Quantitative analysis of cuneate neurone responsiveness in the cat in association with reversible, partial deafferentation

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1. Partial deafferentation, based on peripheral nerve section or local anaesthetic blockade, has been reported to induce both immediate loss of responsiveness and/or immediate reorganization in receptive fields of neurones in the somatosensory system. In the present study, in anaesthetized cats, we have used a rapid, reversible deafferentation procedure based on cold block of the median nerve in order to evaluate quantitatively the response characteristics of cuneate neurones ($n = 39$) *before*, *during* and *after* partial deafferentation.

2. The first hypothesis tested was that cuneate neurones with input from ulnar or superficial radial nerve fields in the vicinity of the median nerve field should undergo, in association with median nerve blockade, an increased level of responsiveness to tactile stimuli within the ulnar or radial nerve zone, and an expansion of their cutaneous receptive fields. However, among eighteen cuneate neurones of this type, there was no evidence for any systematic enhancement of responsiveness nor, in at least sixteen of the eighteen neurones, any evidence for receptive field expansion.

3. The second hypothesis tested was that cuneate neurones whose input came from *both* the median nerve and another peripheral nerve source should undergo, in association with median nerve blockade, an increase in responsiveness to the remaining input and an expansion of the receptive field into the field of that remaining nerve source. However, in none of thirteen neurones of this type tested was there evidence of such a change.

4. The third hypothesis was that cuneate neurones whose 'control' receptive fields were *within* the median nerve zone of deafferentation should show an emergence of novel receptive fields and responsiveness from areas around the field of innervation of the median nerve. However, in none of eight neurones of this type was there evidence for such changes in adjacent skin areas.

5. In conclusion, with the use of cold block of the median nerve for partial deaffferentation, the present study has confirmed previous findings of denervation-related loss of responsiveness in dorsal column nuclei neurones. The conflicting findings in studies of central nervous system plasticity indicate the need to understand better factors that do and do not lead to acute central changes.

The consequences of partial deafferentation for central neurones of the somatosensory system are complex and varied. Many studies report an immediate reorganization of body maps in response to partial deafferentation at the levels of the dorsal horn (e.g. Basbaum & Wall, 1979; Devor & Wall, 1981; Lisney, 1983; Koerber & Brown, 1995), the dorsal column nuclei (Dostrovsky, Millar & Wall, 1976; Millar, Basbaum & Wall, 1976; Pettit & Schwark, 1993, 1996; Panetsos, Nunez & Avendano, 1995), the ventrobasal thalamus (Nicolelis, Lin, Woodward & Chapin, 1993; Rasmusson, Louw & Northgrave, 1993) and the somatosensory cortex (Metzler & Marks, 1979; Merzenich, Kaas, Wall, Sur, Nelson & Felleman, 1983; Rasmusson & Turnbull, 1983; Kelahan & Doetsch, 1984; Calford & Tweedale, 1990, 1991*a*,*b*; Turnbull & Rasmusson, 1990). However, in contrast, other studies have reported an absence of reorganization, or at least the presence of substantial unresponsive zones in response to deafferentation of the dorsal horn (Brown, Brown, Fyffe & Pubols, 1983; Brown, Fyffe, Noble & Rowe, 1984; Wilson, 1987), the dorsal column nuclei (McMahon & Wall, 1983; Northgrave & Rasmusson, 1996) and somatosensory cortex (Merzenich *et al.* 1983; Li,

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Waters, Oladehin, Johnson, McCandlish & Dykes, 1994). Whether the differences are related to the method or extent of the deafferentation, the species used, or other factors remains a matter of conjecture. Where immediate reorganization has been described it is apparent as an expansion in central representation for those body regions around the peripheral zone of deafferentation, which come to occupy part or all of the area of the central somatosensory map that was previously allocated to the deafferented zone. As reorganization is immediate (within minutes of deafferentation) it cannot be based on collateral sprouting of axons and the establishment of new connections. Instead, it has been suggested that the deafferentation may remove a source of background inhibition that ordinarily masks the central actions of adjacent peripheral inputs on neurones beyond their main excitatory target territory (Merzenich *et al.* 1983; Calford & Tweedale, 1990).

Reports of this form of reorganization have been based on analysis of the central body maps with single or multiunit recording following partial deafferentation induced by local anaesthetic or capsaicin blockade of peripheral nerves (Metzler & Marks, 1979; Calford & Tweedale, 1991*a*,*b*; Nicolelis *et al.* 1993; Pettit & Schwark, 1993, 1996; Panetsos *et al.* 1995; Northgrave & Rasmusson, 1996), peripheral nerve or dorsal root sectioning (e.g. Dostrovsky *et al.* 1976; Devor & Wall, 1981; Lisney, 1983; Merzenich *et al.* 1983; Koerber & Brown, 1995), spinal cord cooling (Dostrovsky *et al.* 1976) or amputation of digits (Rasmusson & Turnbull, 1983; Kelahan & Doetsch, 1984; Calford & Tweedale, 1990, 1991*a*,*b*; Turnbull & Rasmusson, 1990). The implications of these reports are first, that individual central neurones whose peripheral input ordinarily comes from within the zone of deafferentation will show a loss of responsiveness from that zone and an emergence of responsiveness from a novel field in a new region of skin outside the boundary of the deafferentation. Second, for neurones with receptive fields just outside the deafferentation zone, responsiveness may be enhanced and receptive fields expanded on account of the loss of the proposed tonic inhibitory action. Although the receptive fields of *individual* cuneate neurones have been examined in previous studies *with* and *without* deafferentation (Pettit & Schwark 1993, 1996; Northgrave & Rasmusson, 1996), any alterations in responsiveness associated with the deafferentation have not been evaluated quantitatively for individual cuneate neurones. In the present study, we have set out to test the hypothesis that there are deafferentation-induced alterations in cuneate neurone responsiveness by means of objective and quantitative procedures. This was done, after mapping the receptive fields of individual neurones with a series of von Frey hairs, by constructing stimulus–response relations for the neurone based on testing with controlled forms of tactile stimuli. Furthermore, in order that these measures of responsiveness could be made on *individual* neurones in both the control and test circumstances, we have employed a rapid, reversible method for partial deafferentation which is based on cold block of the median nerve. As this procedure permits nerve block within 1–2 min and equally rapid restoration of nerve conduction, it enables the receptive field and responsiveness of central neurones to be evaluated *before*, *during* and *after* the partial deafferentation. We chose to study neurones in the cuneate nucleus, as the extent to which different levels of the somatosensory pathway contribute to reorganizational changes described previously is still unclear, and the dorsal column nuclei represent the first relay level in the major tactile sensory pathway to the cortex. A preliminary report has been presented in conference proceedings (Zhang, Bahramali & Rowe, 1995).

METHODS

Animal preparation

Experiments were carried out in fifteen adult cats of either sex and conformed with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Twelve animals were anaesthetized with sodium pentobarbitone (40 mg kg^{-1} I.P. initial dose and later maintenance dose of 1·5–3 mg kg^{-1} h^{-1} I.V.), one with a combination of ketamine (35 mg kg^{-1} I.M.) and xylazine (5 mg kg^{-1} I.M.) followed by intravenous infusion of ketamine as needed, and two underwent a mid-collicular decerebration under halothane anaesthesia. All animals were given an initial subcutaneous injection of atropine (50 μg kg^{-1}) to reduce respiratory secretions. The trachea was cannulated, as were the femoral vein and artery, and an indifferent electrode (Ag–AgCl) was placed under the skin of the head. Intermittent intravenous administration of a neuromuscular blocking agent gallamine triethiodide (3–5 mg kg^{-1}) was used in seven experiments in association with positive-pressure ventilation to stabilize respiratory movements and recording conditions. Rectal temperature was held at 37·5 ± 0·5 °C. End-tidal P_{CO_2} was adjusted to 3·75 ± 0·25% in experiments in which artificial ventilation was used, and blood pressure, heart rate and pupil size were monitored continuously to ensure that surgical anaesthetic depth was maintained. At the end of experiments, animals were killed by I.V. administered anaesthetic overdose.

