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RESPONSES OF THE RAT DORSAL COLUMN SYSTEM TO MECHANICAL STIMULATION OF THE HIND PAW

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Several recent investigations have been concerned with the response of cells in the gracile nucleus to mechanical stimulation of the skin (Gordon & Paine, 1960; Kruger, Siminoff & Witkovsky, 1961; Perl, Whitlock & Gentry, 1962). The aim of the present study has been to explore the nucleus, using inputs which were not only reproducible and of known characteristics, but in which each primary receptor unit fired only one impulse. Particular attention has been paid to the suggestion that the nucleus is functionally organized in its rostro-caudal axis (Gordon & Paine, 1960) and, since the stimulus parameters and the activity in the primary units were determined in these experiments, it has been possible to investigate the modification of signals from receptors by the nucleus. In this study the problem of setting up a known input in primary neurones has been approached by stimulating a small but fairly uniform area of skin with mechanical pulses which had a short rise time, were brief in duration, and were adequately damped so that each fibre fired only one impulse at a predictable time after each pulse; the amplitude of the pulses could be controlled. The results of this study are related to those obtained by Armett, Gray, Hunsperger & Lal (1962), who have used a similar technique to investigate dorsal horn cells responding to mechanical stimulation of the cat pad.

A preliminary account of some of this work has already appeared (McComas 1962).

METHODS

Preparation and fixation of animals. Records were obtained from 50 albino rats (weight range 153–260 g) which were anaesthetized with urethane injected intraperitoneally (1·3–1·8 g/kg). The dorsal column nuclei were exposed by removing parts of the occiput, atlas and axis, dividing the dura, and carefully sucking away the posterior part of the cerebellum. When recordings were to be made from the dorsal column, the arches of the first six cervical vertebrae were removed. The exposed areas were covered with paraffin oil contained in a Perspex loop around which the skin was fastened. The head was held by an insulated metal pin inserted into each external auditory meatus and the snout fixed by a

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clamp. The hind paw was embedded horizontally in soft plasticine with the plantar surface uppermost, taking care not to stretch the skin. The animal was warmed with radiant heat and a record kept of the rectal temperature (36–39° C).

Stimulation. Mechanical pulses were applied to the plantar surface of the hind paw; in the rat this surface is hairless but is complicated by six 'walking pads' of horny skin (Fig. 1*a*). Pulses were produced from 2·5 cm square Rochelle salt crystals which had glass styli of approximately 0·35 mm diameter attached to the moving corners. The crystals were mounted on a holder which could be moved with a micromanipulator. The tip of a stylus was lowered 0·5 mm into the paw below the point of contact. Rectangular pulses of up to 120 V were applied to the crystal, giving displacements of approximately 0·3 μ/V when

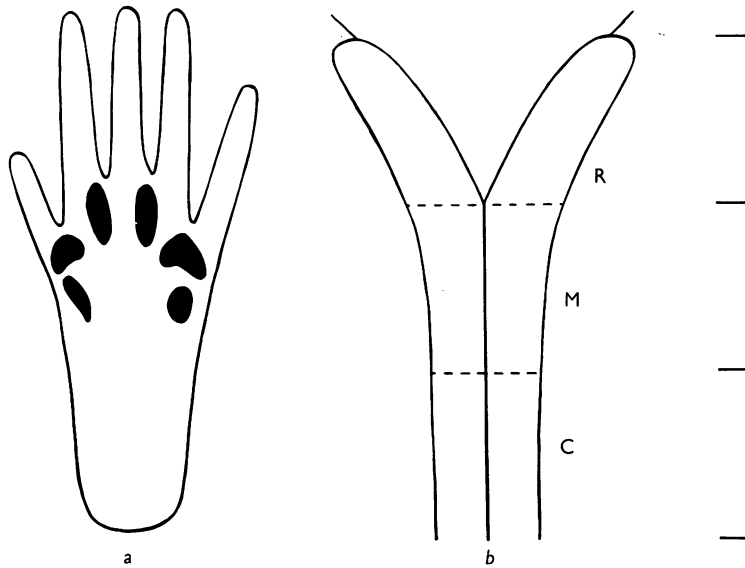

Fig. 1.*a* Plantar surface of rat hind paw (left) showing approximate situation of walking pads (black areas). *b* Dorsal aspect of gracile nuclei. Interrupted lines mark division of nucleus into rostral (R), middle (M) and caudal (C) sections (see text). Each division of vertical scale represents 10 mm (*a*) and 1 mm (*b*).

