J. Exp. Biol. (1963), 40, 15–21
With 5 text-figures
Printed in Great Britain

THE RELATION BETWEEN STIMULUS PARAMETERS AND CURRENT FLOW THROUGH STIMULATING ELECTRODES

By C. H. FRASER ROWELL
*Department of Zoology, Cambridge University**

(*Received* 19 *August* 1962)

INTRODUCTION

During electrical stimulation of the insect brain with chronically implanted electrodes, measurements have been made of the thresholds of behavioural responses. These thresholds are in terms of the voltage across, or the current through, the preparation as measured by the usual type of monitoring circuit.

When pulse length and amplitude are constant, the threshold commonly varies with the pulse repetition rate (p.r.r.) of rectangular stimulating pulses, especially over the

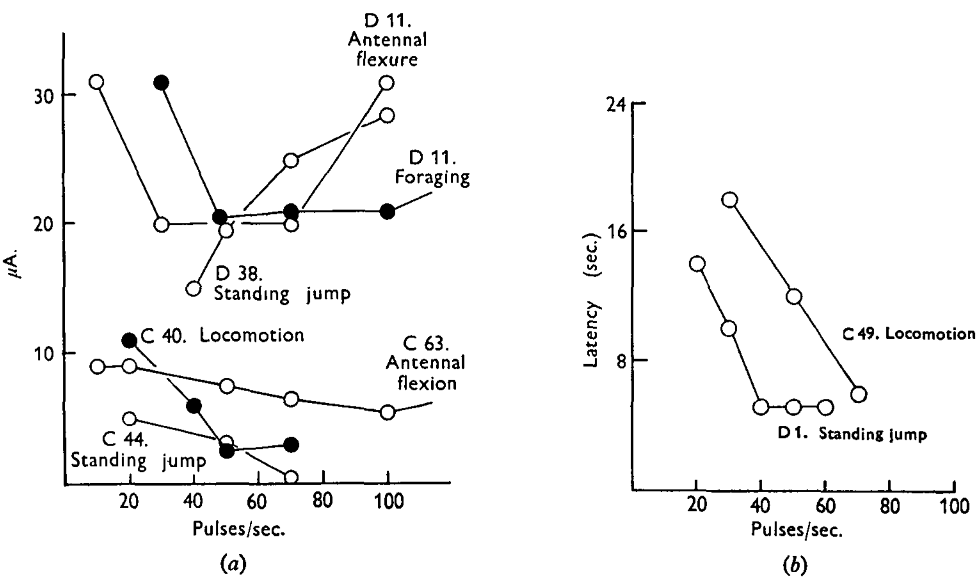

Fig. 1. Typical variations in thresholds of behavioural responses to electrical stimulation of the brain in *Schistocerca* with different pulse repetition rates. (*a*) Thresholds measured in terms of current through insect by a measuring circuit similar to that of Fig. 2, but without isolation units. (*b*) Thresholds measured in terms of latency of response. Stimulus parameters other than pulse repetition rate constant throughout.

range 10 to 100 pulses/sec. (Fig. 1.) Most frequently the threshold falls with increasing p.r.r.; responsiveness to one particular frequency also occurs, and also rising threshold with increasing p.r.r., though this last could easily be due to fatigue. Similar results have been reported by many previous workers with cerebral electrodes from both

* Present Address: Department of Zoology, Makerere University College, Kampala, Uganda.

vertebrate and invertebrate material, and have also been found in studies in which stimulation and recording from nerves took place through external hook electrodes.

An investigation was made to see whether this was necessarily a characteristic of the biological system, or whether artifacts were present in the form of changes in the physical conditions of stimulation. The results suggest that the latter is true.

METHOD

Negative rectangular pulses with a rise time of less than 10 μsec. and a duration of about 1 msec. were applied from a stimulator of low output impedance. The stimulator had a considerable capacitance to earth, and for critical measurements it was necessary to isolate it, not only from the preparation but from the whole measuring circuit, by

