The effects of neonatal median nerve injury on the responsiveness of tactile neurones within the cuneate nucleus of the cat

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1. The capacity of cuneate neurones to attain normal functional properties following neonatal median nerve injury was investigated with single neurone recording in anaesthetized cats, 12–24 months subsequent to a controlled crush injury. Effectiveness of the peripheral nerve injury was confirmed by the abolition of the median nerve compound action potential following the crush.

2. Cuneate recording was carried out after denervation of the forearm, apart from the median nerve, to ensure that neurones studied had receptive fields within the distribution zone of the regenerated median nerve. Controlled and reproducible tactile stimuli were used to evaluate the functional capacities of neurones to determine whether they were consistent with those reported earlier for cuneate neurones in cats that had normal peripheral nerve development.

3. Twenty-two cuneate neurones with well-defined tactile receptive fields within the distribution zone of the regenerated median nerve were classified according to their adaptation characteristics and functional properties. Slowly adapting neurones responded throughout static skin indentations and had graded and approximately linear stimulus–response relations over indentation ranges up to 1·5 mm. Rapidly adapting neurones responded to the dynamic phases of skin indentations and could be divided into two broad classes, one most sensitive to vibrotactile stimuli at 200–400 Hz which appeared to receive a predominant input from Pacinian corpuscle receptors, and a non-Pacinian group that included neurones most sensitive to skin vibration at 5–50 Hz which appeared to receive glabrous skin input from the rapidly adapting class of afferent fibres.

4. Based on the stimulus–response relations and on measures of phase locking in the responses to vibrotactile stimuli, it appears that the functional properties of cuneate neurones activated from the field of a regenerated median nerve subsequent to a neonatal nerve crush injury were consistent with those reported previously for 'control' cuneate neurones. The results indicate that cuneate neurones can acquire normal tactile coding capacities despite the disruption caused by prior crush injury to their peripheral nerve source.

Sensory disturbances and losses associated with peripheral nerve injury may be severe after transection or traumatic injury to the nerve, but are much more likely to be reversible in the case of crush injury (Sunderland, 1978). Although the time course of recovery after crush injury varies, depending upon the site of injury and the distance over which nerve regeneration and regrowth take place, recovery appears to be more or less complete in the adult animal according to clinical (Sunderland, 1978) as well as anatomical and physiological evidence (Burgess & Horch, 1973; Dykes & Terzis, 1979; Horch, 1979; see reviews by Guth, 1956; Sunderland, 1978; Munger, 1988). However, in the neonatal animal, recovery after crush injury is anatomically incomplete as there are long-term reductions in the size and fibre spectrum of the peripheral nerve and its associated dorsal roots and dorsal root ganglia (Bueker & Myers, 1951; Risling, Aldskogius & Hildebrand, 1983; Jenq, Jenq & Coggeshall, 1987; Hník, Vejsada & Palecek, 1992). These impairments may be due to constraints imposed by permanent reductions in the endoneurial tubes which limit the effectiveness of axonal regeneration along the distal nerve segment (Sunderland, 1978). Nevertheless, clinical

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data suggest that the recovery of sensory capacities following peripheral nerve injury is no less successful in young children than in adults (Sunderland, 1978). However, the clinical assessments are often based on simple detection tasks and may not reveal more subtle discriminative disturbances that reflect impaired transmission and signalling, and which might result either from the incomplete anatomical regeneration of the peripheral nerve, or from some form of functional reorganization or 'plasticity' within the central pathways that has been reported to follow partial deafferentation brought about by nerve injury or local anaesthetic blockade (e.g. Dostrovsky, Millar & Wall, 1976; Lisney, 1983; Merzenich, Kaas, Wall, Nelson & Sur, 1983; Calford & Tweedale, 1988, 1990; Wall, Huerta & Kaas, 1992; Nicolelis, Lin, Woodward & Chapin, 1993; Pettit & Schwark, 1993; Koerber & Brown, 1995). The reorganization described involves an alteration in body maps at the levels of the dorsal horn, the dorsal column nuclei, thalamus and cortex, in which there is an expansion in the central representation of body regions around the zone of deafferentation into areas of the map that previously represented the deafferented body part. In the present study, the pattern or map of body representation has not been studied but instead we have analysed the transmission and coding capacities of cuneate neurones whose input came from the peripheral field of the regenerated median nerve. The analysis was carried out in adult cats, 12–24 months after a crush injury to the median nerve in the forearm of the neonatal kitten or, in one case, an adult cat. The aim was to determine whether peripheral nerve injury, in particular in the neonate, leads to permanent impairment in sensory transmission across the cuneate nucleus. A preliminary report of the present work has been presented in conference proceedings (Murray, Taub, Mackie, Zhang, Ghosh & Rowe, 1994).

METHODS

Median nerve injury

The left median nerve underwent controlled crush injury at the mid-forearm level in four neonatal kittens (age <1 week) and one adult cat, each under full surgical anaesthesia (induced in the neonates with an I.M. mixture of ketamine at 15 mg kg^{-1} and xylazine at 0·2 mg kg^{-1} and in the one adult with ketamine at 30 mg kg^{-1} and xylazine at 1 mg kg^{-1}, with supplements if needed) under aseptic conditions. Had the functional properties of cuneate neurones been markedly affected subsequent to neonatal nerve crush, additional experiments would have been conducted on animals that underwent nerve injury as adults to determine whether functional disruptions were less marked. However, as cuneate functional properties subsequent to the neonatal crush overlapped data from equivalent analyses in normal cats (Douglas, Ferrington & Rowe, 1978), we did not extend the observations on adult nerve crush beyond the initial single animal.

