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PROPERTIES OF SPINAL INTERNEURONES

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Several studies of internuncial neurones (interneurones) in the spinal cord have been made. Lloyd (1942) recorded discharge in such cells related to long spinal reflex activity, and Renshaw (1946) studied the activity of interneurones activated by antidromic impulse conduction in motoneurones. After the introduction of the technique of intracellular recording by micropipettes, Woodbury & Patton (1952), Eccles, Fatt & Koketsu (1954), Frank & Fuortes (1955), and Suda, Koizumi & Brooks (1958) have made observations on interneurones as well as other elements within the cord. The most systematic studies of unitary responses of interneurones have been those of Frank & Fuortes (1956*a*), Kolmodin (1957) and Haapanen, Kolmodin & Skoglund (1958).

From the above studies certain general features of interneurones have been characterized. Among these are the frequent occurrence of repetitive discharge to single afferent volleys, the presence in many units of spontaneous or background discharge and the frequent occurrence of convergence from multiple afferent sources on individual interneurones. Frank & Fuortes (1956*a*) emphasized the considerable variation in behaviour among different interneurones and the fact that information about them is limited, although they were able to establish certain general features of their response patterns. Both Frank & Fuortes (1956*a*) and Haapanen *et al.* (1958) described certain characteristic features of membrane and action potentials of interneurones recorded by intracellular micropipettes. Both groups described responses to nerve volleys and to natural stimulation. Kolmodin's study (1957) was concerned principally with the responses of interneurones to stretch-evoked afferent discharge from muscle. Eccles *et al.* (1954) investigated principally the activity of interneurones activated by collaterals of motoneurones.

The present study is concerned with further characterization of the properties and functional organization of interneurones in the lumbosacral cord

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of the cat. This paper is devoted principally to the general properties of such cells; subsequent papers deal with response patterns to afferent input from various sources and the responses to natural stimuli.

METHODS

Adult spinal cats were used, acutely decapitated under preliminary ether anaesthesia (as described by Hunt & Paintal, 1958). After lumbosacral laminectomy and limb dissection, exposed tissues were covered by pools of paraffin oil equilibrated with O_2 95% + CO_2 5%. Body temperature was usually maintained between 36 and 38° C. For intracellular recording glass micropipettes filled with 3M-KCl were used. Relatively high-resistance electrodes were found necessary to minimize

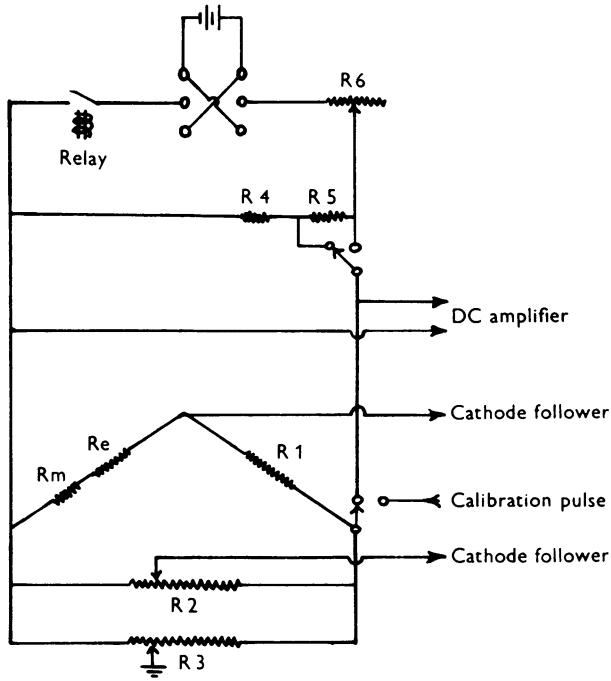

Fig. 1. Schematic view of recording and stimulating circuit. R1 = 500 MΩ, R2 = 10 kΩ, R3 = 10 kΩ, R4 = 100 Ω, R5 = 1000 Ω, R6 = 10 kΩ, Re = electrode resistance, Rm = membrane resistance. Relay is Clare mercury wetted type.

damage to interneurones. Satisfactory electrodes usually had resistances in excess of 30 MΩ. These were connected by a cathode-follower probe, using an electrometer tube (Raytheon 5886) leading to a modified transistor d.c. amplifier of the type described by MacNichol & Bickart (1958).

In many experiments utilizing intracellular recording, the same electrode was used for passing current through the cell and for recording. For this purpose a modification of Araki & Otani's (1955) bridge circuit was used. The final version of the circuit used is shown in the schematic diagram of Fig. 1. This system permitted measurement of electrode resistance, an approximation of the effective recording time constant and correction thereof by capacitative feed back, and the passage of current through the cell. The time constant, when corrected, was less than 50 μsec.

Electrode resistance was estimated from the magnitude of the recorded signal when a known calibrating pulse was introduced. The effective recording time constant was corrected by increasing capacitative feed back until the recorded response to a rectangular calibrating pulse approximated the original wave form. Estimation of current passed through the micro-electrode was made by measurement of the voltage applied across the bridge through the known (500 MΩ) grid leak resistance and estimated electrode resistance.

