A TECHNIQUE FOR DISCRIMINATING DIRECTION OF PROPAGATION OF ACTION POTENTIALS RECORDED FROM AN INTACT MIXED NERVE

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1. INTRODUCTION

A study of the insect central nervous system (Rowell, 1964, 1969) required the impulse traffic in a connective to be resolved into that fraction propagating anteriorly and that propagating posteriorly, and to describe each fraction in terms of the amplitude and frequency of action potentials. The first requirement is usually met by cutting the nerve and recording independently from the two ends. When, however, the nerve joins two ganglia, each of which provides an important input to the other, cutting the nerve may greatly alter the working conditions of the ganglia, and thus their outputs. The device described in this paper allows the action potentials propagating in opposite directions in the nerve to be measured separately in the intact nerve. It also enables one to measure conduction velocities, to count action potentials of any given conduction velocity, and to assess the signal-to-noise ratio of low-amplitude nervous activity.

2. PRINCIPLES

The principle is to apply two external electrodes to the nerve a known distance apart, and to use the signal recorded from one to gate the output from the other. (Fig. 1.) Consider a propagated action potential (PAP) of conduction velocity \( v \) propagating in a nerve, passing electrode A and electrode B which are a distance \( d \) apart. On arrival at electrode A a pulse is generated, and this, after a delay slightly less than \( d/v \), causes a gate to open in the channel connected to electrode B. On arrival at electrode B, the PAP generates a pulse in channel B, which passes through the open gate to the output of the analyser, thence to a high-speed counter or other display. If, conversely, the PAP is propagating in the reverse direction it arrives at electrode B before electrode A. The gate in channel B is shut against it, and no output is counted; it then reaches electrode A and causes the gate to open in channel B, but as it has already passed electrode B it is not counted. By reversing the connections of channels A and B to the signal input of the transmission gate and selector-pulse circuit respectively, PAPS propagating in the reverse direction are differentially selected and counted. The sum of the two counts should, of course, then agree with a count made from either electrode with the gate permanently open, and this gives a check on the accuracy of the method.

A block diagram of the system is given in Fig. 2.

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3. MATHEMATICAL CONSIDERATIONS

A. Random distribution of PAPs. The distribution of PAPs in a single axon is non-
random in time, as the lower limit on the interval between successive pulses is set
by the maximum repetition rate of the axon; many axons show a patterning of their
discharge, which will further preclude a random distribution of intervals. When,
however, the recording electrode includes in its field a number of axons, the distri-

![Diagram](image-url)

Fig. 1. Principle of discrimination of direction of propagation in a nerve. The device is set
to count PAPs propagating from electrode A to electrode B, i.e. channel A gives rise to
selector pulses, channel B gives rise to signal at the gate, and thus to the counter.
A. PAP propagating from electrode A to electrode B.
B. PAP propagating from electrode B to electrode A. Further explanation in text, section 2.

![Diagram](image-url)

Fig. 2. Block diagram of system: (i) Arrangement for recording; (ii) analyser.
bution of the PAPS recorded will approach random, assuming that the firing of the axons is not interdependent. The larger the number of axons recorded by the electrode, the more likely it is that this condition is fulfilled, and the greater the approximation to a random distribution of inter-spike intervals. It has been found empirically that results obtained from wire-hook electrodes and insect connectives agree with predictions made on an assumption of random intervals. For the remainder of this discussion it will be assumed that the distribution is random.

B. Ideal recording conditions. It is assumed for the moment that both electrodes sample the same axons (i.e. no axons are recorded by one electrode and not by the other) and that the same axon records at the same amplitude at both electrodes. The significance of practical deviations from this ideal state is examined further in section 4.

C. Expression for a nerve with PAPS propagating in only one direction. In a nerve in which all PAPS are propagating in the same direction each channel input will receive; (a) the PAPS; (b) other electrical potentials of various origins (e.g. muscle potentials, magnetic and electrostatic interference); (c) thermal noise.