The crush injury was carried out according to the method of Gutmann, Guttmann, Medawar & Young (1942) and was based on firm compression of the median nerve with a pair of smooth-faced watchmaker's No. 5 forceps at a point where the forceps width was 1 mm. The effectiveness of the crush was confirmed electrophysiologically by observing the abolition, proximal to the crush site, of the compound action potential evoked by electrical stimulation (100 μs, single pulse) of the distal segment of the median nerve (Fig. 1). The compound action potentials in the left column of Fig. 1 were recorded at stimulus strengths of 2, 5 and 7 times threshold intensity for the nerve prior to nerve crush. The traces in the right-hand column of Fig. 1 were recorded after the nerve injury and confirm the absence of conduction over the proximal segment of the median nerve at any of the three strengths of nerve stimulation. The region of forearm surgery was carefully sutured and the animals allowed to recover under antibiotic protection.

Cuneate recording procedures

Regeneration of the crushed median nerve should have largely taken place within 1–3 months but can require longer periods (Gutmann *et al.* 1942; Sunderland, 1978). However, to ensure that recovery had stabilized, electrophysiological recordings were not carried out until 12–24 months subsequent to the median nerve injury. These were performed in animals anaesthetized with pentobarbitone sodium (40 mg kg^{-1} I.P. initial dose and ~2 mg kg^{-1} h^{-1} I.V. for maintenance of anaesthesia). The trachea was cannulated (for monitoring end-tidal P_{CO_2}) and the femoral vein and artery catheterized for anaesthetic administration and blood pressure monitoring. The head was secured stereotaxically before exposing the dorsal brainstem above the cuneate nucleus by removal of the atlas and a small part of the occipital bone.

The left forearm was denervated except for the regenerated median nerve by sectioning the ulnar, radial and musculocutaneous nerves at, or proximal to, the elbow. The forepaw was positioned with pads uppermost in a Perspex trough and secured with paraffin wax to allow accurate positioning of a mechanical stimulator. Recordings were made from single neurones of the left cuneate nucleus by means of tungsten microelectrodes. As both the mid-line and obex provided visible reference locations on the exposed dorsal surface of the brainstem we were able from these co-ordinates to reliably specify recording positions and be quite confident that these were within the main cuneate nucleus. The neurones studied were 1–4 mm caudal to the obex and 1–2·3 mm lateral to the mid-line. Their depths ranged from 230 to 2100 μm with a mean of 1140 $\pm$ 500 μm (mean $\pm$ S.D.). Although lesions were not made and histological verification not carried out, the above locations are all entirely consistent with the middle or cluster zone of the main cuneate nucleus (Hand & Van Winkle, 1977). Further support for this conclusion came from the detection, 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.

All neurones isolated electrophysiologically for study had receptive fields within the area of innervation of the regenerated median nerve. Their fields were delineated by gentle tapping or brushing with a small probe. Those whose fields included the glabrous skin were examined with controlled and reproducible forms of mechanical stimuli delivered by means of small, 1–4 mm diameter circular probes driven by a servo-controlled mechanical stimulator (Douglas *et al.* 1978; Ferrington & Rowe, 1980).

Neurones were classified into two broad functional groups of slowly adapting and dynamically sensitive neurones. Stimuli used for studying slowly adapting neurones were steady indentations of a rectangular form lasting 1–1·5 s (amplitude up to 1·5 mm). For studying dynamically sensitive neurones, a train of sinusoidal vibration lasting 1 s was superimposed on, and started 300 ms after the onset of, a 1·5 s rectangular indentation, usually at an amplitude

of 400 μm. Impulse activity was displayed on an oscilloscope and fed to a differential amplitude discriminator from which constant output pulses were relayed to a counter unit and a laboratory computer. This was used to construct cycle histograms from which it was possible to evaluate quantitatively the extent of phase locking in the responses of cuneate neurones to vibrotactile stimuli. The cycle histograms use a pulse associated with the onset of each successive cycle in the vibration train as a stimulus marker and display the incidence of impulse occurrences at different times throughout the period of the vibration cycle (Douglas *et al.* 1978; Ferrington & Rowe, 1980; Ferrington, Horniblow & Rowe, 1987*a*; Ferrington, Rowe & Tarvin, 1987*b*; Vickery, Gynther & Rowe, 1994). The analysis period for the cycle histogram corresponds to the *cycle period* of the vibration. When there was no phase locking of impulse activity, the cycle histograms were approximately rectangular in shape, whereas a grouping of impulses within a restricted segment of the histogram indicated that the neurone had a preferred point of discharge and its response was therefore phase locked to the vibration waveform (see Fig. 5). A quantitative measure of phase locking, the *percentage entrainment*, was derived from the cycle histogram by obtaining the maximum proportion of impulses that occurred within any continuous half-cycle period of the histogram (Douglas *et al.* 1978). Its value ranged from a minimum of 50% (for a rectangular distribution) to a maximum of 100% when all impulse occurrences fell within a half-cycle segment of the histogram.

The experimental work conformed with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Animals were killed at the end of experiments by an I.V. administered anaesthetic overdose.

