EFFECTS OF DIRECT CURRENT ON MOTOR NEURONES

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Different biological structures show a typical activity when a direct current (d.c.) is applied to them. Direct current stimulation of the central ganglia in insects produces rhythmic movements of the wings (Fessard, 1936). Barron & Matthews (1936, 1938) found that a continuous response, of a frequency depending on the current intensity, was produced in the motor neurones by d.c. It is also known that a cathodal current causes a sudden rise of the excitability of the tissues to which it is applied (cata-electrotonus) as shown by Hill (1936a, b), Rosenberg (1937), Hodgkin (1937) on nerve. Katz (1939) was able to show facilitation of the transmission of the nerve-muscle preparation, applying a subthreshold cathodal current to the receptive region of the muscle fibres.

Taking into account these facts, the present work was done with the aim of studying the response of motor neurones to d.c. in more detail to see how the depolarization by d.c. changes the excitability and other properties of the neurones when stimulated via sensory nerve endings, and to see whether the effects are confined to those neurones traversed by the d.c.

METHOD

The experiments were carried out on frogs (*Rana temporaria* and *R. esculenta*). The spinal cord was exposed under ether and either the VIIth, VIIIth or IXth ventral root was freed up to its point of entry into the cord. The spinal cord was then transected at a high cervical level and the immobility of the animal secured by rigid fixation. Once the frog had recovered its excitability the chosen ventral root was raised into air or paraffin oil and set up on the electrodes for stimulation and recording (see Fig. 1). A pair of chlorided silver electrodes were used for leading off and another pair (Zn–ZnSO$_4$–Agar–cotton-wool) for stimulation by d.c. Condenser-coupled amplifiers and a double-beam cathode-ray oscilloscope were used for recording. The interdigital membrane of the foot of the frog (ipsilateral to the ventral root from which the recording was made) was stimulated either mechanically by a bristle driven by motor or electrically by condenser discharges at a constant frequency. The reflex activity of the spinal cord remains substantially constant for

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some hours if irrigated with oxygenated Ringer solution. The d.c. was delivered by a circuit similar to the one used by Barron & Matthews (1938). In addition, experiments were carried out with the dorsal skin-nerve preparation, to see if similar responses could be evoked from a peripheral receptor, the stimulation of a single end organ being effected mechanically with a fine bristle, attached to a lever with very small excursion. After fixing the skin on a rigid platform the nerve and skin were kept in a small moist chamber at room temperature (15–20° C). The placing of the electrodes on the sensory nerve was the same as that used on the motor roots which is shown in Fig. 1.
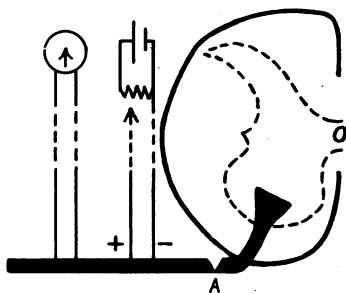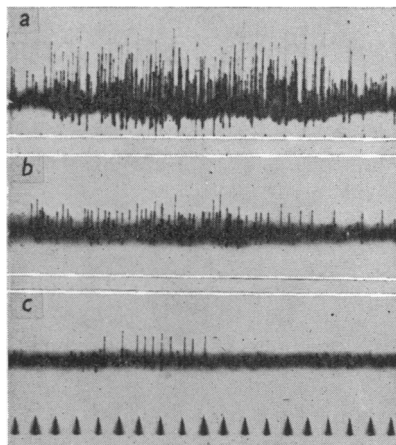

Fig. 1.

Fig. 2.

Fig. 1. Diagram to show placing of the electrodes for recording and stimulation of central structures by steady current flow. *A*: point at which the nerve has been subdivided to cut off all active fibres but one. Placing of the electrodes for the experiments on skin receptors was similar (cathode of the stimulating electrodes being towards the receptors).

Fig. 2. *a*, action potentials recorded from a ventral root in response to mechanical stimulation of the skin of the ipsilateral foot of the frog; *b*, recording from the same root after partial section close to the spinal cord; *c*, record after subdivision until a single fibre record was obtained. Time marker: 60 msec.

