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A GROUP OF NEURONES IN THE DORSAL HORN ASSOCIATED WITH CUTANEOUS MECHANORECEPTORS

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Slow potential changes, resulting from stimulation of cutaneous and muscle afferent nerve fibres, can be recorded from spinal cords extracellularly through glass capillary micro-electrodes (Eccles, Fatt, Landgren & Winsbury, 1954; Coombs, Curtis & Landgren, 1956; Fernandez de Molina & Gray, 1957; Bravo & Fernandez de Molina, 1960). These potentials are a resultant of the activity of all the cells in the neighbourhood of the electrode tip and may be termed mass responses. Fernandez de Molina & Gray (1957) recognized three phases of the mass response produced on electrical stimulation of the sural and the saphenous nerves in the cat, each phase having a characteristic time course and distribution in the cord. Analysis of their properties led the authors to attribute all the phases to activity in post-synaptic elements and to suggest that synchronous synaptic potentials made a major contribution.

The experiments described in the present paper were done on cats. The activity of single spinal units, which could be excited both by electrical stimulation of the medial plantar nerve and by mechanical stimulation of the foot, was recorded through micro-electrodes; when electrical stimulation was used the impulses were seen superimposed on the mass response. The properties of spinal interneurones have been studied by several workers using intracellular micro-electrodes; under such conditions certain properties of the impaled cell may be accurately determined (Woodbury & Patton, 1952; Frank & Fuortes, 1955, 1956; Haapanan, Kolmodin & Skoglund, 1958; Hunt & Kuno, 1959*a*, *b*). In the present experiments the activity of the individual cell was superimposed on a potential change which reflected the electrical activity of a whole population of cells; it was therefore possible to relate the discharge of a single unit to the activity of the total population and to analyse the properties of groups of cells from consideration of both single unit recordings and mass responses. This work was done during the course of an investigation into the interaction

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of primary signals at synaptic levels in the cord and consequently activity was recorded from only about 150 units, of which 75 were suitable for analysis. The purpose of the analysis was to begin the specification of interneurones, particularly second-order neurones, associated with mechanoreceptors in the pad. The work deals with the input to the interneurones studied and the properties of their discharge, but does not take into account the destination of their axons. More information on the latter aspect of the problem has been obtained by Dr R. M. Eccles.

A preliminary account has appeared elsewhere (Armett, Gray & Palmer, 1958).

METHODS

Most experiments were performed on cats anaesthetized with chloralose (0·05 g/kg) and urethane (0·5 g/kg), injected intravenously after induction with ethyl chloride and ether. In a few experiments the dissection was done under sodium pentathone anaesthesia, after which the animal was decerebrated and the anaesthetic allowed to wear off. The spinal cord was exposed from S1 to L5 and divided in the thorax; the dura was also divided in order to reduce the movements of the cord due to respiratory activity. The medial plantar nerve was exposed for electrical stimulation but was not divided. The preparation was then mounted rigidly in the manner described by Fernandez de Molina & Gray (1957). The spinal cord was protected by a paraffin pool. The medial plantar nerve was placed over a pair of platinum electrodes; the nerve and electrodes were buried in paraffin wax (m.p. 39° C).

Pulses of about 100 μsec duration from a low impedance source were applied to the nerve through the platinum electrodes.

The spinal cord potentials were recorded through glass capillary micro-electrodes of tip diameter *ca.* 1·0 μ filled with NaCl solution 10 g/100 ml. Electrodes of resistance greater than 5 MΩ were rejected. During an experiment the electrode was held in position by a manipulator; this was graduated along three axes the angles between which were variable; adjustments to about 50 μ were thus effected. There was also a fine downward movement operated on a hydraulic principle and this could provide movements down to the order of 1 μ. The electrode was connected to a high-impedance recording system by means of a Ag-AgCl contact. The indifferent electrode, a Ag-AgCl-NaCl (in agar, and cotton wool) system, was inserted between the skin and the back muscles of the cat and connected to the other side of the recording system. The animal and the holding frame were earthed independently. The potentials were amplified with an instrument of suitable band-width, and displayed on one beam of a cathode-ray oscilloscope.

In some experiments the dorsal root volley was recorded. A filament of the dorsal root was cut as near to the spinal cord as possible and its peripheral end placed over a pair of platinum electrodes; the potentials were amplified and displayed on the second beam of the oscilloscope.