RESULTS

Identification and classification of cuneate neurones

Twenty-two single cuneate neurones responsive to light tactile stimulation of forepaw regions within the area of innervation of the previously crushed median nerve were identified, functionally classified, and their receptive fields mapped manually with a fine probe or brush. Sixteen neurones came from animals in which the median nerve injury was imposed in the neonate and six from the animal with adult nerve injury. The receptive fields of the twenty-two neurones were mainly on distal regions of the forelimb. They were small, well defined and usually confined to the glabrous and/or hairy skin of one or two digits or the central pad. Two such receptive fields are plotted in Fig. 2. However, we were unable to make quantitative comparison of receptive field sizes between 'control' and 'nerve-injury' animals as the receptive field mapping was not undertaken in earlier studies, or in the present one with a standardized, quantified stimulus (e.g. a specific von Frey hair of fixed force value). Nevertheless, within the limits of our receptive field mapping we observed no qualitative abnormalities in terms of overall sizes or the extent of discontinuities in the fields. In many ways this is to be expected as the regeneration following crush injury leads to orderly re-innervation according to the original pattern in contrast to the more haphazard pattern of re-innervation following nerve transection (Horch, 1979).

Neurones were classified initially into two broad categories (Bystrzycka, Nail & Rowe, 1977; Douglas *et al.* 1978), the slowly adapting (SA) neurones (4 out of 22; ~20% of the sample) that responded throughout a step indentation of the skin lasting 1–1·5 s, and the purely dynamically sensitive neurones (18 out of 22; ~80% of the sample) that responded only to the 'on' and 'off ' phases of cutaneous step indentations (e.g. upper traces in Fig. 4). The latter group could be divided into two principal classes, one most sensitive to high-frequency (200–600 Hz) vibration whose input appeared to come selectively from fibres associated with Pacinian corpuscle (PC) receptors, and another, non-Pacinian group that included neurones sensitive to hair follicle afferent input and neurones that were most sensitive to low frequencies (5–50 Hz) of vibration, whose input appeared to come from the rapidly adapting (RA) class of

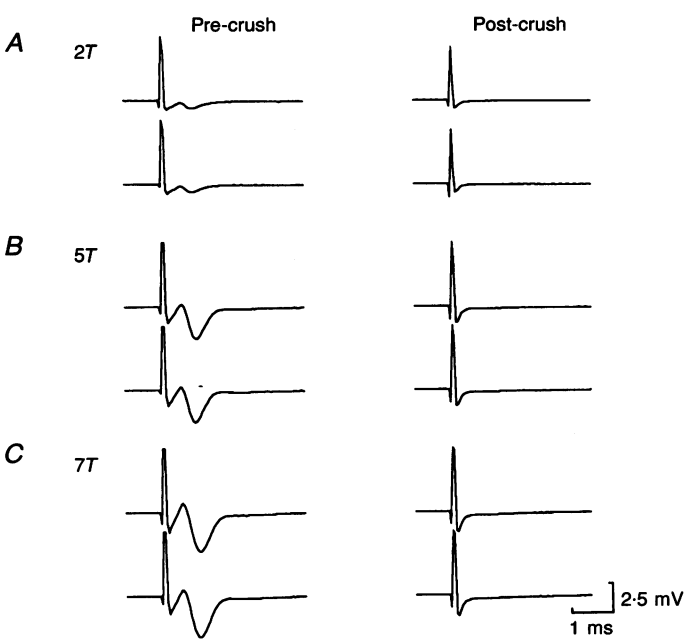

Figure 1. Abolition of compound action potential following median nerve crush

Electrical stimulation from threshold (*T*) to 7*T* (0·2–1·4 V; 0·05 ms duration) to the distal segment of the median nerve evoked compound action potentials (latency to onset, ~0·35 ms) which were recorded proximally near the elbow at 2*T* (*A*), 5*T* (*B*) and 7*T* (*C*) (Pre-crush). The compound action potentials were abolished (Post-crush) following median nerve crush in the mid-forearm.

tactile afferent fibres (Jänig, Schmidt & Zimmermann, 1968; Iggo & Ogawa, 1977; Bystrzycka *et al.* 1977; Douglas *et al.* 1978; Ferrington & Rowe, 1980, 1982). Nine neurones were identified in the latter class, three of which were analysed quantitatively; nine were identified in the PC-related class, of which six were subject to detailed analysis; and four were in the SA class, of which two were analysed quantitatively for the construction of full stimulus–response data. The limited overall sample of neurones, and the small sample subjected to detailed quantitative analysis (because of limitations of recording stability), did not permit reliable conclusions about the actual proportions of neurones in these three classes for the cuneate nucleus in receipt of input from a regenerated median nerve. However, as no equivalent data exist specifically for cuneate neurones with median nerve input in the 'normal' nucleus, it would not be possible to say whether the breakdown into classes was affected by the prior injury and regeneration of the median nerve. The small sample of neurones studied also precludes any statistical comparison of control and nerve-injury related neurones. The purpose of the study therefore was to determine qualitatively whether there was any clear segregation in responsiveness and functional capacities of neurones from the control and nerve-injury animals.

