THE LABORATORY SETUP

Each laboratory setup is different, reflecting the requirements of the experiment or the foibles of the experimenter. This chapter describes components and considerations that are common to all setups dedicated to measure electrical activity in cells.

An electrophysiological setup has four main requirements:

(1) *Environment:* the means of keeping the preparation healthy;
(2) *Optics:* a means of visualizing the preparation;
(3) *Mechanics:* a means of stably positioning the microelectrode; and
(4) *Electronics:* a means of amplifying and recording the signal.

This *Guide* focuses mainly on the *electronics* of the electrophysiological laboratory setup.

To illustrate the practical implications of these requirements, two kinds of "typical" setups are briefly described, one for *in vitro* extracellular recording, the other for single-channel patch clamping.

The *In Vitro* Extracellular Recording Setup

This setup is mainly used for recording field potentials in brain slices. The general objective is to hold a relatively coarse electrode in the extracellular space of the tissue while mimicking as closely as possible the environment the tissue experiences *in vivo*. Thus, a rather complex chamber that warms, oxygenates and perfuses the tissue is required. On the other hand, the optical and mechanical requirements are fairly simple. A low-power dissecting microscope with at least 15 cm working distance (to allow near-vertical placement of manipulators) is usually adequate to see laminae or gross morphological features. Since neither hand vibration during positioning nor exact placement of electrodes is critical, the micromanipulators can be of the coarse mechanical type. However, the micromanipulators should not drift or vibrate appreciably during recording. Finally, the electronic requirements are limited to low-noise voltage amplification. One is interested in measuring voltage excursions in the 10 µV to 10 mV range; thus, a low-noise voltage amplifier with a gain of at least 1,000 is required (see **Chapter 6**).

The Single-Channel Patch Clamping Setup

The standard patch clamping setup is in many ways the converse of that for extracellular recording. Usually very little environmental control is necessary: experiments are often done in an unperfused culture dish at room temperature. On the other hand, the optical and mechanical requirements are dictated by the need to accurately place a patch electrode on a particular 10 or 20 µm cell. The microscope should magnify up to 300 or 400 fold and be equipped with some kind of contrast enhancement (Nomarski, Phase or Hoffman). Nomarski (or Differential Interference Contrast) is best for critical placement of the electrode because it gives a very crisp image with a narrow depth of field. Phase contrast is acceptable for less critical applications and provides better contrast for fine processes. Hoffman presently ranks as a less expensive, slightly degraded version of Nomarski. Regardless of the contrast method selected, an inverted microscope is preferable for two reasons: (1) it usually allows easier top access for the electrode since the objective lens is underneath the chamber, and (2) it usually provides a larger, more solid platform upon which to bolt the micromanipulator. If a top-focusing microscope is the only option, one should ensure that the focus mechanism moves the objective, not the stage.

The micromanipulator should permit fine, smooth movement down to a couple of microns per second, at most. The vibration and stability requirements of the micromanipulator depend upon whether one wishes to record from a cell-attached or a cell-free (inside-out or outside-out) patch. In the latter case, the micromanipulator needs to be stable only as long as it takes to form a seal and pull away from the cell. This usually tales less than a minute.

Finally, the electronic requirements for single-channel recording are more complex than for extracellular recording. However, excellent patch clamp amplifiers, such as those of the Axopatch series from Axon Instruments, are commercially available.

A recent extension of patch clamping, the patched slice technique, requires a setup that borrows features from both *in vitro* extracellular and conventional patch clamping configurations. For example, this technique may require a chamber that continuously perfuses and oxygenates the slice. In most other respects, the setup is similar to the conventional patch clamping setup, except that the optical requirements depend upon whether one is using the *thick-slice* or *thin-slice* approach (see *Further Reading* at the end of this chapter). Whereas a simple dissecting microscope suffices for the thick-slice method, the thin-slice approach requires a microscope that provides 300- to 400-fold magnification, preferably top-focusing with contrast enhancement.

Vibration Isolation Methods

By careful design, it should be possible to avoid resorting to the traditional electrophysiologist's refuge from vibration: the basement room at midnight. The important principle here is that prevention is better than cure; better to spend money on stable, well-designed micromanipulators than on a complicated air table that tries to compensate for a micromanipulator's inadequacies. A good micromanipulator is solidly constructed and compact, so that the moment arm from the tip of the electrode, through the body of the manipulator, to the cell in the chamber, is as short as possible. Ideally, the micromanipulator should be attached close to the chamber; preferably bolted directly to the microscope stage. The headstage of the recording amplifier should, in turn, be bolted directly to the manipulator (not suspended on a rod), and the electrode should be short.