Spinal interneurones appear to be much more easily damaged by movement relative to the micro-electrode tip than do motoneurones. Hence prevention of movement is a prime problem. Muscle contraction was avoided by administration of tubocurarine or occasionally of Flaxedil (gallamine triethiodide; May and Baker). Spinal cord fixation was achieved by paired pins in the ilia, a dorsal spinous process clamp just above the laminectomy site, and by two pairs of rigid steel angulated members grasping the vertebral bodies near the recording site. The array of fixing devices was attached to a heavy aluminium base which also held the micromanipulator. Respiratory movements were minimized by using artificial ventilation by a Palmer Ideal pump at rapid rate (44 min) and low tidal volume (30–40 ml.). In most experiments bilateral pneumothorax was also employed.

Recording sites were known only approximately from the point of entry of the micro-electrode into the cord, the direction of the electrode track and the depth to which the electrode was inserted by the micromanipulator. No attempt was made to locate electrode tracks by histological methods. The majority of units were in segments L7–S1.

RESULTS

Criteria for identification

Several types of neural elements are encountered by a micro-electrode inserted into the spinal cord. Primary afferent fibres may be identified by single impulses evoked by stimuli to a particular peripheral nerve, when the latency can be clearly accounted for by conduction time. Identification is aided, when the peripheral nerve is intact, by discharge patterns characteristic of peripheral receptors. Motoneurone somata were identified on the basis of inflexion on the rising phase of the spike, post-spike hyperpolarization, synaptic potentials, and limited capacity to respond to repetitive antidromic stimulation. The latter was associated with separation of the impulse into two distinct components (Brock, Coombs & Eccles, 1953; Frank & Fuortes, 1955). Motor axons were distinguished by impulses of singular configuration evoked by antidromic stimulation, a capacity to respond to such stimulation at high frequencies, lack of prominent post-spike hyperpolarization, and synaptic potentials of much smaller amplitude than in motoneurone somata.

Most of the remaining units were classed as interneurones. In general, the latter could be easily distinguished from the other types of neurones mentioned by their repetitive response to volleys in more than one peripheral nerve (convergence) and their lack of a directly conducted response to antidromic stimulation of ventral root fibres. Our criteria for identification were generally similar to those used by Frank & Fuortes (1955). An attempt was also made to distinguish between recording sites in interneurone somata and the axons (see below, p. 349).

When glass micropipettes were used, elements were encountered which showed steady membrane potentials but no response to nerve volleys. Frank & Fuortes (1955) reported similar findings. In some cases direct stimulation via the same micropipette as that used for recording evoked an impulse. In others direct stimulation failed to produce a spike. The latter may have been non-neural elements. Non-responsive elements of both types were classed as unidentified.

Recording from interneurones

Recording from interneurones has been more difficult than from motoneurones. Many units were impaled only transiently as a micropipette was advanced into the cord, the abrupt appearance of a resting membrane potential being followed by a series of injury discharges and rapid decline of the membrane potential. Relatively few units could be held for long periods with stable membrane potentials. With KCl-filled micropipettes variations in potential were seen during advance along a track, some of which were abrupt and clearly associated with entry into a cell. However, slower variations, usually 10–20 mV in magnitude, were often observed without evidence of cell penetration but related to movement of the micropipette. Such potential changes probably result from alterations in junctional potential and were associated with measurable changes in electrode resistance (cf. del Castillo & Katz, 1955; Frank & Fuortes, 1955). Potentials occurring with apparent cell impalement varied widely in magnitude from about 20 mV to nearly 100 mV but were usually in the range of 40–60 mV. In view of the difficulties in impaling interneurones without obvious damage and the variations in potential due to the micropipettes themselves, no reliable estimate can be given as to the normal level of membrane potential in interneurones (cf. Frank & Fuortes, 1955; Haapanen *et al.* 1958).

Spike potentials recorded with KCl-filled micropipettes showed considerable variation. On approaching a cell spike potentials could often be recorded in the absence of a recorded membrane potential. These were usually diphasic in positive–negative sequence (cf. Haapanen *et al.* 1958), although at times impulses of simple monophasic positive configuration were seen. After impalement spikes appeared as positive deflexions. In most units spike amplitude approached or exceeded the size of the recorded resting membrane potential. However, certain interneurones showed spike potentials far smaller than the resting membrane potential, often with a relatively slow falling phase of approximately exponential form. In such cases the membrane in the region of the micro-electrode, because of injury, apparently does not undergo the active regenerative changes associated with the impulse, but the spike is recorded electrotonically at some distance.

An attempt has been made to distinguish between recording sites in interneurone somata and axons. Recordings in which synaptic potentials a few

millivolts or larger were observed were considered to be from interneurone somata. In contrast, recordings which displayed very small synaptic potentials or no detectable synaptic potentials were considered to be from interneurone axons (Fig. 2). With the high-resistance micro-electrodes used in the present study, primary afferent fibres, whose conduction velocities indi-

