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## BACKGROUND DISCHARGE AND EVOKED RESPONSES OF SPINAL INTERNEURONES

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The preceding paper (Hunt & Kuno, 1959) dealt with properties of spinal interneurones and mechanisms of synaptic excitation of such cells. The present study is concerned with an analysis of the background discharge of interneurones, the modulation of discharge by natural exteroceptive stimulation, and the response to nerve volleys from various peripheral sources. All these features of interneurone behaviour have been studied previously. Background discharge of interneurones has been noted by a number of authors (Frank & Fuortes 1955, 1956; Kolmodin 1957; Haapanen, Kolmodin & Skoglund 1958; Kolmodin & Skoglund 1958). Kolmodin (1957) has studied extensively the patterns of response of interneurones to muscle stretch and to a lesser extent to natural exteroceptive stimulation. Finally, several authors (Woodbury & Patton 1952; Frank & Fuortes 1955, 1956; Haapanen *et al.* 1958) have published some examples of the responses of interneurones to afferent volleys. The present study provides new information on these aspects of interneurone behaviour. The background discharge has been studied by an analysis of frequency distribution of impulse intervals, responses to natural exteroceptive stimulation have been subject to more extensive study, and a systematic study has been made of responses to nerve volleys from various peripheral sources. The interneurones that respond by high-frequency trains of impulses to antidromic volleys in ventral root fibres have been studied in detail (Renshaw, 1946; Eccles, Fatt & Koketsu 1954) and are not included in the present report.

### METHODS

Intracellular recording was carried out in spinal cats by the methods described in the previous paper (Hunt & Kuno, 1959). For extracellular recording, glass micropipettes filled with indium were employed; these had tip diameters of 10-20 $\mu$  and d.c. resistance of 8-12 k $\Omega$ . They were

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connected by a cathode follower to an a.c. preamplifier. Unless otherwise specified, nerve volleys were delivered at a rate of one every 2 sec. Other details were described in the previous communication. Over 270 spinal interneurons have been studied. The majority of units were recorded from segments L7 and S1, chiefly in dorsal horn. No histological control was carried out.

## RESULTS

### *Background discharge*

A large proportion of the interneurons examined exhibited background discharge. About 75% of the units studied by extracellular recording, and a slightly smaller percentage of those studied by intracellular recording, showed background discharge in the absence of added stimulation. Some examples

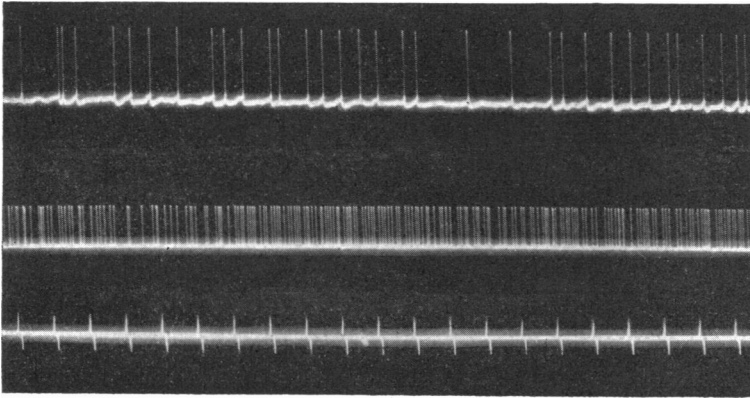


Fig. 1. Background discharge, intracellular records. Upper trace, from interneurone soma; note prepotentials and base-line fluctuation of membrane potential. Lower trace, from interneurone axon; no prepotentials were observed. Time marker 0.1 sec.

of background discharge in interneurons are shown in Fig. 1. The upper record shows the potentials recorded intracellularly by a micropipette from a position judged to be in an interneurone soma. Impulses recurred at irregular intervals and were associated with progressive depolarizations before impulse initiation (prepotentials). In the intervals between impulses other fluctuations of membrane potential occurred. These subthreshold variations did not recur at regular intervals. The lower trace of Fig. 1 shows an intracellular recording from a position judged to be in an interneurone axon. In this case no appreciable prepotentials were seen before the 'spontaneous' impulses. The justification for considering recording sites as being from soma or axon was given in the preceding paper (Hunt & Kuno, 1959).

In a number of units continuous recordings of about 200–600 background discharges were made and each impulse interval was subsequently measured. A frequency distribution of impulse intervals was then constructed. Figure 2

shows some representative samples. In A the mean impulse interval was 35.6 msec and the total number of intervals counted was 391. The observed distribution in this case has been compared with the curve which indicates the expected distribution for random events given by the formula (Feller, 1950):

$$n = N \frac{\Delta t}{t} \exp\left[-\frac{t}{T}\right],$$

where  $n$  is the number of occurrences of any interval between  $t$  and  $t + \Delta t$ ,  $N$  is the total number of intervals, and  $T$  is the length of the mean interval. The frequency distribution of impulse intervals of this unit was distributed in a manner suggesting random discharge. Had sufficiently small class intervals

