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PRESYNAPTIC AND POST-SYNAPTIC INHIBITION ELICITED IN THE CAT'S DORSAL COLUMN NUCLEI BY MECHANICAL STIMULATION OF SKIN

By P. ANDERSEN, B. ETHOLM and G. GORDON
From the Institute of Neurophysiology, University of Oslo, Norway

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SUMMARY

1. Primary afferent depolarization (PAD), with a time course comparable with that of the PAD following limb nerve stimulation, was produced in the cuneate nucleus by mechanical stimulation of the skin of the ipsilateral forepaw. Brushing or blowing on hairs was as effective as any other form of stimulation and there was a rapid adaptation to a sustained stimulus. Up to 55 % increase in excitability was produced by blowing on hairs.

2. The P-wave and the PAD produced by mechanical stimulation were at a minimum in the rostral part of the cuneate nucleus and at a maximum 2–6 mm caudal to the obex.

3. The distribution of the PAD produced in the gracile nucleus by blowing on the skin of the hind foot was studied by a technique allowing measurement of excitability changes near the terminals of single fibres. Minimal values were obtained rostral to the obex and maximal values 1–4 mm caudal to the obex, a distribution matching that previously determined for single cells subject to surround inhibition.

4. Post-synaptic inhibition was produced in the cuneate nucleus by gentle blowing on the ipsilateral forepaw. Up to 20 % fall in excitability occurred in populations of cells tested by direct electrical stimulation. IPSPs lasting up to 160 msec were observed in single cells following light mechanical stimulation in the immediate neighbourhood of these cells' receptive fields. Blocking of antidromic invasion of single cells was occasionally produced by mechanical skin stimulation.

5. It is concluded that both pre- and post-synaptic inhibition must be concerned in the phenomenon of afferent surround inhibition, though there was no evidence to indicate their relative roles, qualitatively or quantitatively.

6. It is shown that up to 20 % reduction in transmission through the

gracile or cuneate nucleus could be produced by blowing on the ipsilateral hind paw or forepaw respectively, measured as reduction in the area of the monophasically recorded lemniscal response. A single electrical stimulus to the skin of the contralateral forepaw reduced transmission through the cuneate nucleus by less than 5%.

INTRODUCTION

Presynaptic and post-synaptic inhibitory actions have been described in the cat's cuneate nucleus following electrical stimulation of nerves in the ipsilateral forelimb (Andersen, Eccles, Schmidt & Yokota, 1964*a*, *b*; Andersen, Eccles, Oshima & Schmidt, 1964). The dorsal column nuclei offer a specially favourable site for investigating the P-wave and the primary afferent depolarization, which provide evidence for presynaptic inhibition, because of the regular geometry and well defined origin and destination of the incoming and outgoing fibre tracts. They are less favourable for obtaining direct systematic evidence on post-synaptic inhibition because pulsation and the small size of the cells make intracellular recording difficult.

Powerful inhibitory action has also been observed on single cells in these nuclei following gentle mechanical stimulation of the skin hairs, using extracellular recording (Gordon & Paine, 1960; Perl, Whitlock & Gentry, 1962; Gordon & Jukes, 1964). Commonly the excitatory region of such a cell's receptive field is partly or completely surrounded by a region from which inhibition of the resting or evoked discharge of the cell is easily elicited by displacing a few hairs or blowing gently on the hairy skin. The inhibitory effect diminishes with distance from the excitatory zone. In the gracile nucleus, cells with these properties are most common in a region 2–5 mm caudal to the obex, and virtually all project into the contralateral medial lemniscus (Gordon & Jukes, 1964).

The main object of the present experiments was to investigate the effectiveness of mechanical stimulation of the skin in eliciting presynaptic and post-synaptic inhibition in these nuclei and to get some evidence as to the type or types of skin receptor concerned. The quantitative distribution of presynaptic inhibition in the nuclei has also been studied and compared with the distribution of surround inhibition, in an attempt to evaluate the suggestion of Andersen *et al.* (1964) that the two are related. Finally, an experimental estimate has been made of the power of skin stimulation in inhibiting transmission through these nuclei.

A preliminary account of this work has already been published (Andersen, Etholm & Gordon, 1968).

METHODS

All experiments were done with cats anaesthetized with sodium pentobarbitone (Nembutal). Anaesthesia was maintained at a level sufficient to prevent spontaneous movement or movement in response to any experimental procedure. The dorsal column nuclei were exposed by removing the laminae of the first two vertebrae and some of the occipital part of the skull, and enough of the posterior cerebellum was removed by suction to allow direct access to the rostral parts of the nuclei. The nuclei were usually covered with liquid paraffin. When attempts were made to record from single cells under stable mechanical conditions and to make intracellular records, the nuclei were covered with 4% agar in Ringer solution, introduced at about 42° C and becoming firm on cooling; the small area intended for exploration by the electrode was kept clear of agar by previously inserting a conical plug of Plasticine and removing it after the agar had hardened.

Limb nerves, appropriate to the experiment, were prepared for stimulation or recording. For experiments on the cuneate nucleus, the median (M), ulnar (U) and superficial radial (SR) nerves in the forearm were usually prepared on one side, and the SR alone on the other side so as to leave most of the skin innervation intact. In experiments on the gracile nucleus the sural (SUR) nerve was prepared in the lower leg. Each nerve was lifted on to a pair of electrodes, cut and if necessary crushed distally so that its antidromic response was recorded monophasically; and all nerves were kept in paraffin pools warmed by radiant heat.

The rostral end of the medial lemniscus (ML) on each side was exposed for monopolar stimulation or monophasic (killed-end) recording by sucking out part of the thalamus, following the technique described by Anderson, Eccles, Schmidt & Yokota (1964*c*).

Mechanical stimuli synchronized to the time-base sweep (referred to in Figures as MECH) were provided by a converted moving-coil loud-speaker operating a cylindrical Perspex probe of 9 mm diameter. The operating pulses were long compared with the responses studied so that only the onset phase of the mechanical pulse was relevant: this had a very rapid rise to a maintained level about which there was some damped oscillation. Other forms of mechanical stimulation are described in the text. Pairs of needles with about 3 mm separation were inserted in the skin in some experiments and used for stimulation.

Tests of presynaptic inhibition. Cuneate positive (P) and negative (N) waves were always monitored with a focal electrode on the surface of the nucleus: this electrode could be moved systematically to investigate spatial distribution of the P-wave. Changes in the P-wave with depth were investigated with micropipettes of 0·5–1·0 MΩ resistance filled with 4 M-NaCl ('coarse' electrodes). Recording time constant was 1 sec.

Primary afferent depolarization (PAD) was investigated by testing the excitability of primary afferent terminals. Test stimuli were delivered either through an electrode on the surface of the nucleus or through an inserted 'coarse' micropipette, and the antidromic response was monitored by recording from a limb nerve (Wall, 1958). Conditioning stimuli were either electrical (to limb nerves or skin) or mechanical (to skin surface or hairs). For the *cuneate* nucleus the effect of conditioning, based on the size of the compound action potential (in, e.g. the SR nerve) is expressed as a percentage increase in excitability relative to a control calibration curve constructed by using a series of test voltages (Eccles, Magni & Willis, 1962). For the *gracile* nucleus the effect of conditioning is expressed here as a percentage reduction in the threshold of individual fibres for the test shock: in this case test *current* was monitored in order to discount errors introduced by changes in resistance of the test stimulating electrode.