RESULTS

I. *Response of motor neurones to direct current*

Concerning the response of the motor neurones to d.c., the present results confirmed the findings of Barron & Matthews (1938) that the motor neurones response is proportional to the intensity of the current employed. The ventral root was subdivided to cut all fibres but one. This is controlled by observing the simplification of the response which occurs as dissection proceeds to the point where reflex stimulation via the skin gives a single rhythmic response (Fig. 2) and stimulation by d.c. gives also a simple rhythmic response. The neurones examined showed a decline in frequency of response to d.c. with time. This is shown in Fig. 5*b*.

II. *Localization in the spinal cord of direct current effects*

In order to find out how the current was spreading and the pathways taken by the d.c. applied to the motor roots, the following procedures were performed: (*a*) Simultaneous recording of the neighbouring ventral roots and the root where the d.c. is applied. Only in the latter was it possible to get propagated responses. (*b*) When a ventral root is subdivided into several
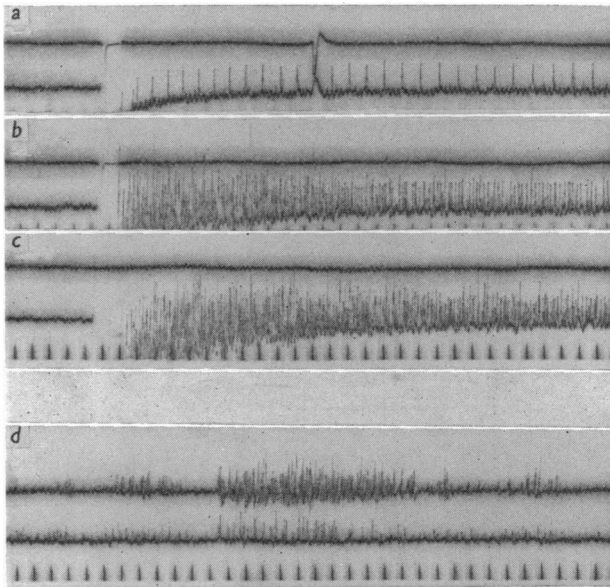

Fig. 3. Ventral root recording of action potentials from motor neurones provoked by direct current. Recording of two filaments of the same root. Direct current is applied on one of the filaments (lower records). *a*, 1 μA cathodal current; *b*, 2 μA; *c*, 3 μA. Although the frequency of the motor neurones discharge goes up when the intensity of the current is increased no propagated activity is seen in the other filament. *d*, mechanical stimulation of the skin of the ipsilateral foot. The response of motor neurones appears in both filaments showing that neurones and nerve fibres are in good physiological condition. Time marker: 60 msec. The stimulus artifact displaces the record. Deflexion in centre of (*a*) is also an artifact caused by dropping of Ringer.

filaments, and records are made from two of them at the same time, the action potentials are present only in the filament on which the stimulating electrodes are placed. If the intensity of the depolarizing current is increased, the frequency of the response goes up without showing propagated responses in the other filaments of the same root; that these are in good physiological condition, is shown by the fact that stimulation of the skin produces responses in many motor neurones and these are recorded in both filaments (Fig. 3). (*c*) If the distance of the stimulating electrodes from the spinal cord is varied,

keeping the intensity of the current constant, the frequency of discharge of the motor neurones is greater when the distance of the cathode from the spinal cord is shorter.

III. *Direct current on peripheral receptors*

In view of the many parallel properties of motor neurones and sensory nerve endings, the response of cutaneous nerve endings in the frog to the stimulation used above was tested. A cutaneous nerve was set up on two pairs of electrodes as in Fig. 1, on stimulation with d.c. a discharge of impulses is seen (Fig. 4)
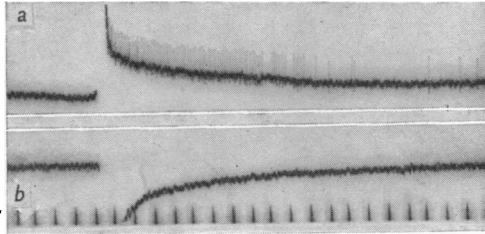