Procedure. Those segments of the spinal cord activated by electrical stimulation of the medial plantar nerve were located in the manner described by Fernandez de Molina & Gray (1957). The afferent volley was recorded at points along the length of the cord through a micro-electrode placed over the dorsal root entry zone, and the region of the cord opposite the rootlet that gave the maximum evoked response was judged to be that containing most activity. Two distinct elevations were usually seen in the primary fibre potentials but the maxima were found within 2 mm of each other. The pia was opened in the active region and the micro-electrode lowered on to the mid line of the cord. The co-ordinates were noted and tracks were then made into the cord at intervals not less than 0·1 mm apart in both head-to-tail and side-to-side axis. In each track a record of the mass response was taken routinely

(*a*) 1 mm below the surface of the cord, (*b*) as near as possible to the position of maximum amplitude of the main early response (phase II in the terminology of Fernandez de Molina & Gray (1957)), and (*c*) at the point where the sign of the phase II potential reversed. The co-ordinates of these points and of any single unit investigated were recorded. At the end of a series, the electrode was left in the cord and the positions of the units were identified by the histological techniques described in a previous paper (Fernandez de Molina & Gray, 1957).

RESULTS

General

The mass responses recorded through micro-electrodes in the dorsal horn on stimulation of the medial plantar nerve were similar to those described by Coombs *et al.* (1956) and Fernandez de Molina & Gray (1957). The main early response (phase II in the terminology of Fernandez de Molina & Gray) had a maximum in a position medial to that of the equivalent potential on sural stimulation; the small early phase I was not investigated.

Fernandez de Molina & Gray concluded that the phase II potential was the mass response of a population of neurones, at least some of which were monosynaptically connected with the lowest-threshold afferent fibres from the skin. Since the primary object of the present experiments was to find units in the spinal cord that were second-order to mechanoreceptors in the pad, the region searched was that in which the main early phase (phase II) of the mass response was large. The units considered are therefore not, and were not intended to be, a random sample of interneurones with cutaneous connexions. However, all units which were found in this region and which could be excited both by electrical stimulation of the medial plantar nerve and by mechanical stimulation of the foot are included in the analysis.

The activity of the single units was seen as a spike discharge superimposed on a mass response (Fig. 1). The latency of the peak of the first impulse of the discharge was measured from the negative peak of the incoming volley, and the values obtained in the anaesthetized preparations have been plotted as a frequency histogram in Fig. 2. The histogram shows two peaks, at 1 and at 3 msec, and a dip at about 2 msec. In certain experiments recordings were made from a dorsal rootlet (Fig. 1 top beam) and the area of the primary fibre volley could then be used as a measure of the threshold for a response from the unit. The relation between the area of the incoming volley at threshold and the latency of the first impulse (measured under suprathreshold conditions) is shown for 25 units in Fig. 3. The units in the earlier hump of the latency distribution (Fig. 2), that is units with a latency less than 2 msec, all had low thresholds (see Fig. 1*a*). The other units, with one exception, had higher thresholds. The units