Response features of slowly adapting cuneate neurones

Cuneate neurones of the SA class whose input came via the regenerated median nerve after the neonatal crush injury responded with a brief high-frequency burst of impulses to the onset of the step indentation of the skin and a maintained response throughout the steady component of the indentation (Fig. 2, inset). The response was graded as a function of indentation amplitude over the 1·5 mm range used, as shown by the impulse traces and the stimulus–response relations of Fig. 2 for two neurones whose receptive fields were on the glabrous skin of toes 1 and 3, respectively. Relations *a* and *b* plot the mean ± S.D. response of ten successive responses obtained at each of the indicated step indentation amplitudes. At each amplitude the variability in the response level is low, as reflected in the small values for the standard deviation, in particular for relation *a* in which some values fell within the bounds of the symbols used for plotting the graph. The slope over the major part of the stimulus–response relation (~35–40 impulses s^{-1} mm^{-1}), together with the low response variability at a given indentation amplitude, indicates that these cuneate neurones are able to provide a reliable signal of skin indentation over the 1·5 mm range tested.

Functional capacities of dynamically sensitive cuneate neurones

Most observations on dynamically sensitive cuneate neurones were made on the Pacinian-related neurones as their responsiveness to high-frequency vibrotactile stimuli provides a more stringent index of transmission efficacy across the cuneate relay and therefore a more sensitive index of any permanent impairment occasioned by the prior nerve injury. Nevertheless, RA-related neurones were encountered subsequent to neonatal and adult nerve crush where the

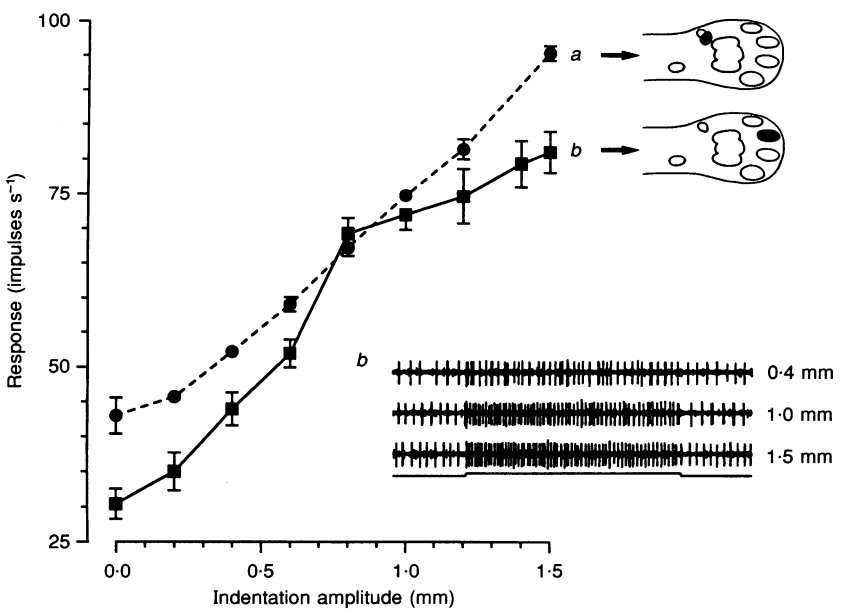

Figure 2. Stimulus–response relations for two (*a* and *b*) slowly adapting 'tactile' neurones of the cuneate nucleus recorded >12 months after neonatal nerve crush

Both neurones exhibited a graded response as a function of indentation intensity over a range of 1·5 mm. Each point represents the mean ± S.D. for 10 responses (impulses s^{-1}) to the first 1 s of indentation (total duration of indentation, 1·5 s). The stimulus site for each neurone was the centre of each receptive field, indicated as the filled area in each paw. Impulse records in the inset show 3 traces from stimulus–response relation *B* obtained in response to step indentations of 0·4, 1·0 and 1·5 mm.

lowest thresholds to vibration were found around 20 Hz (for example, Fig. 3) and the stimulus–response relations rose steeply over the amplitude range, 2–50 μm. Although the neurone whose data are plotted in Fig. 3 was also quite responsive at 5 and 50 Hz, the thresholds at these frequencies were clearly higher than at 20 Hz. In contrast, there was no response at amplitudes up to 25 μm at either 100 or 300 Hz and, indeed, little evidence of a response at 300 Hz over the whole range of amplitudes tested up to 120 μm.

The identification of the other class of cuneate neurones whose footpad input came via the regenerated median nerve as Pacinian related was based on the following: first, the absence of a static, or slowly adapting response to step indentations of the skin; second, their relative insensitivity to low-frequency ($\leqslant$50 Hz) vibrotactile stimuli; and third, their exquisite sensitivity to vibration over a broad range of high frequencies, from ~100 Hz to >600 Hz (Fig. 4). The impulse records in Fig. 4, obtained from a cuneate PC neurone subsequent to neonatal nerve crush and regeneration, show that responses occurred to the 'on' and 'off' phases of the 1·5 s step indentation and within the segment between the arrows when a 1 s train of 400 Hz vibration was delivered at 1, 5 and 75 μm amplitudes in the three examples shown. The plots of mean ± S.D. response ($n = 10$) for this neurone as a function of vibration amplitude at six vibration frequencies, in the lower part of Fig. 4, show that the relations rose steeply at the 'best' vibration frequencies (>200 Hz) where threshold responses were obtained at amplitudes of <1–2 μm. At these frequencies, a plateau in response level appeared to be reached at vibration amplitudes of 25–50 μm at 400 Hz, but whether this was so at 600 Hz could not be determined because of power limitations in the mechanical stimulator. The neurone was unresponsive at low frequencies (30 and 50 Hz) at amplitudes up to 100 μm. Vibrotactile sensitivity in all six PC neurones examined was similar to that shown in Fig. 4 in thresholds, bandwidths, 'best' frequencies and in the form of the stimulus–response relations.