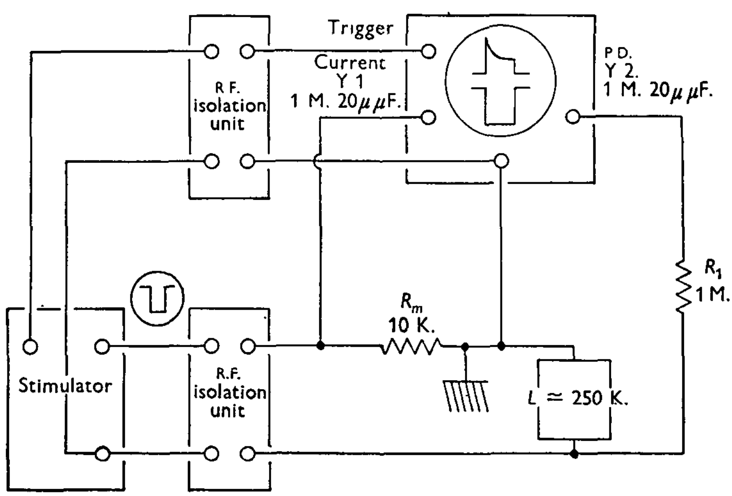

Fig. 2. Experimental arrangement required to measure true current through and potential difference across, a load L connected to a square wave stimulator. The 1 M. resistor R_1 increases the input resistance of the Y2 channel of the oscilloscope, decreasing the shunt across the load L. The oscilloscope is triggered before the stimulus in order that the initial part of the wave-form may be seen. Further explanation in text.

means of an R.F. head. The voltage across, and the current through, the load L were measured as shown in Fig. 2. The measuring resistor R_m must be small compared to the impedance of the load to avoid a significant reduction of the initial pulse voltage across the load. Component values were arranged so that the input impedances and capacitances of the oscilloscopes did not significantly affect the measurements. The load was (*a*) a passive RC network, (*b*) electrodes dipped in saline or water, or (*c*) an insect. In the last case the stimulating electrode was of insulated stainless-steel wire 20 μ in diameter, presenting a surface of approximately 3×10^{-4} mm.^{2}, and the indifferent electrode was of stainless-steel wire and of comparatively large surface, about 3 mm.^{2}. The electrode was implanted in the brain by a method to be described elsewhere (Rowell, unpublished.)

RESULTS

The current wave-form seen when a single pulse is applied to the brain is complex, and varies with the size of the electrodes and the impedance of the preparation, but the most important elements produced by the electrodes described are shown in Fig. 3. An identical wave-form is seen if the same electrodes are placed in a saline of the same impedance as the preparation. The wave-form closely resembles that produced by the passive network shown in Fig. 3. An exact circuit analogue would, however, be much more complex, as the observed wave-form shows non-linear change with almost every parameter, and it is probably unsafe to identify any parts of the analogue with parts of the actual system. The initial current spike, equivalent to the parallel capacitance C_p,

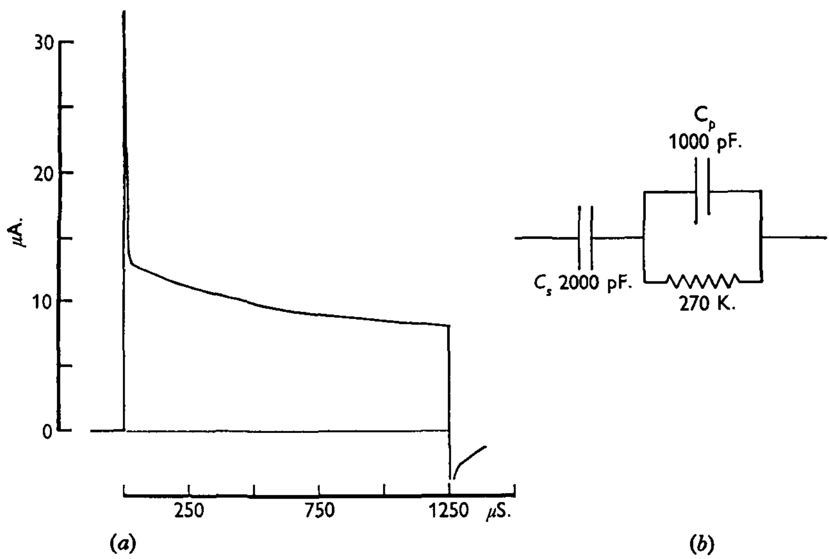

Fig. 3. (*a*) Current wave-form through a *Schistocerca* brain during a 1·25 msec., 3 V. pulse. (*b*) Analogue circuit giving a closely similar wave-form.

is usually neither high enough nor long enough to be physiologically effective, and the remaining wave-form during the pulse approximates to that of a series RC model (though not, of course, between pulses, as the series capacitance would imply no overall DC component). This agrees with the analogue circuit proposed by Sander & Yates (1953), for the polarization effect seen when similar pulses are applied to dilute electrolytes in electrolytic tanks.