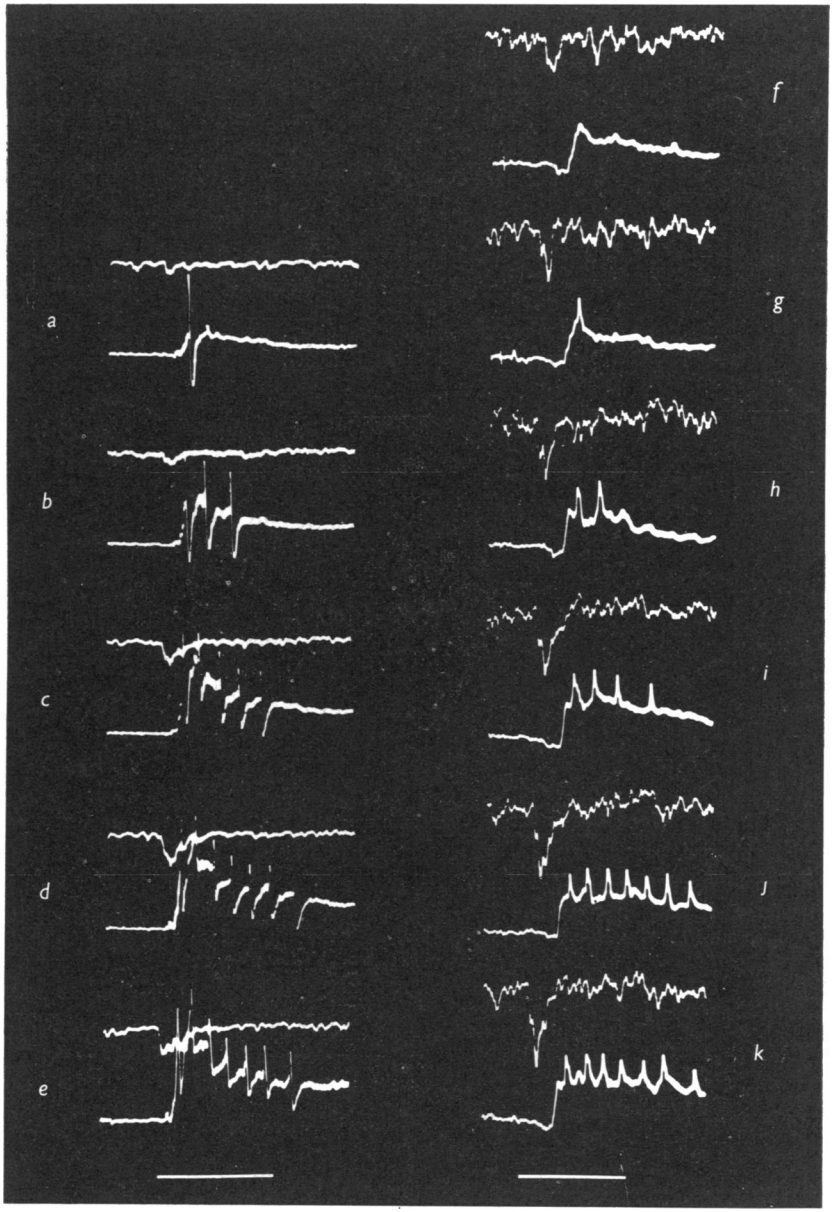

Fig. 1. Effect of increasing the strength of stimulus applied to the medial plantar nerve on the response of a single unit in the dorsal horn. Upper beam, record from dorsal rootlet; lower beam, record from unit in the grey matter. *a–e*, effect of increasing stimulus strength on a unit responding at a short latency. *f–k*, a comparable set of records for a unit responding at a longer latency. Negative deflexions in this and all subsequent records are upwards. The horizontal bar represents 10 msec.

with low thresholds and short latencies appear to form some sort of a group and their properties are considered in the next section.

Short-latency units

The mean value of the latency of these units in anaesthetized animals was 1·1 msec (standard deviation 0·34 msec, number 35). A few units were isolated in decerebrate animals and these had significantly shorter latencies (mean 0·76 msec, S.D. 0·22 msec, number 10).

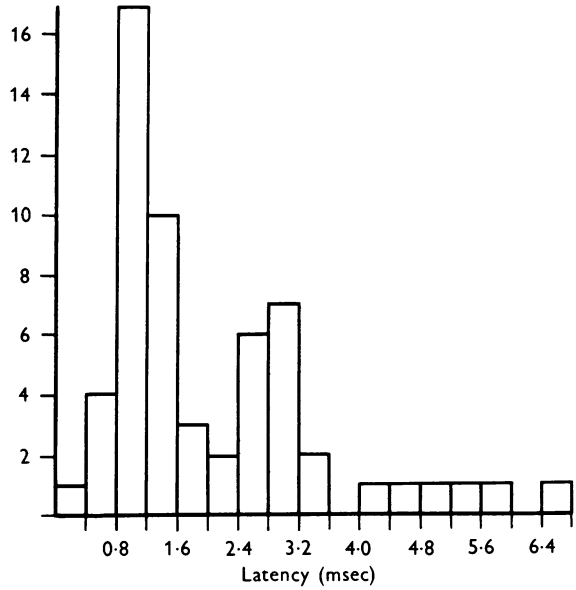

Fig. 2. Frequency distribution of latency of all units isolated from anaesthetized animals and analysed. Latency (msec) from negative peak of incoming volley to the peak of the first impulse, with stimulus strength much greater than threshold.