Coding of information about the frequency of vibrotactile stimuli

The extent to which cuneate responses mediated via the regenerated median nerve were phase locked to the vibration waveform, and therefore able to encode in their impulse patterns some signal of vibration frequency (Douglas *et al.* 1978), was examined by constructing cycle histograms (see Methods) from which quantitative measures of phase locking (*percentage entrainment*) were derived. The cycle histograms of Fig. 5*A* were obtained for a PC neurone from an animal that had undergone neonatal nerve crush. The neurone was most responsive at vibration frequencies of $\geqslant$200 Hz and showed a decline in the tightness of phase locking over the range 100–400 Hz. However, even at 400 Hz, the cycle histogram shape and percentage entrainment value (65%) indicates some phase locking, and is therefore not apparently different from 'control' PC neurones (Douglas *et al.* 1978).

Percentage entrainment values were obtained for each of the six PC neurones studied and have been plotted as a function

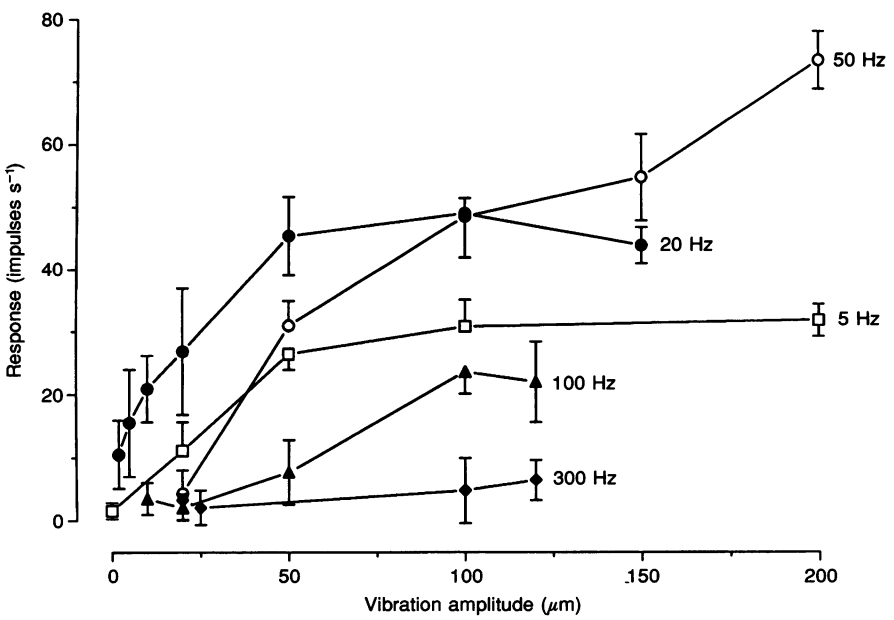

Figure 3. Stimulus–response relations for a cuneate neurone sensitive to low-frequency cutaneous vibration

Stimulus was applied to digit 3 (receptive field: glabrous skin of digits 3 and 4) and responses recorded in the adult-crush animal. The mean (± S.D.) for 10 responses is plotted against peak-to-peak vibratory amplitude at 5 different frequencies.

of vibration frequency in Fig. 5*B*. Considerable variability exists from neurone to neurone, in particular at low vibration frequencies; however, this was also the case for control PC neurones (Fig. 11*A* in Douglas *et al.* 1978). When the mean percentage entrainment values were plotted in Fig. 5*C* (continuous line), the tightest phase locking was found in the frequency range 50–200 Hz, as was also the case for the control PC neurones ($n = 5$), whose mean percentage entrainment values have been included in this graph as the dashed line (data from Fig. 11*B* in Douglas *et al.* 1978). For both nerve-injury and control data, the percentage entrainment declined from values of 80–100% at 50–200 Hz to lower values as the vibration frequency rose from 200 to 700 Hz. The relations in Fig. 5*C* are similar, and show clearly that the measures of phase locking are no poorer in PC neurones, whose input came via the regenerated median nerve, than in control cuneate neurones.

DISCUSSION

Cuneate neurone classes responsive to regenerated median nerve input

The division of cuneate neurones responsive to footpad inputs into three major classes whose properties were consistent with inputs that came respectively from the SA, RA or PC class of tactile afferent fibres is in accord with observations on cuneate neurones in control animals (Bystrzycka *et al.* 1977; Douglas *et al.* 1978; Dykes, Rasmusson, Stretavan & Rehman, 1982; Ferrington & Rowe, 1982; Connor, Ferrington & Rowe, 1984). This finding is perhaps not unexpected as the prior crush injury should enable peripheral nerve fibres to regenerate along their original Schwann tubes in the distal segment of the nerve and re-establish contact with the receptors they had originally innervated (Horch, 1979). However, the major aim of the study was to evaluate whether cuneate neurones whose input came via the regenerated median nerve acquired the normal repertoire of functional properties and coding capacities. The purpose of the present study therefore was to determine whether disruption to the normal inflow of tactile sensory information, in particular in the early postnatal period, might lead to permanent impairment in the coding and processing of tactile sensory information within the cuneate nucleus, the first central relay in the principal pathway for tactile sensation associated with the forelimb. As the crush injury was applied in the mid-forearm, the regeneration of the median nerve to supply the glabrous footpad region would have taken place over a period that could be as long as several months (Gutmann *et al.* 1942; Sunderland, 1978). During this time the developing cuneate nucleus would have been deprived of normal tactile inputs via the median nerve.

