A versatile, inexpensive, easily constructed suction electrode system is described that can be used for routine exercises by the student on, or for demonstrations of, the electrophysiology of frog sciatic nerve and gastrocnemius and sartorius muscles. A disposable plastic syringe and a disposable pipette with Ag-AgCl wire comprise the suction electrode. Six readily manipulable electrodes are installed in the walls of the Plexiglas preparation bath, and an arbitrary number of additional “free” electrodes can be placed where desired. An optional small chamber, formed from a disposable plastic culture dish and Gootch rubber tubing, provides for “barrier” recording within the larger chamber. Interpretations are given of the electrical responses of nerve and muscle in terms of longitudinal currents along the preparation within the suction electrode tubes.

Many students of physiology find their first lessons in the scientific study of excitability and contractility in the sciatic nerve, the gastrocnemius muscle, and sometimes the sartorius muscle of the frog. Reliable and easy-to-operate electronic equipment is now available, but the instructional value of the electrophysiological exercise is too often diminished by avoidable inconveniences and difficulties. Salts must be weighed out for the physiological solutions, and the pH must be adjusted. Records taken with the usual methods may be bedeviled by artifacts. In air, the preparation dries out and dies.

Although instructive and convenient, audiovisual aids and computer simulations are inadequate responses to these tribulations, for the real physiology is found only in the actual nerve and muscle. Suggestions made here are intended to alleviate some of the technical uncertainties and encourage the use of real nerve and muscle in introductory electrophysiology exercises. In addition to descriptions of the devices, discussions and interpretations of typical records are provided. Some of the ideas may also be helpful in more advanced explorations.

The core of the proposal is a simple suction electrode system used in a versatile chamber. The devices have been designed to introduce two fundamental aspects of excitability: propagation of impulses along nerve and muscle and the transmission of impulses across the neuromuscular junction as an example of a synapse. Typical records are shown from the sciatic nerve, the gastrocnemius muscle (with its nerve), and the sartorius muscle and nerve of the common frog, *Rana pipiens*. The apparatus will serve as well for any nerve or muscle preparation of similar dimensions.

**PHYSIOLOGICAL SALT SOLUTION**

A practical consequence of the fact that the ratios of salts in the body fluids are essentially similar to those in seawater (5) is that excellent physiological solutions can be made by suitable dilution of natural or artificial seawater. Table 1 shows approximate
TABLE 1.

Physiological salt solution from seawater

<table>
<thead>
<tr>
<th>Ion</th>
<th>Seawater</th>
<th>Frog</th>
<th>1/4 Seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>450</td>
<td>110</td>
<td>112</td>
</tr>
<tr>
<td>K⁺</td>
<td>10</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>10</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>50</td>
<td>1.2</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Concentrations of cations in mm/l are compared in seawater, frog blood (equivalent to ‘standard frog Ringer’s”), and seawater diluted 1:3 with distilled water.

ratios of various major salt constituents in frog blood (7) and the physiological salt solution that can be constructed by dilution of seawater. A concentration of one part seawater plus three parts distilled (or tap) water (1/4 seawater) serves as an excellent physiological solution for frog nerve and muscle, and the same concentration is appropriate for freshwater teleosts. For marine teleosts, a ratio of 1:1 is suitable, and for mammals, 1:2.

The only major discrepancy is Mg²⁺, and for ordinary purposes, the excess of this ion species poses no problem. After a session of stimulation in diluted seawater, a frog sciatic nerve remains quite responsive after 1 wk of refrigeration in the same solution. By reducing the effectiveness of synaptic transmission, high Mg²⁺ reveals the end-plate potentials (4), and in the present context that effect is an advantage. The anesthetic effects of Mg²⁺ can be counteracted by about one equivalent of Ca²⁺ to every seven equivalents of Mg²⁺ (6).

Natural seawater has its own pH buffer system, ~pH = 8, somewhat alkaline for the usual tissue conditions but apparently without deleterious effects on nerve and muscle function. The use of diluted seawater simplifies the problem of making physiological salt solutions in the elementary laboratory.

