PHYSICAL TECHNIQUES IN BIOLOGICAL RESEARCH

edited by

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PREFACE

In preparing material for this volume, authors were asked to provide instruction for graduate students and for more experienced workers who would be entering the field of electrophysiology. I believe that these readers and others will find much of both theoretical and practical value in this volume. Original information has been included which cannot be found elsewhere. Authors were asked to limit their references to selected works which were judged to be especially useful. The goal was to help the reader enlarge his background and to avoid bewildering him.

The preparation of this volume extended over a considerable period of time and involved much painstaking effort on the part of all concerned. I wish to express my appreciation to the contributors, all of whom generously maintained attitudes of patience and cooperation during the entire process.

A number of colleagues provided critical comments on various parts of the book. Some of these were given directly to me and some directly to the authors. I am grateful for this valuable aid and wish to thank E. Amatniek, W. H. Freygang, Jr., M. G. F. Fuortes, K. Frank, J. Y. Lettvin, J. C. Lilly, W. R. Loewenstein, D. P. Purpura, and W. Rall.

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LIST OF ABBREVIATIONS

atm—atmospheres
cm—centimeter
cos—cosine
coul—coulomb
cps—cycles per second
°C—degrees Centigrade
d—dyne
deg—degree
log—logarithm_{10}
ln—logarithm_e
gm—gram
in.—inch
kc—kilocycle per second
kg—kilogram
mc—megacycle per second
m—meter
msec—millisecond
mw—milliwatt
pps—pulses per second
sec—second
v—volt
w—watt
μ—micron
μsec—microsecond
μv—microvolt
μw—microwatt
Ω—ohm

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CHAPTER 1

STIMULATION

E. E. Suckling

I. Introduction

Stimulation as the term is used in this chapter involves the supply of energy in some form either to a receptor organ or to excitable tissue. The amount of energy which is needed at the actual site of stimulation is always extremely small. However, losses of various sorts occur and in experimental stimulation a substantial amount of energy is usually supplied to insure that some of it reaches the excitable area.

In some basic experimental work precise knowledge of the stimulating mechanism is of interest. In other cases the stimulus is merely a means to produce some desired result such as a limb movement or systolic action in the heart. This chapter will be concerned with the aspects of stimulating technique which are more or less common to the above two objectives. For further details concerning excitable tissue and its responses to applied stimuli, the reader is referred to standard textbooks such as *Medical Physiology* (Bard, 1961) which has a good treatment written by Davies (Chapters 50, 51). For sense organ function and the relationship of sensory receptors to applied stimuli, a useful reference is the *Handbook of Physiology* (Field, 1959).

II. Electrical Stimulation

1. Parameters of an Electrical Stimulus

An electrical stimulus comprises one or more pulses of electric current flowing into excitable tissue. The most important parameters of an electrical stimulus from a physiological point of view are (a) strength of the current which flows into the tissue, (b) the variation of the current with time, (c) repetition rate of the individual pulses of current, and (d) total number of pulses supplied.

It is often difficult to evaluate parameters (a) and (b) as they actually exist at the sites in tissue where they are effective. One reason for this is that excitable tissue contains resistive and reactive elements and the potential difference across these elements changes when electric current flows through the tissue. A second reason is that between the electrodes supplying current and the excitable tissue itself there may be series and shunt paths whose electrical impedance possesses resistive and capacitive components. Frequently parameters of stimulation are measured at the electrodes and the values obtained commonly lie in the following ranges.

Current strengths: These are of the order of milliamps with the driving voltage between 1. and 100 v. In exceptional circumstances values outside this range may be necessary.

Shape of the current pulse: In one commonly used electrical stimulus the current rises to its maximum value in a fraction of a millisecond. It may then be required to endure for periods less than $1/10$ msec or for periods up to a second or more depending on the circumstances.

Repetition rate: It is seldom necessary to repeat stimuli at a rate greater than about 1000 times per second. Frequently individual stimulus pulses must be delivered on demand. Trains of pulses may be required at various times.

When an electric current is used to stimulate tissue, most of the energy is wasted. A very small proportion of the energy acts in a useful manner, that is directly across the excitable membranes, while the remainder generates heat and causes electrolysis. One should keep in mind that unwanted byproducts of stimulation are likely to interfere with the experiment. For this and often for other technical reasons it is nearly always desirable that a stimulating current be kept at as short a duration as will be effective.