For the electrophysiological analysis it was essential to ensure that the tactile inputs were confined to the

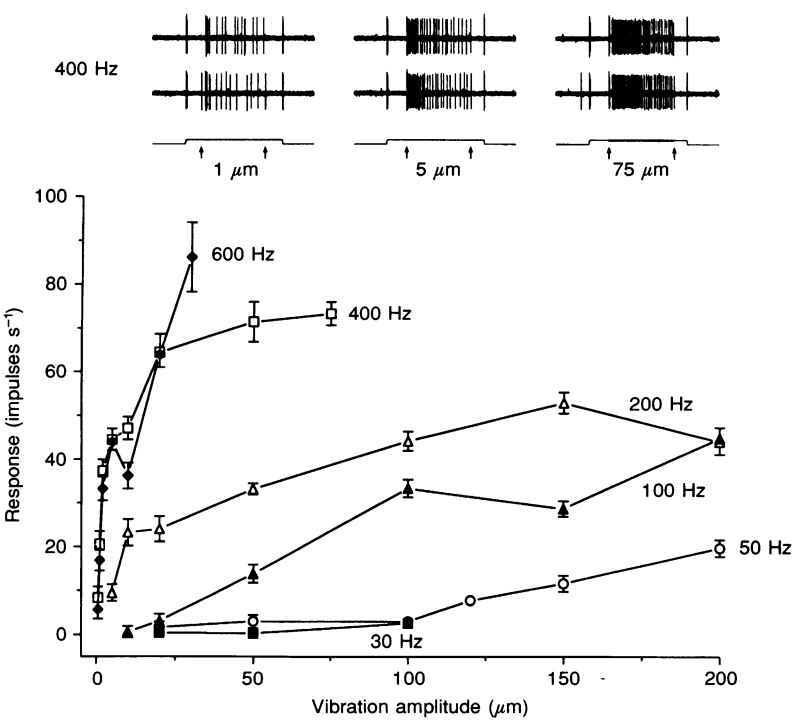

Figure 4. Stimulus–response relations at 6 different vibration frequencies (30–600 Hz) for a PC-related cuneate neurone recorded subsequent to neonatal nerve crush

Each symbol in the graph represents the mean ± S.D. of 10 responses to vibration amplitudes ranging from 0·5 to 200 μm. Sample impulse traces are plotted at the top of the figure in response to 2 trials each of 400 Hz vibration at 1, 5 and 75 μm amplitude; vibration applied to wrist.

regenerated median nerve and did not traverse other peripheral nerves. This was especially important when vibratory stimuli were used as these can spread readily through the skin. For this reason, other forearm nerves were acutely sectioned in each experiment. We therefore cannot be certain whether some of the sampled neurones may have ordinarily received convergent inputs from the regenerated median nerve and from one or more of the acutely sectioned nerves. However, as most neurones had receptive fields well within the zone of the median nerve distribution this appears unlikely (e.g. see Fig. 2).

In order to evaluate whether the cuneate neurones that received input via the regenerated median nerve displayed normal response properties and coding capacities, we compared their attributes with those of cuneate neurones characterized in an earlier study from our laboratory (Douglas *et al.* 1978). We believe the comparisons are valid as the analysis of stimulus–response data was conducted in both studies with the same type of feedback-controlled mechanical stimulators that provide precisely quantified and reproducible stimuli whose parameters can be specified fully. The servo-controlled operation of the mechanical stimulators used in both studies ensured that stimulus amplitudes, whether step indentation or vibration, were accurate, reproducible and independent of the loading imposed by the skin and underlying tissues of the footpads and distal forelimb. Furthermore, in both studies where vibrotactile stimuli were used, the frequency parameter was set with complete accuracy by means of a precision function generator and, for the analyses of phase locking, the initial movement in the vibratory displacement always occurred from the null position in a fixed direction, a requirement that was crucial for the quantitative evaluation of the tightness of phase locking in the responses to vibrotactile stimuli. Where direct comparisons between control and nerve-injury data are not possible, as indicated in the Results, is in the circumstance where non-reproducible stimuli have been used, for example, in the mapping of receptive field sizes.