The number of pulses formed in each channel as a result of these inputs will be equal, but the different categories are differentiated by their time relations. The PAPS will always be separated by a time interval at the two channels, interference potentials will be simultaneous in both channels, and thermal noise is random. In the NOT GATED mode of operation (i.e. when the gate in the counting channel is locked open) the total recorded number of pulses \( F \) will be the sum of these sources

\[
F = f_{\text{nerve}}(\text{PAPS}) + f_{\text{interference}} + f_{\text{noise}}
\]

In the GATED mode (i.e. the gate in the recording channel is opened only by a pulse at the other electrode) the gate will be opened \( F \) times, as the input to both channels is numerically equal. By design, all PAPS will be recorded by passing at the appropriate speed between the electrodes. A simultaneous interference potential at both electrodes cannot be recorded, as there is a delay before the gate opens. Therefore, only those interference potentials which occur by chance when the gate is open will be recorded; assuming that these potentials occur at random, then \( Fg/T. f_{\text{interference}} \) will be recorded, where \( g \) is the length of the gate pulse, and \( T \) is the total duration of the counting period. Similarly, \( Fg/T.f_{\text{noise}} \) will also be recorded. The recorded count in the GATED mode made in the accepted propagation direction \( (F') \) will then be the sum of

\[
F' = f_{\text{nerve}} + (Fg/T) (f_{\text{interference}} + f_{\text{noise}}).
\]

\( Fg/T \) is always less than 1, and is usually small (typically \( F/T < 100, g < 400 \mu\text{sec.}, Fg/T < 0.04 \)). Under good recording conditions interference is small, and at all but very high gains thermal noise is negligible, so that

\[
F' \approx f_{\text{nerve}}.
\]

When the channel connexions are reversed a count is made of PAPS propagating in the reverse direction. In a one-way nerve (e.g. one cut, or a pure motor or sensory nerve) this figure should be zero, but a spurious count is registered because of the chance coincidence of a potential derived from any of the above three sources and an open gate in the counting channel. This spurious count \( F' \) is equal to

\[
F' = gF^2/T.
\]
Expression for a nerve with PAPs propagating in both directions (‘up’ and ‘down’).

In the NOT GATED mode the recorded total number of pulses will be

\[ F = f_{\text{nerve UP}} + f_{\text{nerve DOWN}} + f_{\text{interference}} + f_{\text{noise}} \]  

In the GATED mode the recorded totals will be

\[ F'_{\text{UP}} = f_{\text{nerve UP}} + (Fg/T)[f_{\text{nerve DOWN}} + f_{\text{interference}} + f_{\text{noise}}], \]

and

\[ F'_{\text{DOWN}} = f_{\text{nerve DOWN}} + (Fg/T)[f_{\text{nerve UP}} + f_{\text{interference}} + f_{\text{noise}}]. \]

Under ideal recording condition expression (6) and (7) approximate to

\[ F'_{\text{UP}} = f_{\text{nerve UP}} + Fg/T \cdot f_{\text{nerve DOWN}}, \]

and

\[ F'_{\text{DOWN}} = f_{\text{nerve DOWN}} + Fg/T \cdot f_{\text{nerve UP}}. \]

By substitution

\[ f'_{\text{UP}} = \frac{F'_{\text{UP}} - k \cdot F'_{\text{DOWN}}}{1 + k^2}, \]

and similarly

\[ f'_{\text{DOWN}} = \frac{F'_{\text{DOWN}} - k \cdot F'_{\text{UP}}}{1 + k^2}, \]

where \( f'_{\text{UP}}, f'_{\text{DOWN}} \) are the true UP and DOWN numbers, \( F'_{\text{UP}}, F'_{\text{DOWN}} \) are the recorded UP and DOWN counts and \( k = Fg/T \), where \( F \) is the total count recorded in the NOT GATED mode, \( g \) is the gate length and \( T \) the total count time. As \( k \) is small (see above) \( k^2 \) is negligible. As a running check on the analysis, \( f'_{\text{UP}} + f'_{\text{DOWN}} \approx F \).

4. APPROXIMATIONS AND ERRORS

A. Pulse coincidence. All the above expressions neglect coincidence of pulses at one electrode and not the other. This factor is small; assuming a random distribution of intervals and an average pulse frequency = \( f \), the probability that a second pulse will follow a first within time \( t \) is \( p = 1 - e^{-ft} \), or approximately \( p = ft \).