2. Electrical Stimuli of Indeterminate Shape

There are situations in neurophysiological research where the experimenter merely wishes to stimulate excitable tissue and he is not concerned with the parameters of the applied stimuli. In the literature of the past there are many examples in which an inductorium was used to provide such stimuli. For historical as well as for other reasons a short discussion of this device may be useful. Basically this instrument is a transformer in which the coupling between primary and secondary coils can be varied over a wide range. A 1½- to 3-v dc source is commonly used to energize the primary coil and variation in magnetic flux is provided by a magnetically actuated circuit breaker in series with the primary coil. The secondary winding of the inductorium has a large number of turns and it has considerable inductance and self-capacitance with not much resistance. As a consequence, it is possible that the pulse generated in this coil may be oscillatory unless sufficient damping is provided by the tissue resistance which is connected across the secondary terminals.

The secondary winding may be considered as a generator of current which flows through an external circuit (the tissue to be stimulated). The internal impedance of this current-supplying generator is very high, and as a consequence the actual voltage produced at the electrodes depends largely upon the load resistance connected across them. With the secondary open circuited an inductorium may generate 1000-v pulses, whereas when loaded with an average resistance such as exists between two stimulating electrodes in contact with tissue, perhaps only 20 v may be produced.

Modern electronic instruments which provide more ideal characteristics have largely replaced the inductorium. One reason for this change is that the voltage pulses produced by the inductorium are of indeterminate size and shape. Also in the inductorium the output pulse produced on closing the primary circuit ("make") is smaller than that produced on opening the primary circuit ("break").

Despite the above drawbacks the inductorium may occasionally be used in modern research because it allows the user opportunity to alter the distance between primary and secondary windings. By this means the capacitive coupling between primary and secondary may be reduced with a corresponding reduction in recording the artifact originating from this source. (See also Chapter 8, this volume.) Applied in this manner, the inductorium serves principally as an isolation transformer, and as such it may provide better characteristics than can be obtained with the radio-frequency transformers described later in this chapter.

A simple means of energizing the inductorium is shown in Fig. 1. The low impedance of the primary requires that the energy supply also have low internal impedance. In this circuit, the discharge of capacitor C_2, is gated by the thyratron and the resultant current pulse flowing through the primary winding will produce secondary pulses of adequate size. Potentiometer P_1 is used to obtain cutoff bias where external triggering pulses are to

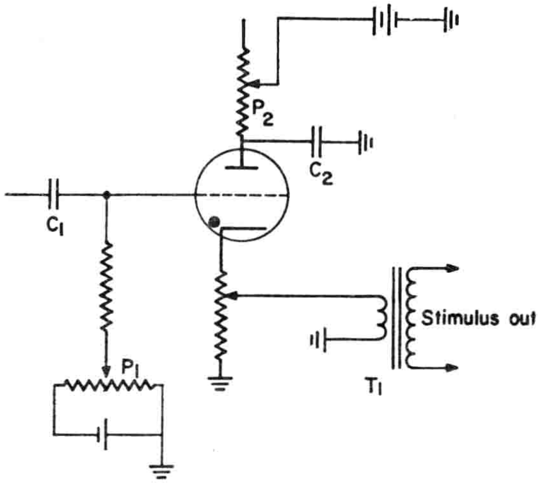

FIG. 1. Circuit of an elementary simulator.

be delivered via C_1. Otherwise, P_1 can be set to produce a free running oscillation whose frequency can be varied by means of P_2.

3. Square-Wave Stimulation

There are many physiological experiments in which it is important to give a quantitative description of the stimulus, and when this is the case the stimulus selected is a rectangular pulse of current whose duration and intensity can be altered.

The use of a pulse of current having a rectangular contour ("square wave") enables one to calculate the total electrical charge or the energy supplied. The total charge in coulombs is equal to average current flowing (amps) multiplied by the time (sec). If voltage and average current are known, the energy can be expressed as watt-seconds. Application of these principles is limited because the stimulus current seldom remains completely constant during the time that stimuli are applied to tissue. Some disturbing factors are chemical changes which occur at the electrodes and the presence of capacitance in parts of the tissues. Even if the total current passing from or into an electrode remains constant during a stimulus one must not assume that the current actually passing across the excitable membrane also remains constant for the reason that the membrane resistance is likely to be altered during stimulus application.

A square wave or rectangular pulse of current can be supplied (a) by switching a battery or power supply on and off by hand, (b) by performing the same action by means of an electromechanical relay, and (c) by using an electron tube or transistor as a gate for the current. In the electrophysiological work of the past, the Lucas spring and the Lucas pendulum have been used as a means of mechanically actuating the switch contacts.