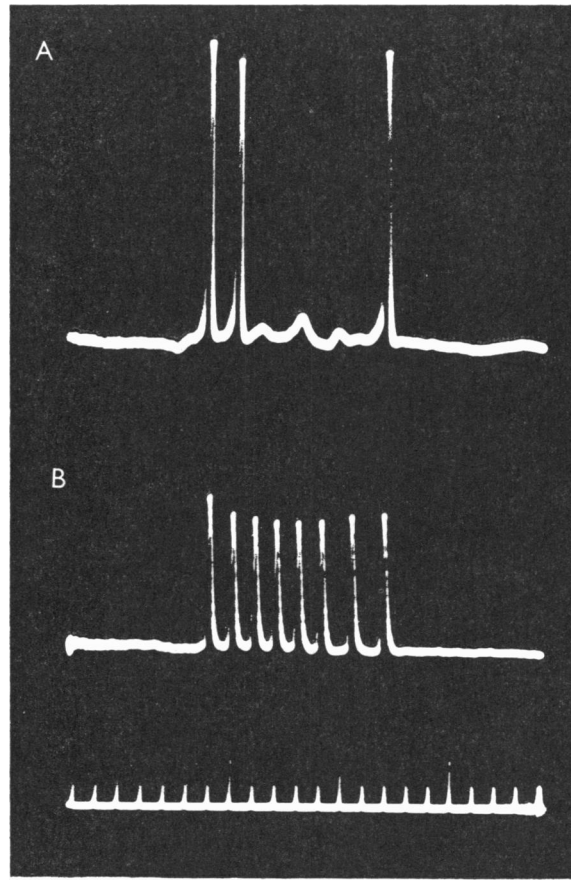

Fig. 2. A, recording from site considered to be interneurone soma; response to volley in plantar nerve. Note synaptic potentials; spike potential 77 mV. B, recording from site presumed to be interneurone axon; response to deep peroneal nerve volley; spike potential 56 mV. Note lack of detectable synaptic potentials. Time marker, 1 msec.

cated diameters measurably less than 10 μ, have been impaled. Therefore it is thought that there is no *a priori* reason why interneurone axons should not have been impaled with the same sized electrodes. Haapanen *et al.* (1958) suggested that they were unable to impale primary afferent fibres smaller than 10 μ in diameter and hence they doubted whether interneurone axons

could be impaled. However, their evidence in this regard was not critical. Unlike motoneurone somata, interneurone somata show no conspicuous 'negative after-potential' and no prolonged after-hyperpolarization. However, a brief post-spike 'positivity' was frequently seen, similar to that observed in squid axon (Hodgkin & Katz, 1949), which may be a consequence of an increased potassium conductance following the spike in cells with a resting potential less than the potassium equilibrium potential. When repetitive response was initiated in interneurone somata the brief post-spike positivity that was frequently observed was followed by a slow depolarization leading to the next impulse. Such a slow depolarization was also seen in interneurones discharging spontaneously. In contrast, spike potentials recorded from interneurone axons usually rose from the base line without detectable synaptic

TABLE 1. Spike duration of spinal neurones, directly stimulated (msec)

	Interneurone		Motoneurone		
	Soma	Axon	Soma	Axon	Primary afferent fibre
	0·65	0·65	1·11	0·52	0·37
	0·38	0·30	0·88	0·47	0·45
	0·38	0·50	0·99	0·44	0·37
	0·67	0·50	1·06	0·48	0·39
	1·00	0·36	0·72	0·69	0·50
	0·39	0·41	0·98	0·65	0·42
	0·48	0·72	1·20	0·61	0·47
	0·39	0·35	—	—	—
	0·28	0·34	—	—	—
Mean	0·51	0·46	0·99	0·55	0·42

potential and returned to the previous potential level without post-spike positivity. When presumably being recorded from interneurone somata, spike potentials have rarely shown an inflexion in the rising phase even in the case of high-frequency discharge and only on one occasion was it possible to separate the spike into two components by repetitive stimulation, as can be done in motoneurones (Brock *et al.* 1953; Frank & Fuortes, 1955). In some units, particularly when deterioration was evident, small spike potentials were seen, sometimes of two or three discrete amplitudes. In some cases spikes of two distinct sizes could be separately evoked by stimulation of two distinct peripheral sources and when coincident the spike amplitudes summed. In such cases it is presumed that the small spikes are recorded electrotonically from a distance, perhaps from different parts of the cell.

Absolute values of spike potential amplitude cannot be given with assurance, in view of the uncertainty of resting membrane potential values. In some units overshoot during the spike was observed, in others not. Frank & Fuortes (1955) and Haapanen *et al.* (1958) reported similar findings. The latter authors noted overshoot in 10% of the interneurones examined. Table 1 presents representative findings on spike duration in interneurone somata and axons

and in motoneurone somata and axons. No clear difference has been seen between the duration of spikes from recording sites considered to be in somata and those considered to be in interneurone axons. On the other hand, the spike duration of the interneurone soma appears to be appreciably shorter