As the pulse length is only 10 \( \mu \text{sec.} \), and \( f \) rarely greater than 200/sec., \( p < 2 \times 10^{-3} \); less than two pulses in a thousand will be subject to a coincidence.

B. Failure of selector-pulse to reset. In the circuit used a second PAP arriving at the selector pulse channel during the previously initiated selector-pulse does not reset the pulse; accordingly, the gate may be shut when the PAP arrives at the recording channel. The proportion of PAPs which will be affected by this error depends on their conduction velocity relative to the gate length. In a typical experiment with an insect connective, in which the frequency and conduction velocities of the PAPs has been measured, the calculated error is about 1.0%.

C. Variation from ideal recording conditions. In practice the two electrodes differ in their receptive fields, and some axons record in one channel and not in the other. This makes the total NOT GATED count \( F \) higher than expected, and this in turn introduces an error into all the other expressions except equations 1–3. It would be a serious error where the nerve trunk twisted between the electrodes, but with care in electrode placement the error is tolerable (section E).

In both channels the PAP waveform triggers a Schmitt trigger circuit which is differentiated to give the 10 \( \mu \text{sec.} \) pulse used. Where the PAP is recorded at a slightly
different amplitude or waveform in the two channels, a timing error is introduced. It can be shown by construction that the maximum error obtainable in this way is to lengthen or shorten the expected interval between the pulses by about 200 \( \mu \)sec. If the conduction velocity is high enough to make the expected inter-electrode interval less 200 \( \mu \)sec., then a certain number of PAPS will arrive at the counting electrode before the gate is open, and \( F' \), the observed gated frequency, will be too small. The error will be most serious in applications where the electrodes are placed very close together, and where the PAPS conduct at a high velocity. Insect work fills the first criterion, but is less liable to the second. In practice, it has been found that serious discrepancies of amplitude can be detected by eye if the two inputs are displayed on a CRO and superimposed at equal gain, but some error due to this source must remain in all counts.

The over-all result of the errors arising from non-ideal recording conditions will be to make the not gated totals \( F \) larger than the gated totals \( F' \).

D. Effect of noise at high amplification. When very small PAPS are analysed there is significant noise input, and an error arises due to this; this condition was largely neglected in the expression derived above. The false count due to noise (and interference) will again be greater in the not gated mode than in the gated one. This error then acts in the same way as that due to departures from ideal recording conditions, but is greater when the spike amplitude is smaller.

E. Assessment of total error. In a one-way nerve, the gated and not gated totals (\( F' \) and \( F \)) were compared over a range of gain settings (Table 1). The results show (i) the observed spurious count in the reverse direction to the direction of propagation (\( F'_{\text{UP}} \)) agrees reasonably with the expected value \( F^0 \) calculated from formula (4). Agreement becomes closer as \( F \) increases, presumably because of nearer truly random interpulse intervals; (ii) for all amplitude categories of PAP except the smallest, \( F' \) was between 77 and 96% of \( F \); that is, up to 96% of all the input potentials recorded were confirmed as PAPS. At the highest gain, \( F' \) dropped abruptly to only 14% of \( F \); this presumably represents the increased element of noise at high gain.

In a two-way nerve (Table 2) values of \( f_{\text{UP}} \) and \( f_{\text{DOWN}} \) calculated according to formulae (10) and (11) were summed and compared with the value \( F \), the total number of pulses recorded at the electrode. Over three different amplitude classes, between 72 and 84% of the total input was confirmed as PAPS.

The discrepancies must derive from potentials of non-nervous origin in the input, inequality of the inputs recorded at the two electrodes, or instrumental error. The best figure of 96% agreement shows that instrumental error is 4% or less. Except at high gain, non-nervous input is unimportant under good recording conditions. The most serious error therefore arises in differences of recording conditions at the two electrodes, and this normally limits performance to 70–90% accuracy.

5. Design considerations

A. For statistical reasons, usually not less than 30 sec. nervous activity are necessary for an analysis. Unless the discriminating and counting apparatus is duplicated many times, an analysis of UP and DOWN signals at several levels of spike amplitude
Table 1. Analysis of one-way traffic (locust cervical connective, pinched posteriorly)

(Gate pulse duration 600 μsec., gate pulse delay 200 μsec., duration of recording 30 sec.)