The current wave-forms in saline and in the insect were examined during pulse trains. In all cases the effects observed in the insect were closely similar to those observed in saline of the same impedance. Change in current wave-form and amplitude occurs in two different circumstances; (*a*) cumulative change with successive pulses, and (*b*) change when the p.r.r. is altered. The current changes which follow change in p.r.r. are reversible.

The cumulative change (*a*) is irregular and often very large over the first few pulses, but thereafter there is usually a slow progressive rise in current. With the electrodes described, this effect was small except in very long pulse trains; for example, 5000 pulses at 100 pulses/sec. might produce a total rise of 10% in current.

The simplest of the changes following change in p.r.r. (*b*) is an increase in current flow with increase in p.r.r. This is the commonest result, but with different electrodes, etc., increase in p.r.r. can produce a decrease in current flow, or an irregularly variable one (Fig. 4). The differences can be up to 50% in current over the range 10–100 pulses/sec. There is also a further effect. As long as the total electrical energy applied in unit time (joules) does not exceed a certain value (which is different for different electrodes and impedances) the relationship between current and voltage across the preparation is more or less linear. If, however, this value is exceeded by increasing pulse length, amplitude, or p.r.r. beyond a certain point, the current flow rapidly increases, and usually regains another stable level two or three times the original. This 'runaway' value varies with electrode size, being about 1–2 μW sec. for the electrodes described, but about 5–6 μW. sec. with a larger indifferent electrode (Table 1). Frequently, alteration of p.r.r. crosses this value, causing large differences in current flow.

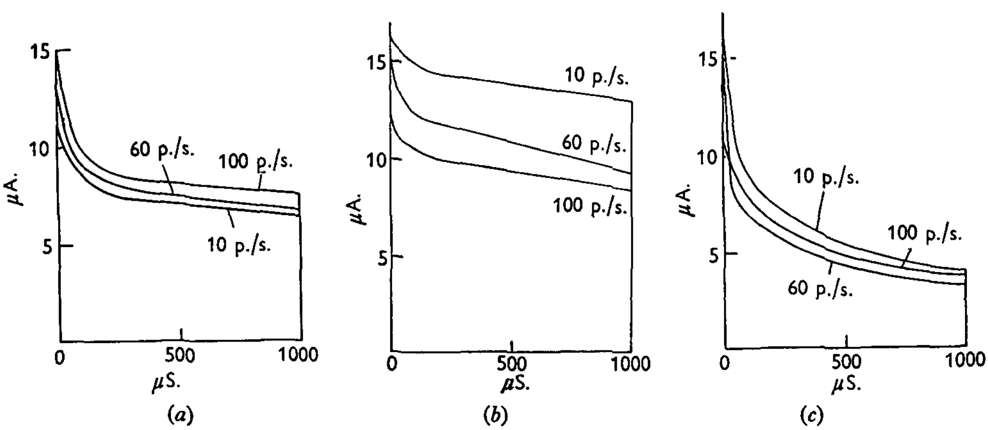

Fig. 4. Current wave-forms through three different electrode arrays in distilled water at 10, 60 and 100 pulses/sec. Negative electrode area 3×10^{-4} mm.^{2} throughout. (*a*) 2·5 V. pulse. Positive electrode area 3 mm.^{2}. Resistance ~300 K. (*b*) 4·5 V. pulse. Positive electrode area 3 cm.^{2}. Resistance ~300 K. (*c*) 2·0 V. pulse. Positive electrode area 3×10^{-4} mm.^{2}. Resistance ~300 K. Initial current spikes not shown.

Table 1. *Conditions producing current runaway at different pulse repetition rates*

p.r.r. (p./s.)	Terminal current (μA.)	Voltage (V.)	Power dissipated (μW. sec.)
100	15	5	4·5
60	18	6	6·5
50	21·5	7	7·3
35	23	8	6·4
25	26	10	6·3

1 msec. pulses throughout. 20 μ negative electrode, large positive electrode, in saline. Resistance between electrodes approx. 320 K.

Similar results were also obtained from current measurements made on two external silver-wire electrodes stimulating an insect abdominal nerve cord. Increase of p.r.r. commonly led to a higher current flow per pulse. It has recently been shown

(Fielden, unpublished) that p.r.r.-sensitive transmission occurs in such preparations with preganglionic stimulation and post-ganglionic recording; however, simultaneous preganglionic recording showed that in some, though not all, cases the higher p.r.r. merely excited more preganglionic fibres. This was probably due to an increase in effective pulse amplitude such as described above.