The animal was placed in a stereotaxic frame with the head flexed by ~30 deg to facilitate access of the recording electrode to the dorsal column nuclei. The dorsal surface of the medulla was exposed by removal of part of the occipital bone. The left or right forelimb was shaved and the paw fixed in a U-shaped Perspex frame by attaching fine strings from the frame to the nail of each digit. The paw was oriented so that the dorsal and ventral surfaces were aligned vertically, allowing access to either side for tactile stimulation. The frame had a removable base that could provide support for either the dorsal or ventral surface of the paw. The median nerve was exposed in the forearm for a length of 5–6 cm by removing the overlying muscles. In each experiment, a bipolar trough electrode was used for stimulation of the median nerve, and, on the other side of the cooling block, a recording electrode placed under the nerve for recording evoked compound action potentials (Fig. 1*A*). In circumstances in which the responsiveness of a cuneate neurone disappeared during median nerve cooling it was possible, with placement of the stimulating electrodes on the proximal side of the cooling block, to verify that this was not attributable to failure of isolation and discrimination of the neurone's activity.

Rapid, reversible deafferentation based on median nerve cooling

The median nerve was placed in a 10 mm long silver trough that had a precision thermistor attached to the inner surface of the base where it was in contact with the nerve (Fig. 1*A*). A 4% agar gel covered the nerve and provided a better equilibration of temperature for the 10 mm length of nerve within the trough (Franz & Iggo, 1968). In order to minimize the spread of cooling to the adjacent tissues of the arm and, in particular, to other nerves, the outside of the silver trough was covered by a thick layer of insulating epoxy (Araldite). The exposed nerve beyond the trough was covered with petroleum jelly. Rapid cooling of the trough and median nerve was achieved by the circulation of cold alcohol (−20 °C) through the trough at a flow rate that could be varied to achieve the desired temperature. Rapid rewarming was achieved by circulation of warm water (~37 °C).

Although cooling has been used widely for the temporary blockade of peripheral nerves, precautions are needed as the blocking temperature for different nerve fibre groups varies. Conduction fails in the majority of myelinated fibres between 9 and 5 °C but temperatures below 5 °C are needed for most unmyelinated fibres (Paintal, 1965; Franz & Iggo, 1968). However, prolonged cooling, for example to 5 °C for 2 h, may cause lasting conduction failure (Kennett & Gilliatt, 1991). Because of these considerations the median nerve cooling was never maintained for more that 30 min and, on average, lasted less than 15 min and in some tests was as brief as 5 min (see Results). The initial experiments were carried out to determine the temperature required, in the present arrangement, to block conduction in myelinated fibres of the median nerve and to evaluate the reversibility of the procedure. No attempt was made to record a C fibre component of the compound action potential. Figure 1*B* shows the compound action potential recorded from the median nerve in response to electrical stimulation <15 cm away from the recording site. The response was markedly attenuated and delayed by nerve cooling to 8·5 °C, and was abolished at 5·5 °C. The compound action potential was fully restored upon rewarming the nerve to 37 °C (lower record in Fig. 1*B*). The complete recovery was confirmed for single sensory fibres in the median nerve by recording from fine fascicles isolated by microdissection of the nerve, in this case proximal to the cooling block. The strand was placed over a silver hook electrode and recordings made between

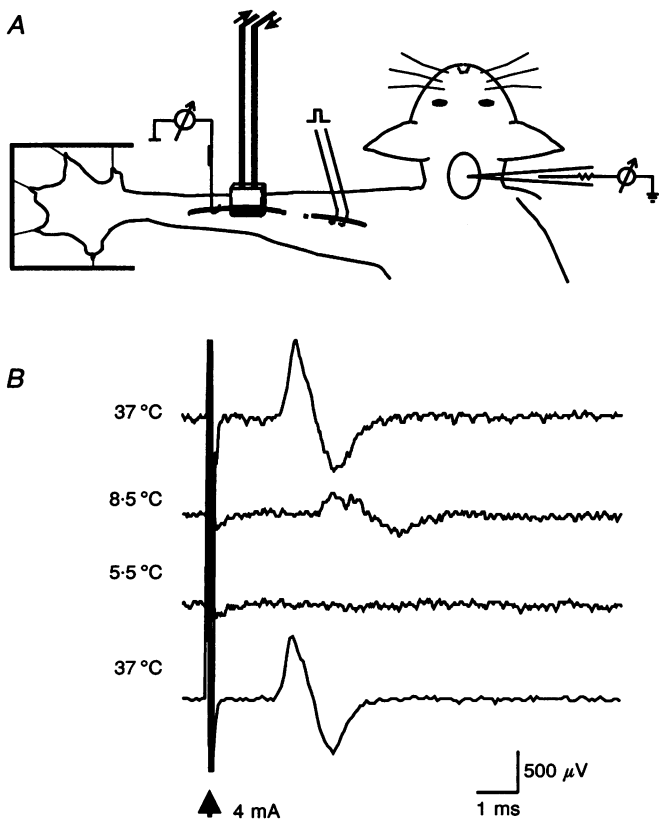

Figure 1. Experimental arrangement for reversible cold block of the median nerve

A, a segment of median nerve in the left forearm was placed in a 10 mm long silver cooling trough through which cold (−20 °C) alcohol or warm (~37 °C) water could be circulated. Bipolar stimulating electrodes and a platinum hook recording electrode were placed under the nerve on either side of the cooling trough, and microelectrode recordings were made from single neurones in the cuneate nucleus. A Perspex frame was used to hold the paw with dorsal and ventral surfaces aligned vertically and available for tactile stimulation. *B*, compound action potential recorded from myelinated fibres of the median nerve when nerve conduction was intact (trough at 37 °C), and when it was impaired (8·5 °C) or blocked (5·5 °C). The single-pulse electrical stimulus to the nerve (50 μs; 4 mA; 4–5 × threshold) was applied at a distance of <15 cm from the recording site.

that and an indifferent electrode in contact with nearby muscle tissue. The tactile afferent fibre whose responsiveness is illustrated in Fig. 2 displayed an exquisite sensitivity to high-frequency sinusoidal vibration (for example, 300 Hz), delivered to the footpad skin of digit 1, which established its association with Pacinian corpuscle (PC) receptors in the footpad. The PC fibre achieved a 1:1 pattern of response (one impulse per vibration cycle) to a 1 s train of 300 Hz vibration at an amplitude of 1–2 μm in the control circumstance prior to median nerve block (○, Fig. 2*B*) and lost its responsiveness entirely when the nerve temperature fell below 10 °C (■, Fig. 2*B*). However, upon rewarming the median nerve, responsiveness recovered fully as reflected in the stimulus–response relation (△) in Fig. 2*B* where the 1:1 threshold was once again below 2 μm. Based on our observations in these initial experiments and the previous studies (Paintal, 1965; Franz & Iggo, 1968; Kennett & Gilliatt, 1991) we employed temperatures in the range 2–5 °C, for the times indicated above, to achieve nerve block and recovery without median nerve impairment. Furthermore, in each experiment, the effectiveness of the cooling for blocking the nerve was verified by observing the abolition of the compound action potential (Fig. 1). Irreversible deafferentation based on median nerve section was also carried out near the end of several experiments (see Results).

Cuneate neurone recording procedures

Impulse activity was recorded extracellularly by means of tungsten microelectrodes from thirty-nine individual cuneate neurones whose locations were specified in relation to the mid-line, obex and the surface, each of which provided a visible reference on the exposed dorsal surface of the brainstem. The thirty-nine neurones studied were, in all but three cases, 1–3·3 mm caudal to the obex, between 1 and 2·3 mm lateral to the mid-line, and at depths below the brainstem surface of 180–1940 μm with a mean of 950 ± 460 μm (mean ± S.D.). Although lesions were not made and histological verification was not carried out, these locations are all entirely consistent with the middle or cluster zone of the main cuneate nucleus (Hand & Van Winkle, 1977) where neurones examined by Pettit & Schwark (1993, 1996) were also reported to be located. Further support for our conclusions about cell locations came from the observation, in more medially located electrode tracks, of neurones whose input came from the hindlimb or trunk and whose locations would have been within the gracile nucleus.

A 4% agar gel was placed over the exposed brainstem during recording periods to minimize cardiac and respiratory pulsations. Recorded signals were amplified, displayed on an oscilloscope and passed to a Schmitt-trigger discriminator unit for counting, and to a laboratory computer and magnetic tape recorder.