ELECTRODES

Commonly the conditions for observing the action potential of the frog sciatic nerve are met by suspension of the nerve over several wires (2), as in the Harvard nerve conduction chamber (Ealing). The thin film of solution adherent to the nerve serves as pathway for the external currents of the impulse to move along the nerve between the recording electrodes. If the resistance of the pathway is high enough, these currents produce a voltage difference that, after amplification, is adequate for display on the cathode-ray oscilloscope (CRO). A particular virtue of the suction electrode system is that it allows continuous immersion of the nerve or muscle in the physiological solution. The required high resistance is along a zone where the volume of fluid external to the nerve or muscle is restricted. The key element is a simple suction electrode evolved from earlier designs (1, 3) and employed in a variety of arrangements. The use of the system presupposes the availability of an electronic stimulator and a CRO together with standard cables and connectors. Two-pulse output capability, as provided by the Grass SD9, ought to be available, and a high-gain input such as that provided by a Tektronix 5111 allows direct recording by the CRO without the necessity for an additional preamplifier. A CRO camera is helpful, and a shielded cage may be necessary.

Construction of suction electrode. Make the suction electrode from a 1-ml disposable plastic tuberculin syringe, a section from a 1-ml disposable polystyrene serological pipette to replace the syringe plunger arm, and Ag wire (e.g., 20 gauge, ~1 mm diam) to serve as conductor (Fig. 1).
Different brands of the disposable pipettes may measure from ~4.6-4.7 mm OD, and the internal diameter of the syringes may also vary slightly in this range. Ream out the slight lip at the inside edge of the syringe barrel (rotating a scissors blade works nicely), and select a pipette that slides easily into the reamed barrel. Cut the pipette into lengths slightly greater than that of the syringe barrel. A flat end is assured if the cutting is done on a lathe.

Slip the rubber tip off the syringe plunger, and punch through its center a hole small enough to hold the Ag wire snugly. A punch can be made from a hypodermic needle (e.g., 16 gauge) squarely cut off and sharpened. Roughen the other end of the rubber tip and attach it to the flat end of the pipette section. A better alternative is to cut off and discard the open end of the tip and cement the remainder into the end of the pipette section, which has been previously milled out sufficiently to receive the truncated tip. In either case, Super Glue will hold the tip in place. Then thrust the Ag wire, preferably already chloridized, into the holder (Fig. 1). Chloridization can be done electrolytically, but a simpler method is to immerse the Ag wire in Chlorox for several hours. (I am indebted to Dr. Stuart Dryer, Florida State University, who credits Dr. A. R. Martin, University of Colorado, for this suggestion).

Complete the suction electrode by adding an extension tube of glass or polyethylene tubing having an internal diameter sufficient to accept the nerve end easily. Attach the extension to the syringe barrel by means of a tightly fitting short length of rubber tubing.

To use the suction electrode, vigorously draw in and expel solution until the system is free of air bubbles. With the tube end submerged, a quick short withdrawal of the syringe plunger, armed with the electrode, draws into the tube the end of the nerve lying close by.

Minimally, two suction electrodes to receive the nerve, plus three more electrodes immersed in the solution, will suffice for recording the action potentials from the expected sciatic nerve (Fig. 2). Draw the nerve end to be stimulated into one electrode tube; draw the end for recording into the other. For each of the two suction electrodes that hold the nerve ends, a second electrode completes the circuit, and a fifth is connected to ground.

PREPARATION CHAMBER

Figure 2 shows construction of a chamber to hold the nerve or muscle preparation and to provide convenient control of the horizontally held suction electrodes. The box can be made from sheets of Plexiglas or can be a prefabricated plastic container. The dimensions are not critical, but the indicated box size is convenient and allows use of various accessory devices (see below). The syringe barrel is held in place, but free movement is allowed, as required, by O rings tightened by screws that press on the retaining plate (Fig. 2, inset). In use, the chamber is filled with physiological solution to a level just above the electrodes. To lower the temperature of the preparation, containers of ice can be set into the corners of the bath. Disposable 100-ml plastic beakers with covers, filled with frozen water, serve well for this purpose.

