There is a rather large gap between membrane properties of individual neurons and behavior or physiological events. To bridge this gap, we need to be able to understand the activity of individual neurons and how that activity contributes to neuronal circuits. Extracellular microelectrode recording, which focuses on neuronal action potentials of individual neurons as the “code” used by the intact nervous system for transmitting information, is the method of choice for investigating questions at this level of analysis.

Extracellular Recording: Neuronal Activity in a Functional Context

Because the extracellular recording method allows an investigator to record the discharges of a single neuron without impalement, a single cell can be isolated and studied for hours, or even days, in chronic recording studies in animals. Individual neurons can thus be characterized comprehensively in terms of physiology and connectivity, and cell activity can be correlated with behaviors or physiological events. Electrical or chemical stimulation of identified cell populations at the recording site can also be used to determine the effects of cell activity on behavior or physiology.

As with any methodology, there are limits to the kinds of questions that can be addressed with extracellular microelectrode recording. At the membrane level, subthreshold events, such as synaptic potentials that might influence cell excitability without producing an action potential, remain hidden. Recording subthreshold potentials requires an intracellular or membrane level approach. Investigators focusing on subthreshold events for the most part have moved to in vitro preparations where intracellular and patch recording techniques are much more easily applied than in the whole animal. At the level of neural circuits, an extracellular electrode samples activity of only one or relatively small numbers of neurons. It can therefore be difficult to develop a realistic picture of the functioning of a large neuronal ensemble or circuit with this approach. Multiunit or slow potential recording and imaging techniques can often be more informative for analyses of distributed circuits. Nevertheless, whether the scientific interest is in events at the cell membrane or in circuits distributed across broad regions of the central nervous system, interpretation of the data will be enhanced by information about the physiology of the relevant neurons functioning within the intact nervous system. Extracellular microelectrode recording can provide this information with high spatial and temporal resolution.

What Is an Extracellular “Spike”? 

For the purposes of extracellular microelectrode recording, our interest is in the action potential as the code used by the neuron for information transmission. The task is thus to detect whether or not an action potential has occurred at a specific time; the waveform itself can be considered to carry no information (other than to serve for spike identification; see below). Indeed, the actual waveform is usually discarded after spike sorting has been completed. It is nonetheless of some interest to consider the basis for the action potential obtained with an extracellular microelectrode.

The action potential or “spike” recorded with an extracellular microelectrode is produced by currents that are induced to flow in the extracellular space around an active neuron. A straightforward approach to these current flows is provided by volume conductor theory. In this approach, the neuron is visualized as surrounded by an extracellular medium with low
uniform resistance; that is, a “volume conductor.” In the simplest case, one can imagine an isolated axon in a saline bath. When the axon is at rest, the membrane potential is uniform, and no current flows. However, if the axon is depolarized at some point along the membrane, the potential difference between the depolarized and resting regions will cause current to flow. Current flows inward at the depolarized region (“sink”) and outward at adjacent regions, which act as a “source” of current for the sink.

At least in theory, one should be able to model the distribution of sources and sinks associated with action potential discharge in an active neuron. This proves to be a straightforward task when considering the isolated axon. As an action potential approaches, reaches, and then moves away from an electrode adjacent to a spot somewhere along the axon, the electrode will at first register a positive potential as the membrane under the electrode serves as a current source for the depolarized membrane some distance away. Then, when the action potential reaches the region underlying the electrode, the depolarized membrane will act as a sink. The extracellular electrode will therefore record a negative potential. Finally, as the action potential continues past the electrode, the underlying membrane is repolarized and once again serves as a current source. The recording electrode will therefore again be positive relative to a distant reference electrode.

The action potential recorded by an electrode adjacent to an isolated axon should therefore be triphasic. This theoretical prediction has been confirmed, as shown in the example in Figure 2–3, which is from an early experiment by Terzuolo and Araki in 1961. They used a double electrode for simultaneous recording of intracellular and extracellular potentials during antidromic activation of a motoneuron in the ventral horn of the cat. As predicted by volume conductor theory, the potential recorded with the

FIGURE 2–1 Volume conductor theory can be used to model current flows around an axon in a uniform, low-resistance bath (a “volume conductor”). (A). When the axon is at rest, the membrane potential is uniform, and no current flows. (B). Current will flow when a segment of the membrane is depolarized. The flow is inward at the depolarized region (“sink”) and outward at adjacent regions, which act as a “source” of current for the sink.