All the units in this group, unlike the remainder, were recorded from positions at which the main early phase (phase II) of the mass response was negative. There was in fact a close relation between the distribution of the short-latency units and the peak of the early phase of the mass response. This was shown quantitatively in some experiments. The distance of the units from the point on the track where the early phase reversed was measured along a dorsoventral line; also in each track the peak of this early mass response was related to the reversal point. The mean position of the units was 0·73 mm dorsal to the reversal point and they were scattered with a standard deviation of 0·25 mm (number of observations 14). The position of the peak was in the mean 0·59 mm (S.D. 0·23 mm, number 15) dorsal to the reversal point. The anatomical positions of some

of the units in the short-latency group are shown as full circles in Fig. 4. These positions were determined by a co-ordinate method and the points have been transferred to an equivalent position on one diagram. The method is liable to error for individual points, but is likely to give a satisfactory picture of the general distribution.

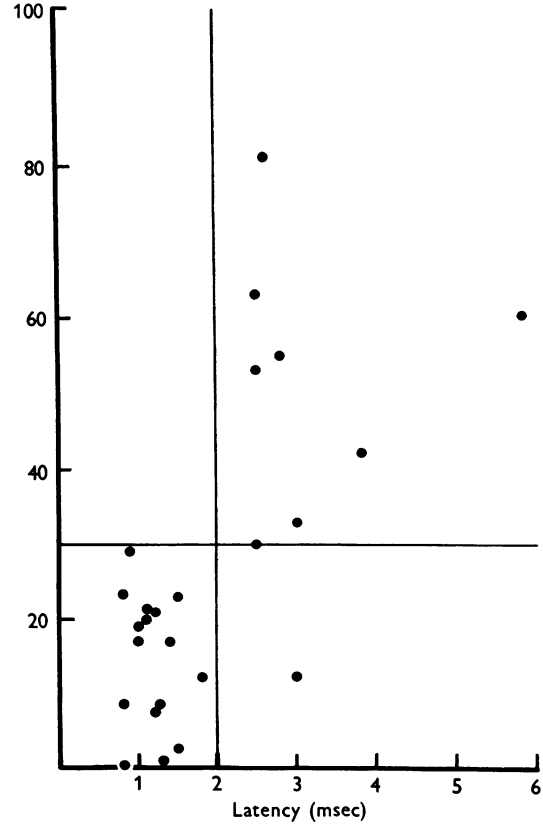

Fig. 3. Latency and threshold scatter diagram. Ordinate, area of the dorsal root volley at threshold, given as a percentage of the maximal area; abscissa, latency.

A repetitive discharge usually resulted from a single stimulus (Fig. 1*a–e*). A frequency distribution of the latencies of all the impulses in the responses to single maximal shocks of all the units was constructed. This temporal distribution of impulse activity was found to be consistent with the time course of the mass response. It showed a break at approximately 3 msec, which is about the time that the later (phase III) part of the mass response breaks the continuity of the falling phase of the earlier part. A break in the sequence of the impulse discharge from individual units has been seen quite frequently; for example, in Fig. 1*c–e* the interval between the

second and third impulses is longer than either the preceding or succeeding intervals. Figure 5 illustrates a particularly clear example of this and shows how regular the gap is for a particular unit. Figure 5*a* shows the pattern of discharge in response to ten different electrical stimuli of approximately equal strength. This pattern is analysed in Fig. 5*b*, in which the preceding interspike interval is plotted against the total time to the spike from the incoming volley in the primary receptor fibres. The third spike (×) occurred consistently at an interval which was appreciably