Tests of post-synaptic inhibition. The first short-latency wave recorded in the ML in response to a stimulating pulse delivered through a coarse micropipette in the cuneate nucleus is attributable to direct stimulation of cuneate neurones or their axons. The depression of this wave by peripheral conditioning was used as an index of post-synaptic inhibition (see Andersen *et al.* 1964). Inhibition is expressed here as a percentage reduction in excitability relative to a control calibration curve formed by using a series of test voltages. Signal detection in ML records was improved by averaging a number of sweeps with an averaging computer.

Any depression produced by conditioning could be read out from the computer by subtracting from the unconditioned test response an equal number of conditioned test responses (see Fig. 10*B* and *C*); or alternatively subtracting unconditioned test responses from conditioned test responses, giving a corresponding reversal of sign in the read-out of the depression (see Fig. 12*D*, *E* and the following section).

Responses of single cuneate cells, most identified as relay cells by their antidromic response to contralateral ML stimulation, were recorded in some experiments with fine micropipettes of 5–10 MΩ resistance filled with 2 M potassium citrate. With extracellular recording, peripheral receptive fields were determined for these cells and any inhibitory components of these fields investigated: any blocking of antidromic invasion by peripheral stimulation provided evidence of post-synaptic inhibition. An attempt was made to obtain intracellular records from each cell, with the particular object of observing any inhibitory post-synaptic potential (IPSP) elicited by peripheral mechanical stimulation. Stable records were rarely obtained because of pulsation in the tissue.

Tests of transmission through the dorsal column nuclei. The response in the contralateral ML to stimulating the skin of the forelimb or hind limb depends largely on transmission through the cuneate or gracile nuclei respectively, though a small part of the response must be attributed to the output of the spino-cervico-thalamic system. In this case we used electrical skin stimuli through needle electrodes for test stimulation, and studied the depression of the monophasic (killed-end) ML response, measured as percentage reduction in its area and peak amplitude produced by mechanical stimuli in different regions of skin. Detection was improved by computer averaging of a number of sweeps (see previous section).

Conventions followed. In describing positions along the longitudinal axis of the gracile or cuneate nucleus, zero is taken as the position of the obex: positions (in mm) rostral to the obex are prefixed with plus (+) symbols and positions caudal with minus (−) symbols. The rostral borders of both nuclei are taken to be at about +2 mm.

In all Figures, negative deflexions at the active electrode are upwards for extracellular records, and depolarization is upwards for intracellular records.

RESULTS

Presynaptic depolarization of cutaneous fibres terminating in the gracile and cuneate nuclei

Adequate stimulus. A single shock delivered to an ipsilateral forelimb nerve causes a response, which can be recorded from the surface of the cuneate nucleus, consisting of a brief triphasic deflexion representing the volley in the cuneate fasciculus, followed by a larger negative deflexion (the N-wave) and finally a still larger and prolonged positive wave (P-wave). Such a response is illustrated in Fig. 1*A*. The N-wave has been

shown to represent the depolarization of cuneate cells by the afferent volley, and the P-wave to represent prolonged depolarization of the synaptic terminals of primary fibres in the nucleus (Andersen *et al.* 1964*a*, *b*). Fig. 1*B* shows that a similar response was produced by delivering a fast-rising mechanical stimulus of about 1 mm amplitude to the extremity of the limb: the N-wave is smaller, probably because of the less synchronous activation of afferent fibres by the stimulus, but the P-wave is similar both in size and duration to that produced by nerve stimulation.

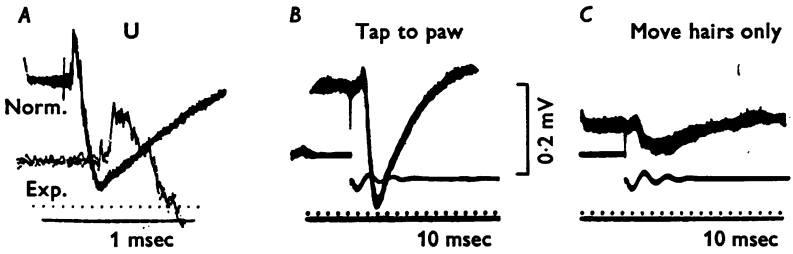

Fig. 1. Records taken from the surface of the cuneate nucleus in response to stimulation of ulnar nerve (*A*), a strong tap to the tip of the ipsilateral forepaw (*B*), and exclusive movement of a cluster of five to ten hairs (*C*). In each trace, three records are superimposed. Middle trace in *A* shows expanded (exp.) sweep of the initial part of the normal (norm.) trace. Middle traces in *B* and *C* indicate onset of the mechanical stimulation, expanded sweep.

It is certain that fast-rising mechanical pulses of this amplitude will excite a variety of types of receptor, possibly in parts of the limb remote from the point of application of the stimulus. Considerable restriction in spatial spread was achieved by applying the probe to hairs only, avoiding contact with the skin surface. N- and P-waves were still produced by such a stimulus, the latter now much smaller but following approximately the same time course (Fig. 1*C*). Detectable P-waves could be produced by restricting the stimulus to the movement of one or two hairs. When recorded through a coarse micropipette inserted in the nucleus, the P-wave produced by mechanical stimulation was found to reverse its electrical sign at a depth of approximately 1 mm, the inverted counterpart reaching its maximum amplitude at a depth of about 1·4 mm: in these respects it behaved exactly as the P-wave elicited by nerve stimulation, and there is no reason to doubt that the mechanism of its production is the same in either case.

Investigation of the excitability changes in the terminals of primary afferent fibres provides a much more sensitive index of primary afferent depolarization than the P-wave; and, as would be expected, fast-rising

mechanical stimuli to the forepaw caused an increase in the excitability of terminals of cutaneous (SR) fibres comparable with that produced by electrical stimulation of a limb nerve, up to 50 % (for technique of estimation, see Methods). An increase in excitability occurred in both the fast and slower components of the SR antidromic action potential. This increase had a long time course matching that of the P-wave, with a maximum at 25–30 msec and a total duration 100 msec or more (see Fig. 2).