Fig. 4. *a*, response of a single end organ of the skin of the frog to direct current (cathodal, $2 \mu A$). The intensity of the direct current was decreased until only a single unit discharged; *b*, anodal current of the same intensity applied to the preparation evokes no response. Time marker: 60 msec.
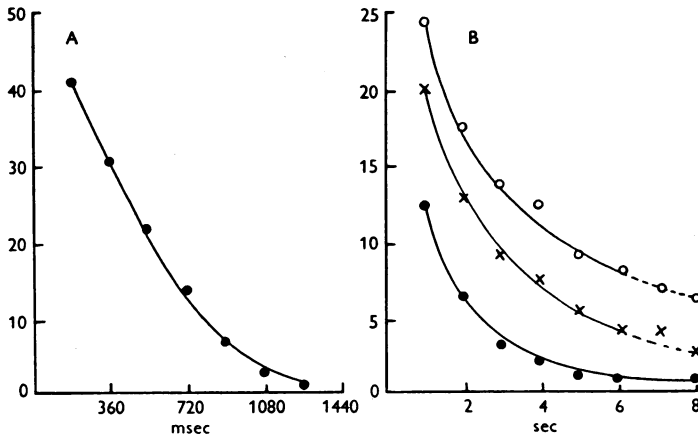

Fig. 5. A: adaptation curve of a skin receptor to a cathodal direct current ($2 \mu A$). Ordinate: frequency of discharge (n/180 msec). Abscissa: Time in msec. B: adaptation curves of a single motor neurone to different intensities of direct current. ●, $2 \mu A$; ×, $4 \mu A$; ○, $5 \mu A$. Ordinate: frequency of discharge (n/1 sec). Abscissa: Time in seconds.

and the frequency of such discharge increases with the intensity of the current used. If a single cutaneous receptor preparation is used and d.c. applied to its nerve, the frequency of discharge plotted against time (Fig. 5a) gives a curve resembling that of motor neurones but with a change of the time scale, the skin receptor adapting 7–8 times more rapidly than the motor neurone.

When a single end-organ preparation was stimulated mechanically (bristle) at a constant frequency before, during and after the application of a sub-threshold d.c. to the fibres coming from the cutaneous receptors, the response increased immediately when the current was switched on and fell to its previous value as soon as the current was switched off (Fig. 6).

As the d.c. interacts with the mechanical stimulation we must presume a common locus of action and suppose therefore that the d.c. takes effect on the nerve ending rather than provoking this discharge from the unspecialized parts of the nerve fibre.
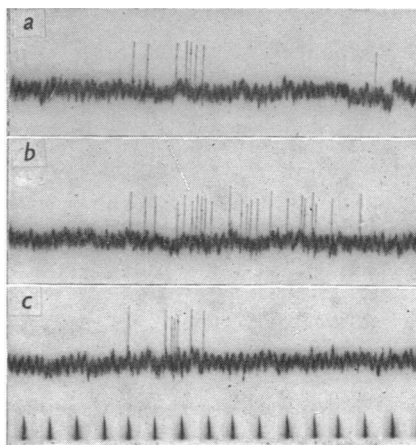

Fig. 6. Single end-organ response to mechanical stimulation. A fine bristle was applied at a constant frequency to the same spot on the skin of the frog. *a*, before; *b*, during; *c*, after application of direct current (cathodal, $2\,\mu$A), which alone provoked no response. Time marker: 60 msec.

IV. *Interaction of the effects of sensory stimulation and of direct current*

Adrian (1931), Eccles (1936) and others have demonstrated a relationship between the slow potential changes in ganglionic systems and propagated activity. A like relationship has been found in sense organs (Hartline & Graham, 1932; Bernhard, 1942; Katz, 1950) where a change which spreads up the fibres by electrotonic propagation precedes impulses discharge. Barron & Matthews (1938) have interpreted the slow potentials recorded in the spinal roots to indicate a depolarization of the motor neurones and find that these do not discharge before that depolarization reaches a certain value, and also that the rate of rhythmic response is related to the magnitude of the depolarization; this has been confirmed by Bremer (1941), and also by intra-cellular recording by Brock, Coombs & Eccles (1952), and Alanis & Matthews (1952).

It is possible to test whether interaction occurs between the normal depolarization produced by sensory impulses and that resulting from applying d.c. to the axons of the motor neurones. The latter may be expected to spread by electrotonus to the neurones in the same way that their own depolarization appears to spread to the ventral roots.