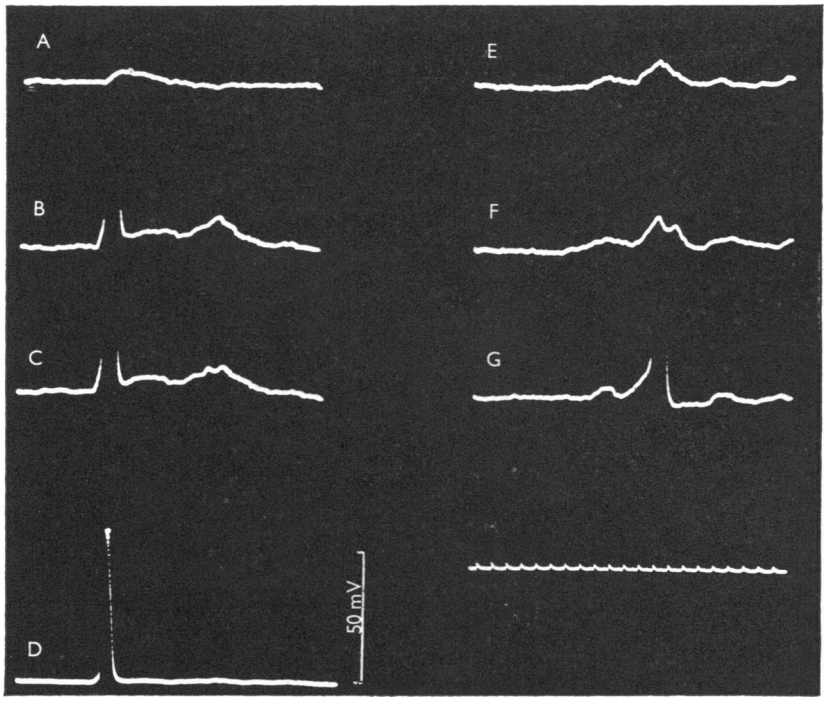

Fig. 3. Synaptic potentials of interneurone soma. A–D are responses to deep peroneal volleys; stimulus strength increased from A to C; D same strength as in C. E–G are responses to sural volleys, stimulus strength increased from E to G. Amplification in A–C and E–G was 5 times that in D; stimuli at beginning of sweeps; time marker, 1 msec.

than that of the motoneurone soma. Our results differ from those of Frank & Fuortes (1955), who considered the spike duration of impulses from interneurone somata to be longer (*ca.* 1 msec) than those from interneurone axons (0·3 msec).

Synaptic potentials

Figure 3 illustrates some features of synaptic potentials recorded from an interneurone soma. In A–D the responses to volleys in the deep peroneal nerve are shown (A–C being at 5 times the amplification of D). Stimulus strength was successively increased from A to C. In A an excitatory synaptic potential is evoked which is subthreshold for initiation of an impulse; this has a fairly simple wave form and decays to half amplitude in about 2·4 msec.

However, the excitatory synaptic potential was usually more complex (as in E and F) and showed components which decayed more rapidly. Although in the latter records contamination by inhibitory actions cannot be excluded, it seems likely that the synaptic potentials shown result from prolonged synaptic action, probably due to summation of temporally dispersed synaptic events. In view of the effective time constant of the interneurone soma (see below), the rate of decay of the synaptic potential shown in A is much slower than would be expected from passive decay of membrane potential change to a brief synaptic action. It has not been possible to record excitatory synaptic potentials considered to result from brief synchronous presynaptic actions as can be done in motoneurones. Even with weak afferent volleys measurable excitatory synaptic potentials were obviously compounded of temporally dispersed events.

In Fig. 3 B and C the excitatory synaptic potential evoked an impulse. This was followed by a maintained synaptic potential which failed to evoke another spike although the level of depolarization associated with the initiation of the first impulse was exceeded. A large and prolonged post-spike hyperpolarization that is so conspicuous a feature of motoneurone somata is obviously lacking in records from interneurone somata. Impulses initiated by direct stimulation were also not followed by prolonged hyperpolarization (see below, p. 361). The threshold for initiation of an impulse in the interneurone of Fig. 3 occurred when the cell was depolarized from 2·5 to 4 mV by the synaptic potential. The level of depolarization by the synaptic potential which was associated with impulse initiation ranged from 2·5 to 10·9 mV, the average value being close to that found in association with impulse initiation in motoneurone somata (Brock, Coombs & Eccles, 1952). In addition to the excitatory synaptic potentials already considered, synaptic potentials characterized by an increase in membrane potential and inhibition of interneurone responses were also observed, but these have not been systematically studied. Haapanen *et al.* (1958) have observed synaptic potentials in interneurones, both excitatory and inhibitory, and associated with depolarization and hyperpolarization respectively. They have studied in particular the synaptic potentials and firing levels in response to natural stimulation.

Responses to current pulses

Using the modified bridge circuit of Araki & Otani (1955) described in Methods, anodal or cathodal current pulses were passed through the same electrode as was used for recording. Measurements were made of the strength–latency relations by recording the latency to onset of spike potential and the current intensity. The time constant was derived from the following equation:

$$I_0/I = 1 - e^{-t/\tau}$$

where I_0 is rheobasic current, I current, t latency, and τ the time constant

(Frank & Fuortes, 1956*b*). Figure 4 shows an example of recordings from an interneurone soma used for strength–latency measurements, and Fig. 5 a plot of the relation for this unit. Table 2 presents findings on the time constant of interneurones and other units encountered in the spinal cord. The time constant of interneurone axons was brief and comparable to that of primary afferent fibres and motor axons. The average time constant of interneurone

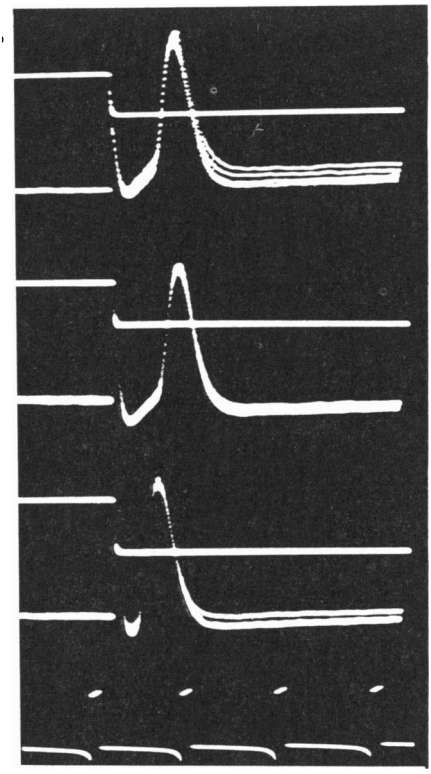

Fig. 4. Strength–latency measurements by direct stimulation of interneurone soma at three current strengths. Upper beam, current; lower beam, micro-electrode recording; time marker, 1 msec.