calibrated under a microscope with the stylus moving in air. The terms 'stimulus strength' and 'size of stimulus' are used interchangeably in the text and refer to the voltage applied to the crystal. Some idea of the mechanical displacement was obtained by using another crystal as a mechano-electric transducer; after it had been damped with silicone grease pulses were produced such that their displacement was 90% complete in 200 μsec and restoration was at least 67% complete in 700 μsec. The frequency of stimulation used in the experiments was in the range 1–3/sec, except when the maximum frequency of following was investigated.

Recording. Extracellular recordings from cells were made with glass micro-electrodes of 1 μ or less outside tip diameter, filled with NaCl solution, 10 g/100 ml., and having resistances of 2–10MΩ in Ringer's solution. For recording from the dorsal column fibres, units were more easily found by using platinum electrodes insulated to within approximately 20 μ of the tip with glass, leaving a metal core of 10 μ diameter which tapered to a point. An indifferent Ag-AgCl electrode was inserted into the neck muscles and the animal, which was insulated from its holder, was earthed. The input from the recording electrodes was fed through a cathode follower to a push-pull capacity-coupled amplifier, displayed on a cathode-ray

oscilloscope, and photographed. A short coupling time constant of either 3 or 30 msec was used because of marked vascular and respiratory pulsation of the brain stem and cervical cord.

Location of units. The insertion of the micro-electrode into the gracile nucleus was controlled under a dissecting microscope, the superficial boundaries of the nucleus having been previously identified (Fig. 1*b*). The position of a unit was recorded by measuring the distance of the micro-electrode tip from three planes of reference: (1) the mid line, (2) a line drawn transversely through the point where the two gracile nuclei separated at the caudal limit of the fourth ventricle and (3) the surface of the nucleus. In only a minority of animals was it possible to determine with any certainty a raised rostral boundary of the nucleus, and in these its distance from the point where the two nuclei separated varied from 0·8 to 1·0 mm. In four rats (weight 175–235 g) 15 μ thick sections were cut at intervals along the rostro-caudal axis of the nucleus and stained alternately with Heidenhain's iron haematoxylin and thionine. From these sections it seemed that units within 400 μ of the surface could reasonably be regarded as lying within the confines of the nucleus. In fact, it was unusual to record evoked activity deeper than this and all but three of the units included in this series were more superficial.

RESULTS

General characteristics of evoked activity

On exploration of the area outlined in Fig. 1*b* 264 single unit responses were obtained. In general these had amplitudes of up to 5 mV but showed considerable variation in form. In the course of this paper these units will be classified according to position into three groups, corresponding to the three sections of the nucleus shown in Fig. 1*b*; this division of the nucleus into three sections along the rostro-caudal axis was made on the basis of differences in receptive field areas (see below). It appeared that the responses in the rostral section of the nucleus were more labile than those in other parts; in some preparations good activity was obtained caudally but not rostrally, though the reverse was never found (cf. Gordon & Paine, 1960). Some organization was also apparent in the transverse axis of the nucleus; this consisted of a topographical representation in which the medial part of the nucleus received inputs from the tail and the lateral part from the flank, while the projection from the hind limb occupied a comparatively large region in between. Recordings were also made in the dorsal column at the level C3–C6 where no cells could be demonstrated histologically; these responses were of small amplitude (200 μV maximum, main deflexion negative).

Size of receptive fields

A receptive field was defined as the total area of skin which could be stimulated mechanically to excite a particular cell. Only those cells with fields which included the plantar aspect of the hind paw were studied, and since many of these fields extended into hairy skin where the crystal could not be used satisfactorily their full extents were measured by lightly

brushing the fur or gently prodding the skin with a glass rod. The results obtained resembled those described for the cat by Gordon & Paine (1960) in showing that cells in the middle part of the rostro-caudal axis of the nucleus tended to have smaller fields than those in the rostral and caudal parts (Fig. 2). It was also possible to confirm the observation made by these authors, but not by Kruger *et al.* (1961), that the background activity of cells in the middle, and less commonly in the rostral, sections could often be inhibited by stimulating skin outside the receptive fields. On the basis