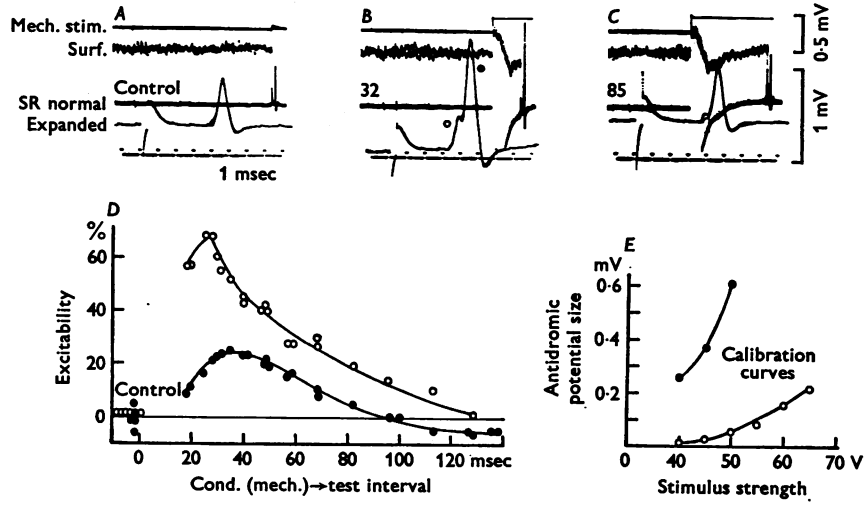

Fig. 2. Time course of primary afferent depolarization produced by mechanical stimulation. In the specimen records *A* to *C*, reading from the top, the first trace shows the onset of a mechanical stimulation (Mech. stim.) of the ipsilateral forepaw, and the second a surface (Surf.) record taken from the central region of the cuneate nucleus. The third and fourth traces show the antidromic response in the superficial radial nerve produced by a test shock delivered through an inserted micro-electrode in the central region of the cuneate nucleus on normal and expanded sweep, respectively. In *B*, the test shock was preceded by 32 msec by a mechanical stimulation (tap) of the dorsal surface of the ipsilateral paw. Both components of the antidromic nerve action potential were increased (open and filled circles). *C*, similar to *B*, but the conditioning-test interval was 85 msec. *D*, the excitability of the superficial radial nerve terminals ending in the cuneate nucleus plotted against the conditioning-test interval. The excitabilities were calculated using the calibration curves in *E* according to the method given by Eccles *et al.* (1962).

This time course for the mechanically produced excitability change matches that determined by Andersen *et al.* (1964*b*) for the excitability change produced by nerve stimulation.

Fast-rising mechanical stimuli, synchronized to the time base, offer advantages in the timing of events relative to the stimulus, for example,

in determining the time course of excitability changes. But even when such a stimulus is restricted to a few hairs, a number of types of receptor must be excited locally. Short of using more sophisticated stimulating equipment allowing generation of a large variety of pulse forms, we thought it more informative to apply stimuli by hand-held probes (stroking hairs, or pressing lightly on the skin) or by blowing gently on the hairy skin, thereby allowing approximate determination of the character of the receptors concerned.

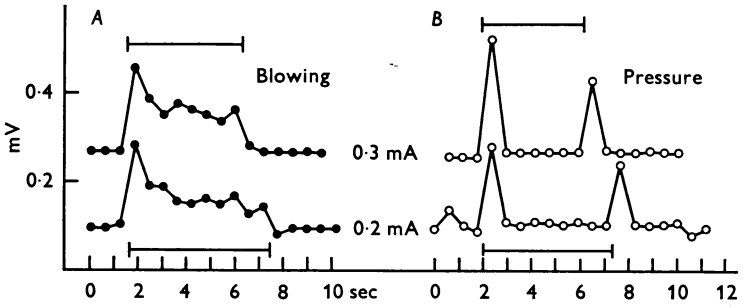

Fig. 3. Excitability changes of fibres of the superficial radial nerve terminating in the cuneate nucleus, induced by blowing and pressure on the ipsilateral forepaw. *A*, filled circles give the size of the antidromic action potential in the superficial radial nerve in response to stimuli of 0·2 and 0·3 mA delivered by a micro-electrode inserted into the cuneate nucleus. The horizontal bars indicate the duration of gentle blowing near the tip of the ipsilateral paw. *B*, similar to *A*, but the stimulus was firm pressure on the dorsal surface of the ipsilateral forepaw. Responses were evoked every 0·6 sec.

Fig. 3 shows the effect of such conditioning stimuli on the size of the antidromic response in SR to test stimuli of constant (but well below maximal) voltage repeated every 0·6 sec through the conditioning period.

Fig. 4 shows original records from such an experiment. It can be seen that light blowing gave a large on-effect and a much smaller off-effect (Figs. 3*A*, 4*A*) with a low plateau of maintained effect during the stimulation period. These effects must have depended almost entirely on receptors in the hairy skin, because there was no detectable difference if the hairless pads were screened from stimulation. Light pressure on hairy skin of the forepaw gave large on- and off-effects, but little or no maintained plateau in between (Figs. 3*B*, 4*B*). Increase in stimulus amplitude by applying heavy pressure or a prolonged pinch did not increase the amplitude of effect and the effect still adapted rapidly. It was concluded that the largest part of this effect was derived from rapidly adapting receptors in hairy skin, probably from hair receptors themselves. The low maintained plateau during steady blowing can be attributed to turbulence

in the air current causing small hair movements: it was confirmed in records from primary fibres from hair receptors entering the cuneate nucleus that such a discharge occurs during prolonged blowing.

Quantitative estimates of the excitability change in SR terminals produced by light blowing on the forepaw were made by measuring the maximal increase in amplitude of the SR response produced by blowing at a number of test stimulus voltages (see Methods): examples of such observations are shown in Fig. 6. The greatest observed increase in excitability caused by blowing on the skin occurred in this experiment and was estimated as 55% (Fig. 6*F*).

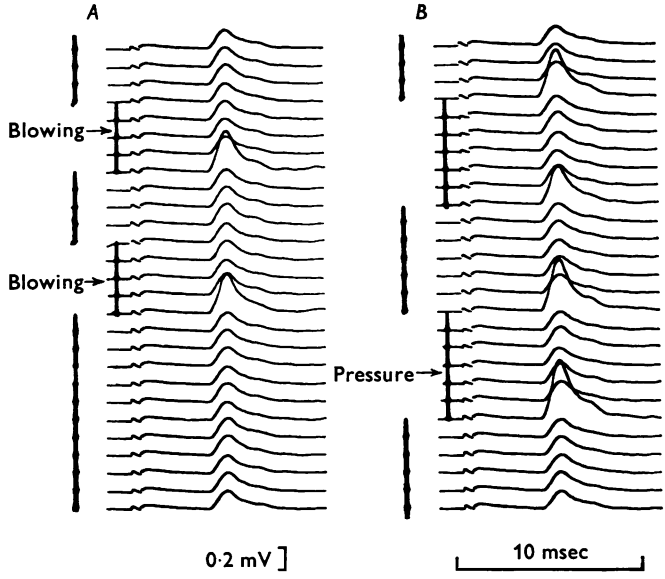

Fig. 4. Antidromic potentials recorded from the ipsilateral superficial radial nerve in response to stimuli of constant size delivered through a micro-electrode inserted in the cuneate nucleus. The records should be read from below upwards. The deflexions of the vertical line on the left indicate the duration of gentle blowing (*A*) or firm pressure (*B*) delivered to the ipsilateral forepaw. The traces occurred every 0·6 sec.

Distribution

(*a*) *Cuneate nucleus.* Fig. 5*A* shows the distribution of the N- and P-waves, elicited by single pulses to different forearm nerves, along the surface of the cuneate nucleus. It is seen that both waves tended to a peak at about −3 mm, decrementing slowly in the caudal direction and rapidly in the rostral direction. This agrees with the distribution found by Andersen *et al.* (1934*a*). Fig. 5*B* shows the distribution of N- and P-waves

elicited in the cuneate nucleus of the other side in the same animal by fast-rising mechanical stimuli applied to the forepaw of that side. The peak amplitude of the P-wave occurred at about the same place as that for nerve stimulation: the peak amplitude of the N-wave was very low for all points.