TABLE 2. Time constant (msec)

Interneurone		Motoneurone		
Soma	Axon	Soma	Axon	Primary afferent fibre
0·80	0·14	0·66	0·28	0·25
0·48	0·40	0·53	0·27	0·20
0·69	0·19	0·74	0·22	0·13
0·42	0·71	0·71	0·27	0·29
0·27	0·29	0·59	0·42	0·24
0·28	0·19	0·57	0·39	0·35
0·26	0·20	1·48	0·42	0·34
—	0·21	—	0·25	—
Mean 0·46	0·29	0·75	0·31	0·26

somata was longer than that of interneurone axons but shorter than that of motoneurone somata, but the data are too limited to have statistical significance. Since the only criterion for identification of recording site as interneurone soma was the presence of synaptic potentials of some magnitude,

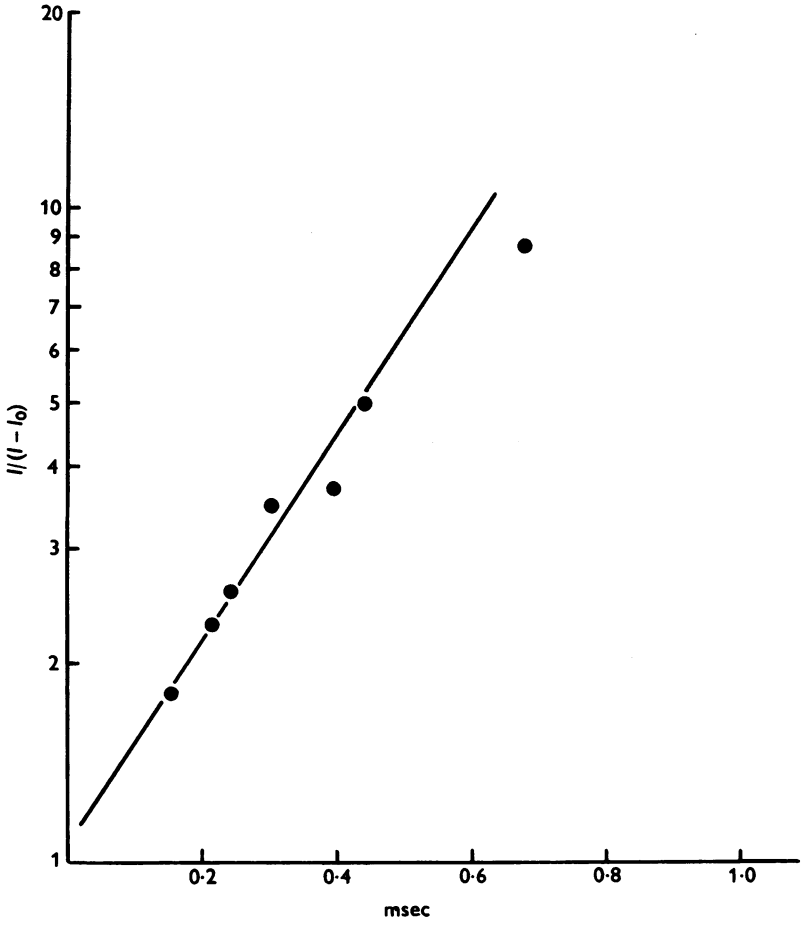

Fig. 5. Plot of relation between $I(I-I_0)$ and latency for unit of Fig. 4, where I = current, I_0 = rheobasic current. Semi-log. scale

some of the recording sites classified as interneurone somata might have been from interneurone axons in close proximity to the cell body. For this reason, and because of the comparatively small number of observations, one cannot be certain that there is a significant difference between the time constant of an interneurone soma and that of a motoneurone soma. Average rheobasic current for interneurone somata was $2{\cdot}5 \times 10^{-9}$ A.

The high resistance of electrodes needed to record from interneurones and

the variation of electrode resistance with movement prevented measurement of the time constant in the direct manner employed by Araki & Otani (1955) for toad motoneurones, that is, by the time course of membrane potential change following the application or withdrawal of current. A further difficulty in the use of this technique was the presence of a considerable capacitative artifact when the bridge was resistively balanced.

Interneurone somata and axons responded to current pulses of long duration and sufficient intensity by discharging repetitively. Figure 6 illustrates

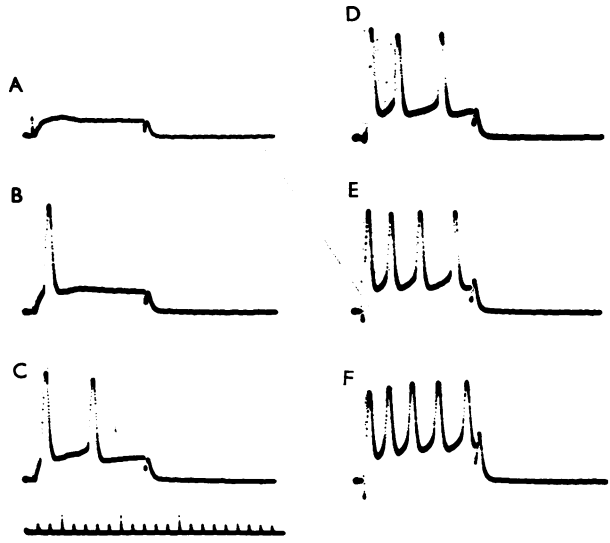

Fig. 6. Responses of interneurone soma to current pulses (9·2 msec duration). Current intensity increased stepwise from A to F. Time marker, 1 msec.