For most fine work, such as patch clamping, it is preferable to use remote-controlled micromanipulators to eliminate hand vibration (although a fine mechanical manipulator, coupled with a steady hand, may sometimes be adequate). Currently, there are three main types of remote-controlled micromanipulators available: motorized, hydraulic/pneumatic and piezoelectric. Motorized manipulators tend to be solid and compact and have excellent long-term stability. However, they are often slow and clumsy to move into position, and may exhibit backlash when changing direction. Hydraulic drives are fast, convenient and generally backlash-free, but some models may exhibit slow drift when used in certain configurations. Piezoelectric manipulators have properties similar to motorized drives, except for their stepwise advancement.

Anti-vibration tables usually comprise a heavy slab on pneumatic supports. Tables of varying cost and complexity are commercially available. However, a homemade table, consisting of a slab resting on partially-inflated inner tubes, may be adequate, especially if high-quality micromanipulators are used.

Electrical Isolation Methods

Extraneous electrical interference (not intrinsic instrument noise) falls into three main categories: radiative electrical pickup, magnetically-induced pickup and ground-loop noise.

Radiative Electrical Pickup

Examples of radiative electrical pickup include line frequency noise from lights and power sockets (*hum*), and high frequency noise from computers. This type of noise is usually reduced by placing conductive shields around the chamber and electrode and by using shielded BNC cables. The shields are connected to the signal ground of the microelectrode amplifier. Traditionally, a Faraday cage is used to shield the microscope and chamber. Alternatively, the following options can usually reduce the noise: (1) find the source of noise, using an open circuit oscilloscope probe, and shield it; (2) use local shielding around the electrode and parts of the microscope; (3) physically move the offending source (*e.g.*, a computer monitor) away from the setup; or (4) replace the offending source (*e.g.*, a monochrome monitor is quieter than a color monitor). Note that shielding may bring its own penalties, such as introducing other kinds of noise or degrading one's bandwidth (see **Chapter 12**). Do not assume that commercial specifications are accurate. For example, a DC power supply for the microscope lamp might have considerable AC ripple, and the "shielded" lead connecting the microelectrode preamplifier to the main amplifier might need additional shielding. Solution-filled perfusion tubing entering the bath may act as an antenna and pick up radiated noise. If this happens, shielding of the tubing may be required. Alternatively, a drip-feed reservoir, such as is used in intravenous perfusion sets, may be inserted in series with the tubing to break the electrical continuity of the perfusion fluid. Never directly ground the solution other than at the ground wire in the chamber, which is the reference ground for the amplifier and which may not be the same as the signal ground used for shielding purposes. Further suggestions are given in **Chapter 6**.

Magnetically-Induced Pickup

Magnetically-induced pickup arises whenever a changing magnetic flux cuts a loop of wire, thereby inducing a current in the wire. It most often originates in the vicinity of electromagnets in power supplies, and is usually identified by its non-sinusoidal shape with a frequency that is a higher harmonic of the line frequency. This type of interference is easily reduced by moving power supplies away from sensitive circuitry. If this is not possible, try twisting the signal wires together to reduce the area of the loop cut by the flux, or try shielding the magnetic source with "mu-metal."

Ground-Loop Noise

Ground-loop noise arises when shielding is grounded at more than one place. Magnetic fields may induce currents in this loop. Moreover, if the different grounds are at slightly different potentials, a current may flow through the shielding and introduce noise. In principle, ground loops are easy to eliminate: simply connect all the shields and then ground them at one place only. For instance, ground all the connected shields at the signal ground of the microelectrode amplifier. This signal ground is, in turn, connected at only one place to the power ground that is provided by the wall socket. In practice, however, one is usually frustrated by one's ignorance of the grounding circuitry inside electronic apparatuses. For example, the shielding on a BNC cable will generally be connected to the signal ground of each piece of equipment to which it is attached. Furthermore, each signal ground may be connected to a separate power ground (but not on Axon Instruments' amplifiers). The loop might be broken by lifting off the BNC shielding and/or disconnecting some power grounds (although this creates hazards of electrocution!). One could also try different power sockets, because the mains earth line may have a lower resistance to some sockets than others. The grounds of computers are notorious for noise. Thus, a large reduction in ground-loop noise might be accomplished by using optical isolation (see **Chapter 12**) or by providing the computer with a special power line with its own ground.

The logical approach to reducing noise in the setup is to start with all equipment switched off and disconnected; only an oscilloscope should be connected to the microelectrode amplifier. First, measure the noise when the headstage is wrapped in grounded metal foil; microelectrode headstages should be grounded through a low resistance (for instance, 1 MΩ), whereas patch-clamp headstages should be left open circuit. This provides a reference value for the minimum attainable radiative noise. Next, connect additional pieces of electronic apparatuses while watching for the appearance of ground loops. Last, install an electrode and add shielding to minimize radiative pickup. Finally, it should be admitted that one always begins noise reduction in a mood of optimistic rationalism, but invariably descends into frustrating empiricism.