Assessment of tactile receptive fields and responsiveness for cuneate neurones

All observations were confined to single cuneate neurones whose signal-to-noise ratios permitted unequivocal isolation of the impulse activity before, during and after cooling-induced blockade of the median nerve. Multiunit activity was not accepted for study. Tactile receptive fields for single cuneate neurones were identified by gentle tapping with small probes, and were delineated in the three circumstances – before, during and after median nerve blockade – by means of calibrated von Frey hairs, usually in the force range 0·05–1·0 g wt. The hair was applied to the skin normal to the surface to determine the point of maximum sensitivity and the area from which responses were obtained, which was then mapped on an enlarged sketch of the distal forelimb (see Figs 3–7). The responsiveness of each neurone was studied by applying tactile stimuli that were derived from a servo-controlled mechanical stimulator used in many of the previous studies from this laboratory (Ferrington & Rowe, 1980; Ferrington, Rowe & Tarvin, 1987; Turman, Ferrington, Ghosh, Morley & Rowe, 1992; Vickery, Gynther & Rowe, 1994). Controlled stimuli of this type are required for evaluating whether alterations occur in neural responsiveness as a result of partial deafferentation. They were delivered (at a repetition rate of 1 per 10 s to allow recovery of skin position) to the point of maximum sensitivity in the receptive field by means of circular probes, 0·25–4 mm in tip diameter. The assessment of responsiveness was limited to this single point, the subjectively determined 'best' point of the receptive field, although ideally the assessment should also be made at weakly responsive sites within the receptive field. However, we did not attempt this as the quantitative assessment of responsiveness at two or more points in the receptive field would require movement and repositioning of the mechanical stimulator on the skin between the pre-cooling control assessment and the test assessment (during deafferentation) and again for the post-cooling assessment. Any repositioning of the mechanical stimulator in this way would introduce inherent variability or non-reproducibility in the stimulus conditions. The only solution to this problem would be to have two or more independently controlled mechanical stimulators, each in place at different sites in the receptive field for all phases of the assessment.

Neurones studied were initially classified as rapidly or slowly adapting according to their response to a steady rectangular indentation of the skin that was 1·5 s in duration and < 1 mm in amplitude. Slowly adapting neurones were tested with 1 s long step indentations at a range of amplitudes, and rapidly adapting neurones with 1 s trains of vibration superimposed on, and starting 300 ms after, the onset of 1·5 s background rectangular displacement that was normally 400 μm in amplitude (see waveform in Fig. 2*A*).

Stimulus–response relations for cuneate neurones were constructed by plotting the mean ± S.D. impulse rate for 5–10 repetitions of the fixed, 1 s long stimulus against the amplitude of the stimulus, which was either a steady indentation or a train of vibration (Figs 2–7). The mean response (impulses s^{-1}) at each amplitude on the stimulus–response relation was compared for the three relations constructed before, during and after median nerve cooling by means of a Student's unpaired two-tailed t test. Differences were accepted as significant, *at a particular amplitude*, if $P < 0{\cdot}05$. An effect of the partial deafferentation on the neurone's responsiveness was accepted if the mean response level during cooling-induced nerve block was different from pre- and post-cooling controls for more than half of the stimulus amplitudes tested over the stimulus–response relations.

RESULTS

The effects of median nerve deafferentation were tested for thirty-nine neurones whose original receptive fields were on the distal forelimb. Thirty-two neurones were studied in cats under pentobarbitone anaesthesia, three with ketamine anaesthesia and four in decerebrate cats. However, they are considered together as there was no indication of systematic differences in the effect of deafferentation among these preparations.

The cuneate neurones studied could be placed in three groups according to the effects of median nerve blockade. One group, made up of thirteen out of the thirty-nine neurones, showed a reduction in receptive field size and/or

responsiveness. A second group, made up of eight neurones, underwent an abolition of peripheral-induced responsiveness, and a third group (eighteen neurones) was unaffected in any systematic way in terms of receptive fields and responsiveness. Each group was made up predominantly of neurones with receptive fields that were confined to, or included the glabrous skin of the footpads. There were no apparent differences among these three groups in terms of the functional classes of cuneate neurones represented. Each group included neurones of the three major tactile classes (Douglas, Ferrington & Rowe, 1978) whose glabrous skin input appears to come predominantly from one of the following classes of tactile afferent fibres: Pacinian corpuscle (PC) associated fibres, rapidly adapting (RA) fibres, or slowly adapting (SA) afferent fibres.

Neurones that display a reduction in receptive field size and responsiveness with median nerve blockade

The thirteen cuneate neurones that underwent a reduction in cutaneous receptive field size and in responsiveness with the partial deafferentation presumably received convergent input from the median nerve and at least one other cutaneous nerve, such as the ulnar, which supplies the ventral side of the paw adjacent to the median nerve field of innervation, or the superficial radial which supplies the skin on the dorsal side of the paw. In this group, there was a reduction in *both* receptive field size and measures of responsiveness for six neurones, a reduction in receptive field size for five neurones and, in two neurones, a reduction in responsiveness but no change in receptive field area. The extent of the deafferentation-induced receptive field shrinkage for one neurone is illustrated in Fig. 3*A*. This neurone had a rapidly adapting response to skin deformation on the pad of toe 4 (indicated by the fine oval outline) and was most sensitive to vibratory stimulation at low frequency (<100 Hz). Its toepad input was therefore presumably derived from the RA class of tactile afferent fibres. In the control circumstance, its receptive field occupied most of this toepad when evaluated with a 1 g wt von Frey hair (Fig. 3*A*, ▨), but the focus was shown to be on the distal half of the pad when finer von Frey hairs were used (0·44 and 0·3 g wt, ▥ and ■, respectively, in Fig. 3*A*). When the median nerve was blocked, the neurone was unaffected by the strongest of these von Frey hairs (1 g wt) except from a punctate locus confined to the area of the pad at the point where the mechanical stimulator probe was positioned. This corresponded to the original point of maximum sensitivity, and this residual input presumably came via the ulnar nerve. It is probable therefore that, in the control circumstance, the convergence and summation of this ulnar input with the median nerve input from toepad 4 confers on this cuneate neurone its peak sensitivity at this locus on the pad. Upon rewarming of the median nerve, the receptive field, as mapped by the three von Frey hairs (Fig. 3*A*), was similar to the pre-deafferentation control field. The stimulus–response relations constructed in Fig. 3*B* by

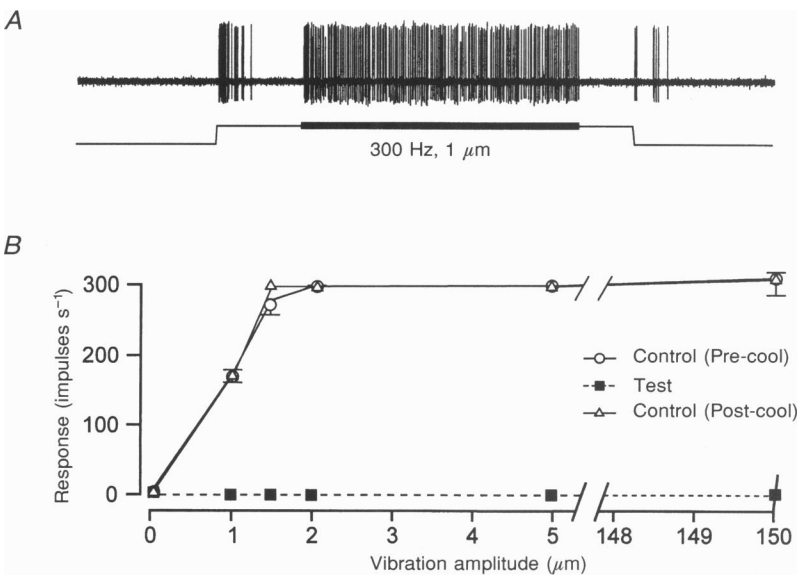

Figure 2. Reversible, cooling-induced conduction block of the median nerve demonstrated at the single-fibre level

A, response of a PC-related sensory fibre recorded from the teased nerve fascicle proximal to the cooling block. The vibrotactile stimulus consisted of a 1 s train of sinusoidal vibration at 300 Hz (1 μm) superimposed on a 400 μm, 1·5 s step indentation of the glabrous skin of toe 1 (the waveform below the response trace shows the vibration amplitude in an exaggerated form for illustrative purposes). *B*, stimulus–response relations constructed from the responses of the PC fibre to the 300 Hz vibration (amplitudes, 0–150 μm) before (○), during (■) and after (△) the cooling-induced nerve blockade. Each point represents the mean of 10 responses at a fixed amplitude and S.D. values are indicated where they extend beyond the symbol.

plotting the mean response (impulses s^{-1}) to five repetitions of a 1 s long vibrotactile stimulus at a series of amplitudes at 50 Hz show that responsiveness for this neurone was attenuated during the median nerve blockade. This is also shown, at three vibration amplitudes (100, 150 and 200 μm), in the impulse traces in Fig. 3*C* which also show on an expanded time scale on the right-hand side, superimposed spike records that confirm the identity of the unit studied before, during and after median nerve cooling and demonstrate, along with the response traces, the unequivocal single unit isolation for the study of this neurone.