the response of an interneurone soma to current pulses of 9·2 msec duration, with current intensity increasing from A to F. In A the current is subthreshold for initiation of a spike, whereas in B a single impulse is initiated. Further increase in current strength (C–F) decreased the latency of the first impulse and caused repetitive discharge. In F the maximal frequency reaches approximately 650/sec. It may be noted that the brief post-spike hyperpolarization, relative to the firing level, was followed by a slow depolarization leading to the next impulse (see especially D). The slope of the slow depolarization was the greater the shorter the impulse interval. The impulse frequency was highest between the first and second impulses and decreased slightly thereafter (F). Figure 7 shows the relationship between frequency of discharge of an interneurone soma during passage of an outward current pulse and the time after the first impulse. It may be noted that the impulse frequency decreases for successive impulse intervals. However, adaptation to prolonged current pulses was not great and maintained direct current was capable of

eliciting a continued high-frequency discharge for many minutes. Interneurone somata showed a greater tendency to repetitive discharge than did primary afferent fibres or motor axons. Motoneurone somata showed the least tendency to discharge repetitively, and even strong current pulses evoked only a low-frequency discharge. Figure 8 compares the repetitive discharges initiated in an interneurone soma and in a motor axon. In this plot the number of impulses initiated during a 14 msec current pulse is shown as a function of applied

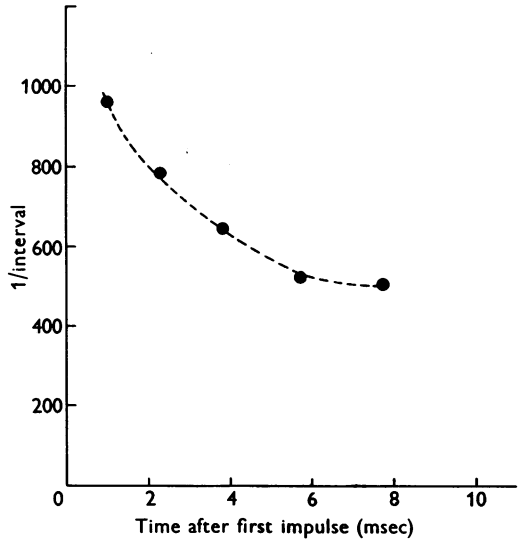

Fig. 7. Frequency of successive impulses in repetitive discharge set up by a prolonged constant current pulse.

current. The slope of the relation is much greater for the interneurone than for the motor axon. In addition to the features described above, some interneurone somata showed oscillatory fluctuations in potential during passage of depolarizing current pulses, which were similar to those found in squid neurones by Hagiwara & Oomura (1958), but less marked.

Even strong current pulses, when made suitably brief, initiated only one impulse, which was not followed by detectable synaptic potential. Impulses were not initiated after the cessation of outward current pulses. On the other hand, on cessation of strong inward current pulses a single impulse was sometimes evoked, an effect analogous to anodal break excitation in axons. As noted above, impulses evoked by direct stimulation of interneurone somata were not followed by a conspicuous prolonged hyperpolarization.

Inward current pulses to interneurone somata caused interruption of spontaneous discharge and blocked initiation of reflexly evoked discharge, leaving

only synaptic potentials evident. In recording sites considered to be from interneurone axons inward current of graded strength caused an increase in amplitude of spike potential and then, with further increase in strength, an abrupt decrease. In the latter case the hyperpolarization was apparently sufficient to block conduction of impulses into the region near the microelectrode. Consequently only attenuated spikes were recorded by electrotonic spread from a distance.

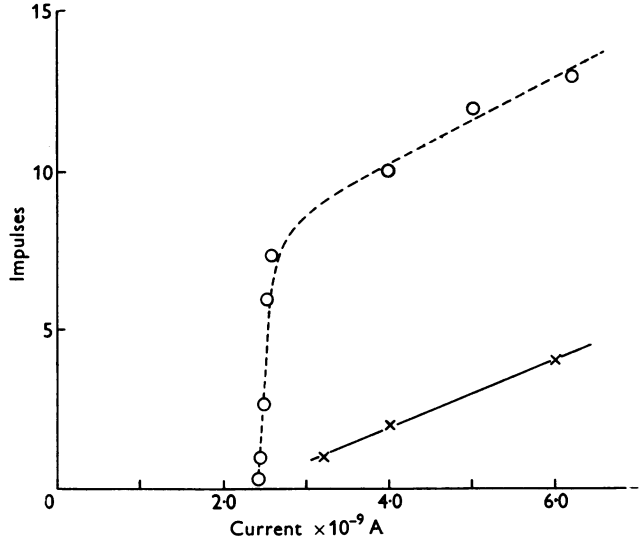

Fig. 8. Repetitive response of a motor axon (———) and interneurone soma (– – – –) to current pulses of 14 msec duration. Rheobasic current: for interneurone soma $1{\cdot}5 \times 10^{-9}$ A, for motor axon not known accurately but approximately the same.

Post-anodal enhancement was also noted in interneurone somata. In some units showing spontaneous discharge this consisted of an increased rate of discharge after cessation of an inward current pulse.