The interaction of sensory stimulation and the effects of d.c. are seen in Fig. 7. The skin of the foot was stimulated repeatedly at a constant frequency while recording the resulting response of the motor neurones appearing in the ventral root. When the sensory stimuli no longer evoked action potentials in
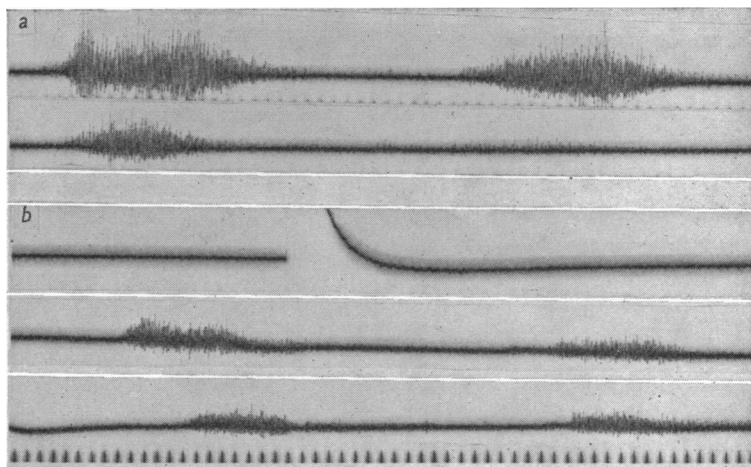

Fig. 7. *a*; action potentials recorded from a motor root evoked by a mechanical stimulus applied at a frequency of 1/sec to the skin of the foot of the frog. The responses produced decrease progressively in frequency and amplitude and finally no response is seen. *b*; cathodal current (1 μA) is applied to the same motor root and the reflex response reappears. A direct current of the same intensity used alone did not produce any response of the motor neurones. Time marker: 60 msec.

the motor neurones (Fig. 7 *a*), the application of subthreshold d.c. which had been previously set below the level at which it evoked a response, made the reflex response reappear (Fig. 7 *b*) after a variable latency.

Facilitation to the peripheral stimuli takes place both when the d.c. applied is subthreshold or threshold for provoking responses by itself, but the time for its appearance changes and the latency is longer the lower the intensity of the d.c. The latency is also shortened when the d.c. is applied a second time within 3–8 sec of a previous application. On a few occasions it was possible to observe the summation of peripheral stimuli subthreshold for reflex response with subthreshold d.c., the two resulting in a discharge of impulses by the motor neurones. Often the facilitation disappears while the current is on, but some-times it is possible to see facilitation lasting 1 to 2 sec after the cathodal

current is off. The discharges recorded during the facilitation have the same temporal course as the ones recorded without the d.c.

An anodal current of the same intensity produced no facilitation, and a few cases appeared to reduce the reflex response, but it has not been possible to control whether this was due to anodal block of conduction.

When the root is subdivided as described in § II, the facilitation is only observed in the filament that is being stimulated and does not appear in the other filaments of the same root.

DISCUSSION

The experiments described lead to the conclusion that the effect of the direct current is exerted on the central parts of the motor neurones and not on the nerve fibres, which can under some conditions give repeated impulses to a d.c. stimulus (Skoglund, 1942), for the following reasons: (1) The d.c. does not provoke any response of the motor neurones of the neighbouring roots nor in the other filaments of the same root, with the intensity of the d.c. used. (2) For a given current the frequency of the action potentials coming from the motor neurones is in inverse relation with the distance of the cathode of the stimulating electrodes from the spinal cord. (3) The facilitation to sensory stimulation is observed only in the motor neurones corresponding to the root where the d.c. is applied. (4) When the ventral root is progressively severed central to the stimulating electrodes the total discharge of action potentials recorded diminishes in proportion to the extent of the cut. (5) When the root has been separated completely by cutting all the fibres the propagated impulses cannot be evoked by the values of d.c. used, which can therefore only provoke the discharge seen when the central structures are in continuity with the fibres.

It must be concluded that an appreciable fraction of the current entering the axons travels in them up the root and leaves by the cell bodies and dendrites. The central spread of current is then by electrotonus and this clearly agrees with (2) for electrotonic potentials fall off exponentially, and so for a constant intensity of d.c. we should expect the effect to be reduced as the cathode is moved away from the spinal cord as is found to be the case.