ISOLATED NERVE

The sciatic nerve of the frog is usually dissected from the origin of the plexus at the spinal column to the entry of the nerve into the gastrocnemius muscle. Ordinarily, in vitro, the stimulus is delivered to the proximal end. A stimulating tube ~1.0 mm ID and for the smaller distal end a recording tube ~0.5 mm ID are usually suitable. Both ends can be freely moved, but the nerve can be better controlled if the proximal end is tied (e.g., with 0–6 surgical thread) to the end of the stimulating electrode wire. If the end of the wire is flattened, a small hole can be drilled to receive the thread.

The stimulation, propagation, and recording lengths can be measured with a millimeter rule resting next to the nerve at the bottom of the bath. Stimulation length is the length of nerve within the stimulating tube. Actual stimulated zone is the region of nerve through which current passes outward through the nerve fiber membrane (see Fig. 3A). Propagation distance is the length of nerve between the entry of the stimulating tube (at which region the current exits the nerve) and the entry of the recording tube (where the propagating currents are first detected).
Recording distance is the length of nerve within the recording tube. A voltage change is detected during the time that currents of the impulse are in this zone.

In demonstrations of the action potential (AP) of isolated nerve, stimulus artifact control is often a problem. In the suction electrode bath, the size of the stimulus artifact can be controlled by positioning of the electrodes and virtually eliminated if the ground electrode tube is located close to the tip of the stimulating electrode. Thus the action potential is well resolved, even when the propagation distance is small.

At the stimulated end the high resistance, within the tube and external to the nerve, directs current into the fibers, and excitation occurs just outside the tube end, where current exits from the fibers (when the end electrode is positive). The effect of stimulus direction is dramatic in the tube system (Fig. 3A). When stimulus direction is reversed, the AP seen at the recording end is diminished in amplitude and delayed. At the recording end, the impulse is detected as a voltage difference due to currents flowing external to the fibers, in the high resistance of the limited volume of solution within the tube.
Stimulus-Response Relation

A typical example of a stimulus-response relation is shown in Fig. 4A. Fig. 4B explains the decreased latency as stimulus intensity is increased and shows why time between stimulus and AP should not be used to calculate impulse velocity.

The conductance velocity of the impulse is commonly estimated from the ratio of the length of nerve between the stimulating and recording electrodes to the time between stimulus artifact and AP. Application of that rule to Fig. 4B yields the incorrect conclusion that velocity increases with stimulus intensity. There is a simple way to remove from the measurement of velocity the influence of events at the stimulus zone. If the record is taken with a short and then a longer length of nerve in the recording tube, the difference in distance is the actual conduction distance associated with the time difference in latencies between artifact and AP in the two records. (See Fig. 10 for another method: the AP can be recorded at two points along its course. The separation of the two peaks is easier to see in the muscle AP, which travels more slowly than the nerve impulse.)

Injury Potential

Recorded 0.5 h after the nerve has been dissected, the APs will ordinarily be diphasic in form (Fig. 5). The initial upward peak arises from the leading-edge current of the impulse, which is detected when that current flows from the confines of the tube toward the “center” of the propagating impulse as the impulse approaches the tip of the recording tube. The downward deflection that follows represents the currents of the trailing edge as the impulse “center” moves beyond the tube tip and toward the nerve end.