The mechanism of repetitive discharge

One of the striking features of the reflex response of spinal interneurones is the tendency for repetitive discharge to a single orthodromic volley. Of the various possible mechanisms for the production of repetitive discharge, two major types may be recognized: (1) self re-excitation of the cell by after-currents or by activity in reverberating circuits initiated by recurrent collaterals or axons of the same cell, and (2) prolonged synaptic excitation. Self re-excitation as the sole basis for repetitive discharge in interneurones may be ruled out by the fact that direct stimulation by a brief depolarizing pulse elicits only one impulse and no subsequent depolarization is observed. Furthermore, by weak orthodromic stimulation a single impulse can also be evoked. It

has been shown that the frequency of discharge to a long outward current pulse can be graded by current intensity (Fig. 6). If prolonged synaptic excitation were responsible for repetitive discharge one would expect the frequency and duration of the reflexly evoked repetitive discharge to be related to the magnitude and duration of the synaptic potential. Figure 9 illustrates an experiment designed to explore this relationship. In A the repetitive response to a volley in the superficial peroneal nerve is shown. In B the same volley was delivered when the cell was hyperpolarized to an extent

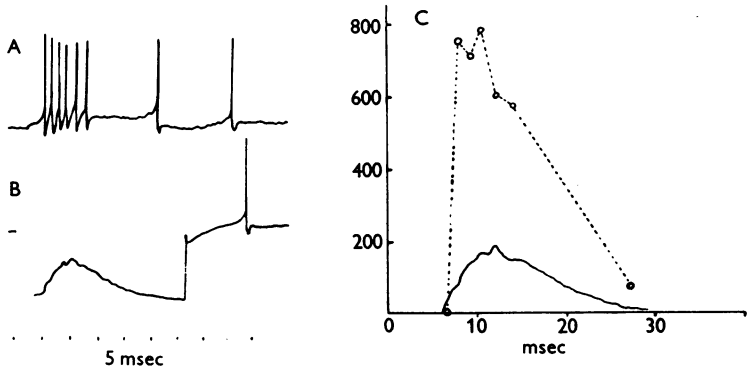

Fig. 9. Synaptic potential and repetitive discharge in an interneurone soma. A, response to a volley in deep peroneal nerve. B, repetitive response to same volley during passage of hyperpolarizing current; only synaptic potential evoked. C, plot of synaptic potential observed in B and inverse of interval between impulses in response train of A. Tracings from original records.

sufficient to prevent initiation of discharge, and only a prolonged synaptic potential was observed. The recording site was considered to be in the soma of the interneurone. C shows a plot, on the same time scale, of the inverse of interval between discharges in the response train, together with a tracing of the synaptic potential recorded during hyperpolarization. The possibility exists that some of the synaptic potential observed during hyperpolarization might have been due to inhibitory synaptic potential causing depolarization if the membrane potential had been greater than the equilibrium potential for that process. On the other hand, the fairly close correspondence between duration and amplitude of synaptic potential and discharge frequency supports the idea that prolonged synaptic excitation is a major factor in causing repetitive discharge. The lack of a prolonged subnormality and the rather small degree of adaptation permit the interneurone to discharge repetitively to prolonged synaptic excitation. This type of experiment cannot, of course, distinguish between a prolonged transmitter action following individual presynaptic impulses (cf. McIntyre, Mark & Steiner, 1956), or a briefer transmitter action resulting from temporally dispersed presynaptic impulses.

The latter, however, seems more probable, in view of the fact that weak afferent stimuli can evoke only a single impulse in an interneurone which responds by a repetitive train to stronger afferent stimuli. Furthermore, the 'spontaneous' synaptic potentials, presumably due to background impingement of presynaptic impulses as recorded by Haapanen *et al.* (1958), are of relatively brief duration.

DISCUSSION

Although interneurones in the spinal cord may not form a homogeneous group with respect to their properties, there are certain features which are common to units identified as interneurones in the present study. In particular, there are striking differences between interneurones and motoneurones. The latter cells have been more extensively studied, but behaviour of the type exhibited by spinal interneurones is much more common in the central nervous system. The difficulties of recording from interneurones by present techniques have been a limiting factor in studying their properties in detail but sufficient information has been obtained to indicate some of their general features.

Unit recordings from interneurones by intracellular micropipettes may be classified into two types, from axon or from cell body. The distinguishing feature is the presence of synaptic potentials of some magnitude (more than 1 mV) in the latter. In the present study KCl-filled micropipettes of high resistance were used. With such electrodes successful impalement of axons of primary afferent fibres and of motor axons occurs frequently; therefore a presumed recording site in interneurone axon is feasible. This identification is also suggested by the shorter average time constant from units considered to be interneurone axons than from interneurone somata, but the data are not conclusive. Unfortunately, other criteria for distinguishing axon from cell-body recording, such as are present in motoneurones (after-potential and double component spike) are lacking in interneurones. However, the assumption of different recording sites from interneurones is reasonable and avoids the necessity of postulating that some interneurone somata do not exhibit excitatory synaptic potentials (Haapanen *et al.* 1958). The same conclusions were implied in Frank & Fuortes's (1955) interpretation of potentials recorded from interneurones.