The distribution of presynaptic depolarization in the cuneate nucleus, using excitability testing of the terminals of a cutaneous nerve (SR), was

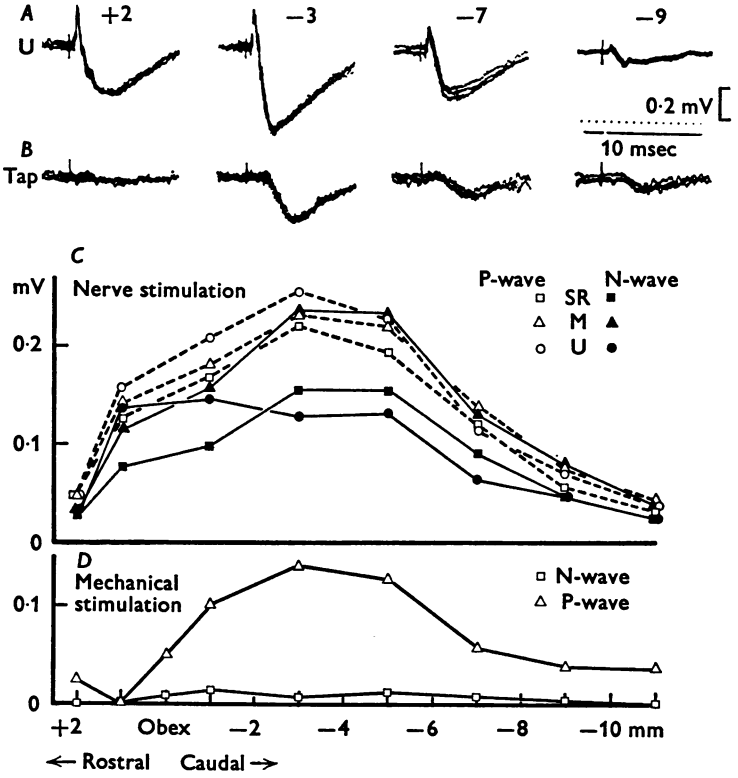

Fig. 5. Distribution of N- and P-waves along the cuneate nucleus in response to stimulation of various forelimb nerves and to mechanical stimulation. The records in *A* are taken from various positions along the right cuneate nucleus in response to stimulation of the ipsilateral ulnar nerve: each is composed of three superimposed traces. The figures above indicate the distance from obex in mm, + indicating rostral to and − indicating caudal to obex. *B*, records similar to those in *A*, taken from corresponding points on the left cuneate nucleus in the same animal in response to a strong tap delivered to the second and third toe of the left forepaw. *C*, size of N- (filled symbols) and P-waves (open symbols) induced by stimulation of three forelimb nerves in relation to recording location along the cuneate nucleus. *D*, size of N- (open squares) and P-waves (open triangles) produced by mechanical stimulation of ipsilateral forepaw in relation to the recording location along the right cuneate nucleus.

investigated in three experiments. Difficulties were encountered in estimating the excitability changes systematically through the length of the nucleus with an inserted test micropipette: the nucleus is up to 2 mm wide, and to obtain the maximum change in excitability at each position in the long axis requires many insertions of the electrode. The consequent damage and deterioration in the nucleus probably accounted for our being unable to obtain consistent repeat readings by this method.

The experiment illustrated in Fig. 6 was done with the test micropipette pressing on to the surface of the nucleus, using blowing on the hairy skin as the conditioning stimulus. The observations from *A* to *G* (+1 to −8 mm

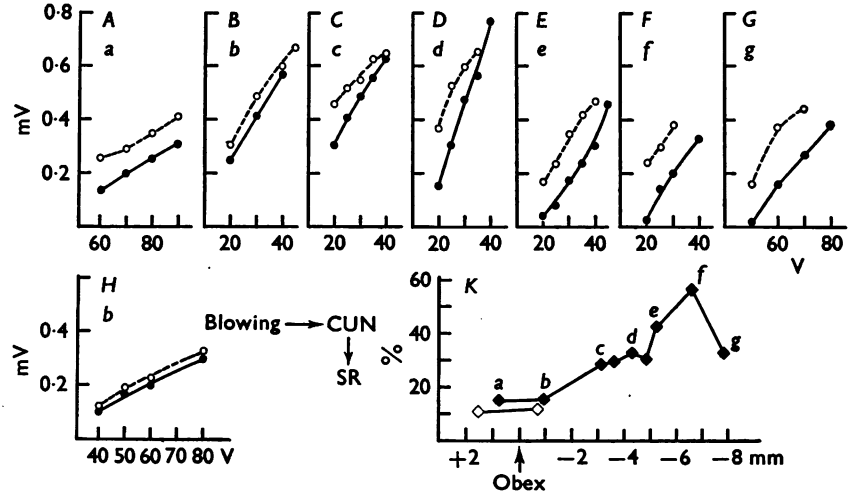

Fig. 6. Distribution of primary afferent depolarization in superficial radial nerve terminals ending in the cuneate nucleus. In *A* to *G* the filled circles give the size of the antidromic responses in the superficial radial nerve produced by control stimuli of increasing strength. Simultaneous blowing on the tip and dorsal surface of the ipsilateral forepaw increased the excitability of the superficial radial terminals as indicated by a larger antidromic response to the test stimuli (open circles). The excitability changes, calculated according to the calibration technique of Eccles *et al.* (1962), have been plotted in *K* as a function of longitudinal distribution along the cuneate nucleus. The labelling *a* to *g* indicates the points from which the graphs in *A* to *G* were derived in succession. *H* is a control run to test the excitability of fibres ending near point *b*: this and one other control were made about one hour after the first test run (points marked ◇ in *K*).

in the long axis) were made in reasonably quick succession, moving caudally: finally, two repeat sets of observations were made at the rostral end which gave values agreeing well with the original ones at this level. The repeat values are plotted as open symbols in Fig. 6*K*, and the data on which one

of them is based are shown in Fig. 6*H*. It is seen that the maximum change in excitability determined by this method came at about −6 mm (around 50 %), and that the value fell rostrally to the low value of 10–15 % which was maintained between −1 and +1·7 mm. This distribution may have been slightly distorted by the test electrode being on the surface, the whole curve in Fig. 6*K* possibly being displaced caudally with respect to the position of the actual terminals where depolarization occurred. In another

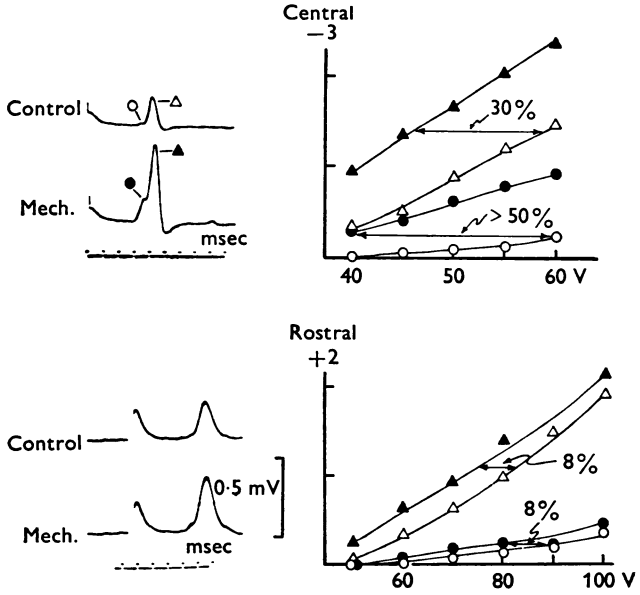

Fig. 7. Testing of excitability of superficial radial fibres in the central and rostral regions of the cuneate nucleus. The tracings in the upper left corner indicate the antidromic response to a test stimulus delivered to the cuneate nucleus 3 mm caudal to obex (Control) and the response induced by the same stimulus when a mechanical (Mech.) tap was given the tip of the forepaw 20 msec earlier. To the right are calibration curves where the test responses (open symbols) and mechanically conditioned responses (filled symbols) are plotted against stimulus strength. The figures indicate the percentage increase of stimulus strength necessary to equal the increased antidromic responses produced by the mechanical conditioning stimulus. In the lower part of the figure the test stimulus was delivered to the rostral region of the nucleus (2 mm rostral to obex).