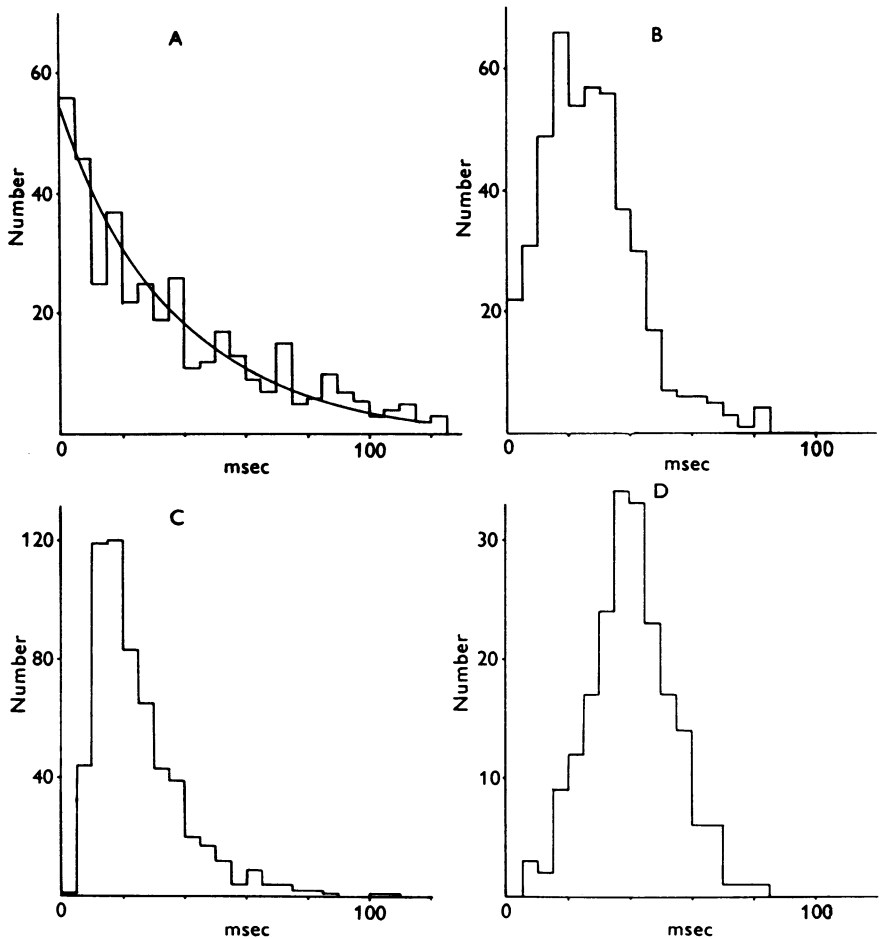


Fig. 2. Frequency distributions of 'spontaneous' impulse intervals in four interneurons (A, B, C, D) characterized by different mean impulse intervals. In A the curve represents distribution expected on random basis (see text).

been used, one would have expected a smaller number of occurrences for the least interval because of refractoriness.

The units of Fig. 2B, C and D have frequency distributions of impulse intervals which obviously depart from that expected with purely random discharge and which indicate a rhythmic generation of impulses subject to considerable fluctuation. The histogram of Fig. 2D shows a nearly normal distribution, while those of Figs. 2B and C show considerable skewness. When the mean interval was longer, the distribution was more nearly normal. These results may be interpreted as indicating a rhythmic excitation of interneurons subject to random fluctuation. A similar analysis of spontaneous discharge of Betz cells in cortex has been reported by Martin & Branch (1958). The present observations provide little direct evidence about the mechanism of generation of 'spontaneous' discharge; that is, whether it is caused by autorhythmic activity in individual cells or by excitation in networks of interneurons. In these acute spinal preparations there is a very considerable background of afferent activity entering the cord from various receptors. The large variation in impulse interval, even in interneurons exhibiting a tendency toward rhythmic discharge, suggests that the background impingement of synaptic influences on the interneurone plays an important part in the initiation of 'spontaneous' discharge.

#### *Natural stimulation*

Kolmodin (1957) has investigated in detail the responses of spinal interneurons to proprioceptive natural stimuli. The time course of frequency changes of interneurone discharge to muscle stretch has been investigated and the convergence patterns from various muscles, as well as from exteroceptive sources, has been described. The interaction between proprioceptive and exteroceptive natural stimulation on interneurons was also described. The emphasis in Kolmodin's study was on proprioceptive stimulation and responses to exteroceptive natural stimulation was not reported in detail. In the present study stimulation of skin and paw areas of the lower hind limb was carried out using light touch, bending of hairs, pressure, or pinching. By exploring the peripheral receptive field to which the natural stimulus was effective, the majority of units studied were found to be affected by stimulation of skin over a wide area. This might be expected from the large amount of convergence shown in response to nerve volleys (see p. 376). However, a number of interneurons were found which showed an increase in discharge only to stimulation of a discrete and small receptive field. Such units were usually excited specifically by bending of hairs in the receptive area and showed a high-frequency burst on bending the hairs and another burst on release, with little or no discharge when the hair was held in the bent position. The adaptation in this case may be attributed largely to the receptor, since a

rapidly adapting discharge of this type is seen in afferent fibres from hair receptors.

The response of most interneurons to touch or pressure stimuli was maintained for the duration of the stimulus. Although it was not possible to be certain of constancy of stimulus intensity, there appeared to be relatively little adaptation in the interneurone response to maintained pressure or touch (Fig. 3). Kolmodin (1957) also noted, in studying the interaction between proprioceptive stimulation and pressure to the pad of the foot, that the effect from the latter showed little adaptation. Slowly adapting receptors responding to touch were noted in the foot pad by Adrian & Umrath (1929).

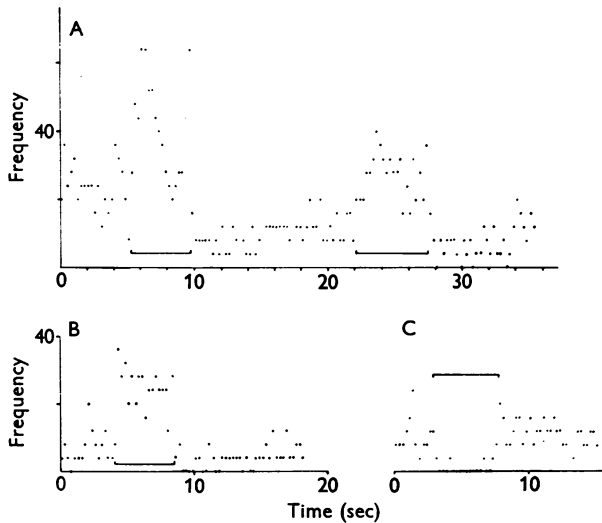


Fig. 3. Frequency changes in discharge of a spinal neurone to (A) pressure first to ipsilateral paw then to contralateral paw, (B) squeezing skin over tendo Achillis (ipsilateral) and (C) touch of skin over trochanter of ipsilateral femur. In this and subsequent records bar indicates approximate duration of stimulus.