The fact that repeated peripheral stimuli become ineffective for evoking response of the motor neurones but that the response reappears as result of a further depolarization of the motor neurones when the direct current is applied to them, raised the question of what factors are responsible for the failure of the response. It is clear that adaptation of the peripheral receptors to repeated stimuli does not occur in a way that can account for this (Cattell & Hoagland, 1931). The irresponsiveness of the motor neurones might be due to a failure of impulses to reach them or to their own depressed excitability. The

time relations make it impossible for the normal excitability cycle following the absolute refractory periods to be the cause of this (Erlanger & Gasser, 1937).

The results given above show that the motor neurones adapt to a constant current 7–8 times more slowly than nerve endings in the skin and if we are right in regarding this depolarization as acting in the same way as the depolarization seen before their reflex discharge, their irresponsiveness might be due to adaptation of the motor neurones to the prolonged depolarization produced by sensory impulses. The application of d.c. leads to an immediate short-lasting increase in the responsiveness of the motor neurones and also to a later slowly developing rise in the discharge which they give. There thus appear to be two facilitatory processes, the first acting immediately and being a subthreshold manifestation of the process giving discharge to large values of d.c. acting alone. This can perhaps be attributed to the cathodal polarization of the neurone membrane and leads to facilitation as described above and by Renshaw (1940) and Bremer (1941).

The second has a quite different rate of development and decline; it could either be attributed to the effect of the current on other parts of the neurone, e.g. dendrites, or perhaps it is more probable that it is due to the current taking effect on interneurones, but if this is so its effect must be restricted to the interneurones synapsing with the motor cells through which the current is passed, for the facilitation of the discharge is only seen in these.

Facilitating effects of previous activity lasting many seconds have been described in the spinal cord by Sherrington & Sowton (1915), Ranson & Hinsey (1930) and many others. One hypothesis suggested to account for the persistence of facilitation is that continuing activity might occur in chains of neurones, 'reverberating circuits' (Ranson & Hinsey, 1930) or 'internuncial neurones' (Lorente de Nó, 1938) set into action by a stimulus. But the present results appear to be much more in accord with the earlier concept of 'central excitatory state' acting on responsive structures. Central excitatory state may be regarded as comprising the physico-chemical changes resulting from the arrival of sensory impulses which lead to the observed depolarization of the motor neurones. The close relationship described above between the latency for full facilitation to appear, and the intensity of the depolarizing current, and also the observation that this latency is shortened when a second depolarization is applied a few seconds after the first one, suggest that, during this time, the excitatory state is becoming threshold for the new condition of the neurones. The slow building up of that excitatory state is regarded as the cause of gradual increase of the frequency and number of motor neurones discharging. Barron & Matthews (1938) suggested that we may regard '...the secondary neurone as a sense organ responding to the level and rate of change of the central excitatory state in its immediate vicinity rather than as

a structure simply conducting on the impulses arriving by the primary neurones'; and the present observations appear capable of simple interpretation in these terms.

SUMMARY

1. Direct current (d.c.) applied to a ventral root of the spinal frog spreads by electrotonus and affects only the neurones that supply that root.

2. Adaptation curves of the responses of a single motor neurone and a single end-organ (skin receptor) to the same d.c. stimulus are compared and found to be similar apart from their time scales, the motor neurone adapting 7–8 times more slowly than the skin receptor.

3. When d.c. is applied to a cutaneous nerve, the response of the skin receptors is proportional to the intensity of the current.

4. A subthreshold d.c. applied to a single end-organ through its nerve fibre causes facilitation of the skin receptor response to a mechanical stimulus, shown by increasing the frequency of its discharge. The facilitation appears without any appreciable delay and falls as soon as the current is switched off.

5. Threshold and subthreshold d.c. applied to a ventral root facilitate the response of the motor neurones when stimulated via sensory organs (skin receptors). The facilitation of the sensory impulses take place after a variable latency and the duration of this latency is in inverse relation to the intensity of the depolarizing current and it is shortened by a second depolarization applied a few seconds after the first one is off.

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