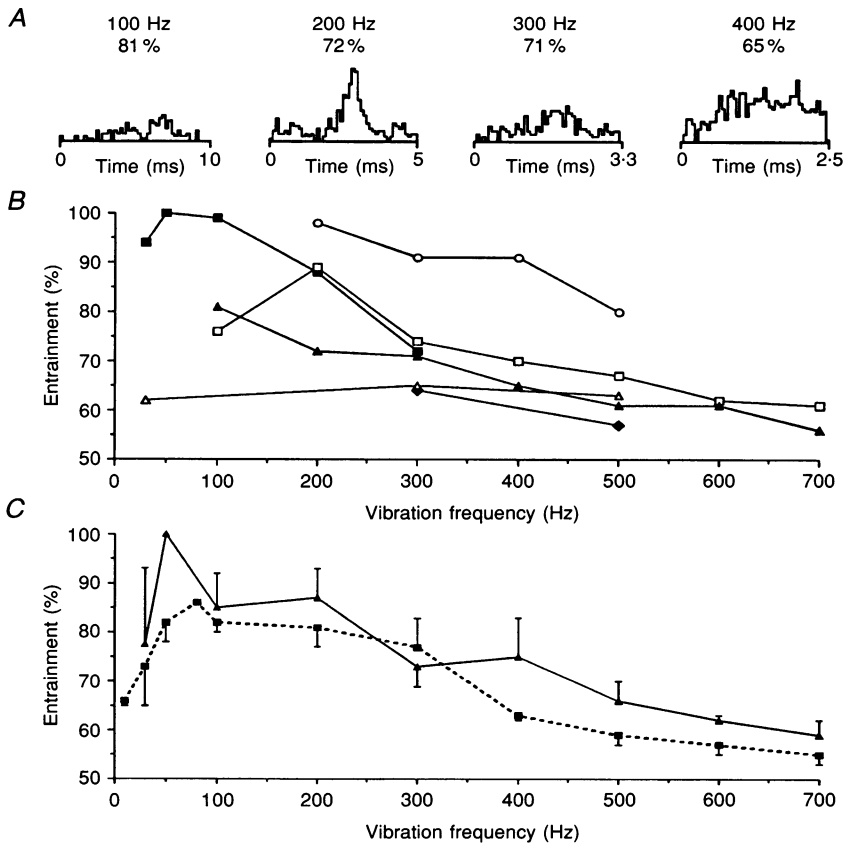

Figure 5. Phase locking of cuneate responses to vibrotactile stimuli

The percentage entrainment (see Methods) was obtained from cycle histogram distributions (*A*) as a quantitative measure of phase locking and plotted in *B* for 6 PC-related neurones from the present crush experiments as a function of vibration frequency; vibration amplitude at each frequency was between 20 and 50 μm. The sample cycle histograms are for the neurone, from a neonatal crush animal, whose data are represented by ▲ in *B* (curves in *B* for neurones from neonatal crush animals were plotted as △, ◆ and ▲, and those from adult crush animals were ■, □ and ○. *C*, continuous line shows mean ± S.E.M. entrainment values for the 6 PC-related neurones in *B*. The dashed line shows mean ± S.E.M. for 5 PC-related neurones reported in our control experiments (Douglas *et al.* 1978). At some low frequencies, impulse counts in the cycle histograms were too low for reliable quantification of entrainment.

Functional capacities of slowly adapting cuneate neurones

The stimulus–response relations for the SA neurones were roughly linear or sigmoidal (Fig. 2) and therefore corresponded in form with those constructed previously under equivalent stimulus conditions for the control cuneate neurones (see Fig. 1 in Douglas *et al.* 1978). Furthermore, all other response features derived from the stimulus–response relations were consistent with those of SA cuneate neurones described in the earlier study. First, the variability in response level at a fixed indentation amplitude (measured by the S.D. for the mean response at each intensity) was as low or lower than values plotted in control relations (Fig. 1 in Douglas *et al.* 1978). Second, the slopes of stimulus–response relations, which over the major part of the relations in Fig. 2 were ~35–40 impulses s^{-1} mm^{-1}, were in the range of those for control relations (Fig. 1 in Douglas *et al.* 1978), as were maximum response levels (75–100 impulses s^{-1}) attained over the 1·5 mm range of static indentations. Thresholds, based on the increment in response over background activity levels, were <200 μm for the relations in Fig. 2, which is also consistent with values of ~100–300 μm in the earlier, control stimulus–response relations. Furthermore, the relations remained graded over an indentation range of at least 1·5 mm, as did control relations (Douglas *et al.* 1978). The observed functional properties of these two cuneate SA neurones, whose stimulus–response relations are plotted in Fig. 2 and whose input came from a regenerated median nerve that had undergone neonatal nerve crush, indicated that they were able to contribute discriminative information about indentation intensity on the glabrous skin and that their capacities were indistinguishable from those of normal cuneate SA neurones.

Functional capacities of dynamically sensitive cuneate neurones

Dynamically sensitive cuneate neurones that were selectively sensitive to low frequencies of cutaneous vibration on the footpads presumably derived their input from the RA class of tactile afferent fibres thought to be associated with the Krause corpuscles in the footpads (Jänig *et al.* 1968; Iggo & Ogawa, 1977; Ferrington & Rowe, 1980; Ferrington, Hora & Rowe, 1984). Their functional properties appeared consistent with their counterparts studied previously in the 'control' cuneate nucleus (Bystrzycka *et al.* 1977; Douglas *et al.* 1978) in that they were purely dynamically sensitive, and in response to vibrotactile stimulation of the footpads had 'best' frequencies in the range 5–50 Hz, and were unresponsive or had high thresholds to vibration at high frequencies ($\geqslant$100 Hz).