A variety of patterns of convergence of excitatory and inhibitory influences from different peripheral sources was observed. One type pattern is shown in Fig. 3. Impulse intervals were measured from continuous photographic records and the inverse of interval (or frequency) calculated. Fig. 3A shows the increase in discharge frequency that followed pressure to the ipsilateral paw and then to the contralateral paw. Fig. 3B illustrates the increase in discharge that accompanied squeezing the skin over the ipsilateral tendo Achillis, and Fig. 3C shows the inhibition of spontaneous discharge that occurred with touch of the skin over the trochanter of the ipsilateral femur. A different pattern was found in the unit of Fig. 4. In this case pressure to the ipsilateral paw caused an inhibition of base-line discharge maintained for the duration of stimulation (A), but similar stimulation of the contralateral

paw increased the discharge frequency (B). The converse of this pattern was also observed.

The responses of 75 interneurons to stimulation of ipsilateral or contralateral paw have been tabulated in Table 1. The patterns most frequently observed were excitation from ipsilateral paw and inhibition from contralateral paw, or excitation from ipsilateral paw and no effect from contralateral

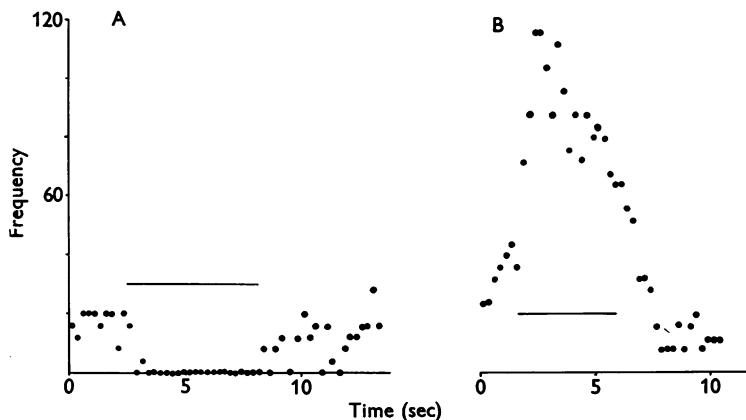


Fig. 4. Frequency changes in discharge of an interneurone to (A) pressure to ipsilateral paw and (B) pressure to contralateral paw.

TABLE 1. Effects of paw stimulation

Number of units	Contralateral paw	Ipsilateral paw
9	+	+
22	-	+
6	+	-
4	-	-
21	0	+
11	0	0
1	+	0
1	-	0
0	0	-

paw. Excitation from both paws was fairly common. Inhibition from ipsilateral paw and contralateral excitation was not infrequent. No effect from either paw was seen in eleven units. In general, ipsilateral inhibitory effects were usually associated with demonstrable contralateral effects, but ipsilateral excitatory action could often be found with no evident crossed effect. Frank & Fuortes (1956) have also described an interneurone with excitation by contralateral skin stimulation and inhibition by ipsilateral skin stimulation, as well as one which was excited by both.

Responses to natural stimulation sometimes showed a mixture of excitatory and inhibitory effects. In the unit of Fig. 5, pressure to the ipsilateral paw caused initial inhibition of discharge followed by increase in frequency above

the base-line level. The interneurone of Fig. 6, on the other hand, responded to pressure to the ipsilateral hind paw by an initial acceleration of discharge leading to a marked inhibition (A). This unit is also of interest in that an excitatory effect of ipsilateral forelimb stimulation could readily be shown (B).

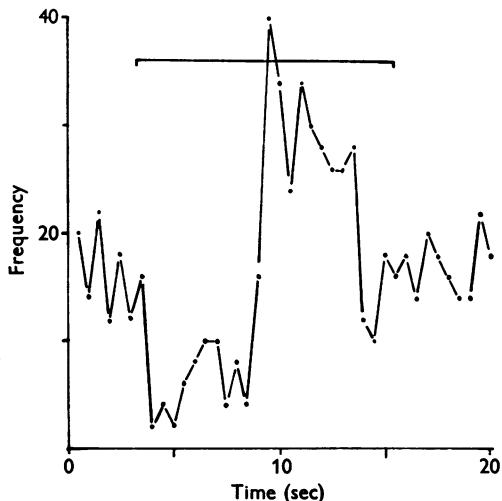


Fig. 5. Frequency changes in discharge of a spinal interneurone to pressure to ipsilateral paw.

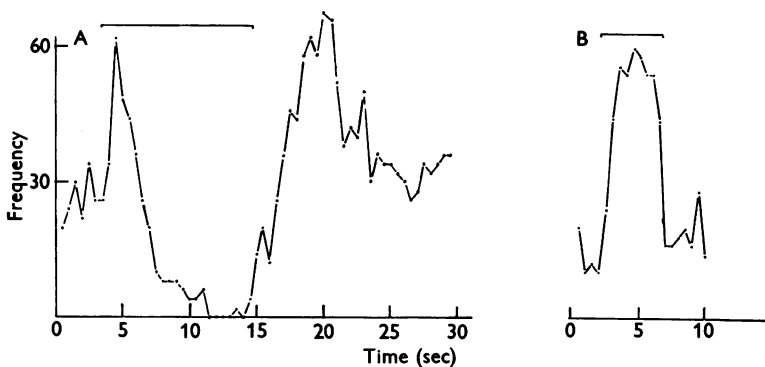


Fig. 6. Frequency changes in discharge of a spinal interneurone to (A) pressure to ipsilateral hind paw and (B) squeezing ipsilateral forelimb.

A few units showed clear evidence of having a discrete receptive field causing excitation while stimulation of nearby areas produced inhibition. One such interneurone (Fig. 7) had an increase in discharge frequency in response to touching the 5th digit of the ipsilateral hind paw (B) yet touch of the 2nd digit produced inhibition of the base-line discharge (A). This pattern bears some similarity to lateral inhibition in the *Limulus* eye (Hartline, Wagner & MacNichol, 1952), on units with 'off' surround in the cat eye (Kuffler,

1952), and to response of cortical neurones to natural stimulation in which there is an excitatory peripheral field with an inhibitory surround (Mountcastle, 1957).