<table>
<thead>
<tr>
<th>Gain setting</th>
<th>$F_{DOWN}$</th>
<th>$F_{UP}$</th>
<th>$F'_{DOWN}$</th>
<th>$F'_{UP}$</th>
<th>Calculated (formula 4)</th>
<th>Number of PAPS in each amplitude category</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (lowest)</td>
<td>400</td>
<td>437</td>
<td>348</td>
<td>1</td>
<td>3</td>
<td>$&gt; 5$ (greatest) 400 348 87</td>
</tr>
<tr>
<td>B</td>
<td>800</td>
<td>840</td>
<td>708</td>
<td>5</td>
<td>14</td>
<td>4-5 400 360 90</td>
</tr>
<tr>
<td>C</td>
<td>1900</td>
<td>1850</td>
<td>1553</td>
<td>40</td>
<td>68</td>
<td>3-4 1100 845 77</td>
</tr>
<tr>
<td>D</td>
<td>3600</td>
<td>3700</td>
<td>3185</td>
<td>180</td>
<td>260</td>
<td>2-3 1700 1632 96</td>
</tr>
<tr>
<td>E (highest)</td>
<td>5050</td>
<td>5800</td>
<td>3532</td>
<td>612</td>
<td>672</td>
<td>1-2 (least) 2350 337 14</td>
</tr>
</tbody>
</table>

Table 2. Analysis of two-way traffic (locust cervical connective)

(Gate pulse duration 800 μsec., gate pulse delay 200 μsec., duration of recording 60 sec.)

<table>
<thead>
<tr>
<th>Gain setting</th>
<th>$F_{UP}$</th>
<th>$F_{DOWN}$</th>
<th>$F'_{UP}$</th>
<th>$F'_{DOWN}$</th>
<th>Calculated (formulae 10 and 11)</th>
<th>Number of PAPS of each amplitude category</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>419</td>
<td>399</td>
<td>6</td>
<td>334</td>
<td>4 334</td>
<td>$&gt; 5$ 399 337 84</td>
</tr>
<tr>
<td>B</td>
<td>1363</td>
<td>1288</td>
<td>54</td>
<td>938</td>
<td>39 937</td>
<td>4-5 889 639 72</td>
</tr>
<tr>
<td>C</td>
<td>4302</td>
<td>3866</td>
<td>297</td>
<td>2979</td>
<td>148 2965</td>
<td>3-4 2578 2137 83</td>
</tr>
</tbody>
</table>
Propagation of action potentials

requires a fairly large number of repeated input sequences; it is therefore almost essential that the input data are stored on magnetic tape.

The magnetic tape recorder has proven to be the most critical link in the system; especially it is difficult to obtain tape systems which show no phase shift between their channels, which is essential for present purposes. A wide range FM instrumentation recorder (Precision Instruments Type 6200) has been found satisfactory. In this machine phase shift is caused only by tape skew, which is equivalent to less than 5 μsec. at its recording speed of 190 cm./sec. Further to eliminate phase shift, the input signals are filtered of frequencies less than 1 kcyc./sec. and greater than 10 kcyc./sec.

B. In many nerves the maximum possible spacing of the recording electrodes is less than the wavelength of the action potential: that is, the time taken for the action potential to traverse the distance between the electrodes is less than the duration of the action potential. When the gate opens at the passage of an impulse in the rejected direction of propagation, the tail of the original PAP is therefore still at the recording electrode, and results in a spurious count. To eliminate this effect, it is necessary that the action potentials be transformed into short pulses; this is accomplished by applying them to a Schmitt trigger circuit in both recording channels, and differentiating the resulting square wave, giving pulses of approximately 10 μsec. duration. Schmitt circuits differ slightly in their hysteresis characteristics, and provision has to be made for matching their outputs. In the block diagram (Fig. 2) this is accomplished by the control labelled SCHMITT A COMPENSATION.

C. It is convenient to provide the instrument with phase-splitting circuits to cope with inputs of either polarity, and to provide a series of monitor points (not shown on Fig. 2) for setting up the machine. These monitor points are all available at either of two sockets, selectable by rotary switches:

1, 2  Signal input, both channels.
3, 4  Input to Schmitt trigger, both channels.
5, 6  Output from Schmitt trigger, both channels.
7    Signal input to transmission gate.
8    Output from transmission gate.
9    Gate delay pulse.
10   Selector pulse.