The major point to be emphasized for this neurone is that, during the partial deafferentation achieved by means of median nerve blockade, there was no cutaneous activation of the unit apart from the localized point on the pad of toe 4, despite testing with the 1 g von Frey hair and with firmer wooden probes within the ulnar and superficial radial fields of innervation that were medial and dorsal, respectively, to the original receptive field locus (Kitchell, Canton, Johnson & Maxwell, 1982).

Two other examples of this type of behaviour, in which the median nerve blockade led to some reduction in

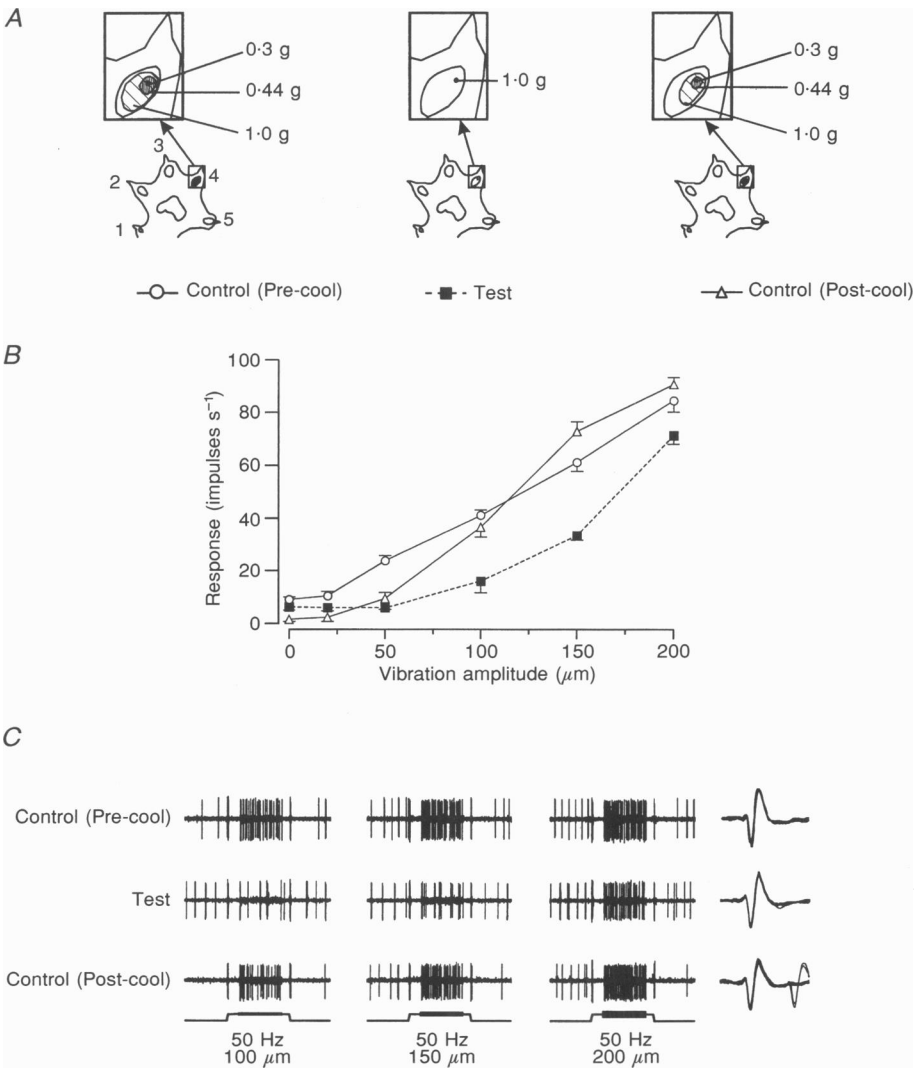

Figure 3. Reduction in receptive field size and responsiveness of a cuneate neurone during reversible median nerve block

A, the receptive field mapped on toepad 4 (of left arm) by means of 0·3, 0·44 and 1·0 g wt von Frey hairs is plotted prior to, during and after median nerve blockade. *B*, stimulus–response relations constructed before (○), during (■) and after (△) median nerve block by plotting the mean response (impulses s^{-1} and S.D.) to 10 repetitions of a 1 s train of 50 Hz vibration superimposed on a 1·5 s step and delivered by a 2 mm probe to the most sensitive point of the receptive field on the pad of toe 4. The stimulus waveform is shown beneath the response traces in *C*. *C*, impulse traces showing the response of the neurone at 3 vibration amplitudes (100, 150 and 200 μm) before, during and after median nerve block. Ten superimposed spikes are shown on an expanded time base on the right-hand side for unequivocal confirmation that the same single unit was studied throughout the 3 phases of the analysis.

responsiveness and/or the receptive field size, and no expansion of responsiveness into a new cutaneous territory, are shown in Fig. 4. For Fig. 4*A*, the neurone was activated by a 450 mg wt von Frey hair from the rather diffuse shaded areas shown on the ventral and dorsal surfaces of the paw in the uppermost pair of sketches in Fig. 4*A*. It displayed rapidly adapting responses to skin stimulation, was most sensitive to high-frequency cutaneous vibration when tested from the most sensitive point in its receptive field marked by the black dot on the distal margin of toepad 3, and therefore appeared to be activated by PC sources. The stimulus–response relations in Fig. 4*A* were constructed from responses to 300 Hz vibration applied at a series of amplitudes to this point on toe 3. The relations plotted with the open symbols were the pre- and post-cooling control relations respectively, and the relations plotted with the filled symbols were obtained during median nerve blockade, either by cooling (■) or by nerve section (◆) when the receptive field (mapped with the 450 mg wt von Frey hair) had shrunk largely to the glabrous regions on the ventral surface and was still present in a fragmented, incomplete form on the dorsal surface.

The data in Fig. 4*B* come from a cuneate neurone whose cutaneous input appeared to come from hair follicle-

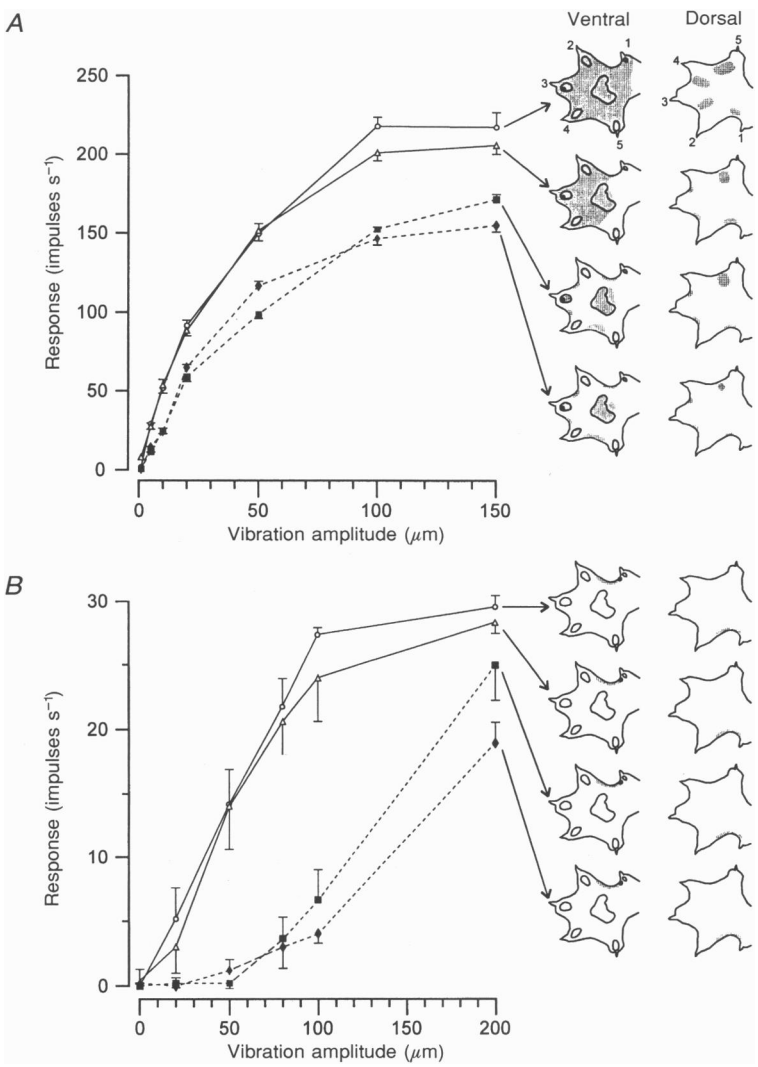

Figure 4. The effects of cold block and transection of the median nerve on the responsiveness and receptive fields of two cuneate neurones (*A* and *B*)