The discharge frequency of an interneurone could be graded by intensity of natural stimulation. Maximal frequencies varied widely among different units: in some, natural stimulation caused discharge frequency to rise above 250/sec (cf. Frank & Fuortes, 1956). In general, excitatory responses to natural stimuli were at lower frequencies than those to nerve volleys (see below).

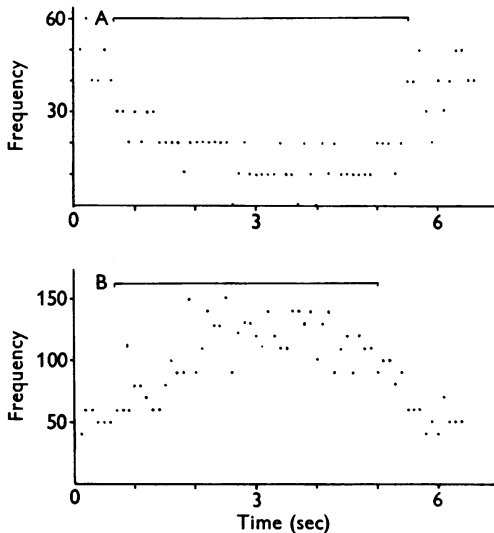


Fig. 7. Frequency changes in discharge of a spinal interneurone to (A) touch of 2nd digit and (B) touch of 5th digit of ipsilateral paw.

#### *Responses to nerve volleys*

As has been noted above, the response of a spinal interneurone to a single volley in a peripheral nerve usually consists of a repetitive train of impulses. The number and frequency of impulses in the response train varies among different units and with the size and source of the afferent volley. In spite of the variation seen, certain general features of the response pattern can be typified, with a caution that there is no clear-cut delineation between categories.

*General features of interneurone discharge.* Figure 8 illustrates the response of an interneurone to volleys of graded size in the plantar nerve. The number of discharges can be seen to increase with afferent volley size, the latter being recorded at the root-cord junction. Also, as the volley size was increased the latency of the first impulse was shortened and the frequency of discharge during the response train increased. In order to present these findings in a



manner that permits easier comparison a graphic representation has been used. Each impulse was plotted as a point on the graph, the abscissa indicating latency from stimulus (or from volley entry at cord) and the ordinate the reciprocal of the interval between the impulse being plotted and the preceding one. The data of Fig. 8 are presented in this way in Fig. 9, latencies being plotted from the time of arrival of the most rapidly conducting afferent impulses at the root-cord junction. At stimulus strength 1.00, two impulses were evoked, the latency of the first being 1.4 msec. At strength 1.45, two

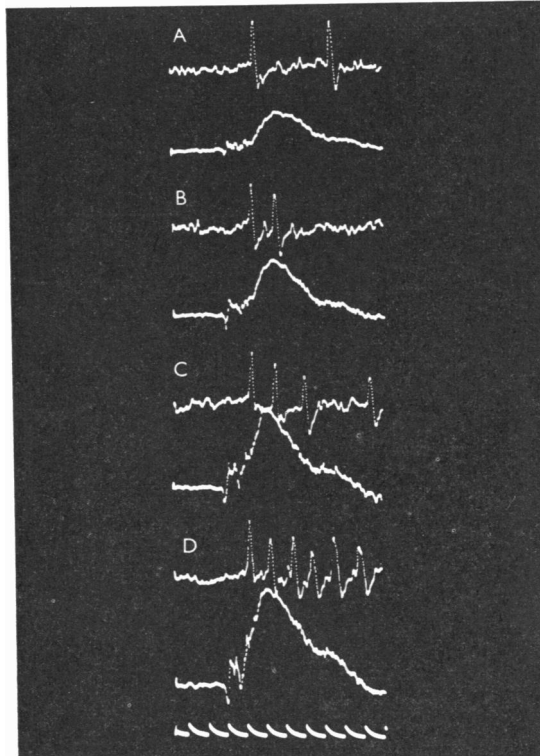


Fig. 8. Interneurone response to graded volleys in plantar nerve. Stimulus strength increased from A to D. Each record shows, on upper trace, recording of interneurone response by indium electrode; on lower trace potential recorded from lead at root-cord junction. Time marker, 1 msec. (Spikes retouched.)

impulses again were evoked but the latency of the first was briefer and the impulse interval shorter. At strength 2.24, four impulses were evoked, the first two being nearly identical in latency with those at strength 1.45. At stimulus strength 2.56, six impulses were evoked, the highest frequency being attained between the third and fourth impulses. In the latter case the response was not of greater duration than at strength 2.24, but was of higher frequency.

Input-output studies were carried out on a number of interneurons that responded to sural volleys. Input size was measured by monophasic recording of spike potential amplitude in the distal cut end of the sural nerve. At the conduction distance used, the initial component of the compound spike potential was produced by fibres conducting at 30–70 msec. The number of discharges in the response train of interneurons could be finely graded by size of this initial component. Further, in the units examined input had to exceed 10% of the initial spike amplitude before interneurone discharge resulted. This suggests that summation is required for initiation of discharge in the

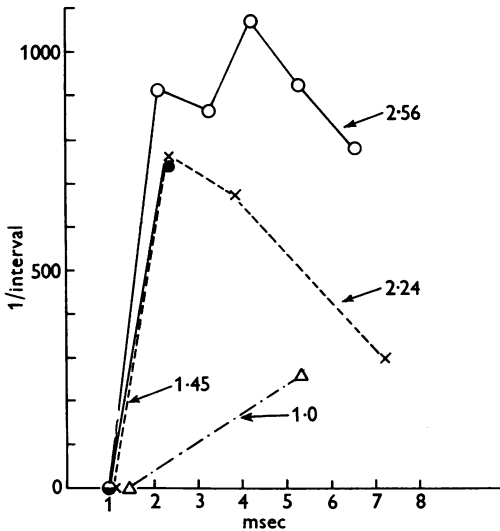


Fig. 9. Inverse interval-latency relation of responses shown in Fig. 8. Each impulse plotted on abscissa as latency after arrival of fastest component of plantar volley at cord, on ordinate as interval from preceding impulse. Stimulus strength in arbitrary units. See text.

neurons examined. Increase in stimulus strength, to bring in more slowly-conducting sural afferent fibres, often further increased the number of impulses in the response train. In view of the limited sample it is not possible to generalize as to the need for summation in interneurons.