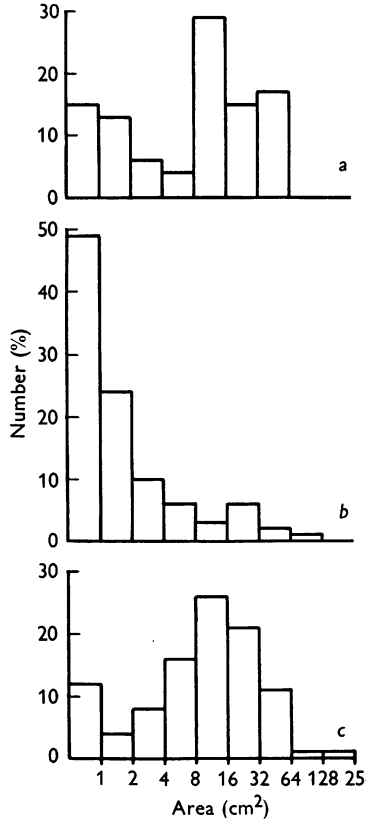

Fig. 2

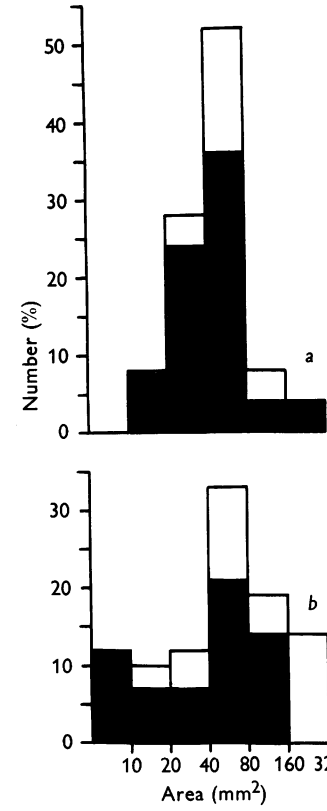

Fig. 3

Fig. 2. Sizes of receptive fields to crude stimulation for cells in different sections of the gracile nucleus. Note geometrical scale of abscissa. *a* Rostral section; 52 cells, mean 16·7, S.E. ± 2·3 cm². *b* Middle section; 113 cells, mean 4·4, ± 1·0 cm². *c* Caudal section; 77 cells, mean 17·6, ± 2·6 cm².

Fig. 3. Sizes of receptive fields to stimulation with Rochelle salt crystal. Black areas represent full extents of receptive fields confined to plantar surface of the hind paw; white areas represent fields spreading into hairy skin, full extents uncertain. *a* Results for 25 primary units: *b* results for 42 cells in the middle section of the nucleus.

of the differences in receptive field areas the gracile nucleus of the rat was divided into three sections along its rostro-caudal axis. The rostral section thus refers to that part of the nucleus rostral to the point where the two gracile nuclei divide (Fig. 1*b*); the middle section refers to the next 1 mm; and the caudal section comprises the remainder of the nucleus. The results of other investigations carried out in this study have also been related to the position of the cells in these three sections.

Figure 3 compares the receptive fields of 42 cells in the middle section and of 25 primary units; these results were obtained by exploring the entire plantar surface of the paw with the crystal. Although some of the fields were incomplete because of extension into hairy skin, it was not possible to investigate the primary units satisfactorily except with the crystal; the small responses could then be superimposed so as to make their observation easier. An interesting finding was that five of the cells in the middle section, but none of the primary units, had fields of 10 mm^2 or less. Ten of the cells encountered in the caudal section also had their fields measured in this way and 7 were found to cover all the plantar surface (approximately 3 cm^2); on the other hand, none of the primary units had fields as large as this.

A more detailed examination of the receptive fields was made by measuring the threshold for excitation at different points on the paw, taking the threshold to be the size of pulse required to excite a cell with a probability of 0·5. Figure 4 shows typical results for five cells when measurements were made at 1 mm intervals along the largest diameters of the receptive fields. *A* and *B* were both primary units, but while the receptive field of *A* was confined to the hind paw, that of *B* extended into hairy skin. The caudal cell *C* responded to gentle manual stimulation of the entire hind limb; the receptive field of this cell, like that of many others with large fields, showed more than one point of low threshold. Finally, *D* and *E* were two cells in the middle section with small fields.