On the left, stimulus–response relations constructed by plotting the mean response (impulses s^{-1}; ± S.D.) to 10 repetitions of a 1 s train of vibration at 300 Hz (*A*) or 10 Hz (*B*). Pre- and post-cooling control relations are plotted with ○ and △, respectively. On the right, the receptive fields of the 2 neurones (determined with a 450 mg wt von Frey hair for *A*, and a 230 mg wt von Frey hair for *B*). Similar changes in the stimulus–response relation and in the receptive field size were obtained by cold block (3 °C, 10 min in *A* and 5 °C, 12 min in *B*; ■ in *A* and *B*) or by transection of the median nerve (◆ in *A* and *B*). The black spots on the outlines of the cat paw (right arm) indicate the locations of the stimulus probe (2 mm probe on toe 3 for *A*, and 1·5 mm probe near toe 1 for *B*), from which stimulus–response relations were obtained.

associated afferents on the dorsal and ventral skin margin between toes 1 and 2. The receptive field as mapped with a 230 mg wt von Frey hair showed no apparent change in the four circumstances tested in Fig. 4*B*. However, the responsiveness of the neurone to controlled 10 Hz vibration was reduced, as demonstrated by the lower stimulus–response relation obtained during blockade of the median nerve by cooling (■) or by sectioning (◆) compared with pre- and post-cooling controls (○, □). The principal conclusion to be drawn from the analysis for both neurones in Fig. 4 is that, once again, as in Fig. 3 there was no emergence of a novel receptive field or enhancement of responsiveness in association with the partial deafferentation.

In neurones of this group, which displayed attenuated responsiveness during median nerve cooling, it was important to control for two possible artefactual effects. First, it was necessary to show that the cooling led to complete removal of that component of the cuneate neurone response mediated by the median nerve and therefore confirm that the residual response was not attributable to incomplete blockade of the nerve. For the two neurones whose data are illustrated in Fig. 4 this was done subsequent to the cooling analysis at the end of the two experiments, by sectioning of the median nerve. When this was done for the neurone in Fig. 4*A*, the receptive field, based again on the 450 mg wt von Frey hair and shown in the lowest set of paired sketches in Fig. 4*A*, was almost identical to that obtained during median nerve cooling. Furthermore, the stimulus–response relation obtained after nerve section (■) was almost indistinguishable from that obtained during median nerve cooling. This was also the case for the neurone whose data are illustrated in Fig. 4*B*. For both neurones, the residual responsiveness that survives median nerve block or sectioning was shown to come via the superficial radial nerve, as sectioning of this nerve at the end of the study in each of these experiments abolished the residual response.

The second potential artefact to be eliminated was the possibility that the cooling may spread to affect other nerves such as ulnar or superficial radial. If this were so, the

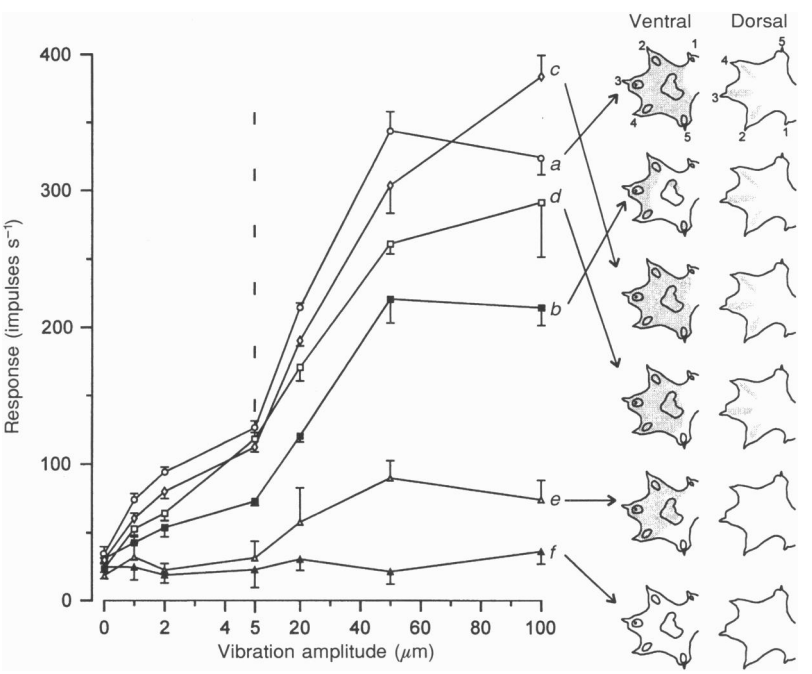

Figure 5. Effect of cold block and transection of the median nerve on the responsiveness of a cuneate neurone that received convergent forelimb input from the median, ulnar and radial nerves

The 6 stimulus–response relations were constructed in *a*–*f* by plotting the mean ± S.D. response (impulses s^{-1}) to 6 repetitions of a 1 s train of vibration at 300 Hz delivered by means of a 2 mm diameter probe to the point of maximum sensitivity indicated by the black dot on the pad of toe 3. The abscissa is plotted on a more expanded scale at amplitudes of $\leqslant 5$ μm in order to prevent overlap and congestion among the plotted values at these low amplitudes. The neurone's receptive field was mapped with a 500 mg wt von Frey hair and indicated by the stippled areas on the sketches of ventral and dorsal surfaces of the paw on the right-hand side of the figure. Relations *a* and *c* and the associated receptive field estimates were obtained as pre- and post-cooling controls for relation *b* obtained when the median nerve was blocked by cooling to 3 °C. Once median nerve conduction was restored, relation *d* was obtained after sectioning of the ulnar nerve, and relation *e* after both the ulnar and radial nerves had been sectioned. Relation *f* was then obtained after median nerve conduction was again blocked by cooling.

reduction in response for neurones such as those in Figs 2–4 may not be a consequence of loss of median nerve input but instead an impairment of input from these other nerves. However, this could not be the case as the median nerve section had the same effect on responsiveness as the cooling (Fig. 4*A* and *B*). In addition, direct temperature measurements in the tissue between the cooling device and the other nerves indicated that the spread of cooling was insufficient to affect refractoriness in these nerves (Paintal, 1965).

The effects of partial reversible deafferentation were also examined for cuneate neurones with even more widespread convergent input than those of Fig. 4. The data in Fig. 5 were from a cuneate neurone whose receptive field (based on mapping with a 0·5 g wt von Frey hair) occupied most of the ventral surface and part of the dorsal surface of the paw (upper pair of outlines in Fig. 5). The responsiveness of the neurone was tested quantitatively at its most sensitive point, indicated by the black dot, on toepad 3. The neurone was purely dynamically sensitive and, from its selective sensitivity to high-frequency vibrotactile stimuli, was activated, in part at least, by inputs from Pacinian corpuscles. Stimulus–response relations *a* and *c* were obtained as control relations *before* and *after* the reversible block of the median nerve which led to some fall in responsiveness (relation *b*) and shrinkage of the receptive field on the ventral side of the paw (second pair of outlines in Fig. 5). Transection of the ulnar nerve led to loss of the toe 5 component of the receptive field but had little effect on responsiveness from toe 3 (relation *d*). Subsequent transection of the radial nerve led to disappearance of the receptive field on the dorsal surface of the paw but led to little change on the ventral surface, and a profound fall in responsiveness from the test point on toe 3 (relation *e*). When the median nerve was again blocked by cooling after the transection of both ulnar and radial nerves, responsiveness was abolished (relation *f*) and the receptive field disappeared (lowest pair of outlines). Despite the evidence for convergent inputs for this neurone from median, ulnar and radial nerve fields of distribution, the loss of the median nerve (or other) component of input led to no expansion of the neurone's receptive field in the territory of the residual intact nerves.