If the nerve is expelled from the recording tube, a millimeter or so is amputated, and the nerve end is immediately drawn back into the tube, the CRO beam will be shifted downward (and then will move upward only slowly if DC recording is being used). This downward shift signals the onset of injury current, supplied by the membrane battery and...
**FIG. 4.**
Intensity-response relation of nerve. A: small AP (few fibers activated) evoked by small stimulus. No. of fibers excited, total longitudinal current flowing, and therefore total voltage drop increase as stimulus is increased. Cathode-ray oscilloscope (CRO) beam was incrementally displaced horizontally for each stimulus by adjustment of horizontal position knob, as stimulus intensity was increased. Same display can be obtained by systematically changing stimulus pulse delay on stimulator as stimulus intensity is progressively increased. B: superimposed records showing decreasing latency as stimulus intensity is increased. Each AP is designated by intensity (4–6 V) of stimulus giving rise to it. (Note artifacts preceding each response.) Amplitude increases mainly because larger stimulus voltage excites more nerve fibers. In addition, latency decreases because depolarization extends further along away from stimulus tube end as shown below, where 4, 5, and 6 indicate origins of corresponding APs. With shorter propagation distance, relative dispersion of impulses is less, and this factor also tends to increase AP amplitude. S, length of nerve in stimulating tube. P, propagation distance, length of nerve between stimulus tube end (where impulse ordinarily arises) and end of recording tube, where first indication of AP is seen. R, length of nerve in recording tube.

flowing from the uninjured zone. At the same time, the AP will become monophasic. Only the initial peak remains, for the impulse entering the nerve end encounters a region already depolarized by the currents that flow from intact membrane to the interior via the cut end, and the competing center of the advancing impulse can draw no more current. The magnitude of the injury potential, and thus the observed effect on the diphasicity, depends on the length of the cut end drawn into the recording tube. During the course of 15–30 min, the injury current will disappear as the cut end heals, and the diphasic form of the AP will return.

**NEUROMUSCULAR TRANSMISSION**

**Gastrocnemius Muscle and Sciatic Nerve**
The AP propagated along a muscle can also be recorded in the suction electrode system. The gastrocnemius muscle is a popular object for study at the introductory level, even though its ample girth slows exchange of metabolites and thus encourages deterioration. Use of small- to medium-sized frogs and a lowered temperature will, however, improve the survival of the preparation. The gastrocnemius muscle is easily prepared, along with its nerve. Carefully separate and remove other muscles near
FIG. 5. Injury current and effect on AP form. AP is recorded before and after 1 mm has been cut from nerve end. Note downward displacement of baseline after cut. Initially (healed nerve), there is zero longitudinal current (zero potential drop) at end region. After cut, there is a voltage drop due to injury current. This voltage drop, 1–10 mV, depending on resistance pathway, results from “short circuit” of membrane battery of ~100 mV. Bottom: pattern of longitudinal currents during invasion of impulse to cut end, where leading currents die out as they approach depolarized cut end. Propagation of impulses depends on eddy currents flowing as illustrated: upward deflection L is due to L (leading) currents flowing (external to fibers) from normal membrane (right) toward impulse center (stippled) as impulse advances left to right (direction of large arrows). Downward deflection T is due to T (trailing) currents flowing from recovered zone (left) toward impulse center. At healed end, T currents advance toward end. At cut end, trailing currents disappear because of steady depolarization due to injury (I) currents.

Because the nerve-muscle junction is a region of low safety factor of transmission, the relation of stimulus to response is not the same as in the simple nerve. Single shocks adequate to evoke a response in the nerve may yield a small or no response in the muscle, but repetitive stimulation (e.g., 10/s) will normally evoke large signals.

More careful analysis of the nerve-muscle system can be done with pairs of shocks, preferably delivered to the nerve at regular intervals (e.g., with the stimulator set at a frequency of 0.2/s). Then, as the interpulse interval of each pair of shocks is increased, the second shock is delivered later. The first stimulus establishes at the nerve-muscle junction a condition that is in effect tested by the second stimulus. The muscle AP response amplitude resulting from the second shock will normally reach an early peak when the interpulse interval is short, 1–2 ms, and then will decline progressively as the interval is increased up to 15–20 ms. Such a response pattern shows that depolarization evoked at the nerve-muscle junction by the first incoming nerve impulse of the pair declines with time. The muscle response to double-shock stimulation of the nerve is thus quite different from the two-shock response of the nerve. The nerve shows a refractory period, whereas the nerve-muscle system shows a facilitation effect: the action of a stimulus is made more effective, rather than less, by a preceding stimulus.