Threshold

Figure 5 shows the minimum thresholds of primary receptor units and cells in the gracile nucleus after measurements had been made at several points within the individual receptive fields. An important observation was that different units in both populations of neurones often had differences in threshold for the same position of the stimulus. The five primary units with the highest thresholds gave responses that were characteristic of dorsal column relays (Hursh, 1940; Amassian & de Vito, 1957); they occurred when the rectal temperature was below 37° C and had long and variable latencies. The values of minimum threshold for the remainder of the primary units are probably rather high, because their fields were not

explored as thoroughly as those of most cells in the nucleus. The most sensitive gracile cells were in the caudal section, where some units could respond to displacements of 1 μ. The highest thresholds belonged to cells in the rostral section; 12 % of these responded to prodding of the paw but not to a maximum pulse from the crystal. The rostral cells also differed in that their thresholds tended to rise while observations were being made,

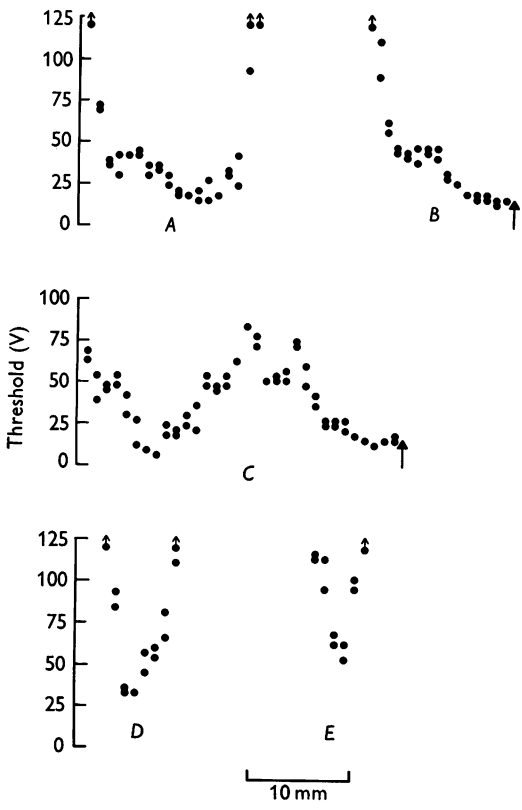

Fig. 4

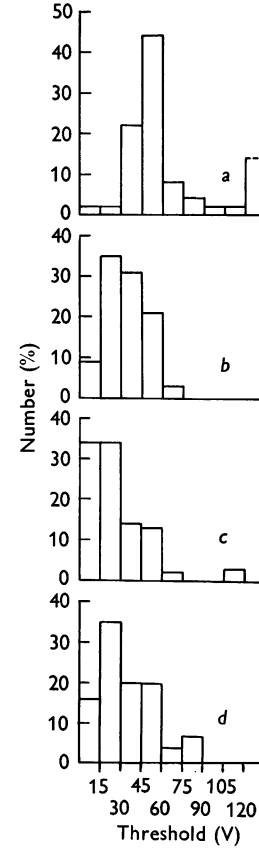

Fig. 5

Fig. 4. Thresholds at different points along the diameter of receptive field for 5 units; *A* and *B* primary units, *C* caudal cell, *D* and *E* cells in the middle section. Ordinate is voltage applied to crystal. Two sets of readings were made so as to give a second measurement for each point approximately 15–30 min after the first. Heavy arrows denote transition into hairy skin.

Fig. 5. Thresholds of primary units and cells in gracile nucleus. Abscissa is voltage applied to crystal (lowest value of several measurements at different points in receptive field). Cells with thresholds in excess of 120 V indicated by column drawn in interrupted lines. *a* Rostral section; 50 cells, mean > 65 V. *b* Middle section; 86 cells, mean 36, S.E. $\pm$1·8 V. *c* Caudal section; 64 cells, mean 30, $\pm$2·9 V. *d* Primary neurones; 55 units, mean 37·3, $\pm$3·0 V.

even at low stimulus frequencies (1–3/sec); the increase in threshold could be as much as 50% in the space of a few minutes. This lability made accurate determinations of threshold and receptive field size impossible for most rostral cells. A further observation was that the response of these cells, unlike those of high-threshold units investigated in the dorsal column, occurred at normal body temperatures.