experiment a direct comparison was made between the maximum excitability change that could be detected, following a fast-rising mechanical conditioning stimulus, in a rostral area (+2 mm) and in a central area (−3 mm) of the nucleus, using an inserted test micropipette. In this case the maximum increase for the rostral area was 8 %, and that for the

central area 50 %, determined for terminals of the fast-conducting component of the SR nerve. This experiment is illustrated in Fig. 7.

(*b*) *Gracile nucleus.* The excitability of primary fibres in the gracile nucleus, under the influence of conditioning stimuli delivered to the skin of the ipsilateral hind leg, was investigated in five experiments. A single pulse stimulus given through a coarse micropipette inserted 0·5–1 mm into this nucleus gave an antidromic response in the ipsilateral sural nerve in which individual fibre-spikes could be recognized (see Fig. 8): this

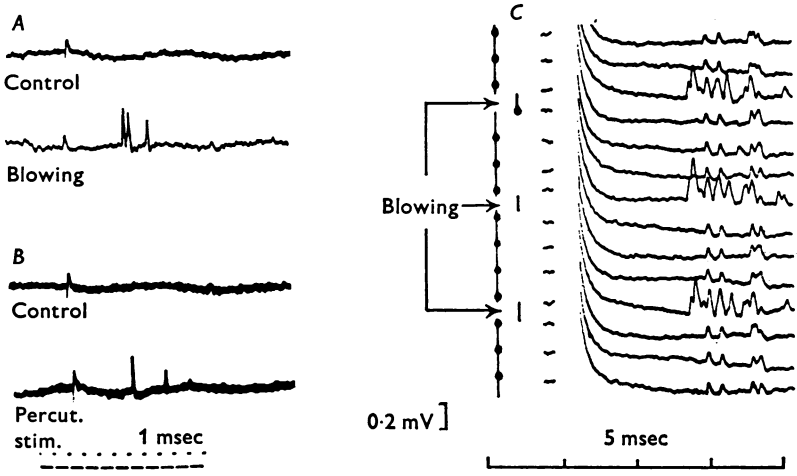

Fig. 8. Increased excitability of sural nerve terminals in the gracile nucleus induced by blowing on the skin or by electric stimulation of the ipsilateral hind paw. *A*, upper trace shows the response of the sural nerve to a test stimulus to the gracile nucleus which was just below the threshold of all fibres in the sural nerve. The lower trace shows the occurrence of three unitary action potentials when the test stimulus coincided with gentle blowing on the tip of the ipsilateral hind paw. *B*, similar to *A*, but conditioning stimulus was now a percutaneous stimulus delivered by a needle electrode inserted under the skin of the plantar surface of the ipsilateral hind paw. *C*, consecutive records taken every second in response to a standard shock delivered to the central region of the gracile nucleus. Normally, four individual unit discharges are seen. Every fourth test stimulus coincided with gentle blowing to the ipsilateral hind paw, resulting in the occurrence of several new action potentials, two of which had a shorter latency than those produced by the test stimulus alone.

results from the smaller number of fibres available for excitation compared with the response in SR to cuneate stimulation, and the much greater temporal dispersion in the longer pathway. With stimuli just above threshold for the sural response, particular fibres could be identified by their all-or-nothing response and characteristic latency, so that excita-

bility changes could be studied in such fibres individually. Each value for change in excitability was determined by successive approximation during a number of trials with conditioning, and is expressed as a percentage reduction in threshold current (see Methods). Five or six fibres could usually be studied in this way at a single micropipette position without risk of confusing them: Fig. 8 shows several fibres which were just below

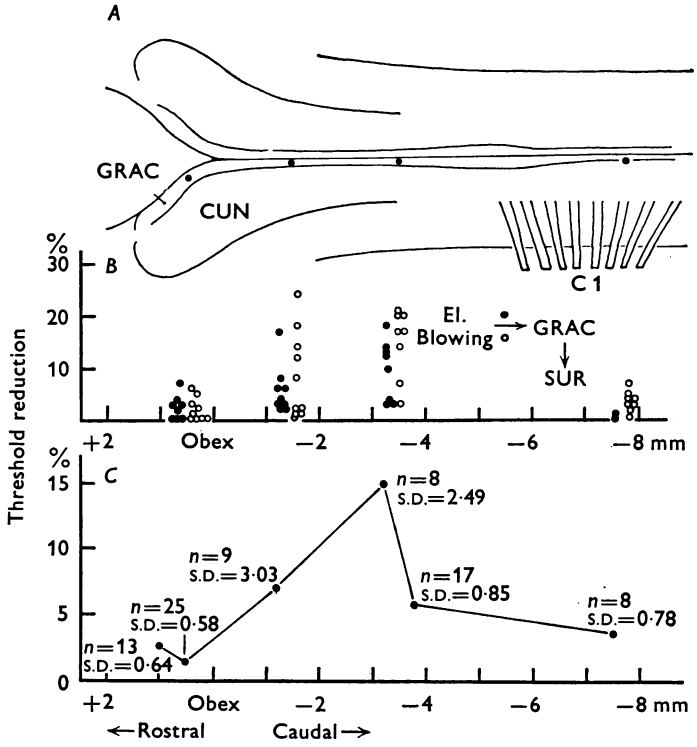

Fig. 9. Distribution of primary afferent depolarization in the gracile nucleus produced by electrical percutaneous stimulation or gentle blowing to the ipsilateral hind paw. *B*, the thresholds of individual sural fibres were tested by stimulation through a small surface electrode, located at indicated distances along the longitudinal extension of the gracile nucleus. Filled circles give the reduction of the threshold of individual fibres produced by a percutaneous electrical stimulus, delivered 20 msec earlier than the test shock. Open circles give a similar threshold reduction of the same responses produced by gentle blowing on the ipsilateral hind paw. *A* indicates the electrode positions, *C*, pooling of all results from three different experiments of this type. The average reduction of threshold of individual units by blowing on the skin is plotted against distribution of points along the longitudinal axis of the gracile nucleus. Points less than 0·5 mm apart were regarded as one location. At each point, the number of fibres (n) and the standard deviation (S.D.) are indicated.

threshold for the control test shock responding upon conditioning by blowing on the skin. Parallel observations were usually made with single electrical conditioning pulses through needles in the skin, and with light blowing on the skin, the stimulus in either case delivered to the plantar side of the hind foot. These two forms of stimulus were comparable in effectiveness (see Fig. 9*B*). In all experiments the micropipette was inserted successively at different points in the long axis of the nucleus.