Neurones that displayed an abolition of responsiveness with median nerve blockade

A complete loss of receptive field, and therefore disappearance of tactile responsiveness during median nerve cooling, was observed for eight cuneate neurones, which are assumed to receive input exclusively from the median nerve. Each of these neurones was tested carefully during the median nerve blockade for the appearance of any novel receptive field on either the ventral or dorsal surface of the distal forelimb. However, despite the use of stronger

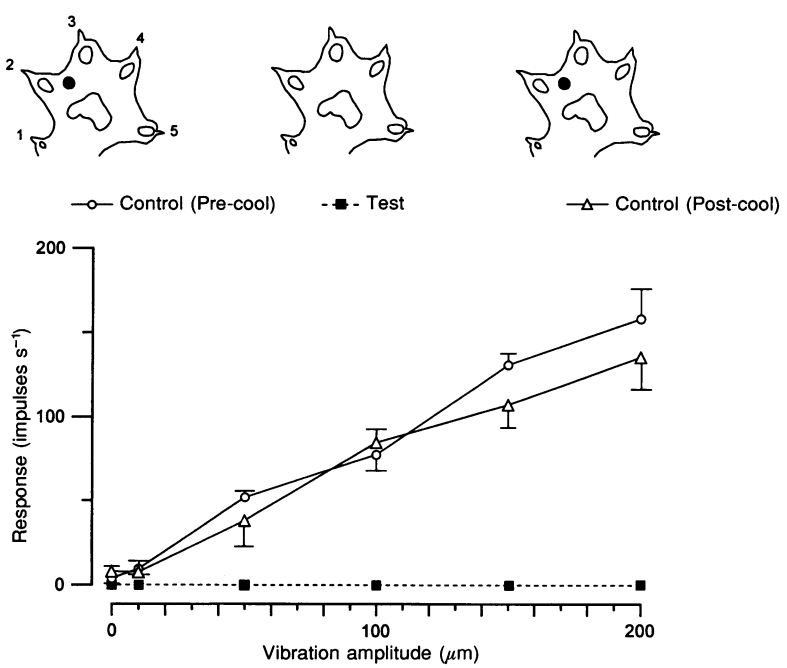

Figure 6. Effect of reversible median nerve blockade on the receptive field and responsiveness of a cuneate neurone

The receptive field, mapped with a 440 mg wt von Frey hair during the pre- and post-cooling control periods, is indicated on the sketches by the black area between toes 2 and 3. Responsiveness (mean ± S.D., $n = 6$) to a 100 Hz train of vibration lasting 1 s is plotted as a function of vibration amplitude in the graph. Stimulus–response relations plotted with ○ and △ represent pre- and post-cooling controls, respectively; that plotted with ■ was obtained when the median nerve was blocked and both responsiveness and the receptive field were abolished.

tactile stimuli than the von Frey hairs, for example brushing, the use of small wooden probes and direct manual tapping, there was no emergence of novel fields.

The data in Fig. 6 came from a dynamically sensitive neurone whose receptive field (mapped with a 0·44 g wt von Frey hair) was confined to the area indicated on the hairy skin between toes 2 and 3. The neurone, whose input came presumably from hair follicle afferent fibres, was most sensitive to skin vibration at frequencies of ~100 Hz. It had a graded response as a function of vibration amplitude in the two control stimulus–response relations constructed at 100 Hz (Fig. 6) before and after the median nerve block. During the nerve blockade, responsiveness to the 100 Hz stimulus was abolished (■, Fig. 6) and the receptive field disappeared, only to reappear upon restoration of median nerve function.

Neurones essentially unaffected by median nerve blockade

The remaining eighteen neurones with receptive fields in the innervation zones of the ulnar or superficial radial nerves showed no systematic changes during median nerve blockade in their stimulus–response relations (Fig. 7) or, with the possible exception of two neurones, in receptive field size. Figure 7*A* shows results for a neurone representative of this group where the receptive field area, mapped with three von Frey hairs, and the stimulus–response relations obtained in response to 30 Hz vibration at the toe 3 focus, were apparently unchanged in association with median nerve blockade. This neurone had its receptive field on the hairy skin on the side of toe 3, when tested with von Frey hairs of 0·1 and 0·23 g wt, but with a stronger 0·45 g wt hair, the field included a discontinuous region on the edge of toe 2. The outlines in the inset of Fig. 7*A* show the extent of the field (which is located on the margin of the innervation field for the lateral branch of the superficial radial nerve; Kitchell *et al.* 1982) as a function of von Frey hair force. When mapped in this way the receptive field was indistinguishable in the control circumstances (before and after median nerve blockade) and during the blockade.

Among the eighteen neurones in this group that showed no systematic change in responsiveness based on their stimulus–response relations there were two that appeared to have a slight expansion in their receptive field. One of these, shown in Fig. 7*B*, had a slowly adapting response to static displacement within its small receptive area in hairy skin on the dorsal surface of the paw near toe 1. There appeared to be a slight enlargement of the field during median nerve blockade but no consistent effect on the stimulus–response relation (see criteria in Methods). As there were only two neurones that had any sign of an expanded receptive field, and as the extent of expansion was so slight, it appears

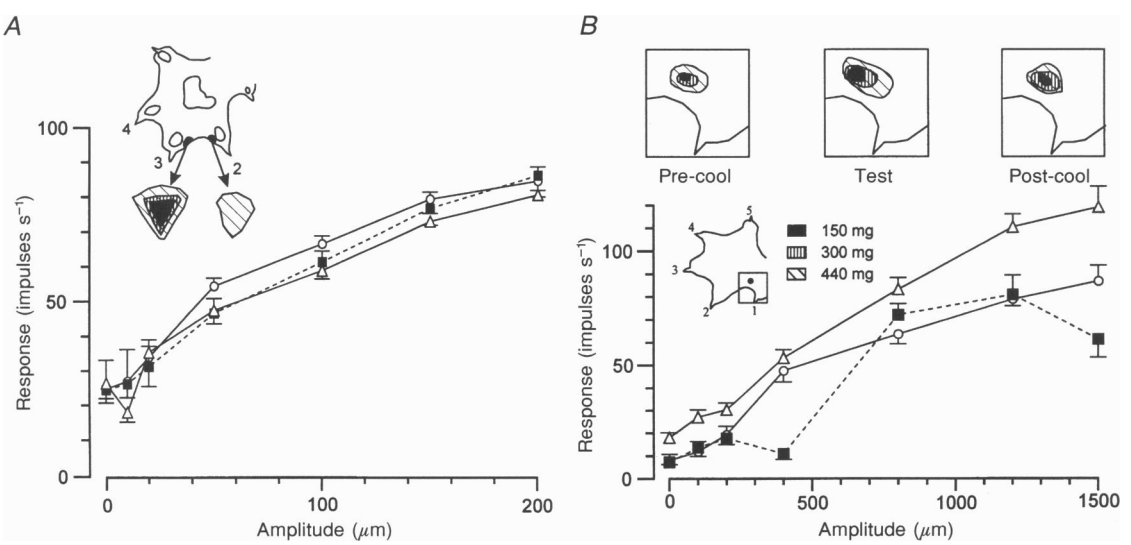

Figure 7. Absence of effect of median nerve blockade on cuneate responsiveness

A, the 3 stimulus–response relations plot the mean ± S.D. response (impulses s^{-1}, $n = 6$) to a 1 s train of vibration at 30 Hz delivered by means of a 2 mm diameter probe to the hairy skin near toe 3. Relations plotted with ○ and △ represent pre- and post-cooling controls, respectively, and that plotted with ■ represents the relation obtained when the median nerve was blocked by cooling to 3 °C. The receptive field of the neurone appeared indistinguishable in the 3 circumstances and is plotted on the inset sketch of the paw on which toes 2, 3 and 4 are indicated. The enlarged sketches show the receptive field mapped with 0·1 (■), 0·23 (▥) and 0·45 g wt (▨) von Frey hairs. *B*, the 3 stimulus–response relations plot the mean ± S.D. response (impulses s^{-1}, $n = 10$) to a 1 s step indentation delivered by a 1 mm diameter probe to the hairy skin between toes 1 and 2. Relations plotted with ○ and △ represent pre- and post-cooling controls, respectively, and that plotted with ■ represents the relation obtained during median nerve block. The sketches in the boxes above the graph and in the inset show the receptive field location mapped with von Frey hairs at the indicated strengths.

unlikely to represent any significant or systematic deafferentation-related reorganization. Instead, it may reflect inherent inaccuracies from trial to trial in the receptive field mapping procedure. Although the von Frey hairs were calibrated for a particular force value, these values were based on 'static' force measures, and cannot indicate the transient onset force and the fluctuations in this that arise from trial to trial related, for example, to variations in the rotational position of the experimenter's grasp of the von Frey support rod and the consequences of this for hairs that are not straight, variations in the angle or velocity of application of the hair to the skin, and variations in the exact sites of application of the von Frey hair to the skin from one test to another. These factors will contribute to trial-to-trial fluctuations in estimates of receptive fields and, as we did not systematically and repeatedly assess the stability of receptive fields prior to cooling as was done by Calford & Tweedale (1990) and Pettit & Schwark (1993), it is difficult to specify rigorous criteria for the identification of genuine deafferentation-related changes from the background fluctuations in receptive field estimates. For these reasons our receptive field assessments must be regarded as less quantitative and rigorous than our measures of neuronal responsiveness.