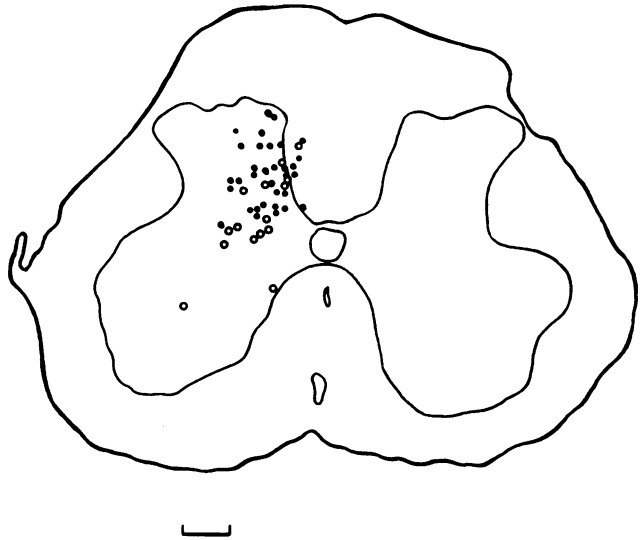

Fig. 4. Diagram of a transverse section of the spinal cord at the level of L 6, to show the distribution of the units in the dorsal horn. ● short-latency units; ○, long-latency units. Points have been found by a co-ordinate method and then transposed to corresponding positions on this standard diagram of the cord; the positions of individual points should therefore be regarded with reserve. Horizontal bar represents 0·5 mm.

longer than the preceding interval and, in about half the trials, longer than subsequent intervals. In this particular experiment there seemed to be a high probability of a fourth (●) or fifth spike (□) occurring at about 8 msec. Figure 5 may be compared with certain figures shown by Hunt & Kuno (1959*b*). The precise timing and significance of these irregularities in the discharge pattern need more investigation; a few internal recordings obtained incidentally by R. M. Eccles and ourselves do, however, show that the synaptic activity of a single cell may show phases of activity, which could well be associated with the discharge patterns observed. Figure 6 shows records obtained internally: in *a–e* the size of the synaptic activity is of the right order of magnitude, although the impulse is very

small and slow, indicating a low membrane potential; *f–g* were taken later and the cell was then in very poor condition, although it was still able to fire an impulse. *a* is a record of spontaneous activity and probably