Latency

The latencies of single unit responses were measured as the time elapsing between the start of a mechanical pulse and the appearance of activity under the recording electrode. The measurements were made to the nearest 0·25 msec and the values for 50 primary units and 173 gracile cells after pulses of twice threshold strength are given in Fig. 6; typical responses of

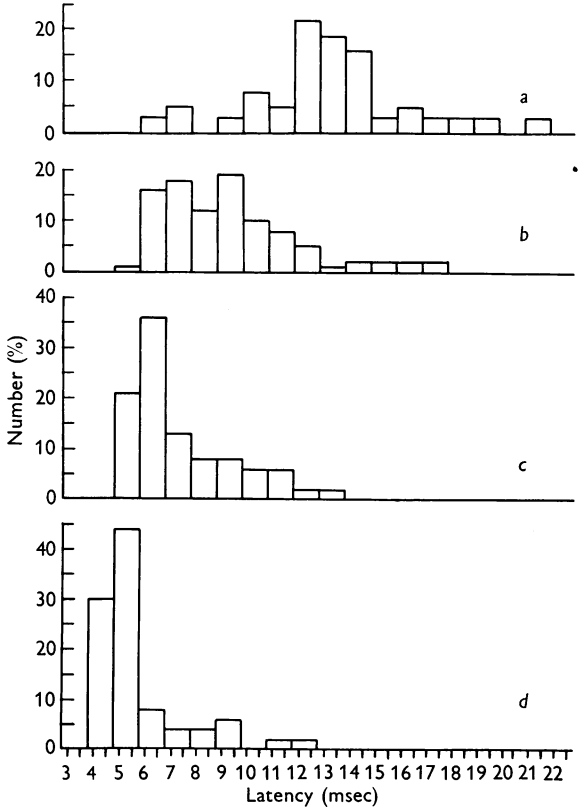

Fig. 6. Mean latencies of primary units and cells in gracile nucleus to mechanica pulses of twice threshold strength. *a* Rostral section; 37 cells, mean 13·0, S.E. ±0·5 msec. *b* Middle section; 83 cells, mean 9·3, ±0·3 msec. *c* Caudal section; 53 cells, mean 7·2, ±0·3 msec. *d* Primary units, (C3–C6), 50 units, mean 5·7, ±0·3 msec.

single units from the same preparation are shown in Fig. 7. The results did not allow an accurate estimate of the conduction velocity of first-order axons to be made, since the time occupied by the initiation of impulses was not measured; for this reason the conduction velocity of the fastest axons must have been greater than 50 m/sec, as the distance to C4 level was roughly 20 cm (19–21 cm in four animals) and the earliest activity in this

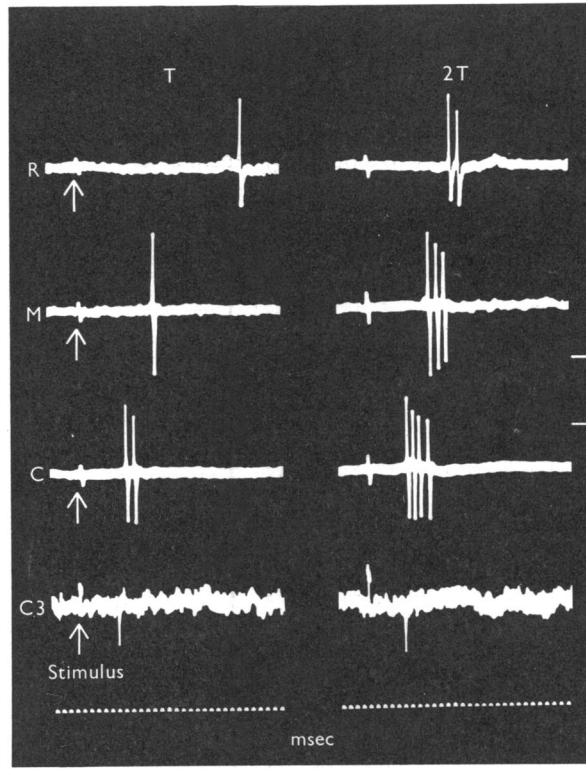

Fig. 7. Typical responses of 3 cells in rostral (R) middle (M) and caudal (C) sections of the gracile nucleus together with a primary unit at C3 level (C3) in the same preparation to pulses of threshold (T) and twice threshold (2T) strength (see text). Vertical bar represents 2 mV for records R, M, C and 150 μV for record C3. Positivity upwards. Arrows mark stimulus artifact for threshold pulses. Spikes retouched.