Current pulses lasting a few milliseconds or less can be produced using some types of modern electromechanical relays.[1] The fact that the relay contacts and a battery current source can be well isolated from ground or from any other electrical equipment is an important advantage in experimental work. It is, of course, necessary that the rather delicate contacts of a very fast acting relay be protected from arcing or overload. Usually a current-limiting resistance is adequate for this purpose.

An electronic system can be used to generate rectangular stimulus pulses whose duration can be made as short as practical circumstances require. Commercially manufactured stimulators seldom supply pulses shorter than a few microseconds but this is far from the technological limit. Pulses developed by electronic means can also be seconds in duration when that is necessary. For very long current pulses electromechanical methods are probably preferable to purely electronic ones.

The output of the circuit shown in Fig. 1 is isolated from ground so that either terminal can be connected to a grounded point in the biological preparation. In an electronic circuit it is usually not practical to use a totally ungrounded or "floating" system and, in general, the negative side of the stimulus generator is permanently connected to ground. Most electronic stimulators produce pulses which are positive (or anodal) relative to ground but this arrangement is not mandatory and stimulators can be designed to produce pulses which are negative relative to ground. It is usual to isolate the stimulator from the biological preparation by means of a transformer as shown in Fig. 1, or by means of a radio-frequency isolation unit (see page 12), and when this is done the output polarity of the stimulator itself is of no importance.

It is usually necessary that the strength of an applied stimulus be continuously variable over the range between a few tenths of a volt and a hundred or more volts, and decade steps as well as a smoothly varying control are used to achieve this. When the threshold of a preparation is being measured it can happen that a change of stimulus level of one or two per cent will make the difference between a liminal and a subliminal condition. For this reason the output level of a stimulator must remain constant wherever it is set and must not show instabilities caused by electronic factors, power line variations, etc.

Two classes of electronic stimulators are available commercially. There are (a) the relatively inexpensive stimulators frequently specified for student use and (b) the more complex stimulators generally used for

[1] General Electric Company, type CR2791K110, Stephens Arnold millisec 328, etc.

research purposes in conjunction with an oscilloscope. Most of the simpler stimulators[2] will supply rectangular pulses and can be adjusted for pulse frequency over a range between about one to a thousand pulses a second and for pulse duration between one second and one millisecond. Output voltages are usually adjustable up to one hundred or more volts. This type of stimulator is intended to give pulses continuously once it has been turned on. It is therefore useful for many purposes such as stimulating the vagus nerve to slow the heart. These units are also useful in experimental arrangements where, for instance, single pulses must be delivered to a nerve but there is usually no arrangement for triggering these pulses externally for synchronization to an oscilloscope trace. For long pulses most student stimulators have a key which can be held down for as long as a continuing current is required to flow.

The majority of research stimulators in use are manufactured by the Grass Instrument Company, by the American Electronics Laboratories, Inc. and by Tektronix, Inc. The longest established of these, the stimulator produced by the Grass Company, gives continuous adjustment of pulse-repetition frequency and of pulse duration and also provides an adjustable time delay between the application to the stimulator of an external synchronizing pulse and the generation of a stimulus. The unit will itself supply a synchronizing pulse which can be made to start an oscilloscope sweep and when so used the time-delay unit is interposed between this synchronizing pulse and the commencement of the stimulus. Pulse durations and also delays from 10 μsec to 1 sec are available, with repetition frequency limits of one pulse in ten seconds to 10,000 pulses a second. Output voltage is adjustable in several ranges up to 150 v maximum. Pulses are positive relative to the chassis of the stimulator. Trains of pulses (for instance, 50 pulses, each of 200 μsec duration spaced 1 msec from the previous pulse and with one train every second) can be obtained if two Grass stimulators are interconnected and appropriate dial adjustments are made or, in some models, such operation is obtained by means of special connections on a single stimulator.

The stimulator of the American Electronics Laboratories has parameters of its output which are similar to those of the Grass stimulator. Its output voltage is higher and pulse trains can be obtained from a single stimulator. The stimulator supplied by Tektronix is inexpensive and is very precise in in its settings. It must, however, be used in conjunction with other Tektronix equipment (wave form generator) in order to obtain triggering and delay facilities.