Altogether 105 fibres were investigated in the region between +1·0 and −7·5 mm in the long axis. The greatest mean increase in excitability upon conditioning and the highest individual values were observed with the electrode in a region between approximately −1 and −4 mm. The least mean increase in excitability was consistently found in the rostral part of the nucleus, above the obex: in this region no detectable effect (less than 1%) was observed on twenty-seven out of forty-three (or 63%) fibres investigated with electrical skin stimulation with needle electrodes, compared with one out of twenty-five (4%) in the region between −3 and −4 mm.

Fig. 9*C* shows the mean increase in excitability produced by blowing on the skin, expressed as a function of rostro-caudal position, pooling the data from all observations where this position was accurately known. Fig. 9*B* shows the data from individual fibres where four regions were compared in the rostro-caudal axis in a single experiment.

Post-synaptic inhibition in the cuneate nucleus by cutaneous stimulation

Tests of excitability of cuneate cell populations. Post-synaptic inhibition in a population of cuneate cells can be detected by a fall in their excitability to direct electrical stimulation (see Methods). The response recorded from the contralateral ML to a test shock delivered in the nucleus is shown in Fig. 10*A*. The component attributable to direct stimulation of the cells is the first downward deflexion, which starts about 1·0 msec after the stimulus. Fig. 10*B* (upper trace) is an averaged record of 200 test responses. The test response was then conditioned by gentle blowing on the forepaw with a continuous jet of air circling over the palmar surface. The lower trace, derived by subtraction of an equal number of conditioned test responses from the averaged record in the upper trace, shows the deficit produced by this conditioning. Part of this deficit must have been due to occlusion, as some of the cuneate cells responding to the test shock will have been excited by the conditioning stimulus and may have been refractory at the moment the test shock was given: this effect of the conditioning stimulus can be gauged by the increased thickness of the baseline in the lower trace, and it is clear that the effect of occlusion must have

been small compared with the total deficit observed. The amount of depression in this case was 21 %, of which almost all must be attributed to post-synaptic inhibition of cuneate cells by blowing on the forepaw. Fig. 10 (*C* and *D*) shows that the effect was greatest when elicited from the most distal part of the paw and decremented rapidly in a proximal direction. These observations were made by blowing through a slit in a piece of card and restricting the area of skin receiving the stimulus to a transverse band occupying, in sequence, the positions *a* to *d* on the dorsal surface of the forepaw.

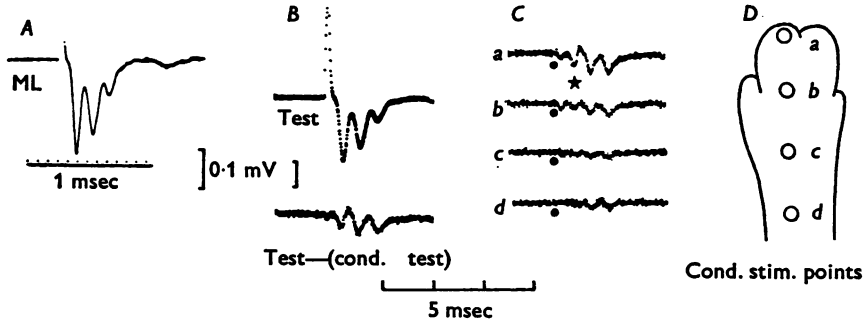

Fig. 10. Excitability changes of a population of cuneate neurones produced by blowing on the ipsilateral forepaw. *A*, response from 'killed ends' of medial lemniscal fibres (ML) recorded with a coarse electrode in response to micro-electrode stimulation within the cuneate nucleus. The initial deflexion (latency 0·9 msec) indicates the direct stimulation of post-synaptic elements, whereas the second and third deflexions indicate trans-synaptic activation. *B*, upper trace shows averaged records of 200 responses like the one described in *A*. Lower trace indicates the subtraction from the above of 200 responses in which the test stimulus coincided with gentle blowing on the ipsilateral forepaw. The remaining positive (downward) deflexions indicate that post-synaptic excitability was decreased by the conditioning blowing stimulus. *C*, records similar to those in the lower trace in *B* were taken following conditioning blowing stimuli delivered to the various points indicated in *D*. • marks the point of test stimulation and ⋆ the direct response.

Post-synaptic inhibition produced by blowing on the skin was demonstrated by this method in two experiments. In one of them it was observed qualitatively that an effect was still present after section of the ipsilateral dorsolateral fasciculus of the spinal white matter at the level of C3, so that it does not require any pathways outside the dorsal column–lemniscal system. The inhibition was only studied with test stimuli in the central region of the cuneate nucleus (-1 to -4 mm) which contains the biggest population of cells: it was thought that comparisons between the central and rostral regions would not be valid with this method because the test

stimulus will excite, in addition to the cells, the lemniscal axons of cells lying more caudal.

Observations on single cuneate cells. Records were made from single cells in the cuneate nucleus for some part of the experimental period in five animals. The region of the nucleus mainly explored was -2 to -4 mm in the long axis. Altogether the receptive characteristics and receptive fields of twenty-seven cells were satisfactorily investigated, of which nineteen were excited by hair stimulation at high sensitivity, and gave a rapidly adapting response; six responded with a slowly adapting discharge to light pressure on the skin; and two gave a slowly adapting discharge to skin pressure but had subcutaneous receptors. Thirteen of the hair-sensitive cells (out of seventeen tested) had a clear-cut inhibitory component in the outlying parts of their receptive fields, where stroking or lightly blowing on hairs caused inhibition of the cells' resting discharge. The two cells with subcutaneous excitatory fields were inhibited by moving hairs in the overlying skin; but none of the cells with cutaneous pressure-sensitive fields had any apparent inhibitory component in their fields. These observations suggested that this region of the cuneate nucleus has similarities with the central part of the gracile nucleus, where hair-sensitive cells subject to surround inhibition form the biggest part of the population (Gordon & Jukes, 1964). Such observations, in experiments in which investigations of other kinds were also made (for instance, of primary afferent depolarization) were useful in confirming that the condition of the nucleus was such that the mechanisms of surround afferent inhibition were active.

There was clear evidence that post-synaptic inhibition must have played some part in the total mechanism of inhibition operating on some of these cells. Antidromic invasion was blocked by mechanical stimulation in the inhibitory part of the receptive field in two hair-sensitive cells. This effect is clearly not common: it must be due to post-synaptic inhibition, though the test is only a qualitative one. Further evidence comes from the detection of IPSPs in intracellular recordings. IPSPs, alone or preceded by EPSPs, were observed following mechanical stimulation in the neighbourhood of the receptive field in altogether eight hair-sensitive cells: we have no evidence from other types of cell. Observations could usually only be made for a matter of seconds, during which it was not possible to plot out the area of skin responsible for generating IPSPs and EPSPs. It was assumed, and sometimes confirmed, that the area generating EPSPs approximately coincided with that previously determined with extracellular recording as the excitatory part of the field. Otherwise blowing on the skin or brushing hairs in the neighbourhood of the receptive field were as much as time allowed. Most of the cells had very low membrane poten-

tials after impalement, and spike production had often ceased. The best records and investigation were from a cell which had a hair-sensitive excitatory field of about 2 cm^2 on the tips of two adjacent digits on the forepaw, and could be inhibited by blowing on skin proximal to this, either on the palmar or volar surfaces of the paw. During the period of observation it had a membrane potential of 40 mV and generated spikes of about 20 mV. In response to electrical stimulation through a pair of needle electrodes, previously inserted through the skin into the approximate position of the median nerve in the proximal palm, the cell gave a discharge of one to two spikes followed by a large IPSP of about 160 msec