DISCUSSION

The physical causes of the changes in current flow which have been described above are not at all clear, though there are many factors which might be important. For example, the equivalent circuit of the preparation is a very complex array of series and parallel resistances and capacitances. The rectangular voltage pulses applied can be considered as a mixture of sine waves of varying frequency and phase, with limiting values set by the rise and fall times of the pulse, the pulse length and p.r.r. The transmission characteristics of the circuit with respect to each component would differ, and thus change in p.r.r. will cause a change in overall transmission. Rough calculations indicate that this change is unlikely to be significant.

The electrodes used are of course subject to polarization, and as the voltage across them exceeds the gas overvoltage, hydrogen and oxygen will be formed at the cathode and anode, respectively. Whether or not a gaseous phase exists is determined by very complex factors. The current density at the stimulating electrode tip is high, diffusion from the tip is probably poor, and the calculated spreading resistance of the tip is only of the order of one-tenth the total resistance of the preparation. Calculations by Dr J. N. Agar indicate that the conditions of stimulation are at just about the critical level for the nucleation of hydrogen bubbles at the cathode. If this in fact occurs, there will be marked effects on current flow.

The results demonstrate changes in current flow which are in turn dependent on the size and shape of the electrodes used. This in itself suggests that polarization effects at the electrode are significant factors. The reversibility of changes with p.r.r. suggests a dynamic equibilibrium in these effects. A possible explanation would involve the setting up of concentration gradients of dissolved O_2 and H_2 around the electrodes, facilitating or slowing down the electrolysis reaction, as in the use of oxygen-recording electrodes. The current flow through stimulating electrodes is certainly markedly affected by the concentration of dissolved gases. Fig. 5 shows that a given combination of electrodes and pulses allowed 25% more current flow per pulse in aerated than in boiled saline. It cannot therefore be doubted that the production of oxygen at the anode, whether or not it ever forms bubbles, can influence current flow.

If bubbles ever do become nucleated at the stimulating electrode, the volume of gas produced is likely to be large, even at what are considered low stimulating currents. 1 μC. is equivalent to more than 1×10^{-4} mm.^{3} H_2 at the cathode; thus a 10 sec. train of 1 msec., 10 μA. pulses at 100 pulses/sec. could produce a spherical bubble 100 μ in diameter if there was no diffusion. With the small currents used in insect work, the effect in practice allowing for diffusion must be unimportant or absent. With the much larger and more prolonged currents commonly used to stimulate mammalian brains, however, the results could be serious, and cause gross mechanical deformation of the tissue at the electrode tip. Assessment is difficult, as sine waves, paired alternate pulses, and other wave-forms specifically designed to minimize polarization are frequently used, and also the electrodes present a much greater surface. However, it is interesting to examine a typical and well-documented case. Roberts (1962) has described stimulation of the dorsomedial thalamus of cats with monopolar electrodes and unidirectional pulses. The parameters, and those of a typical locust experiment, are given in Table 2. The average current applied per unit area is virtually the same in both cases (cat, $3\cdot2 \times 10^{-3}$ A./mm.^{2}, locust, $2\cdot2 \times 10^{-3}$ A./mm.^{2}) which suggests that