region had a latency of 4 msec. This value of conduction velocity may be compared with that found by Armett & Hunsperger (1961) for phasic mechanoreceptor units in the pad of the cat (46–85 m/sec).

It will be seen from Fig. 6 that about 1 msec elapsed between the arrival of the afferent volley at C3–C6 and the onset of activity in the caudal section of the nucleus. Part of this time must have been occupied by

conduction in the remainder of the dorsal column (approximately 20 mm at C4) and it seemed reasonable to attribute the rest of the delay to transmission across one synapse. On the other hand, only a small fraction of the difference in latency between caudal and rostral cells can be explained by intranuclear conduction, since nearly all the cells investigated were within 3 mm of each other. The latency measurements also revealed other differences between the gracile cells. Thus the latencies of caudal cells were remarkably constant if the stimulus was kept at threshold strength, nor was there usually shortening of more than 1 msec if the strength was increased. In both respects they resembled primary units in which the decrease in latency was never more than 0·5 msec. At the other extreme the latencies of rostral cells to successive threshold stimuli could vary by several milliseconds, and appreciable shortening occurred if the stimulus strength was then doubled, the maximum decrease being 17 msec.

Number of impulses fired

The brief mechanical pulses employed in these experiments usually evoked only one impulse in single primary units once threshold had been reached or passed. This was true of all but 7 of the 55 units investigated, and 5 of these had responses typical of dorsal column relays. By contrast, the majority of cells in the gracile nucleus responded to a suprathreshold stimulus with a repetitive discharge consisting of up to 9 impulses. For a given cell, however, the number of impulses evoked by stimuli of the same strength repeated at 1–3/sec tended to vary; in such cases it was found, by averaging the responses, that most cells fired more impulses to a stronger stimulus. This is shown in Fig. 8, which contains the pooled results for 57 cells with low thresholds, of which 24 were in the caudal section and 33 in the middle section. The 57 cells include 10 which did not alter their output when the stimulus strength was varied; 7 of these units were in the middle section with small receptive fields and latencies of 7 msec or less. The results also included the responses of a caudal cell which fired fewer impulses when the stimulus strength was increased; such behaviour was noted in only two other cells in the whole study, and these were also in the caudal section. When the results for the cells in the two sections were analysed separately, however, no significant difference was found.

Response to repetitive stimulation

Figure 9 shows the ability of 121 cells in the gracile nucleus to follow repetitive stimulation with mechanical pulses of twice threshold strength (measured at 1–3/sec). The main finding was that whereas none of 31 cells in the rostral section could follow at more than 50/sec, 24 of 43 caudal cells

responded to successive stimuli at more than 150/sec for up to 1 sec and sometimes longer. That these were gracile cells and not primary units was shown by their behaviour at low repeat frequencies; they then responded to each stimulus by firing a number of impulses which was proportional to the stimulus strength. Figure 10 shows a cell which fired repetitively