Spontaneous activity in the cuneate neurones studied showed, in agreement with most other studies (e.g. Dostrovsky *et al.* 1976; Pettit & Schwark, 1993; Panetsos *et al.* 1995) no consistent effect in association with the median nerve blockade. There appeared to be no evidence for a change in ~80% of neurones (e.g. Figs 3, 4*B* and 5), some reduction in 15% (e.g. Fig. 4*A* and 6) and an increase in 5%.

DISCUSSION

The present study employed a rapid, reversible method for partial deafferentation based on cooling-induced blockade of the median nerve in the forearm. The procedure allowed deafferentation to be achieved within ~2 min and permitted a similarly rapid restoration of median nerve input. The advantage of this deafferentation procedure over nerve section or local anaesthetic blockade is that it readily permits examination of responsiveness and receptive field characteristics in the one neurone before, during and after the partial deafferentation.

Effectiveness of cooling for median nerve block

Temperatures for median nerve cooling ranged from 2 to 5 °C, with values of ⩽3 °C being used for twenty-two of the thirty-nine cuneate neurones studied. We can be confident that complete inactivation of myelinated fibres took place, as these temperatures are well below those reported (7·2 °C, on average) for myelinated nerve blockade (Franz & Iggo, 1968), and because we confirmed the abolition of the myelinated nerve compound action potential and unitary activity (Figs 1 and 2). As we did not attempt to monitor either the compound action potential or unitary activity associated with C fibres, we cannot be certain that *all* C fibre activity was abolished, in particular when C fibres require more severe cooling for inactivation than do A fibres (Franz & Iggo, 1968). Nevertheless, for several reasons, we are confident that minimal or even zero C fibre activity would have survived our median nerve cooling procedure. Although the average temperature required for inactivation of saphenous nerve C fibres was reported by Franz & Iggo (1968) to be 2·7 °C, this occurred at rates of activation no faster than 1 per 10 s. However, they showed that the capacity of C fibres to sustain faster rates of discharge was affected markedly at higher temperatures (for example, a discharge rate of $5\ s^{-1}$ was profoundly disrupted at temperatures above 10 °C in Fig. 7 of Franz & Iggo, 1968). Another factor that influences the blocking temperature is the length of the cooled segments of nerve. When a 6 mm segment of saphenous nerve was cooled, Douglas & Malcolm (1955) observed almost complete disappearance of the C fibre complex of the saphenous nerve compound action potential. As our cooled segment of median nerve was even longer, 10 mm in length, this should have ensured that the temperatures used (2–5 °C, and ⩽3 °C for 22 of the 39 neurones studied) were effective for complete or near-complete block of C fibres as well as A fibres. The remaining evidence against any residual C fibre activity preventing some reorganization of responsiveness was the absence of any differential effect of nerve cooling and nerve sectioning (e.g. for the 2 neurones of Fig. 4).

We do not believe that more certain evidence for the cooling-induced inactivation of C fibres could be offered, for even if we had routinely monitored the C fibre component of the median nerve compound action potential, it is possible that the temporal dispersion induced by nerve cooling may have allowed some residual C fibre activity to remain without detection in the compound recording. We were also unable to risk cooling to lower temperatures because of the danger of causing lasting damage to the nerve by cooling to low temperatures (< 5 °C for more than ~2 h; Kennett & Gilliatt, 1991).

The rapid, cooling-induced deafferentation technique, in combination with the use of controlled methods of skin stimulation, permitted quantitative evaluation of cuneate neurone responsiveness and mapping of receptive field size in the course of deafferentation, and enabled us to test a series of specific hypotheses, arising from previous reports, that partial deafferentation leads to an immediate expansion in the central representation of body regions around the peripheral zone of deafferentation, an effect attributed to removal of background inhibition.

Hypothesis I; for cuneate neurones with receptive fields adjacent to the median nerve field

The first hypothesis tested was that cuneate neurones with input from ulnar or superficial radial nerve fields in the vicinity of the median nerve field should undergo, in association with median nerve blockade, an increased level of responsiveness to a fixed tactile stimulus within that

ulnar or radial nerve zone and an expansion of their cutaneous receptive fields. However, among eighteen cuneate neurones of this type, there was no evidence for any systematic enhancement of responsiveness based on the construction of detailed stimulus–response relations of the type shown in Fig. 7*A* and *B*. In Fig. 7*A* in particular, the neurone's receptive field was at the edge of the distribution zone for the lateral branch of the superficial radial nerve on the border this nerve shares with the median nerve on the ventral surface of toes 2, 3 and 4 (Kitchell *et al.* 1982). Despite this *immediate* proximity of the receptive field to the median nerve field, the responsiveness of the neurone, over the whole of the stimulus–response relation, was unchanged compared with control levels obtained *before* and *after* median nerve blockade (Fig. 7*A*). Furthermore, the receptive field showed no apparent change in size in at least sixteen of the eighteen cuneate neurones of this type.

Hypothesis II, for cuneate neurones with fields that included median and other peripheral nerve fields

The second hypothesis tested was that cuneate neurones whose input came from *both* the median nerve and another peripheral nerve source should undergo, in association with median nerve blockade, an increase in responsiveness to the remaining input and an expansion of the receptive field into the field of that remaining nerve source. However, in none of thirteen neurones of this type tested was there evidence of such a change. The example in Fig. 3 was for a neurone with input from toe 4 at the margin of overlap of the median and ulnar nerves (Kitchell *et al.* 1982). The neurone underwent a shrinkage in its receptive field upon median nerve blockade and there was no suggestion of any expansion to the lateral side of toe 4 or beyond onto toe 5 in a way that might be predicted if the blockade had removed a source of tonic inhibition exerted by the median nerve input upon the adjacent ulnar nerve inputs.

The same observations were made for neurones that received convergent input from the median and superficial radial nerves as illustrated in Figs 4 and 5. With the loss of median nerve input, the receptive field attributable to the superficial radial source on the dorsal side of the paw (Fig. 4*A*) appeared, if anything, to undergo some contraction. In the example in Fig. 4*B*, the neurone had a receptive field on the margin of the innervation fields of both superficial radial and median nerves (Kitchell *et al.* 1982), but the field underwent no expansion upon median nerve blockade. Nor, in Fig. 5, was there evidence of an expansion of the receptive field in the region of superficial radial distribution with median nerve blockade.

Hypothesis III, for cuneate neurones with fields within the medial nerve field

The third hypothesis was that cuneate neurones whose 'control' receptive fields were within the peripheral zone of deafferentation should show an emergence of novel receptive fields and responsiveness from areas around the field of innervation of the median nerve. However, based on testing with von Frey hairs of eight neurones of this type (see Fig. 6), there was no evidence for such changes in the adjacent areas that are supplied by the ulnar or superficial radial nerves.

Relation of results to earlier reports of immediate plasticity in dorsal column nuclei following partial deafferentation

All three hypotheses proposed and examined in the present study were based on the earlier reports that partial deafferentation induces *immediate* plasticity within the adult dorsal column nuclei, involving either an expanded representation of body regions outside the zone of deafferentation (Dostrovsky *et al.* 1976; Millar *et al.* 1976) or, for individual dorsal column nuclei neurones deprived of their 'normal' inputs, an emergence of tactile responsiveness from a *novel* receptive field adjacent to the original field (Pettit & Schwark, 1993, 1996; Panetsos *et al.* 1995). However, as we have been unable to confirm any of the three hypotheses, our findings appear to be in conflict with these earlier reports that have described immediate plasticity in dorsal column nuclei in response to partial deafferentation. Nevertheless, our failure to find deafferentation-induced alterations in cuneate responsiveness is consistent with the reports of McMahon & Wall (1983) for the rat gracile nucleus, Northgrave & Rasmusson (1996) for the raccoon cuneate nucleus, and with the observations of Waite (1984) on the trigeminal nucleus of the adult rat following partial deafferentation induced by infraorbital nerve section. To what extent the discrepancies among different studies are attributable to differences of methodology is unclear. Pettit & Schwark (1993), in their study of cuneate neurones whose normal input was abolished by up to three local anaesthetic injections into the skin within the normal receptive field, observed an emergence of responsiveness from novel receptive fields that were usually continuous with the original cutaneous receptive field. Furthermore, they observed a similar receptive field reorganization following capsaicin injection into the receptive fields of cuneate neurones (Pettit & Schwark, 1996). They interpreted their initial findings with local anaesthetic injections in terms of central changes, attributed to an unmasking of previously ineffective inputs as a result of the removal of tonic inhibition normally exerted by the inactivated afferent fibres on adjacent sources of input to the cuneate neurone. In the case of the capsaicin injections, their interpretation was that the subcutaneous injection of capsaicin caused a desensitization and blockade of C and Aδ afferent fibres from the region of the cuneate neurone's receptive field, and that this loss of fine fibre input allowed a central unmasking of other adjacent tactile inputs to the cuneate neurone. In both series of experiments it was possible, in particular where two or three injections each up to 30 μl in volume were made into the receptive field region, that peripheral factors at the injection site could cause altered mechanical properties associated, first, with the injected fluid volume and second, with a local inflammatory response to the inevitable injury occasioned by the