The time constant of interneurones was derived from measurement of strength–latency relations by direct stimulation. Synaptic potentials were considered too complex to permit estimation of time constant from their rate of decay. Further, such estimates assume displacement of membrane potential by brief synaptic action with subsequent passive decay. Evidence has been presented (Frank & Fuortes, 1956*b*; Eccles, 1957) indicating that the time constant measured by decay of synaptic potential is too long because of persistence of transmitter action. The use of high-resistance micropipettes which showed appreciable resistance changes on movement prevented the use

of the time course of change in membrane potential on application or withdrawal of current for estimation of the time constant of interneurones. Frank & Fuortes (1956*b*) discussed similar difficulties in the use of this method in cat motoneurones, although it has been used successfully by Araki & Otani (1955) in toad motoneurones. In the present study, therefore, only one method of time constant estimation could be used. While the number of observations is limited, the time constant of interneurone somata does not differ greatly from that of motoneurone somata, although it may be slightly shorter. In contrast, the time constant of interneurone axons is similar to that in primary afferent fibres and in motoneurone axons.

Initiation of discharge in interneurones occurred at levels of excitatory synaptic potential of 2·5–10·9 mV (average 6·7 mV). From a limited number of observations this level does not appear to be significantly different from that in motoneurones (Coombs, Eccles & Fatt, 1955). The site of impulse initiation in motoneurones is considered by some authors to be the initial segment (Coombs, Curtis & Eccles, 1957). This view is suggested by the occurrence of an inflexion in the rising phase of the spike, considered to result from impulse initiation in the previous initial segment. In interneurones such inflexions have rarely been observed. If impulse initiation did occur in the axon rather than in the soma of such cells, there could be no appreciable delay in conduction at the axon hillock. In this connexion the results of Haapanen *et al.* (1958) are of interest for they show that the 'firing level' may vary greatly with changes in membrane potential brought about by natural stimulation.

One of the important characteristics of interneurones is the lack of prolonged hyperpolarization after discharge such as is found in motoneurones. This is undoubtedly a factor in determining the frequency at which these neurones can discharge. Subnormality limits the response frequency of motoneurones to about 50/sec, but interneurones can discharge at rates well in excess of 1000/sec. In order to respond at such frequencies, refractoriness following an impulse in interneurones must be nearly as brief as in A fibres.

Repetitive discharge in response to a single afferent volley is a common finding in unit recording from interneurones, the number of impulses depending on the size of the afferent volley. The response to direct depolarizing current pulses of sufficient duration and intensity is also repetitive, whereas brief current pulses can elicit a single impulse. The repetitive response to afferent volleys is due to long-lasting synaptic excitation. The part played by after-currents in the generation of repetitive discharge in spinal interneurones is certainly minimal, if present. The long synaptic excitation that occurs in many interneurones probably results from temporally dispersed presynaptic action. While no direct evidence has been obtained as to the duration of excitatory transmitter action in spinal interneurones, there is no reason to suggest this

is particularly prolonged after impingement of a single presynaptic impulse. Other neurones within the central nervous system that show repetitive discharge may have different mechanisms involved. Some sensory relay neurones have excitatory presynaptic connexions that are probably less complex than the spinal interneurones studied. For example, cells of Clarke's column, certain thalamic relay cells, dorsal column relay cells in the gracilis and cuneate nuclei and neurones of the dorsal lateral geniculate may receive a direct presynaptic input without the interposition of other interneurones. Such relay neurones exhibit repetitive discharge following presynaptic stimulation that may depend upon long-lasting transmitter action or in some cases upon after-current mechanisms (McIntyre *et al.* 1956).

SUMMARY

1. The properties of spinal interneurones have been investigated in the acute spinal cat, using intracellular micropipettes. By means of a bridge circuit, stimulation was carried out through the same electrode used for recording.

2. Recordings from interneurones are considered to be from one of two sites, axon or soma. Those from the latter display conspicuous synaptic potentials. The time constants of interneurone somata and axons, motoneurone somata and axons, and primary afferent fibres are compared.

3. Excitatory synaptic potentials of interneurone somata elicited by nerve volleys are complex and appear to be compounded of temporally dispersed brief synaptic events. Impulses are initiated in association with synaptic potentials of 2·5–10·9 mV.

4. Spike potentials of interneurone somata are often followed by a brief post-spike positivity but lack the prolonged after-hyperpolarization characteristic of motoneurones.

5. Spike duration in interneurone somata does not differ appreciably from that in interneurone axons, but is briefer than that in motoneurones.

6. Interneurone somata show a much greater tendency to discharge repetitively to long depolarizing currents than do motoneurones. The frequency of discharge can be graded by current intensity. Some adaptation of interneurone response to constant current occurs.

7. Repetitive response of interneurones to orthodromic stimulation appears to result from prolonged suprathreshold synaptic excitation. Self re-excitation, by after-currents or by activity in recurrent pathways, appears to play a negligible part in repetitive discharge.

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Note added in proof. Since submission of this paper, Wall (1959, *J. Neurophysiol.* **22**, 305–320) has published a study on the repetitive discharge of neurones, including spinal interneurones. In the latter he noted the greater variation in later impulses of a repetitive response to nerve volleys,

that reduction in volley size reduced the number of discharges and caused slight increase in latency and increased variation of the first response. Our findings confirm these observations. Wall also noted that direct stimulation by an intracellular electrode (presumably with a brief pulse) caused only one impulse to be initiated. He found that a direct stimulus interpolated before the response to a nerve volley could abolish the first response and alter the latency of the second but had no effect on the timing of subsequent impulses. He concluded that the origin of the discharge pattern must be external to the cell, an interpretation which gains support from the present study.

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