In addition, the differentiated output of the Schmitt circuit triggering the selector pulse circuitry is available at a socket for triggering the CRO to display delay and selector pulses. (Fig. 2, TRIGGER CRO.)

The prototype instrument is constructed largely from Venner transistorized circuit modules, and costs less than £100, labour exclusive. The more sophisticated commercial counter/timers with facilities for 2 simultaneous inputs could be modified relatively easily to perform the same functions.

6. APPLICATIONS

A. The prime function of the apparatus is to discriminate direction of propagation in a mixed nerve. However, it lends itself to several other purposes.

B. The device will obviously function as a simple amplitude-selective counter.
Counts of pulses of intermediate amplitude can be achieved either by adding a coincidence circuit, thus transforming it into a kick-sorter, or by making two counts at successive amplitude thresholds and subtracting.

C. **Determination of conduction velocity.** The instrument enables a very precise measurement of the time taken to traverse the distance between the electrodes by the PAP. This is particularly obvious where the amplitude sensitivity is set to respond only to the largest spike in the input signal, but is also often seen where more units are involved, falling into different discrete groups with respect to conduction velocity (Fig. 3).

D. **Differential counting or display of nerve impulses of a stated conduction velocity.** As the selector pulse can be made just wide enough to include any given group of

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**Fig. 3.** Measurement and discrimination of conduction velocity. Electrodes A and B record from a nerve in which two axons are firing repetitively, one giving a large amplitude and one a small amplitude potential, both propagating from A to B.

A. CRO display of these potentials, Y₁ connected to electrode A, Y₂ to electrode B. Sweep triggered on output of Schmitt circuit in channel A. Note that the smaller amplitude PAP propagates at a slower conduction velocity, resulting in a larger delay before its appearance at electrode B.

B. CRO triggered as before; Y₁ displays the input to the transmission gate in channel A; Y₂ displays the selector pulse. \( t \) and \( t' \), which are proportional to the conduction velocity of the two axons, are read directly from the CRO, or from the calibrated delay setting required to bring a narrow selector pulse into coincidence with them; the selector pulse can be set to pass to the counter either or both populations of pulses.
Propagation of action potentials

conduction velocities, and can be positioned by the gate delay control to coincide with them, only this selected group is then passed on to the counter (Fig. 3).

E. The instrument allows one to discriminate between small action potentials, which propagate at a given velocity, and 'grass' or spurious input potentials of similar or identical waveform. Any input which regularly appears at the two electrodes with a delay acceptable in terms of conduction velocity is an action potential, whereas all other signals are probably not (but see section 4C above).

F. Finally, the gated-input pulse counter is useful in analysing the response of a nervous unit to a known stimulus. Thus Rowell & Horn (1967) used it to analyse the response of an optic neurone in the insect brain to a moving stimulus in the visual field. A selector pulse was produced by the movement of the stimulus object, of a length sufficient to include all evoked activity but no more. In this way the response of the unit could be accurately discriminated from its background activity. For this sort of application it is convenient if selector-pulse durations and delay durations are available up to, say, 10 sec.

SUMMARY

1. Activity is recorded in the nerve by two external electrodes spaced a known distance apart. Action potentials trigger 10 μsec. pulses in each channel. Those of one channel generate, after a variable delay, selector pulses which gate the output from the other channel, and this is applied to an electronic counter or other display.

2. As action potentials conduct at finite velocity, gate and delay times can be set to accept and count only impulses of a given range of conduction velocities propagating from the selector electrode to the counter electrode.

3. Reversal of input connexions allows discrimination of impulse traffic moving in the reverse direction. This is best done from a magnetic tape recording of the same activity; the recorder for this purpose must meet stringent requirements.

4. Simultaneous signals at both electrodes are rejected, distinguishing between action potentials and other potential changes, and thus allows retrieval of low amplitude nervous activity from recorded noise.

5. Other applications include selection of impulses of a given amplitude or conduction velocity, and the measurement of conduction velocities of a population of different nerve impulses.

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REFERENCES