injections themselves. As a consequence, the adjacent nerve endings may be sensitized both mechanically and chemically to the hand-held mechanical stimuli, leading to an enhancement of their responses and, in turn, of their probability of discharging the cuneate neurone. However, this peripheral explanation for the observed changes was considered by Pettit & Schwark who observed no expansion in responsiveness in association with a control saline injection into the receptive field in one neurone in the 1993 study and in nine neurones in their 1996 study. Nevertheless, there is a need for interpretative caution in these experiments on several grounds. First, with the local anaesthetic injections, the authors reported that, in the vast majority of cuneate neurones, the altered responsiveness from the novel receptive field 'remained throughout the entire recording period (up to 6 h) even after responsiveness in the original receptive fields had returned'. Clearly this should not have happened if the proposed central explanation were valid. Once the afferents from the original receptive field had recovered from the local anaesthetic blockade they should once again have exerted their putative tonic inhibitory influence on the adjacent sources of input, effectively masking that input.

Second, the assumption in the capsaicin injection experiments (Pettit & Schwark, 1996) was that the subcutaneous capsaicin injection was causing desensitization and blockade of Aδ and C fibres associated with the excitatory receptive field of the cuneate neurone. However, multiple effects will almost certainly result from the capsaicin injection. For example, the capsaicin will cause an initial excitation of the fine-diameter afferents at the injection site with a time course of ~1–2 min. This will be followed by a desensitization of these afferent fibres at the injection site for a period lasting many hours, and this region will be surrounded by a much larger hyperalgesic zone, in part perhaps attributable to sensitization of afferents by lower concentrations of capsaicin that can diffuse from the injection focus (Baumann, Simone, Shain & LaMotte, 1991).

The immediate reorganization within dorsal column nuclei described in earlier reports from Wall's group (Dostrovsky *et al.* 1976; Millar *et al.* 1976) involved an expanded representation of the abdomen and trunk subsequent to partial deafferentation, based on sectioning of dorsal roots caudal to L3 or reversible cold block of the dorsal columns by means of the application of ice made from isotonic saline. Although single-neurone recording was carried out in some experiments by Dostrovsky *et al.* (1976) for selected observations on individual neurones, these two studies were based largely on multiunit recording with, presumably, low impedance, glass-insulated tungsten electrodes. It is possible that if the hindlimb deafferentation led to any decrease in background activity *within* the normal hindlimb representation zone of dorsal column nuclei, the *effective* recording 'pick-up' distance of the low-impedance multiunit recording electrode might be extended to regions some hundreds of micrometres away from the actual electrode tip. Although Dostrovsky *et al.* (1976) reported that there was 'no consistent difference between the on-going spontaneous rate of activity in the deafferented and normal nuclei', no values were given nor is it clear how this could be assessed reliably with multiunit recording. However, as the reported immediate expansion in trunk or abdomen representation appears to take place over distances of ~200–600 μm (Figs 1 and 2 in Dostrovsky *et al.* 1976 and Fig. 6 in Millar *et al.* 1976), the resolution capacity of the multiunit recording procedure may be a factor in the observed reorganization, which at least for 'some of the connections to the expanded abdominal area' was said by the authors to be of a 'tenuous nature' and dependent upon an increased need for 'flick' stimuli to generate gracile responsiveness.

In the case of the single gracile neurones examined by Dostrovsky *et al.* (1976) in association with reversible cold block of the L4 cord, a substantial proportion (10/30 neurones) with receptive fields confined to the leg or foot acquired abdominal fields during deafferentation although, somewhat suprisingly, none of the neurones whose original fields included leg and abdomen displayed any expansion of the abdominal component once the leg component was abolished by the deafferentation. It is not clear why there should be this difference in susceptibility of these two groups of neurones to reorganization, or why in our present study we observed no persuasive evidence for deafferentation-induced reorganization of responsiveness when it has been reported for a significant proportion of dorsal column nuclei neurones in the cat by Dostrovsky *et al.* (1976). It is also puzzling that this capacity for immediate reorganization should be species specific as the same group (McMahon & Wall, 1983) found no evidence for immediate reorganization within the rat dorsal column nuclei subsequent to partial deafferentation.

It seems unlikely that any systematic difference in, for example, anaesthetic agent or depth should account for the discrepancy between our data and many of the earlier studies in the cat, in particular when the present observations and our previous studies on cuneate responsiveness have revealed no obvious differences between pharmacologically anaesthetized animals and those that were decerebrate and therefore free of anaesthetic agents (Ferrington, Rowe & Tarvin, 1987). Although the study by Northgrave & Rasmusson (1996) that found no evidence for deafferentation-induced reorganization in the raccoon cuneate nucleus was conducted under chloralose anaesthesia, which may decrease central inhibition, this anaesthetic agent was not used in the present study or in those by McMahon & Wall (1983) and Waite (1984) which also failed to reveal deafferentation-induced reorganization in adult dorsal column nuclei or trigeminal nuclei. It also appears unlikely that the neurones sampled within the dorsal column nuclei are fundamentally different in the different studies. Those sampled by Pettit & Schwark (1993, 1996) were in the middle or cluster zone of the cuneate nucleus as were the neurones in our sample.

Furthermore, the studies by Dostrovsky *et al.* (1976) and Millar *et al.* (1976) also concentrated on the caudal cell cluster region of the gracile nucleus and did not explore the rostral, 'non-cluster' region. The explanation for the different findings in studies of reorganization in the cat dorsal column nuclei therefore remains uncertain.

Relation of results to reports of immediate plasticity at thalamic and cortical levels in the cat

Where investigations of the effects of partial deafferentation have been conducted at higher levels of the cat somatosensory pathways there have also been differences in the outcomes. For example, Metzler & Marks (1979) have described immediate reorganization within SI after an epidural block of dorsal roots, whereas a recent study by Dykes's group (Li *et al.* 1994) found little or no persuasive evidence for *immediate* reorganization in the SI cortex of the cat after ulnar nerve section. The region of SI served by the ulnar nerve remained largely unresponsive to somatic stimulation immediately after the nerve section (note Figs 6 and 8 in Li *et al.* 1994), and where there was any evidence of novel responsiveness in this cortical region the authors allowed (p. 246 of Li *et al.* 1994) that this might reflect a slight deviation in the replacement of the recording electrode for the cortical remapping after the partial deafferentation, or alternatively that the changes merely reflected moment-to-moment alterations in cortical responsiveness.

Of course, an absence of immediate reorganization as we describe in the present study at lower levels of the cat somatosensory pathway need not be in conflict with any report of immediate reorganization in responsiveness at either thalamic or cortical levels, as these higher levels of the central pathways, in particular the cortical level, may possess greater capacity for reorganizational plasticity. Indeed, studies on the adult visual system in cat (and monkey) indicate that in response to partial retinal lesions, there is an immediate expansion in responsiveness of visual cortical neurones whose receptive fields are near the margins of the representation zone of the retinal scotoma (Gilbert & Wiesel, 1992), whereas equivalent observations at the level of the lateral geniculate nucleus revealed a large silent zone associated with the scotoma. The observations indicate that the changes in visual cortex were intrinsic to the cortex and, in the case of the *immediate* changes, were attributed to an unmasking of previously subthreshold excitatory inputs. The more pronounced chronic reorganization observed was attributed to axonal sprouting of long-range laterally projecting cortical neurones that are responsible for a topographic remodelling of the visual cortex (Darian-Smith & Gilbert, 1995).

In conclusion, observations on the immediate effects of partial deafferentation on the somatosensory pathways in the cat have generated a range of findings both at upper and lower levels of the pathway. One of the clear differences among the studies lies in the type of deafferentation and its extent. Whether these differences, and any differences related to the level of pathway examined, or the sample of neurones studied, can explain the different findings remains unclear. However, we believe that in order to resolve many of the discrepancies there is also a need for unequivocal single neurone analysis and for quantitative response evaluation based upon reproducible forms of tactile stimulation.

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