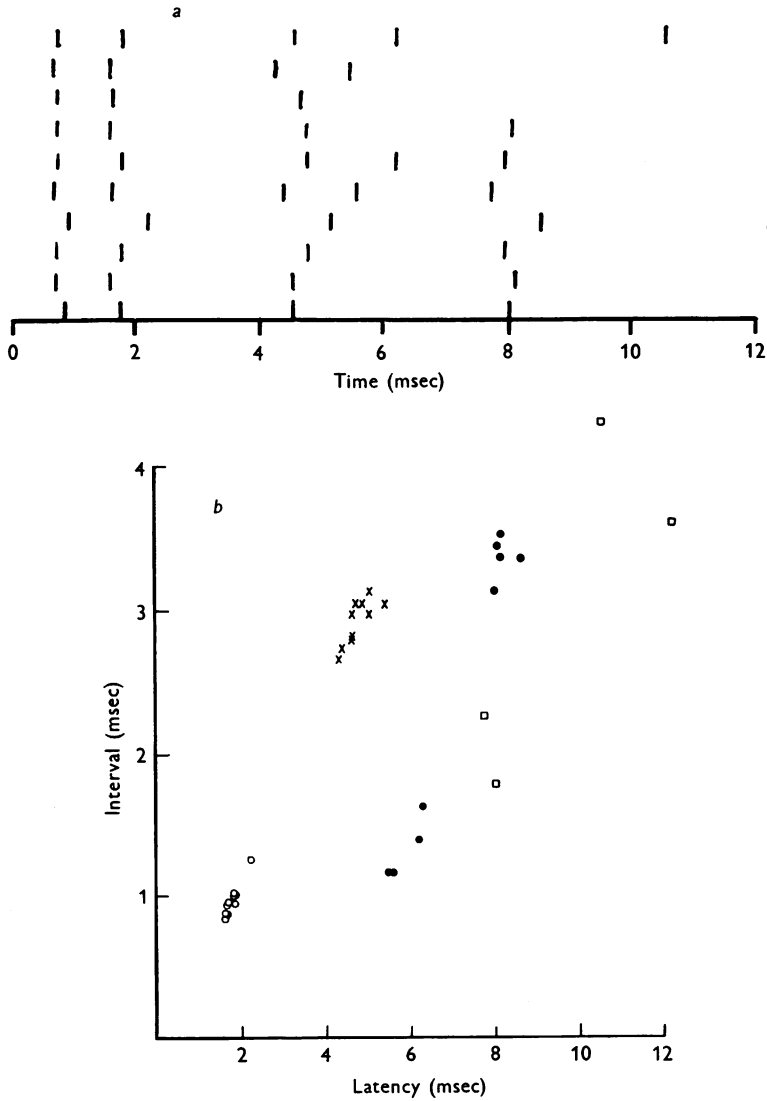

Fig. 5. *a*, diagram to illustrate the pattern of impulse discharge in a single unit. Each horizontal row indicates the time pattern of the impulses resulting from a single shock. The response patterns to ten separate shocks are shown. *b*, the same data as *a* plotted as a graph. Ordinate, interval between a particular spike and the preceding spike; abscissa, time from the incoming volley to the spike. The position of each spike in its own discharge is indicated by the symbol: ○ 2nd impulse, × 3rd impulse, ● 4th impulse, □ 5th impulse.

represents synaptic responses to single presynaptic impulses. *b* and *c* were obtained with very small electrical stimuli and are essentially the same, but for the amplification; in these there is a suggestion of a second deflexion that is more than the random background. In *d*, with a bigger stimulus, a second deflexion is clear; *e* was above but near threshold. *f* and *g* are included to show the effects of big stimuli and are recorded on a slower sweep. With stimulus strengths increasing above the threshold value the later activity increases and the peak of the later hump gets progressively later.

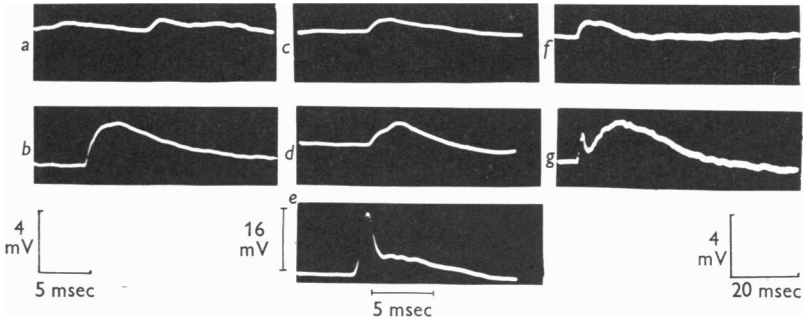

Fig. 6. Intracellularly recorded responses of a cell of the short latency type: *a*, spontaneous activity; *b*, response to a stimulus of low intensity; *c–e*, stimulus strength increased until threshold for spike reached; *f–g*, slow time trace; in *g* the stimulus was many times the threshold value.

Of the units analysed in the short-latency group, 45 % were excited by light mechanical stimulation of the pad. Most of the others were excited by movements of the hairs around the pads; a few had receptive fields on the heel or dorsum of the foot, and two required much bigger forces applied to the whole foot for excitation.

Longer-latency units

Those found are referred to mainly for purposes of contrast with the group just considered. The thresholds of the units were higher than those of the short-latency group; nevertheless, a response could usually be obtained before the main early peak of the dorsal root volley reached a maximum (Fig. 1*g*).

The position in the cord of these units was not related to the position of the early phase of the mass response, in fact 46 % of those recorded were found when the early mass response was positive (Fig. 1*f–k*). The anatomical positions of a sample of these units are shown as open circles in Fig. 4. There is no reason to regard these longer-latency units as a single group; the positions in which the units were found might even suggest the contrary.

DISCUSSION

In order to determine the function of cells in the spinal cord their properties and connexions must be so far determined that it is possible to work on a particular and sufficiently homogeneous group of cells. The purpose of the analysis presented here was to identify a cell group connected mono-synaptically to low-threshold mechanoreceptors in the skin of the foot, and to define some of the properties of the cells.

The units analysed were divided into a short-latency group and a remainder with longer latencies. All units whose responses lay in the short-latency distribution could be fired when the incoming volley was confined to a small proportion of the low-threshold, fast-conducting receptor fibres, whereas the units with a longer latency were only fired when the incoming volley was bigger. Distinctions due to latency and threshold might well be reflexions of the same process if the differences in latency were associated with great enough differences in the conduction velocities of the primary fibres. This would require that the longer-latency unit should in general be excited by fibres conducting at about 40 m/sec, while the short-latency units are excited by the 60 m/sec group (see Fernandez de Molina & Gray, 1957). Longer-latency units could often be excited by stimuli which were not maximal for the faster group. It would seem therefore that, in part at least, the two parameters, latency and threshold, can be regarded as independent.