Figure 10 shows the response of an interneurone to superficial peroneal volleys on three successive trials, the number of impulses evoked varying from five to seven. While the latencies of the first two impulses were quite constant, subsequent impulse intervals showed considerable fluctuation. The interneurone of Fig. 10 showed the highest discharge frequency after the third impulse in the discharge train. In this regard it differs not only from some other spinal interneurons but also from certain sensory relay neurons, which often show the highest frequency between the first and second impulse. In the experiment shown in Fig. 10, the arrival of the superficial peroneal

volley was recorded by a volume lead at the root-cord junction. Its fastest component reached the cord 2 msec after the stimulus, and its arrival is indicated by the arrow on the abscissa. Central delay before initiation of the first impulse was 1.6 msec from the arrival time of the most rapidly-conducting afferent impulses.

Only a few units were found in which the latency of the first reflexly evoked impulse was sufficiently brief— < 1 msec— to indicate monosynaptic excitation from primary afferent fibres. Since stimulation of peripheral nerves rather than dorsal roots was employed, temporal dispersion of afferent volleys

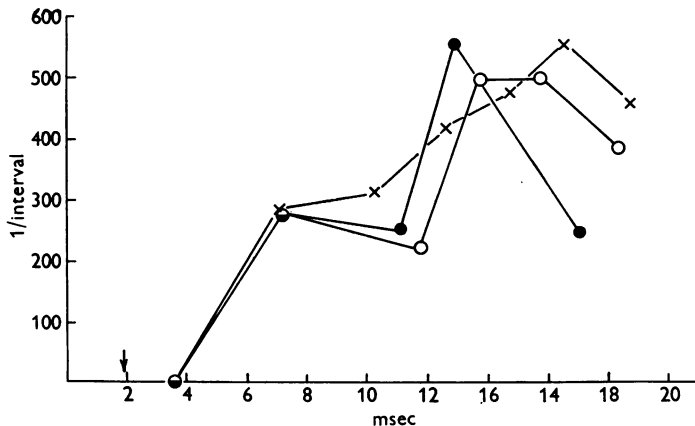


Fig. 10. Inverse interval-latency plot of response of an interneurone to superficial peroneal volleys; latency measured from stimulus artifact. Arrow shows time of arrival of first impulses at root-cord junction.

on arrival at the cord was considerable. Thus monosynaptic excitation of interneurons by slowly-conducting afferent fibres would not have been detected, for the time of volley arrival at the cord was measured from the potential change produced by the most rapidly-conducting fibres at the root-cord junction. The above examples point out certain general features of the response of interneurons to volleys in peripheral nerves: the earlier impulses of a response train show less latency variation on repeated tests than do the later impulses of a train. Increasing the size of an afferent volley may at first diminish the latency of discharge to some minimal value; further increase in size of volley increases the number of impulses in the discharge train to some maximum and shortens the interval between impulses.

The duration of the response train varied between units and also in a given unit with the source of the afferent volley. The examples already shown are of interneurons with moderately short response trains. Figure 11 illustrates a unit that gave a prolonged response to contralateral plantar volleys. An inverse interval-latency plot for two successive responses is shown. The

discharge frequency reaches its peak between the second and third impulses, then gradually falls. Total duration of the response train was about 170 msec. In spite of the long duration of the discharge, it may be noted that the responses to the two successive trials were remarkably similar.

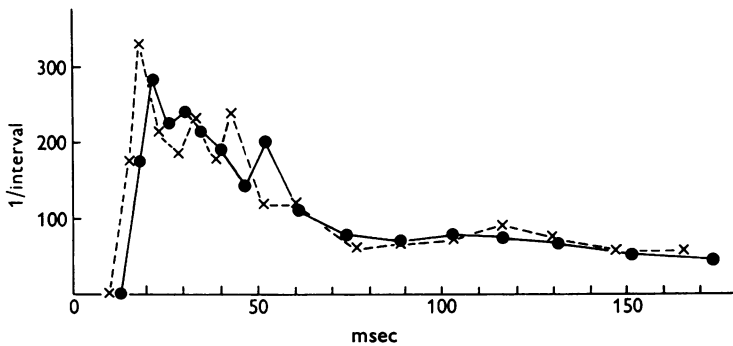


Fig. 11. Inverse interval-latency plot of interneurone to contralateral plantar volleys; latency measured from stimulus artifact.

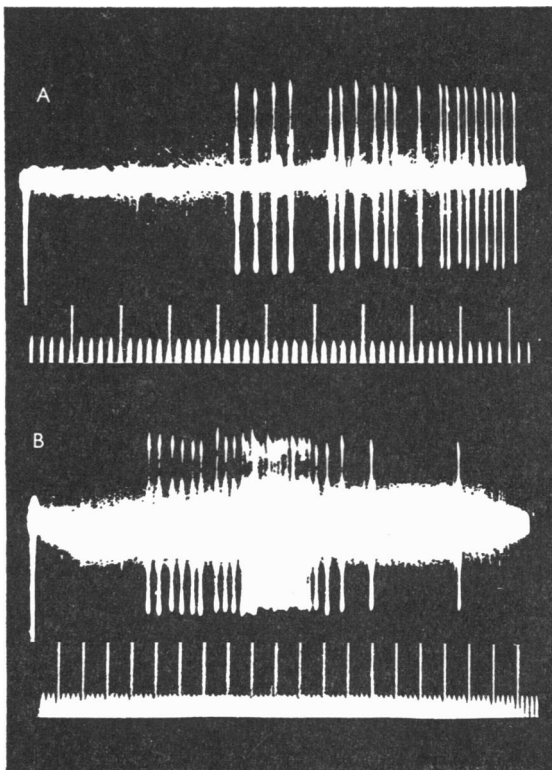


Fig. 12. Response of interneurone to volleys in high-threshold afferent fibres (plantar nerve); single stimulus at beginning of sweep. Sweep speed in A faster than in B. Time marker, 10 + 50 msec.