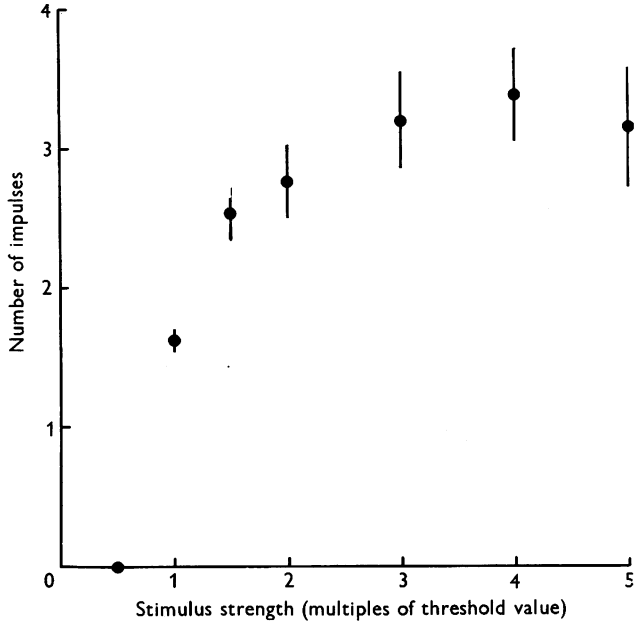

Fig. 8

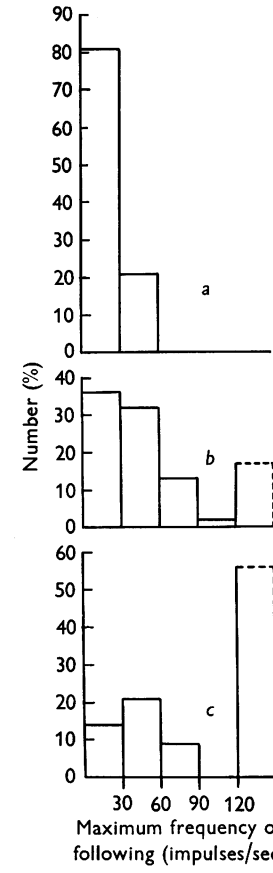

Fig. 9

Fig. 8. Relationship between stimulus strength and the mean number of impulses fired by cells in the gracile nucleus. Negative responses (failure to fire) included in calculation of means. Vertical bars indicate standard errors of means. Stimulus strength shown as multiples of threshold value. Each point represents pooled results for 57 cells except points for 0·5, 1·5 and 5 times threshold strength (20, 24 and 22 of total cells, respectively).

Fig. 9. Maximum frequency of following for cells in rostral (*a*), middle (*b*) and caudal (*c*) sections (31, 47 and 43 cells, respectively). Pulses were of twice threshold strength. Cells able to follow at frequencies in excess of 120/sec indicated by columns drawn in interrupted lines.

while following at 150/sec; the maximum frequency of following observed for a cell in the caudal section was 440/sec. Similar high rates of following have been reported for dorsal column cells by other workers using mechanical or electrical stimuli (Amassian & de Vito, 1957; Gordon & Seed, 1961; Perl *et al.* 1962) but have not been observed for mechano-sensitive dorsal horn cells (Armett *et al.* 1962).

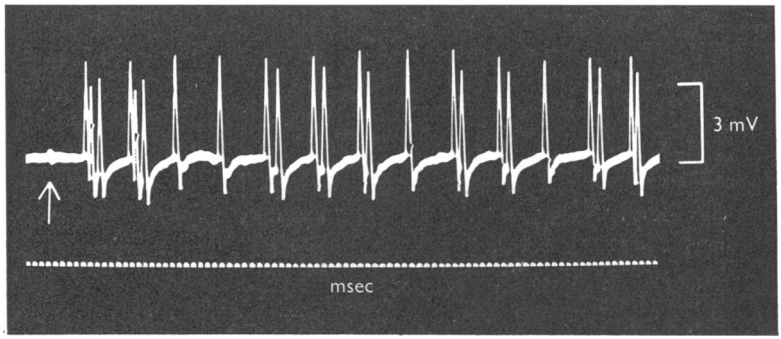

Fig. 10. Response of caudal cell to repetitive stimulation at 150/sec. Note individual responses often consist of more than one impulse. First stimulus artifact shown by arrow, others hidden in discharge. Positivity upwards. Spikes retouched.

DISCUSSION

By using a specific type of quantitatively controlled mechanical stimulus it has been possible to explore the gracile nucleus with inputs which were not only reproducible but in which some of the characteristics were known. These inputs consisted of activity evoked in a population of primary receptor units which had axons running in the dorsal column; each unit could be excited by mechanical stimulation of a certain area of skin to fire most commonly a single impulse which arrived in the vicinity of the nucleus after a measured delay. A more detailed analysis of the patterns of activity evoked in a population of primary receptor units has been made in the cat (Armett & Hunsperger, 1961; Armett *et al.* 1962). One of the results of this study has been to show that cells in the nucleus have functional differences which are not due to changes in the type of stimulus or its position on the body surface. The main findings were that cells in the rostral section of the nucleus, in contrast to those in the caudal section, tended to have high thresholds, long latencies, poor frequency-following ability and an apparent susceptibility to anaesthesia, while the responses of cells in the middle section lay between these two extremes. In addition, it was possible to confirm the observation of Gordon & Paine (1960) that cells in the middle section had the smallest receptive fields and that the

background activity of these cells could frequently be inhibited by mechanical stimulation of adjoining areas of skin.