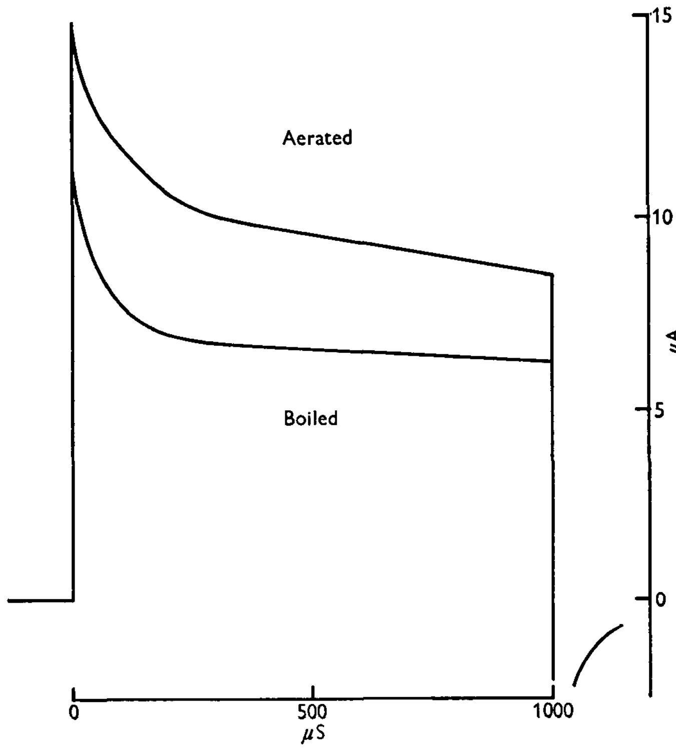

Fig. 5. Current wave-form through (*a*) aerated and (*b*) boiled distilled water during a 1·0 msec., 2·5 V. pulse. Negative electrode area 3×10^{-4} mm.^{2} positive electrode area 3 mm.^{2}. Both wave-forms photographed after several seconds at 100 pulses/sec. Initial current spikes not shown.

Table 2. *Comparison of typical stimulus parameters producing behavioural responses from cat and locust. Chronically implanted monopolar metal electrodes*

Subject	Diameter (μ)	Area (μ^2)	Pulse length (msec.)	Current	Voltage (V.)	P.r.r.	Coulombs/sec.
Cat (Roberts, 1962)	125	10,000	0·2	1·6 mA.	6·5	100/sec.	32×10^{-6}
Locust (Rowell unpublished)	25	465	1·0	20 μA	6·5	50/sec.	1×10^{-6}

the higher currents used in the cat are only a reflexion of the larger number of nervous units which have to be excited. The total quantity of electricity, on the other hand, is thirty-two times greater in the cat experiments. In 10 sec. of this sort of stimulation gas equivalent to a spherical bubble 300 μ across could be produced in the brain. Diffusion must be rapid if damage is to be avoided.

To conclude: the results show that change in p.r.r. produces change in amplitude of the pulse current, which normal monitoring methods do not detect; this causes complex changes in the conditions of stimulation and the effective area of stimulation. The p.r.r. sensitivity of any preparation may well be due to these changes, rather than to a characteristic of the biological system. Such sensitivity can only be thought biological if it can be demonstrated to be independent of these stimulation artifacts, either by accurate monitoring of the current changes, or by further physiological checks, such as pre- and post-synaptic recording.

When the object of an investigation is to explore the qualitative responses of the nervous system to electrical stimulation, these effects can be ignored. When, however, it is necessary to correlate accurately the consequences and parameters of electrical stimulation, the results show that the latter must be measured more accurately than has been usual. They also emphasize that the electrical coupling between extracellular stimulating electrodes and the excitable tissue is complex, especially when the stimulating electrode is embedded in ganglionic material, and that nothing more than correlation is possible without further research into the biophysical systems involved.

SUMMARY

1. The flow of current through a small metal electrode, such as is commonly used in stimulation experiments, has been studied. The wave-form resulting from rectangular voltage pulses has been examined with various electrodes, preparation impedances and analogue networks.

2. Variation of pulse repetition rate, when all other parameters are constant, causes wide variation of current flow and lesser variation of wave-form.

3. An attempt has been made to relate these changes to physical changes occurring at the electrode tip during polarization of the electrodes. Calculations indicate that the amount of gas generated by the currents commonly used in stimulation experiments would produce gross distortion of the tissues in the absence of diffusion, and may do so in practice when large currents are used.

4. It is concluded that changes of threshold with changes of pulse repetition rate are as likely to be due to the physical effects described here as to the physiological characteristics of the stimulated nervous system, to which they have sometimes been ascribed.

It is a pleasure to thank Dr D. Radley, of the Department of Engineering, Cambridge, for constant advice throughout this work, Dr J. A. Ramsay and Dr J. W. L. Beament for reading the manuscript and criticizing it, and Dr J. N. Agar for discussion and advice on the properties of polarizable electrodes. The work was undertaken during the tenure of a D.S.I.R. Research Fellowship.

REFERENCES

Roberts, W. W. (1962). Fear-like behaviour elicited from the dorsomedial thalamus of cat. *J. Comp. Physiol. Psychol.* 55, 191–7.

Rowell, C. H. F. (In preparation.) A method for chronically implanting stimulating electrodes into the brains of locusts, and some results of stimulation.

Sander, K. F. & Yates, J. G. (1953). The accurate mapping of electrical fields in an electrolytic tank. *Proc. Instu Elect. Engrs,* 100, 167–75.