Figure 12 illustrates the response of an interneurone to afferent stimulation of a strength judged sufficient to excite C fibres. Weaker shocks failed to elicit any response. The long latency can be attributed in large part to afferent conduction time. This latency can be seen in A to amount to 214 msec. Record B shows a similar response recorded at a slower sweep speed. The duration of discharge was in excess of 450 msec. It may be noted in B that the frequency of discharge increased to a higher level some 200 msec after the onset of the response train. This unit also showed an occasional irregular spontaneous discharge and the only natural stimulus capable of eliciting discharge was very strong pinching of the ipsilateral paw.

*Convergence.* In many experiments stimulating electrodes were placed on the following peripheral nerves: biceps-semi-tendinosus (BST) triceps surae (TS), deep peroneal (DP), superficial peroneal (SP), plantar (P), and sural (S). Interneurones were then examined for discharge to volleys in each of these nerves. Table 2 presents a summary of the response patterns in 74 units for which complete data are available for all six volleys. The majority of the units examined showed responses to stimulation of more than one nerve. Thus 56 of the 74 interneurones were excited by volleys in three or more of the nerves tested. This indicates the large amount of convergence on many interneurones.

TABLE 2. Patterns of convergence on spinal interneurones

Number of units	Volley					
	BST	TS	DP	SP	S	P
12	+	+	+	+	+	+
12	0	+	+	+	+	+
13	0	0	+	+	+	+
8	0	0	0	+	+	+
7	0	0	0	0	+	+
3	0	0	0	0	0	+
4	0	0	0	+	0	+
2	0	0	+	0	0	+
8	0	0	+	+	0	+
2	0	+	0	0	0	+
1	0	0	0	0	+	0
1	0	0	+	0	0	0
1	0	+	0	+	0	+

For explanation of letters, see text.

Of the nerves tested, volleys in the plantar nerve excited the largest number of units. Sural and superficial peroneal nerve volleys also evoked discharge in a large number of units. In general, the muscle nerves examined excited a smaller number of interneurones. These findings indicate that among the interneurones examined in segment L7 and S1, largely from dorsal horn, the preponderance received excitatory connexions from nerves containing fibres from the paw and skin of the foot.

The temporal relation of discharge of interneurones to volleys from different sources was studied in several units. The latency of the initial impulse of

the response train, the number of impulses and their frequency varied in a given interneurone with the source of the afferent fibres stimulated. Discharge due to volleys from different afferent sources was initiated with nearly the same latency in some interneurones. In others, latency varied widely with source of afferent volley. One factor determining these differences must be the complexity of neuronal connexions utilized in the excitatory pathway.

*Inhibition.* Inhibitory effects of afferent volleys on interneurones were indicated by reduction or cessation of spontaneous discharge or by inhibition of

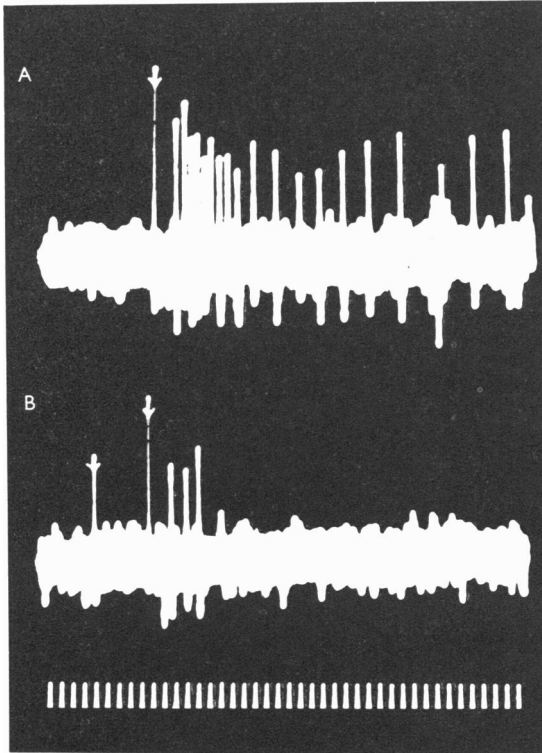


Fig. 13. Inhibition, by conditioning volley in ipsilateral plantar nerve, of the response of an interneurone to a volley in contralateral plantar nerve. A, contralateral plantar volley alone; B, ipsilateral plantar volley initiated 24 msec before stimulation of contralateral plantar volley. Arrows mark shock artifacts. Time marker, 5 msec.

response to an excitatory afferent volley. As was noted by Frank & Fuortes (1956), inhibition sometimes followed discharge to nerve volleys, but inhibition without preceding discharge has also been frequently observed. Figure 13 illustrates the inhibition of response to an excitatory nerve volley. In A, a volley in the contralateral plantar nerve evoked a repetitive response of an interneurone. However, a conditioning volley in the ipsilateral plantar nerve at an appropriate interval beforehand (24 msec) inhibited this response (B).