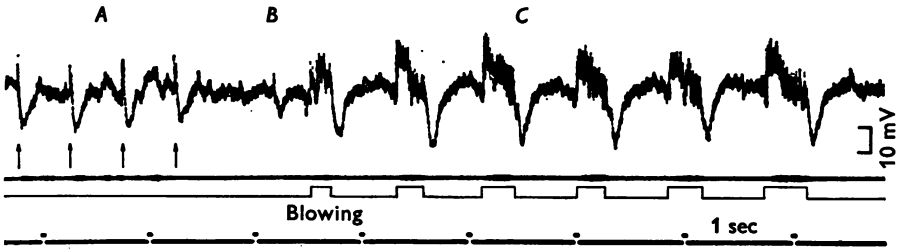

Fig. 11. Intracellular record from a cuneate relay cell. Membrane potential −40 mV. In *A*, single electric shocks were delivered through needle electrodes inserted under the volar surface of the ipsilateral forepaw (arrows). *B* indicates a single blow of air towards the dorsal surface of the paw, whereas *C* indicates six periods of blowing of air towards the tip of the forepaw, lasting from 0·2 to 0·4 sec, indicated by the second and third lines.

duration (Fig. 11, points marked A). In response to blowing on the skin in an area on the volar surface of the paw, mainly proximal to the excitatory field, it gave a similar but smaller response (Fig. 11*B*). Blowing on the skin in an area which must have included some of both the excitatory and inhibitory parts of the field (Fig. 11, points marked *C*) gave a repetitive discharge of spikes superimposed on an EPSP, followed by a large IPSP.

Inhibition of transmission through the dorsal column nuclei by skin stimulation. The response to an electrical stimulus delivered to the skin of the forepaw, recorded monophasically from the contralateral ML, is shown in Fig. 12 (upper traces of *A* and *D*). A corresponding test of transmission to the ML from the hind foot is shown in the upper trace of Fig. 12*E*. Conditioning, by blowing with a circling air-jet on the forepaw or the hind paw respectively during testing, caused a reduction in the size of both single and averaged records (Fig. 12*B*, *C* and *D* for the forelimb system). The actual deficit produced in each case is shown in the difference traces (bottom traces of Fig. 12*D* and *E*). The deficit for the forelimb

system, measured as reduction in the area of the unconditioned monophasic response, was 19% (33% reduction in peak value); and for the hind limb system (see Fig. 12*E*) it was 20% (26% reduction in peak value). These values are averages derived from groups of 200 tests for the forelimb and 500 for the hind limb system. Fig. 13 shows that the reduction in peak value of the ML response produced by blowing on the skin can be larger than this in individual tests. This depended, in experiments where the skin was blown on once for each test, on the accuracy of timing of the blowing, which varied between tests (Fig. 13*A*). When the blowing was continuous, the greatest effect was seen at the onset of the stimulus, indicating some adaptation in the conditioning process (Fig. 13*B*). The

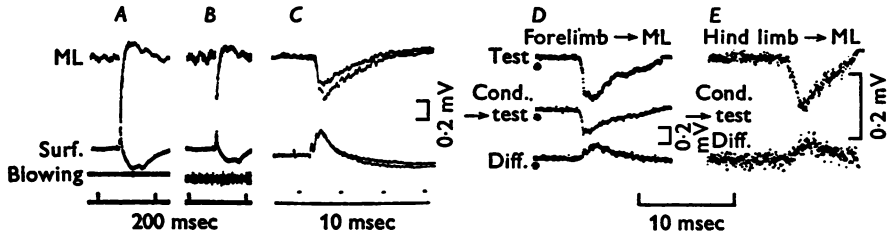

Fig. 12. Effect of mechanical stimulation on transmission of cutaneous signals through the cuneate nucleus. *A*, killed end response from the medial lemniscus (ML) and surface cuneate response (Surf.) produced by a single percutaneous shock to the ipsilateral forepaw. *B*, similar to *A*, but with simultaneous blowing on the tip of the same paw. The third trace is a microphone record detecting the stream of air towards the paw. *C*, as *A* but faster sweep. Two records are superimposed, the larger deflexion being the control record and the smaller a record taken while blowing on the paw. *D*, upper trace is 200 averaged records of the ML response (killed end) to a percutaneous shock to the contralateral forepaw skin. The second trace is similar to the upper but with simultaneous blowing on the same forepaw. The third trace is the difference between the two upper traces, the upper trace subtracted from the middle. *E*, traces recorded from the same location in response to percutaneous stimulation of the hind paw. The two traces correspond to the two lower traces of *D* except that the conditioning and test stimuli were both delivered to the contralateral hind paw.

greatest peak reduction observed in an individual test response was 48%. Only a small part of these depressions of transmission to the ML can be attributed to occlusion. The activity generated in the system by conditioning i.e. by blowing on the skin, which could cause depression by occlusion, can be assessed by comparing the thickness of base line in the conditioned and unconditioned trace in each set of records: a separate control record of conditioning alone is shown for Fig. 13 (in *C*).

Finally, we investigated the effect of stimulating the skin of other limbs

on transmission through the cuneate nucleus to the contralateral ML. As such effects are small, the ipsilateral dorsolateral fasciculus was cut at the level of C 3 to eliminate any contribution to the ML response by the spino-cervico-thalamic system. Electrical skin stimulation with needle electrodes was used for both conditioning and testing. By this means it was found

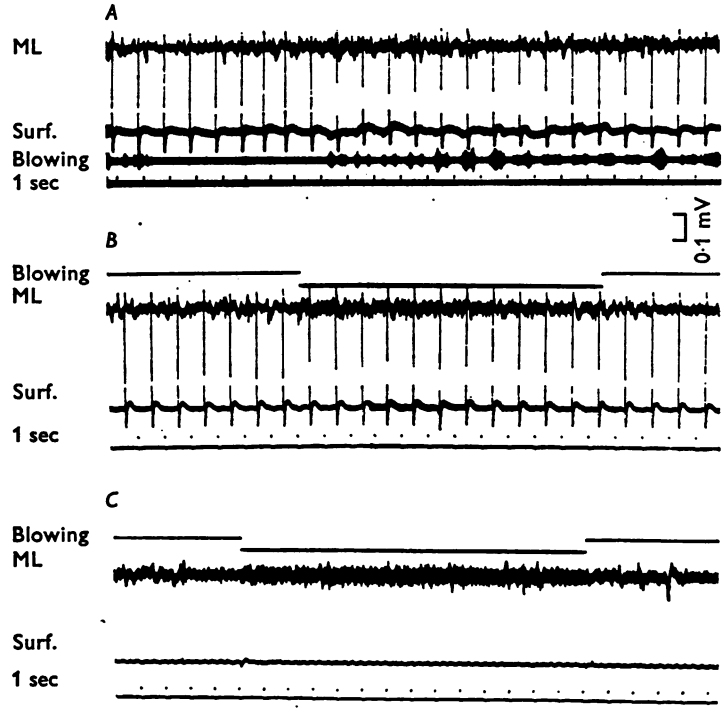

Fig. 13. Effect of interrupted and continuous blowing on transmission through cuneate nucleus. *A*, upper trace: killed end responses from medial lemniscus (ML); second trace: surface responses from the cuneate nucleus; third trace: microphone record indicating the strength and timing of blowing on the left forepaw. *B*, similar to *A*, but continuous blowing, indicated by a deflexion of the upper trace. *C*, similar to *B* but with blowing only and no electrical stimuli.

that the greatest depression in cuneate transmission which could be produced by a single shock to the contralateral forepaw was 5 % (measured as reduction in area of the ML response), at a condition-test interval of 23 msec; and the greatest reduction on giving the conditioning stimulus to the ipsilateral hind paw was of the order of 2 %, at a conditioning-test interval of 27 msec—too small an effect to be measured with any precision by this method. These effects were not attributable to occlusion, because the contralateral forelimb does not have any significant excitatory input to the

cuneate nucleus, and the response in the ML to stimulating the ipsilateral hind limb was completed before the inhibitory depression was detected.