FIGURE 2–2 Model of sources and sinks predicts that a triphasic waveform will be recorded from an isolated axon. (A). As the action potential approaches the region underneath the electrode, that membrane serves as a source, and the electrode sees a positive potential relative to a distant indifferent electrode. (B). When the action potential reaches the membrane underlying the membrane, the electrode records a negative potential. (C). As the action potential continues down the axon, the membrane under the electrode once again acts as a source, and as a consequence, the electrode records a positive potential.
extracellular electrode was triphasic, and the negative phase of the extracellular spike coincided with the depolarization seen by the intracellular electrode. The late positive phase of the extracellular potential corresponded to the repolarization of the membrane recorded intracellularly.

Similar considerations can be applied when modeling the action potential recorded close to the cell soma. As shown in the simple neuron model in Figure 2–4, depolarization at the soma should be reflected in negative potential at the electrode. The soma membrane would subsequently become a source as the action potential moved away down the axon. As a result, the waveform recorded with an extracellular electrode near the soma should in theory be biphasic, with an initial negative component followed by a later positivity. This general pattern has been confirmed for at least some neurons in the central nervous system (Fig. 2–5). However, actual neurons are somewhat more complex than the “lollipop” envisioned in Figure 2–4. Cell morphology and distribution of active conductances on the somatic and dendritic membrane, the location of the electrode relative to the cell body, and the state of cell excitability all complicate the simple picture derived from volume conductor theory. As a result, somatic recordings vary in the relative amplitude of the negative and positive phases of the waveform and often show distinct inflection points on either phase.

The initial segment of the axon generally has the lowest threshold for spike generation, and the initial segment spike can often be seen as a distinct notch on the early negative component of the spike when the electrode is close to the soma (see, e.g., Fig. 2–5B). In addition, the dendrites of many neurons in the mammalian central nervous system are electrically excitable. As a spike initiated at the initial segment back-propagates into the dendrites, the soma will become a source for these active dendritic conductances. Depending on the geometry of the dendrites and the location of the electrode relative to the soma and dendrites, the late positive phase of the spike could be enhanced (if the electrode is influenced mainly by the soma acting as a source) or attenuated (if the electrode...
to some extent “sees” the sink formed by the dendritic membrane). Activity-dependent decreases in extracellular spike amplitude have been documented, and at least in some situations are due to changes in the ability of the axosomatic spike to back-propagate into the dendrites.\textsuperscript{4,7} Spikes may be generated at the dendrites, although whether dendritic spikes are typically sufficient to trigger a somatic or axonal spike is not yet well known.\textsuperscript{8} If a spike were generated at the dendrites rather than at the initial segment, the dendrites would initially act as a sink, then as a source for active membrane at the soma and the axon. Again, the actual impact on the extracellular waveform would depend on geometry of the dendrites and the current density within the dendritic tree, as well as the location of the electrode relative to the cell body and dendrites.\textsuperscript{1,2} Indeed, some somatic recordings are known to be triphasic, rather than biphasic.\textsuperscript{5} It is thus not possible to infer that any triphasic waveform necessarily represents activity of an axon.

Finally, it should be remembered that the extracellular space is not in reality a volume conductor, with a uniform, low resistance. Indeed, there is little vacant space in the nervous system, and discharges of cells surrounding the neuron under study can complicate the extracellular field, adding to or subtracting from the measured potential.

### The Extracellular Electrode

Almost any low impedance material that can be made small enough to get close to a neuron without damaging it can be adopted for extracellular recording. Electrolyte-filled micropipettes, metal etched to a fine tip, and glass-mounted carbon fibers have all been widely used. Metal electrodes are most commonly used in microelectrode recordings in humans. The choice of electrode invariably involves several compromises (Table 2–1). A material sufficiently rugged to pass through neural tissues is an obvious requirement. Metal electrodes can generally penetrate dura, but dura and pia should be removed for insertion of glass micropipettes. Stability of the electrode relative to the neuron under study is also an important consideration. The electrode must be close enough to the cell that its spikes stand out above electrode noise and the undifferentiated activity of other cells. Because current density falls off rapidly with distance from the cell,\textsuperscript{1} minor movement of the electrode can result in loss of the recorded action potential in the noise. Conversely, the cell may be damaged or killed if the electrode is too close. In addition, electrode noise should be low. The amplitude of the extracellular spike is small, and electrode noise can therefore be a significant limitation in trying to isolate a neuron. Electrode noise is positively related to electrode impedance, and lower impedance electrodes usually have a more favorable signal-to-noise ratio. However, there is a trade-off that must be considered when minimizing electrode noise. Lower noise is typically produced by increasing the effective size of the active tip. For example, with a tungsten electrode, this would involve removing more insulation and exposing a larger area of the metal. This produces a lower impedance, but at the same time results in a loss of selectivity because signals are being picked up over a larger area. This may not pose a problem if spikes recorded from the different neurons are very distinct in amplitude or waveform (see Spike Separation and Sorting, below), or if multiunit activity is acceptable. One approach