The spatial distribution of the short-latency units has been given in both electrical and anatomical terms. In electrical terms the units are entirely within the range in which the main early phase of the mass response (phase II potential) is negative and relatively large, the distribution showing a maximum which was not significantly different from the position of the peak of the phase II potential; the anatomical distribution is also similar to that of the phase II potential (but is medial to that found by Coombs *et al.* (1956) and Fernandez de Molina & Gray (1957) for sural responses). The time pattern of the impulses suggests that the phase II potentials are the resultant of early activity in the cells of the short-latency group. The latency of the response, the minimum stimulus required to excite it, and the spatial and temporal distributions of the activity, therefore, all relate the short-latency units to the phase II potential. The spatial and temporal properties of these cells are similar to those of a group of cells identified by Wall (1960) in the dorsal horn of the cat.

When the preparations were not under the influence of an anaesthetic, the average latency of the first impulse in the responses of the short latency cells was 0·76 msec (S.D. 0·22 msec). This figures suggests that some cells of this group must be monosynaptically connected to the

receptor neurones, and if these cells do constitute a related group it seems likely that the cells are all monosynaptically connected with the fastest conducting fibres from the skin. The pattern of the impulse discharge in some experiments, and the intracellularly recorded synaptic potential in others, showed a discontinuity which suggests that the initial phase of monosynaptic activation is followed by later polysynaptic activity; both components can be excited by low-amplitude mechanical stimulation of the skin (C. J. Armett, J. A. B. Gray, R. W. Hunspeiger & S. Lal unpublished) and by electrical stimuli, which are submaximal for the fastest fibres.

In these experiments the cells analysed were all activated by mechanical stimulation of the foot, most of them by small localized displacements of the skin or hairs. The thermal and chemical sensitivities were not tested. It is possible however that these cells, like those analysed by Wall (1960), could be activated by such stimuli. The primary units which are the most sensitive to thermal and chemical events are small, and the evidence presented here and by Fernandez de Molina & Gray (1957) would suggest that these fibres are not necessary to the full development of the early phase of the response of these cells.

These results help to define a group of spinal cord cells not only in terms of their anatomical position but more particularly in relation to the mass responses of the cells in the dorsal horn. Properties of the units have previously been deduced from a study of their mass responses (Coombs *et al.* 1956; Fernandez de Molina & Gray, 1957) and the present experiments confirm suggestions that the cells are second-order to the fastest-conducting afferent fibres, many of which connect with highly sensitive mechanoreceptors in the foot. Neither these results nor those of Wall (1960) tell us anything about the destination of the axons of the cells. Later evidence, however (R. M. Eccles, unpublished), suggests that the cells of this group may be further classified according to the connexions of their axons.

The long-latency units that were found during the course of this study could clearly contribute to the later part of the mass response (phase III). The units were, however, widely scattered and do not seem to represent a homogeneous group. The evidence presented here has shown that the late phase of the mass response is likewise of mixed origin. It includes not only the activity of the long-latency units but also the polysynaptic activity of the short-latency units, and probably activity of yet other types of cell. Further investigation into the properties of this phase of the mass response is unlikely to yield more information concerning cell function in the cord.

SUMMARY

1. Mass responses and single-unit activity produced in the dorsal horn of the cat's spinal cord by electrical stimulation of the medial plantar

nerve were recorded extracellularly through micro-electrodes. An analysis of the activity was made only for those single units that could respond also to mechanical stimulation of the foot.

2. The components of the mass responses were similar to those recorded on sural stimulation; the main negative deflexion (phase II of Fernandez de Molina & Gray, 1957) was, however, found medial to that of the equivalent phase produced on sural stimulation.

3. The single units analysed could be divided into a group that responded at short latencies from the incoming volley when low-threshold afferent fibres were stimulated, and a remainder that responded at longer latencies and to higher stimulus strengths.

4. The spatial and temporal distributions of the activity of single units were examined.

5. It is concluded that the short-latency cells are monosynaptically connected to fast-conducting fibres from mechanoreceptors and that an early phase of their activity gives rise to the main early negative phase of the mass response (phase II); a later phase of the impulse discharge is polysynaptic in origin and is thought to contribute to the later negative phase of the mass response (phase III).

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