The conditioning volley alone evoked no discharge. The duration of such inhibitory effects was studied in a number of interneurons. In general, as the conditioning-test interval was lengthened, the reduction in the number of

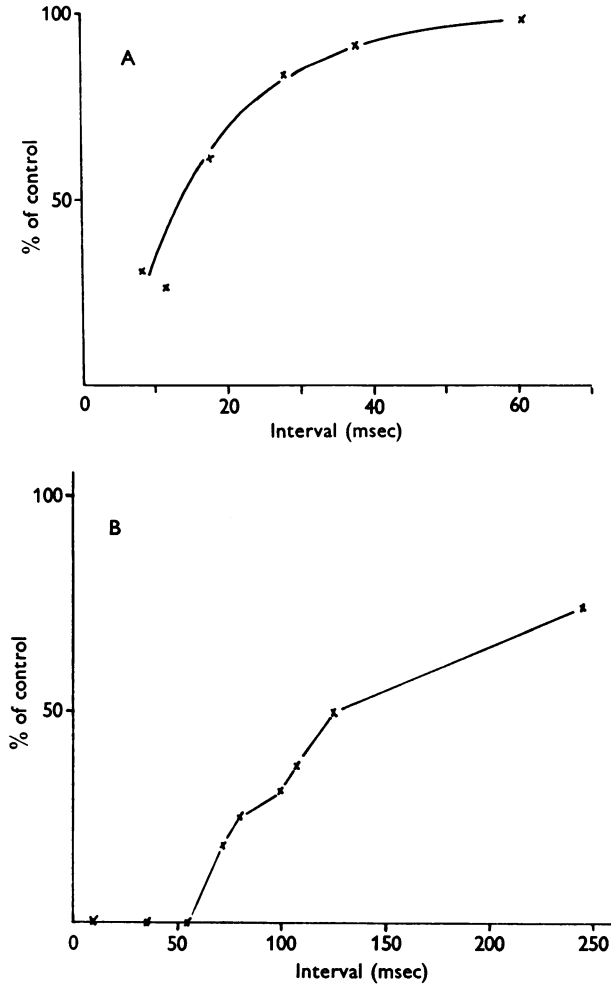


Fig. 14. Time course of inhibition. Ordinates, number of impulses fired by interneurone, expressed as percentage of number of impulses in control responses. Abscissae, interval between inhibitory and excitatory volleys. A, inhibitory volley in plantar nerve; testing excitatory volley in superficial peroneal nerve. B, inhibitory volley in sural nerve; testing volley in contralateral plantar nerve. A and B are from different interneurons.

responses to the test volley diminished. In Fig. 14A the inhibitory effect of a conditioning volley (plantar) can be seen as a decrease in the number of responses initiated by a test volley (superficial peroneal) here expressed as the

percentage of control response. The inhibition decreased as the conditioning-test interval was lengthened, the total duration of the inhibition being about 60 msec. In Fig. 14 B the inhibitory effect of a sural volley on the test response to a contralateral plantar volley is shown for another interneurone. In this case the inhibition had a longer duration, in excess of 250 msec. From these observations it is clear that the number of responses to an excitatory volley can be finely graded by the degree of inhibition.

Volleys often showed a mixed effect on interneurones, usually discharge followed by inhibition. Activation of afferent fibres with dissimilar central effects probably accounts for some of these mixed actions and would also explain the finding of Frank & Fuortes (1956) that variation in frequency of afferent stimulation can alter the response from excitation to inhibition. This may be expected to occur when excitatory and inhibitory actions have different time courses.

Background discharge of a few interneurones was inhibited by ventral root stimulation. The same units discharged in response to volleys in peripheral nerves. The majority of interneurones examined showed no effect of antidromic ventral root volleys. However, a number of Renshaw cells were encountered when recording from the ventral horn; these showed the characteristic responses already described by Eccles *et al.* (1954) and Frank & Fuortes (1956).

*Responses to repetitive stimulation.* As has been recorded above and by other authors, the response of an interneurone to a nerve volley repeated infrequently often consists of a train of impulses. When the rate of repetition is increased above about 2-5/sec the earliest impulse continues to be evoked with little or no change in latency, but later impulses of the response train show greater lability and, with high rates of repetition, fail to appear. In many units the first impulse followed rates of stimulation in excess of 500/sec and in some cases over 1000/sec. To a tetanus of brief and constant duration to a peripheral nerve the number of impulses elicited in an interneurone was often maximal when the stimulus frequency was in the range of 50-100/sec. Although such units followed higher frequencies of stimulation, the number of responses per stimulus decreased. While interneurones varied with respect to the frequency of afferent stimulation that they could follow, the majority showed a much higher frequency limit than do motoneurones. The lack of a prolonged after-hyperpolarization and of consequent subnormality in interneurones as compared with motoneurones must be an important factor determining this difference (Hunt & Kuno, 1959).

#### DISCUSSION

The majority of the interneurones examined in the present study exhibited background discharge. This discharge was generally irregular but analysis of



the frequency distribution of response intervals indicates, in most units, a rhythmic generation of impulses subject to considerable random fluctuation. Kolmodin & Skoglund (1958) recorded the fluctuations in membrane potential of motoneurons and interneurons that occurred 'spontaneously' or in response to natural stimulation. They observed in some motoneurons and interneurons slow oscillations in membrane potential, some of which were not associated with impulse initiation. They suggested that the rhythmic discharge in such neurons is primarily determined by a slow-oscillation tendency intrinsic to the membrane. In the present study oscillatory fluctuations of membrane potential in interneurons have not been found to be regularly recurrent. Furthermore, the background discharge usually displayed impulse intervals that were irregular. Analysis of the frequency distribution of impulse intervals indicates that even in units showing some tendency to rhythmic generation of impulses there is considerable fluctuation of impulse interval. These factors do not exclude inherent rhythmicity of the interneurone membrane as a factor in the generation of rhythmic discharge. They do suggest however, that the background impingement of excitatory and inhibitory effects from presynaptic bombardment plays an important part. A number of interneurons have shown bursts of discharge followed by periods of lower frequency or of silence, but the latter periods have not usually recurred at a regular rate. Regularly recurring cycles of modulation of interneurone discharge frequency, as reported by Frank & Fuortes (1956) in decerebrate cats, have rarely been seen. The difference is probably due to a greater preservation of integrated mechanisms in the decerebrate as compared with the acute spinal preparation. It is also possible that background discharge of spinal interneurons, in preparations in which supraspinal influences are still present, might show less random variation than in the acute spinal cat. No analyses of frequency distribution of response intervals have yet been made in the former.