DISCUSSION

The main fact demonstrated by the present experiments is that both presynaptic and post-synaptic inhibition are generated in the dorsal column nuclei by small mechanical stimuli delivered to the skin of the appropriate ipsilateral limb. When elicited by blowing gently on an area of skin the effects are comparable in size with those produced by electrical stimulation of limb nerves or of skin. This may be partly explained by a wide spatial convergence from the receptors.

Depolarization of the terminals of primary afferent fibres (PAD), indicative of presynaptic inhibition, was found to occur with the same time course whether elicited by mechanical stimuli or by electrical stimulation of limb nerves, with a peak at 25–35 msec and a total duration of 100 msec or more. A similar long duration of the inhibitory effect is seen in surround afferent inhibition, observed as a suppression of the evoked or resting discharge of single cells in the gracile nucleus by a cutaneous stimulus (Gordon & Paine, 1960; Gordon & Jukes, 1964). The quantitative distribution of PAD in single fibres terminating in the gracile nucleus, demonstrated in the present experiments, with the minimum area rostral and the maximum area 2–4 mm caudal to the obex, agrees with the distribution of single cells showing afferent inhibition in response to small mechanical stimuli in the surround of their excitatory receptive fields (Gordon & Jukes, 1964). The distribution of afferent surround inhibition in the cuneate nucleus is not known precisely, though we have seen that it occurs commonly in the central area 1–4 mm caudal to the obex, the region in which PAD is largest. Certainly the change in cytoarchitecture described by Kuypers & Tuerk (1964) occurs at about the same rostrocaudal position in both nuclei, which supports the idea of a general similarity in their organization. Our observations strongly suggest that presynaptic inhibition accounts for at least part of the total mechanism of afferent surround inhibition in these nuclei. Darian-Smith (1965) used similar arguments in proposing an important role for presynaptic inhibition in the mechanism of afferent surround inhibition in the brain-stem trigeminal nuclei: in this case the maxima for the PAD and for the afferent inhibition were found to occur within the rostral trigeminal subnuclei. We have also found the mechanical thresholds for eliciting the two types of phenomenon to be similar: either can be produced by displacing a few hairs. The rapid adaptation of the PAD in response to skin stimulation, and the lack of any evidence for a significant additional contribution from continuous light pressure, or pinching, suggest

hair receptors as the main source; but such minimal stimuli are also capable of activating Pacinian corpuscles, which have been shown to be a source of PAD in terminals of single cutaneous fibres in the spinal dorsal horn (Jänig, Schmidt & Zimmermann, 1968), and a contribution from these receptors is not excluded by our evidence.

The method described here for investigating the PAD in single fibres terminating in the gracile nucleus is a simple application of Wall's technique (Wall, 1958) which takes advantage of the small number of fibres in the nerve used for recording the antidromic response and of the temporal dispersion in the pathway. It is capable of providing evidence of the kind obtained by Schmidt, Senges & Zimmermann (1967*a*) for single fibres terminating in the dorsal horn; but as filaments do not have to be dissected from the nerve it offers the chance of investigating a large number of individual fibres in a single animal.

Our observations make it clear that post-synaptic inhibition must also be involved in surround afferent inhibition. Post-synaptic inhibition of cuneate cells was detected both as a depression of their excitability, and by the presence of IPSPs, in response to displacement of cutaneous hairs; and the time course of these changes, like that of presynaptic inhibitory action, was of the same order as the time course of afferent surround inhibition. Our evidence does not allow any quantitative estimate of the relative effectiveness of these two forms of inhibition in depressing transmission through the nuclei or any qualitative distinction between their roles in normal function. It seems unlikely that they differ widely in spatial organization, because both actions are elicited strongly from the distal part of the ipsilateral limb. Post-synaptic inhibition, observed as an IPSP, was elicited by stimuli in close relation to the excitatory field of the same cell; and presynaptic inhibition, when studied on single fibres, has been shown to be organized on a surround basis with respect to the excitatory field of the fibre, both in the trigeminal nuclei (Darian-Smith, 1965; using the 'trigeminal tract reflex', analogous to the dorsal root reflex) and in the spinal dorsal horn (Schmidt, Senges & Zimmermann, 1967*b*; using the PAD). It is possible that the two inhibitory mechanisms operate differentially with respect to cells innervated by different types of skin receptor; and there is no evidence at present on this point.

We have shown that the total mechanism of inhibition set into operation by light mechanical stimulation of the distal part of the appropriate ipsilateral limb is very powerful in depressing transmission through the cuneate or gracile nucleus into the contralateral medial lemniscus: under the conditions of these experiments averaged depressions of up to 20% in area (or about 30% in the peak value) of the lemniscal response were produced. These observations were made with the ipsilateral dorsolateral

fasciculus intact, so that part of the lemniscal response (about 15%, according to unpublished observations made by L. Fedina, G. Gordon & A. Lundberg) is contributed by the spino-cervico-thalamic system. Our estimate of the deficit in transmission through the dorsal column nuclei would not be greatly affected if a parallel deficit of up to 50% occurred in the spino-cervico-thalamic component. A relatively very small depression, usually less than 5%, was produced by conditioning cuneate transmission by electrical skin stimulation of the contralateral forepaw, such stimuli, as we have shown, being of approximately equal effectiveness to blowing on the skin. The depression of about 2%, produced by a conditioning stimulus to the ipsilateral hind paw after section of the dorsolateral fasciculus, is not valid evidence of inhibition of cuneate transmission, because such a stimulus would be expected to produce prolonged inhibition of the resting discharge of many cells in the gracile nucleus and thus to diminish the lemniscal response recorded. Jabbur & Banna (1968) have shown that single cells in the dorsal column nuclei can be inhibited by activating widespread cutaneous inputs which include the ipsilateral face and hind paw and the contralateral limbs and face, and have shown that these effects are accompanied by PAD in the nuclei affected. They found these effects to be consistently less than those elicited by conditioning the ipsilateral limb supplying the particular nucleus. Under the conditions of our experiments, conditioning stimuli to the ipsilateral limb were at least four times as effective as those to the contralateral limb in depressing transmission into the lemniscus. This contrasts strongly with the spatial convergence of inhibition on the lateral cervical nucleus, where transmission can be depressed with almost equal effectiveness from all the limbs (Fedina, Gordon & Lundberg, 1968). The cells in the lateral cervical nucleus do not show surround inhibition.

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