycle may promote sleep that can aid the process. In general, minimal force should be applied to the implant to prevent loosening of the attaching screws and adhesive. Thus, one strategy is to grasp the

104 | BASIC ELECTROPHYSIOLOGICAL METHODS

base of the implant with the nondominant hand and move along with the animal in the adjustment environment. The adjustment tool in the dominant hand also moves laterally but only rotates when the animal's movement pauses.

Q: How do I know where I am in the brain? How can I tell different types of neurons apart?

A: One of the biggest challenges for a new investigator is to develop an intuition for the different characteristics of relevant brain regions (i.e., the target and the regions above, below, and in close proximity). Some information may be gleaned from atlases (e.g., the presence of a white matter tract) or from the literature (e.g., stereotypical patterns of activity or relevant receptive fields). The presence of characteristic action potentials from pyramidal cells (broad, asymmetric waveforms [Sirota et al. 2008]), fast spiking interneurons (narrow waveforms), or axons (very symmetric or triphasic waveforms) may often be enlightening. However, careful note taking *during the adjustment process* in the first few subjects and postexperiment lesions and histological verification are critical.

Q: What might cause all of my signal to suddenly degrade into noise?

A: Don't panic! Whenever one sees unusually large amounts of noise, it's always best to check the physical connections in the system. Are headstages plugged in securely with the proper orientation? Does signal from an artificial signal generator work? Sometimes static buildup in the recording environment can lead to bad noise characteristics, which can be alleviated with an antistatic spray. In the worst case scenario, if the data acquisition signal pathway is intact, a sudden change in recording quality can sometimes indicate that the microdrive array may be separating from the skull, so extra care should be taken.

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106 | BASIC ELECTROPHYSIOLOGICAL METHODS

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4: RECORDING OF NEURAL ACTIVITY IN AWAKE BEHAVING ANIMALS | 107

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