The second class of purely dynamically sensitive neurones activated from the footpads, whose input appeared to come from PC receptors, also resembled their counterparts in the normal cuneate nucleus. These 'PC neurones' had a broad bandwidth of vibration sensitivity (from ~50 Hz to >600 Hz) with a maximum sensitivity in the range 200–600 Hz where thresholds were $\leqslant 1$ μm (e.g. Fig. 4). As the PC class of cuneate neurones covers the major part of the vibrotactile frequency range, the capacity of these neurones for encoding information about vibration frequency was quantified in terms of measures of phase locking and compared with control data. As these measures reflect the precision of impulse patterning in the cuneate neurones they provide a sensitive index of the capacity of the regenerated median nerve to establish normal transmission efficacy across this sensory relay nucleus. In measures of phase locking derived from the cycle histogram distributions constructed from responses to vibration, the cuneate PC neurones appeared to match the capacities of cuneate PC neurones from control animals. For example, phase locking in the responses to vibration extended into the high-frequency range of 300–500 Hz (Fig. 5) and appeared at least as tight as the control data (Fig. 5, and Douglas *et al.* 1978).

In agreement with the earlier observations on control cuneate neurones of both PC and RA classes, there was no evidence of a plateau in the stimulus–response relations at response levels that correspond to one impulse per vibration cycle, that is, a 1:1 level (or at subharmonics of those levels, for example 1:2 or 1:3), as is found for primary fibres of the RA and PC classes (Jänig *et al.* 1968; Talbot, Darian-Smith, Kornhuber & Mountcastle, 1968; Ferrington & Rowe, 1980; Ferrington, Hora & Rowe, 1984). These attributes of the stimulus–response relations, together with other characteristics of the units studied, for example the biphasic spike configurations, the common presence of background impulse activity, and the levels of dispersion in the cycle histogram distributions, are all entirely consistent with the recorded units being central cuneate neurones and not afferent fibres that were being recorded within the nucleus (Douglas *et al.* 1978; Ferrington & Rowe, 1982; Ferrington *et al.* 1987*a*, *b*, *c*). However, one limitation in our present study is that we cannot be certain whether the full complement of cuneate neurones normally involved in processing median nerve inputs is present subsequent to nerve regeneration. As the regeneration following nerve crush in the neonate may be incomplete (Bueker & Myers, 1951; Sunderland, 1978) there may be fewer input fibres than normal – and perhaps fewer cuneate neurones than normal – involved in the processing of median nerve tactile information. Nevertheless, the sample of cuneate neurones associated with the regenerated median nerve inputs could be classified functionally into the three major tactile classes recognizable in the 'normal' cuneate nucleus. Furthermore, the disruptions to the normal pattern of input occasioned by this form of nerve injury do not appear to cause permanent, marked impairment in the tactile processing capacities of individual cuneate neurones. However, because of the limited sample size, we do not wish to rule out the possibility of some effects of the nerve injury on cuneate response features. Nevertheless, for any such effects to emerge would clearly necessitate a very detailed quantitative analysis on a much larger sample size.

Our study did not examine the issue of whether reorganization of receptive fields and of the cuneate somatotopic map occurred as a consequence of the median nerve crush. This form of reorganization, in which there is an expansion in the central representation of body regions around the zone of deafferentation into areas of the central map that previously represented the deafferented body part, has been reported in response to nerve injury or local anaesthetic-induced deafferentation at each level of the dorsal column–lemniscal pathway, including the dorsal column nuclei, thalamus and cortex (e.g. Dostrovsky *et al.* 1976; Merzenich *et al.* 1983; Calford & Tweedale, 1988, 1990; Wall *et al.* 1992; Nicolelis *et al.* 1993; Pettit & Schwark, 1993). It has also been reported in several studies on the spinal dorsal horn (e.g. Basbaum & Wall, 1979; Devor & Wall, 1981; Lisney, 1983; Koerber & Brown, 1995) but not in others (Brown, Brown, Fyffe & Pubols, 1983; Brown, Fyffe, Noble & Rowe, 1984; Wilson, 1987). In the present experiments, even if such somatotopic reorganization occurred in the short term within the cuneate nucleus in response to the neonatal median nerve injury, the reorganization may have been reversed during the 12–24 months that elapsed before our electrophysiological analysis. In this period, median nerve regeneration would have taken place, allowing re-establishment of median nerve connectivity with the appropriate region of the cuneate nucleus. Reversal of this type of somatotopic reorganization has been observed previously at both spinal and cortical levels. For example, Lisney (1983) found that following sciatic and saphenous nerve transections, the medial region of dorsal horn was taken over within 1 month by inputs from the proximal regions of the leg. However, these changes were not permanent. After 9 months, when at least partial regeneration of the transected nerves to the distal hindlimb would have taken place, the medial region of dorsal horn was again responsive to inputs from the distal limb, though the pattern of connectivity was abnormal, presumably reflecting the somewhat disordered re-innervation of the skin that occurs subsequent to nerve transection (Horch, 1979; Lisney, 1983). Transient reorganization in response to partial deafferentation has also been apparent at the cortical level where Calford & Tweedale (1990) observed that the immediate reorganization of the somatotopic map following amputation or nerve block showed a regression over a 1- to 2-week period. In the present study where the nerve injury was produced by means of controlled nerve crush, it might be expected that more complete and accurate peripheral nerve regeneration might occur than following nerve transection, and that any short-term reorganization in the somatotopic map might have been reversed more completely than following nerve transection, in particular over the 12–24 month interval between the nerve crush injury and the electrophysiological recording study. Nevertheless, as the aims of the present analysis were to evaluate the coding capacity of cuneate neurones, we made no attempt to test for any alterations in the pattern of body representation within the nucleus.

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