Apart from providing further evidence of functional differences amongst cells the present study has also given some information as to the synaptic arrangements within the nucleus. The most conclusive finding, made on the basis of latency measurements, was that most of the cells in the caudal section probably had monosynaptic connexions to primary units. Furthermore, since most primary units responded to each suprathreshold stimulus with a single impulse, whereas the caudal cells fired a number of impulses proportional to the stimulus size, it is clear that convergence must take place; the rather limited observations on receptive field areas support the same conclusion. The longer latencies, greater changes of latency with increasing stimulus strength and poor frequency following of cells in the middle and rostral sections suggest that these cells are probably polysynaptically connected with the primary units. The results do indicate that some cells in the middle section might be monosynaptically connected to fast-conducting primary units and others could have monosynaptic inputs if they were driven from slower conducting fibres which might have been missed by the extracellular techniques employed. However, there is no certain evidence that slower conducting fibres are excited by mechanical pulses of the sort used in these experiments (see also cat's pad, Armett & Hunsperger, 1961; Armett *et al.* 1962). The view taken by Perl *et al.* (1962) is that primary receptor units responsive to hair movement are connected to cells with small receptive fields and that these cells are found predominantly in the middle section of the nucleus. While it is possible that different parts of the nucleus may receive inputs from different types of receptor, a large proportion of the cells studied in the middle section had receptive fields which, although small, were confined to hairless skin, so that the particular suggestion made by these authors seems inadequate, at least in the rat. If, as seems likely, the nucleus does contain polysynaptic pathways, then the cells in the caudal section might direct all or, by means of axon collaterals, part of their output to cells in the middle section for modification. Such a mechanism might explain the observation of Gordon & Seed (1961) that only 60 % of the cells in the caudal section send axons into the medial lemniscus. A related problem, which has not been studied here, is the nature of the inhibitory process responsible for reducing the receptive field areas of cells in the middle section.

The results of this investigation have also thrown some light on the way in which information about the parameters of a mechanical stimulus may be carried initially by primary neurones and subsequently by gracile neurones. An important observation in this context was that cells in both populations had receptive fields which overlapped, but with different

thresholds for given positions of the stimulus. It would be expected that such an arrangement would cause a greater number of cells to be excited in both populations as the stimulus strength was increased. As a result of synaptic mechanisms, however, an increase in size of a stimulus may also be signalled by individual cells in the gracile nucleus discharging more impulses. Similar patterns have been shown to occur in primary neurones and in dorsal horn cells in the cat (Armett *et al.* 1962). The repetitive firing of these second-order cells may conceivably serve to reduce the number of post-synaptic channels required for carrying information. The activity evoked in a system of neurones with overlapping receptive fields can contain information about the position of a stimulus; however, the very small fields of cells in the middle section may put this information in a form which is particularly accessible to higher neurones (cf. Gordon & Paine, 1960).

SUMMARY

1. The responses of single cells in the gracile nucleus to mechanical stimulation of the hind paw have been studied in the rat. The inputs to the nucleus were reproducible and had some known characteristics.

2. The cells in the rostral section of the nucleus, in contrast to those in the caudal section, tended to have high thresholds, long latencies, poor frequency-following abilities and an apparent susceptibility to anaesthesia. The cells in the middle section were characterized mainly by their smaller receptive fields.

3. These differences in the behaviour of cells in the gracile nucleus provide further evidence of functional organization in the rostro-caudal axis of the nucleus.

4. It is probable that cells in the caudal section of the nucleus have monosynaptic connexions to primary units and that convergence occurs. It is likely that more rostrally situated cells have polysynaptic inputs.

5. The way in which the dorsal column system may convey information about a mechanical stimulus is discussed.

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