Equipment Placement

While the placement of equipment is directed by personal preferences, a brief tour of electrophysiologists' common preferences may be instructive. Electrophysiologists tend to prefer working alone in the corners of small rooms. This is partly because their work often involves bursts of intense, intricate activity when distracting social interactions are inadmissible. Furthermore, small rooms are often physically quieter since vibrations and air currents are reduced. Having decided upon a room, it is usually sensible to first set up the microscope and its intimate attachments, such as the chamber, the manipulators and the temperature control system (if installed). The rationale here is that one's first priority is to keep the cells happy in their

quiescent state, and one's second priority is to ensure that the act of recording from them is not consistently fatal. The former is assisted by a good environment, the latter by good optics and mechanics. Working outward from the microscope, it is clearly prudent to keep such things as perfusion stopcocks and micromanipulator controllers off the vibration isolation table. Ideally, these should be placed on small shelves that extend over the table where they can be accessed without causing damaging vibrations and are conveniently at hand while looking through the microscope.

Choice and placement of electronics is again a matter of personal preference. There are minimalists who make do with just an amplifier and a computer, and who look forward to the day when even those two will coalesce. Others insist on a loaded instrument rack. An oscilloscope is important, because the computer is often insufficiently flexible. Furthermore, an oscilloscope often reveals unexpected subtleties in the signal that were not apparent on the computer screen because the sample interval happened not to have been set exactly right. The oscilloscope should be at eye level. Directly above or below should be the microelectrode amplifier so that adjustments are easily made and monitored. Last, the computer should be placed as far as possible — but still within a long arm's reach — from the microscope. This is necessary both to reduce the radiative noise from the monitor and to ensure that one's elbows will not bump the microscope when hurriedly typing at the keyboard while recording from the best cell all week.

A final, general piece of advice is perhaps the most difficult to heed: resist the temptation to mess eternally with getting the setup just right. As soon as it is halfway possible, do an experiment. Not only will this provide personal satisfaction, it may also highlight specific problems with the setup that need to be corrected or, better, indicate that an anticipated problem is not so pressing after all.

List of Equipment

Traditional Patch-Clamp Setup

Item	Suggested Manufacturers
Vibration isolation table	Newport Micro-g (Technical Manufacturing Corp.)
Microscope, inverted	Zeiss Axiovert Nikon
Micromanipulators	
hydraulic	Narashigi
pneumatic	Technical Products International
motorized	Newport
peizoelectric	Burleigh Sutter Instrument Company
Patch-clamp amplifiers	Axon Instruments, Inc.
Tape recorders (VCR-based)	Instrutech Neurodata Bio-Logic
Oscilloscopes	Tektronix Gould
Pipette fabrication	
glass	see Chapter 4
pullers	Narashigi Sutter Instrument
microforges	Narashigi *homemade - based on:* Zeiss metallurgical microscope Olympus CH microscope
coaters	Narashige
hydrophobic coating	Dow Corning Sylgard 184 Q-dope
Microelectrode holders	Axon Instruments, Inc. E. W. Wright
Chamber, temperature control	Medical Systems Corp. Narishige
Computers	See Chapter 8

Patch-Slice Setup

Microscope, low power	Zeiss
Vibratome	Technical Products International

(other requirements as for a traditional patch-clamp setup)

Extra/Intracellular Microelectrode Setup

Item	Suggested Manufacturers
Vibration isolation table	Newport Micro-g (Technical Manufacturing Corp.)
Microscope	Zeiss Nikon Olympus
Micromanipulators	
mechanical	Narashige Prior
hydraulic	Narashige
pneumatic	Technical Products International
motorized	Newport
peizoelectric	Burleigh
Microelectrode amplifiers	Axon Instruments, Inc.
Tape recorders (VCR-based)	Instrutech Neurodata Bio-Logic
Oscilloscopes	Tektronix Gould
Electrode fabrication	
glass	see Chapter 4
pullers	David Kopf Sutter Instrument Company
Microelectrode holders	Axon Instruments, Inc. E. W. Wright
Chamber, temperature control	Medical Systems Corp. Narishige
Computers	See Chapter 8

Optical Recording Setup

Photomultipliers	Hamamatsu Thorn
Imaging systems	Axon Instruments, Inc. ETM Systems

Further Reading

Conventional intra- and extracellular recording from brain slices

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