Several stimulators can be driven from a single wave form generator. However, pulse trains can be obtained by interconnecting wave form gen-

[2] American Electronic Labs., Cambridge Instrument Co., Grass Company, etc.

erators and pulse generators. The Tektronix stimulator gives a maximum output of 50 v. Many publications describing stimulators are found in the literature, but these will not be reviewed here. The interested reader will find more information in *Electronics*, *Electronic Engineering* (England), *Review of Scientific Instruments*, and a number of other journals. Donaldson (1958) has a useful discussion and gives a number of pertinent references and practical circuits.

4. Stimulus Positioning and Delay

It is often necessary to deliver a stimulus at a precise instant of time. An example is the study of the decay of a local excitatory state which is explored by delivering test stimuli at various times. In another as frequently encountered situation, the stimulus evokes a potential change that must be monitored by the oscilloscope and to do this one must be able to choose a convenient position along the rapidly traveling sweep of an oscilloscope. Here the stimulus is delivered at a particular time relative to the oscilloscope sweep in order to insure that the resulting phenomena occur and appear while the trace is near its center point along its traverse.

Stimulators are usually arranged with a delay system so that the actual stimulus pulse is given at some known interval after delivery of a "synchronizing pulse." The synchronizing pulse may be supplied by other apparatus or it may even be of biological origin. For example, the R-wave of the electrocardiogram may be amplified and used for synchronization in order that a stimulus may be delivered in a definite relationship with the heart's normal cycle.

The arrangement described above is shown in Fig. 2. The connection to the oscilloscope can be made to its "synch in" terminal so that the synchronizing pulse generator triggers or synchronizes the beginning of the oscilloscope sweep in addition to synchronizing the stimulator. When this is done the delay setting on the stimulator and the sweep duration setting on the oscilloscope can be adjusted so that the stimulus is applied at any desired point along the sweep.

The preceding arrangement is by no means the only one possible. In some cases it might be desirable to permit the oscilloscope sweep to run free without any external control or synchronization, and then to use a pulse delivered by the oscilloscope at the start of each sweep to trigger the stimulator. Alternatively the stimulator may be allowed to run free and to supply a pulse which synchronizes the oscilloscope. The would-be user who is puzzled about setting up a convenient arrangement is offered the following points of advice.

Go carefully over all designation strips, labels, and control markings on

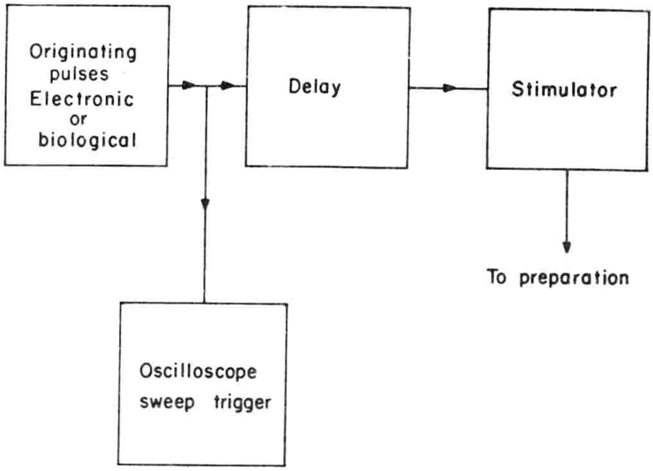

FIG. 2. System for stimulating after oscilloscope spot has started moving.

the apparatus to be used. The logic of any arrangement is often discernible therefrom.

Draw diagrams of the proposed arrangement so that it is clearly decided which piece of apparatus controls the other.

Check manufacturer's instructions to find out about the polarity of synchronizing pulses which are either delivered by one piece of apparatus or required for synchronization by another. Often a transformer will enable polarity reversals to be made where necessary.

Directly connect the output from the stimulator to the oscilloscope to provide visual observation of the stimulating pulse while adjustments are being made.

A system which can be used for positioning a stimulus on an oscilloscope trace is one which employs a "Schmitt trigger" (see Millman and Taub, 1956). The Schmitt circuit is a cathode-coupled multivibrator which generates a pulse when a certain threshold potential is applied to it. In this application, the sawtooth wave of the oscilloscope sweep is applied to the Schmitt circuit with the result that a pulse which actuates the stimulator is produced when the sweep voltage rises past some particular value. As a result the stimulus is triggered at a preset or adjustable point along the sweep. The advantage of this system is that the location of the stimulus along the trace is independent of the sweep coursing velocity (Suckling, 1961).

It is sometimes necessary that time marks appear across the face of the oscilloscope and remain fixed in position during successive traces. In this