Sartorius Muscle and Nerve

A better preparation for observation of neuromuscular transmission is the sartorius muscle. It is thin and nearly transparent, allowing adequate diffusion of metabolites; the muscle fibers are readily distinguished with the help of a low-power dissecting microscope, the innervation zone is relatively re-
Muscle holders. A: retainer for gastrocnemius muscle. Slotted block receives stump of femur and of tibio-fibula, thus fixing muscle origin in place. B: clamp holds firm the sartorius muscle pelvic end. Right: sectional views of clamp. Jaw, held in place and swivelling on pins, is tightened onto pelvic end of sartorius muscle by screw (S). Conduits — (cathode, C) and + (anode, A) drilled in block direct stimulating current (ST) through pelvic end of muscle.

stricted, and the entry of the nerve ramifying over the muscle is visible. It has the added advantage that, in contrast to the gastrocnemius, it can be conveniently arranged for direct stimulation and recording.

In the interests of efficiency and of frog conservation, the sartorius muscle, and especially the muscle with its nerve, should probably be prepared by the instructor (who may need to practice the dissection). The sartorius originates superficially on the ventral aspect of the pelvis and overlies the flat adductor longus, from which it can be distinguished by a faint line at the lateral edge. Separate the muscles along this line, starting at the tendon that inserts distally over the knee. At the medial edge, continue the separation up to the thin whitish nerve, which, along with a more visible blood vessel, comes out at right angles from the deep surface of the sartorius muscle and is adherent to the massive adductor magnus. Into the latter muscle cut along the nerve sufficiently to free it up to its entry into the sartorius. Tie a thread (e.g., 0–6) to the distal tendon, free the muscle up to its origin, and after the nerve has been carefully freed for ≥ 1 cm and severed, cut the muscle close to its origin at the pelvis.

Immerse the preparation and pin it in a dissection dish (with a wax or silicone floor) so that extraneous tissue can be removed; then transfer it to the chamber, deep side uppermost if the nerve entry is
to be accessible. Secure the nerve-free pelvic end in the holding-stimulating clamp (Fig. 6B), tie the distal end to the electrode wire, and draw this end into the extension tube.

CLAMP FOR SARTORIUS MUSCLE

Figure 6B shows the construction of a clamp to hold and stimulate the sartorius muscle directly. Screw S tightens the clamp on the pelvic end of the muscle, and stimulating current is delivered via conduits A (anode) and C (cathode) to two slots in the floor of the groove that receives the muscle.

In the high-Mg\textsuperscript{2+} solution, a single stimulus to the nerve may generate an end-plate potential (Fig. 7A). Stimulation of the muscle directly (Fig. 7B) yields a fully propagated AP that is here recorded at two places in succession, providing two peaks from which the velocity of the impulse can be obtained. The double response is obtained by use of a second zone of high resistance. The small chamber described in the following section is one way to achieve this condition.

The relatively refractory period of the muscle can be determined from the record in Fig. 8, which shows increasing amplitude of response as the conditioning-testing interval is increased. The pattern of response contrasts with that typically seen in muscle records evoked during stimulation of the muscle nerve.

SMALL-VOLUME ACCESSORY CHAMBER

For some purposes, the Plexiglas preparation bath is excessively large. For example, less volume is preferable if the effect of a change in the bathing medium is to be studied. A small chamber set into the larger also helps hold the electrodes in place and serves as barrier to provide additional high-resistance zones where stimulation and/or recording can be done simultaneously at different positions along the nerve or muscle (Figs. 9 and 10).

The muscle clamp can be used inside the small chamber (Fig. 10). In that instance a large round hole is required in the rubber dam for the gastrocnemius, whereas a slit is sufficient for the sartorius.

![Figure 7: Sartorius muscle electrical responses to indirect (nerve) and direct stimulation. A: end-plate potential (EPP) during stimulation (ST) of nerve (SN). Diagram shows direction of currents associated with nonpropagated response. Large arrows show direction impulses would depart from end plates if propagation occurred in muscle. B: AP during direct stimulation of muscle (SM) via clamp at nerve-free end. Interval L\textsubscript{1}–L\textsubscript{2}, in relation to distance D (= 5 mm, below), allows calculation of muscle impulse propagation velocity. Small arrows, direction of leading and trailing currents of impulse. Large arrow, direction of impulse propagation. R, recorder (CRO).]