<table>
<thead>
<tr>
<th>TABLE 2–1 Properties of a Good Extracellular Electrode</th>
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<tbody>
<tr>
<td>• Ruggedness</td>
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<tr>
<td>• Stability</td>
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<tr>
<td>• High signal-to-noise ratio</td>
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<tr>
<td>• High selectivity</td>
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that lowers impedance without reducing selectivity is to plate with platinum black, which can be used with platinum or stainless steel electrodes.\textsuperscript{9}

**Spike Separation and Sorting**

Although volume conduction provides a theoretical framework for thinking about the extracellular spike, most investigators take a rather pragmatic approach to the waveforms that they record. To the extent that there is an interest in the shape of the recorded spike, it is for purposes of cell identification. Indeed, it is actually fortunate for extracellular electrophysiologists that the recorded spikes are not the tidy triphasic and biphasic waveforms derived from the volume conductor approach because differences in waveforms are the basis for separating spikes belonging to different neurons. Because the cell population within a given region of the nervous system can be diverse in terms of physiology and function, careful separation and identification of spikes that might be recorded from more than one neuron with a given electrode are crucial for any quantitative analysis.

With a highly selective electrode, identification based simply on spike amplitude may be adequate. However, one of the technical advances of the last 10 years has been the development and wide availability of software for multidimensional spike sorting. These procedures allow confident identification of an individual cell, as well as simultaneous recording of ensembles of cells, with on-line and/or off-line separation based on waveform parameters. Some packages now include a capability for dynamic modification of parameters, which accommodates activity-dependent changes in amplitude or gradual shifts due to drifting of the electrode. Figure 2–6 shows an example of two neurons sampled with a single microelectrode placed in the medullary raphe of a lightly anesthetized rat. The waveforms are easily distinguished by differences in topography, although there is little difference in amplitude. The importance of distinguishing between the two neurons is demonstrated in the histogram, which shows that while one neuron is inhibited, the other is activated during responses to noxious heat.

**Sampling**

Issues of sampling and bias in the recorded population must always be considered in electrophysiological analyses of neuronal populations. There is no doubt that larger neurons generate a more widely distributed potential field and are simply more likely to be encountered. For example, Towe and Harding,\textsuperscript{10} recording in cats, estimated that the largest 5% of pyramidal tract neurons accounted for ~50% of the sample encountered with an extracellular electrode.

The problem of a search stimulus is more subtle. Many neurons in the central nervous system have little or no spontaneous activity. In a recent study of hippocampal pyramidal cells, for example, only a tiny minority of the pyramidal cells calculated from anatomical data to be within range of an extracellular electrode showed spontaneous activity.\textsuperscript{4} Because an extracellular microelectrode will not “see” an inactive neuron, this presents obvious problems for analyses based on extracellular techniques. It is therefore common to use a “search stimulus” to activate the cell population of interest. Search stimuli can be electrical stimulation (orthodromic or antidromic) or a physiological stimulus thought to be relevant (e.g., light if the population under study is known to be involved in vision). However, it must be recognized that the population revealed will be that which is excited by the stimulus. Unresponsive cell classes, or neurons inhibited by the search stimulus, will be missed. At a minimum, there should be considerable care to ensure that the stimulus is reproducible.

A third source of sampling bias is behavioral state, including anesthesia. Although it is widely agreed that different anesthetics alter the excitability of neural elements and circuits in different and complex ways,\textsuperscript{11,12} it may be less well appreciated that factors

![FIGURE 2-6](image-url) Two distinct neurons recorded with a single electrode can be separated based on waveform topography. Physiological properties of the two cells are quite different as shown in the peri-event time histogram aligned with a spinal reflex executed by the animal in response to application of intense heat to the paw (arrow). One cell (black) shows a distinct reflex-related inhibition, and the second (gray) is activated at the time of the reflex. Inset shows averaged waveforms of the two neurons. (Analyzed using Datawave Software, Datawave Systems, Longmont, CO.)
such as arousal and attention also modulate cell responses, even of seemingly “hard-wired” sensory neurons. Indeed, anesthesia may have the interesting effect of reducing the influence of behavioral variables in some situations.

Although these sources of sampling bias are to some extent unavoidable, recognition of their potential impact will at the least be a source of caution when interpreting extracellular recording data. This is particularly true when making quantitative inferences about numbers or proportions of cells with specific characteristics in a sampled brain region.

**Conclusion**

In sum, extracellular recording is the method of choice when the focus is on the action potential as the code used by the nervous system. The waveform recorded with an extracellular electrode is influenced by a host of factors, and in practice is used by most investigators only as the basis for separation and identification of individual neurons. Finally, it is important to recognize that the neurons encountered with the extracellular electrode cannot be assumed to represent an unbiased sample of the population in that brain region because the search strategy employed will define the population recorded.

**REFERENCES**