Tonic activity in interneurons must influence the background excitability of other interneurons and of motoneurons and hence the capacity of the latter to respond in reflex acts. In the majority of interneurons the effect of natural stimulation is to increase or decrease the discharge frequency. In general, this modulation of frequency of interneurone discharge by activity in primary afferent fibres from exteroceptive sources appears to occur with little adaptation on the part of the interneurone. This appears also to be true in the case of most interneurons excited by proprioceptive input (Kolmodin, 1957). These findings are in keeping with the observation already reported (Hunt & Kuno, 1959) that little adaptation occurs in the response of interneurons to direct current.

A variety of patterns of response to stimulation of different peripheral sources has been noted among the interneurons examined. This is to be

expected from the fact that analyses thus far have dealt with rather heterogeneous collections of interneurons. Since it has not yet been possible to determine the consequences of activity in a particular interneuron it is difficult to relate its behaviour to various types of natural stimulation with its role in reflex function. From knowledge of motoneuron responses and polysynaptic reflexes it is possible to surmise what pattern of interneuron behaviour would be appropriate. Some of the interneurons examined may be concerned with ascending systems; for example, certain units which responded only to bending of hairs in a small area could be concerned with transmission of information relating to location of sensation. The possibility should be kept in mind that the same interneurons may be concerned both with segmental spinal reflexes and ascending systems.

A conspicuous feature of many interneurons is the repetitive response to nerve volleys. The response train evoked by a volley in a peripheral nerve usually attains its peak frequency after the second or later impulses in the train. In contrast, certain sensory relay neurones, which also respond repetitively to incoming volleys, appear most commonly to attain the maximum frequency of response between the first and second impulses of the response train (Rose & Mountcastle, 1954; Laporte, Lundberg & Oscarsson, 1956). The fact that the maximal frequency in the response train of spinal interneurons can occur comparatively late suggests that the intensity of synaptic activation does not reach its maximum until that time. In contrast, the repetitive discharge of at least some sensory relay neurones may depend upon a synaptic excitation which reaches its peak early and declines smoothly thereafter. In sensory relay neurones, such as those of Clarke's column, repetitive discharge may follow a presynaptic input which is considerably more synchronous than that which impinges upon spinal interneurons (Lloyd & McIntyre, 1950; Laporte *et al.* 1956). In such cases the production of repetitive discharge may depend upon a prolonged synaptic excitation due to sustained transmitter action (McIntyre, Mark & Steiner, 1956). As was shown in the previous paper (Hunt & Kuno, 1959), the interneurons now studied exhibit synaptic potentials which appeared to be compounded from temporally dispersed presynaptic impingement, presumably resulting from activation of neuronal networks of varying complexity. The patterns of response described in the present study support this view. It has also been found that the later impulses of a repetitive response train to a peripheral nerve volley show considerably more variation in latency than do the earlier impulses of the train. This difference probably depends upon the fact that the later impulses of the train result from excitation mediated through more complex connexions of the interneurons. The greater lability would therefore be compounded of variability and a number of interneuron junctions serially excited.

The fact of repetitive discharge may be an important factor in the

prolongation of activity in chains of interneurons. For example, if two cells, *A* and *B*, are connected in series, synaptic excitation of *A* for a given period will result in a train of impulses while the excitation is suprathreshold. This train of impulses may be expected to exert a more prolonged synaptic excitation of *B*, in turn generating a longer train of impulses. In this manner prolonged activation of interneurons might occur through a relatively short chain of cells connected in series.

It is common practice to assign a value for synaptic delay and from this to estimate the number of interneurons serially excited in a reflex response from the latency of the reflex less allowances for conduction. However, in the case of a motoneurone caused to discharge in a polysynaptic reflex the repetitive response of interneurons must be considered. The discharge frequency of an interneurone often reaches its maximum between the second and third impulses of its repetitive response train, sometimes later. By temporal summation one may expect the synaptic effect it exerts to reach a maximum some time later than the earliest impulse of the response train. Thus, in the case of a three-neurone reflex arc motoneurone discharge may occur considerably later than would be estimated by the number of synaptic delays. Some earlier experiments of Lloyd (1943) may be pertinent to this argument. He noted that certain polysynaptic reflexes had a latency indicating transmission through an arc of three neurones only when powerfully facilitated. In the absence of added facilitation latency was longer. Presumably only in the facilitated state would the first impulse of the response train of the interneurone be effective in initiating motoneurone discharge. Clearly, in view of the frequent occurrence of the repetitive discharge in interneurons, calculations of the number of neurones intercalated in a reflex response must not be made from central latency simply on the basis of a fixed value of synaptic delay. The high frequency of interneurone discharge observed in many units suggests that temporal facilitation must play an important part in the transmission of polysynaptic reflexes evoked by single afferent volleys.

#### SUMMARY

1. A study has been made of the background discharge of spinal interneurons and their responses to natural stimulation and to nerve volleys in acute spinal cats.
2. Background discharge is characteristically irregular. While frequency distribution of impulse intervals may approach that expected from random probability when mean impulse interval is short, units with longer mean impulse intervals show a distribution indicating rhythmic generation of impulses subject to considerable random variation.
3. Intracellular recording from interneurone somata reveals fluctuation in

membrane potential and prepotentials associated with 'spontaneous' discharge. Such changes are absent in records from axons.

4. Interneurone discharge shows little adaptation to maintained natural stimuli except when activated from receptors known to adapt rapidly.

5. Some interneurones show mixed effects of excitation and inhibition from the same receptive field. Also, certain interneurones have an excitatory receptive field adjacent to an inhibitory one.

6. Repetitive responses to single afferent volleys often display maximal frequency after the second, third, or later impulses.

7. The number of impulses in a response train may be graded by the size of an afferent volley. Increasing strength of afferent stimulation decreases latency of the initial impulse of the response train to some minimum and increases frequency of discharge. Earlier impulses show less latency variation than later impulses of a response train and more readily follow afferent volleys delivered at high repetition rates.

8. Patterns of convergence of afferent volleys from different peripheral sources are described. A large fraction of the select sample of interneurones examined have shown excitatory effects on stimulation of fibres from cutaneous sources.

9. Features of inhibition of response of spinal interneurones by afferent volleys are described.

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