Figures 9 and 10 show the small chamber arranged to allow recording of the AP at two points as the
Sartorius muscle refractory period (direct stimulation). Conditioning-testing pulses \( S_1 \) and \( S_2 \) delivered directly to proximal nerve-free end of muscle; response recorded at distal end. Compare with Fig. 4 A. \( R_1 \) is diphasic response to stimulus \( S_1 \) (see \( L_1, T_1 \), leading and trailing currents in diagram below). Stimulus \( S_2 \) is delivered at a short interval after \( S_1 \) and evokes a small response. When \( S_1-S_2 \) interval is longer, response is larger. \( L_2, T_2 \) represent leading and trailing currents of any 2nd response.

Impulse traverses two zones of high resistance that are spanned by the recording electrodes: the hole in the rubber dam and the entry into the recording tube. The distance between these two zones, taken in conjunction with the time between the peaks, permits assessment of velocity in a single measurement (see Fig. 7). Impulse propagation in both directions along nerve or muscle can be conveniently demonstrated in this arrangement.

A simple barrier arrangement based on a disposable plastic culture dish, 60 × 20 mm, is illustrated in Fig. 9. With a metal rod (e.g., a round file, heated over a Bunsen burner) make several large holes (5–8 mm diam), properly lined up, in the wall of the dish. With a small knife or integral-blade scalpel, scrape off melted plastic and roughen the surface to provide a suitable bond (e.g., with silicone adhesive, such as Borden Stix-All) for a strip of rubber dam. Flat rubber tubing used in Gootch crucibles in chemistry laboratories is excellent for this purpose. After the adhesive has dried, punch (see above) a small hole at the center of each window in the dish to receive the extension tube, the nerve, or the muscle.

Sometimes, as in Fig. 10, electrodes may be required in addition to the six available in the bath. A Plexiglas block holds a (shortened) suction electrode and the extension tube. Soap dish suction cups are split and cemented to both the dish and the block to provide temporary attachment to the floor of the bath.

CONCLUSION

The electrical aspect of excitability is so important that "action potential" has become virtually synonymous with "nerve impulse," although that event also involves many other physical and chemical
Sartorious muscle clamp in small chamber. Muscle is passed through slit in rubber wall of chamber. Record (A–C) is taken across slit near center and near distal end of muscle. Free electrode (FE) can be connected (B–C) to record exclusively along distal end. ST, stimulator; Rec, CRO.

processes. The suction electrode system is not only an inexpensive and convenient technical design for recording some of the electrical events; it also helps the student interpret, in terms of the pattern of external currents, the electrical changes in relation to impulse propagation and synaptic transmission. Don't expect to obtain results identical to those shown in Figs. 1–10. The challenge is to interpret and explain the results in terms of simple principles. Two important matters to keep in mind are Ohm's law and the rule that electric current flows in a positive-to-negative direction. Applying Ohm's law, we see that the eddy currents that flow longitudinally outside the active nerve will produce a voltage drop in the medium proportional to the resistance through which the currents flow. Along the ncvn inside the tube of the suction electrode, the current is essentially unchanged, but the resistance is higher, and thus the voltage drop is great enough to be detected. The direction of the eddy currents is signaled by upward or downward deflection of the CRO beam, and reversal of direction of current flow is shown by the reversal of direction of the deflection with respect to the zero baseline.

The simple suction electrode system has been used successfully for several years in a class of advanced undergraduates. To hold down unnecessary use of frogs and to assure that the preparations will be responsive, the final dissection of the nerve or muscle has usually been done by the instructor. The beginning student is entitled to start out with a system that works; malfunctions will come soon enough!

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References


Teachers and their students may find the following articles from News in Physiological Sciences useful when exploring the physiology of the preceding paper:


