PROCESSING OF VIBROTACTILE INPUTS FROM HAIRY SKIN BY NEURONS OF THE DORSAL COLUMN NUCLEI IN THE CAT

by

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ABSTRACT

The capacity of single neurons of the dorsal column nuclei (DCN) for coding vibrotactile information from the hairy skin has been investigated in anesthetized cats in order to permit quantitative comparison first, with the capacities of DCN neurons responding to glabrous skin vibrotactile inputs, and second, with those of spinocervical tract neurons responding to vibrotactile inputs from hairy skin.

Dynamically-sensitive tactile neurons of the DCN whose input came from hairy skin could be divided into two classes, one associated with hair follicle afferent (HFA) input, the other with Pacinian corpuscle (PC) input. The HFA-related class was most sensitive to low-frequency (<50 Hz) vibration and had a graded response output as a function of vibrotactile intensity changes. PC-related neurons had a broader vibrotactile sensitivity, extending to ≥ 300 Hz and appeared to derive their input from the margins of hairy skin, near the footpads, or from deeper PC sources such as the interosseous membranes or joints.

HFA-related neurons had phase-locked responses to vibration frequencies up to ~75 Hz, while PC neurons retained this capacity up to frequencies of ~300 Hz with tightest phase-locking between 50 and 200 Hz. Quantitative measures of phase-locking revealed that the HFA-related neurons provide the better signal of vibrotactile frequency up to ~50 Hz, with a switch-over to the PC-related neurons above that value. In conclusion, the functional capacities of these two classes of cuneate neuron appear to account for behavioral vibrotactile frequency discriminative performance in hairy skin, in contrast to the limited capacities of vibrotactile-sensitive neurons within the spinocervical tract system.
INTRODUCTION

Discriminative tactile information from the glabrous skin of the limb extremities appears to be conveyed to higher centres of the brain predominantly by the dorsal column-lemniscal system (Brown and Gordon 1977; Douglas et al. 1978; Vierck 1998). There appears to be little representation of these glabrous skin tactile inputs in the spinocervical system (Brown 1981, 1982). Furthermore, some, at least, of the glabrous skin tactile receptor and sensory fiber classes, for example, those associated either with low-threshold slowly adapting, or with Pacinian corpuscle receptors, also have little representation within the spinothalamic system, another of the major ascending somatosensory pathways (Ferrington et al. 1987d, 1988; Surmeier et al. 1988; Willis et al. 1975). There appears to be little doubt therefore, that the major ascending sensory pathway taken by tactile inputs from the glabrous skin is the dorsal column-lemniscal pathway. However, tactile information derived from the hairy skin is well represented within the spinocervical pathway (Brown and Franz 1969; Brown 1981) and in the responses of neurons within its target structure, the lateral cervical nucleus (Craig and Tapper 1978; Downie et al. 1988), in addition to its substantial representation within the dorsal column-lemniscal pathway (Brown et al. 1974; Dykes et al. 1982; Golovchinsky 1980; Gordon and Jukes 1964; Perl et al. 1962).

Although tactile information from both the glabrous and hairy skin regions is conveyed over the dorsal column pathway to higher centres, there are known to be marked differences between these two skin regions in human vibrotactile detection thresholds, with those on the hairy skin of the forearm being approximately an order of magnitude higher than those for the glabrous finger tips (Merzenich and Harrington 1969; Talbot et al. 1968). These regional differences in detection threshold are, in part at least, due to differences in the sensory receptors and afferent fiber classes supplying these different skin areas. At low vibrotactile frequencies (≤100 Hz), the input from hairy skin comes from afferents associated with hair follicles, the hair follicle afferent (HFA) fibers (Burgess et al. 1968; Merzenich and Harrington 1969), whereas
that from glabrous skin arises from rapidly adapting intradermal receptors, known as Meissner corpuscles in primates (Brown and Iggo 1967; Talbot et al. 1968). At higher vibrotactile frequencies (≥100 Hz) the input from both areas of skin appears to be derived from the Pacinian corpuscle (PC)-related class of tactile afferent fibers (Merzenich and Harrington 1969; Talbot et al. 1968). However, although the PC receptors are abundant beneath the glabrous skin of the finger tips and palms in primates, and beneath the footpad skin in the cat, they are either absent or poorly represented in, or immediately beneath, the hairy skin itself (Brown and Iggo 1967; Merzenich and Harrington 1969; Tuckett et al. 1978). As a consequence, whenever PC inputs are recruited by vibrotactile disturbances in hairy skin this may take place from quite remote locations, in particular, if the stimulus occurs in skin overlying substantial muscle tissue. In this circumstance, the soft tissue will provide mechanical insulation to the spread of the vibratory disturbance to remote sites such as the interosseous membrane and to joints and tendons where PC receptors are present (Quilliam 1966). This dependence, in the hairy skin, upon spread of the vibrotactile disturbance to recruit these more remote PC afferents may explain the high vibrotactile detection thresholds for this skin region, in particular, at ≥100 Hz (Merzenich and Harrington 1969). However, it is unclear whether the recruitment of distant receptors may contribute to greater temporal dispersion in the afferent input activity and, in turn, generate less precise temporal patterning in the responses of the related central target neurons than is the case for the central neurons activated by vibrotactile stimulation of the glabrous skin.

In the present study in anesthetized cats, we have investigated the capacity of single neurons of the dorsal column nuclei for coding vibrotactile information that is derived from the hairy skin of the limbs. The response characteristics and coding capacities of these neurons have been quantified in order to permit comparison first, with their glabrous skin counterparts in the dorsal column nuclei (Connor et al. 1984; Douglas et al. 1978) and second, with the capacity of identified spinocervical tract neurons to signal such vibrotactile information (see associated paper by Sahai et al. 2005). Furthermore, quantification of these DCN neuronal coding
capacities permitted the data to be related more closely to psychophysical data on vibrotactile frequency recognition and discrimination in the hairy skin, the subject of the other associated paper (Mahns et al. 2005).

**METHODS**

*Animal preparation*

Data were obtained in ten experiments on adult cats (2–3 kgs in weight) that were anesthetized initially with sodium pentobarbitone (40mg/kg, ip). An intravenous infusion of sodium pentobarbitone (1.4mg/kg/hr) in saline was used to maintain anesthesia, except in one experiment in which the cat was anesthetized with α-chloralose (70mg/kg, ip).

A longitudinal incision was made at the base of the occipital bone to expose the brain stem and hence the cuneate and gracile divisions of the dorsal column nuclei. In each case the forelimb or hindlimb was shaved and placed in a plexiglass trough with the digits tied to the edges and the paw secured with plasticine. This procedure stabilized the limb and permitted accurate positioning of the mechanical stimulator.

During recording sessions the brainstem was protected with paraffin oil or, when it was necessary to minimize respiratory movements, an agar gel (4% w/v) was used. Blood pressure and core body temperature (38±0.5°C) were monitored throughout the experiments. At the termination of experiments an overdose of pentobarbitone was given.

*Recording and stimulation procedures*

The cranium was fixed in a stereotaxic frame and recording electrode penetrations made under micro-manipulator control in the region 1-4mm caudal to the obex which corresponds to the cluster zone of the dorsal column nuclei (Berkley 1975). Extracellular impulse activity was recorded by means of tungsten microelectrodes (impedance 2.5-4.5MΩ) from individual units whose spike configuration and functional properties were consistent with their identity being cuneate neurons rather than primary afferent axons (Coleman et al. 2003; Vickery et al. 1994; Winter 1965). Cutaneous receptive fields for individual neurons were mapped using *von Frey*
hairs, and neuronal responsiveness then examined with the use of precise and reproducible mechanical stimuli that were derived from a mechanical stimulator and were delivered normal to the surface of the shaved skin at the point of maximum sensitivity within the excitatory receptive field of the neuron. The mechanical stimulator probe tips were circular (2-6 mm in diameter) and placed just in contact with the skin surface in the rest position. Stimuli consisted of 1.5s long step indentations for the initial classification of neurons as slowly adapting or as purely dynamically-sensitive neurons. For the study of dynamically-sensitive neurons, a 1s train of sinusoidal vibration, at frequencies of 5-300 Hz, was superimposed on a 400 µm amplitude step indentation and commenced 300 ms after the step onset. Stimulus repetition rate during periods of analysis was one per eight seconds to permit time for recovery of skin position. Response data were collected from five stimulus repetitions at each frequency and amplitude combination.

**Quantitative analysis of phaselocking and impulse patterning in responses to vibration**

Impulse activity was displayed on an oscilloscope and fed to a differential amplitude discriminator from which output pulses could be relayed to a counter unit and laboratory computers that were used to construct impulse records, cycle histograms (CHs), peri-stimulus time histograms (PSTHs), and time interval histograms. The CHs use a pulse associated with the onset of each successive vibration cycle as a stimulus marker and display the probability of impulse occurrences throughout the vibration cycle period. Depending on the vibration frequency, between 25 and 1500 cycles of vibration were used to construct the CHs. The PSTHs use a pulse associated with the start of each train of vibration as the stimulus marker and show the probability of impulse occurrence throughout the vibration stimulus. The time interval histograms displayed the distribution of interspike intervals during responses to vibration.

Two quantitative measures of phaselocking in the vibration-induced responses were derived from the CH data. First, the Resultant (R) was obtained as a measure of *vector strength* in the cyclic distribution (Mardia 1972) and was calculated from each cycle histogram distribution according to the formula
\[ R = \sqrt{\left( \frac{\Sigma cos(x_i)}{n} \right)^2 + \left( \frac{\Sigma sin(x_i)}{n} \right)^2} \]

where \( n \) is the total number of impulse occurrences, and \( x_i (1 \rightarrow n) \) is the phase angle (in radians) of each spike occurrence time relative to the start of the vibration cycle (Zar 1984). It defines the degree of phase coherence or synchronization in the CH distribution and ranges in value from a maximum of 1, for complete phase synchrony, to zero when there is no net phase preference. This measure has been used in earlier studies of phaselocking in somatosensory neurons (e.g. Coleman et al. 2003; Greenstein et al. 1987; Rowe 2002; Zachariah et al. 2001), and in auditory neurons (e.g. Bledsoe et al. 1982; Lavine 1971) where values below 0.3 have been taken to indicate little or no phaselocking, values from 0.3 to 0.7 moderate phaselocking, and values of 0.7 to 1.0 as a high degree of phaselocking. The second measure, percentage entrainment, represents the highest percentage of impulse occurrences that fall within any continuous half cycle of the vibration cycle period and ranges in value from a minimum of 50%, a value that would be obtained with a rectangular distribution in the cycle histogram in the absence of phaselocking, to a maximum of 100% (Coleman et al. 2003; Douglas et al. 1978; Ferrington and Rowe 1980a,b; Rowe 2002; Talbot et al. 1968).

**RESULTS**

**Identification of dorsal column nuclei (DCN) neurons activated by tactile inputs from hairy skin**

Thirty nine neurons, located predominantly or exclusively within the cluster zone of the DCN were isolated electrophysiologically and examined quantitatively for their responsiveness to vibrotactile inputs from the hairy skin. Except for two neurons studied in the gracile nucleus all were in the cuneate division and were activated from the forelimb. Their rostrocaudal locations were between 1 and 4 mm caudal to the obex and mediolateral positions (for the cuneate neurons) were 850 - 2,000 µm from the midline (Fig.1). Neurons activated by tactile inputs from the hairy skin were initially identified following activation by brushing of the skin
either manually, or by means of a camel-hair brush or a fine, hand-held mechanical probe. The neurons were then classified functionally into first, a slowly adapting class (SA neurons) that had maintained responses to static skin displacement and made up 3 of the 39 neurons, and second, a broad group of purely dynamically-sensitive neurons that responded only at the onset and offset of skin indentation applied with the servo-controlled mechanical stimulator at the identified best point of the receptive field. The 36 purely dynamically-sensitive tactile neurons could be subdivided into two classes, one associated with hair follicle afferent (HFA) input, the other with Pacinian corpuscle (PC) input, based upon receptive field characteristics and differential vibrotactile responsiveness (see below).

The receptive fields (RFs) for 37 of the 39 neurons studied were mapped in Fig. 2A-C with calibrated von Frey hairs of different forces in the range 10-120 mg wt. (as indicated by the value adjacent to each RF in the figure), to define the extent of the field and to identify the most effective stimulus focus within the field. Receptive fields in Fig. 2A were on the hairy skin of the dorsal surface of the cat's distal forelimb while those in the two representations of the limb in Fig. 2B were on the ventral surface. The two fields in C were on the lateral hindlimb and were associated with gracile nucleus neurons. Neurons with receptive fields marked by shading appeared to derive their input selectively from HFA fibers, based upon both manual probing with the von Frey hairs and subsequent controlled testing for vibrotactile sensitivity. Those with stippled representation of the fields appeared to be activated by PC inputs (see below), while those with cross-hatched fields were slowly-adapting tactile neurons.

**DCN neurons activated selectively by dynamic components of tactile stimuli**

The majority (21/36 neurons) of the purely dynamically-sensitive DCN neurons activated from the hairy skin responded to brushing of the shaved skin surface and were activated by direct skin displacement or movement of the cut ends of the hairs. As neurons of this class had circumscribed receptive fields that remained stable on the skin surface, even when it was possible to displace the skin across the underlying tissues, and were most sensitive to vibration at
low frequencies (usually <50 Hz), they appeared to derive their input selectively or predominantly from HFA fibers. The remaining dynamically-sensitive neurons (15/36) could often be activated with manual tapping stimuli from widespread regions of the limb or even the experimental table, and in circumstances in which the skin could be displaced it appeared that responsiveness was associated with subcutaneous sources. As these neurons displayed a broader vibrotactile sensitivity, extending up to or beyond 300 Hz, it appears that their peripheral inputs are derived from PC sources. Only with the use of delicate von Frey hairs were the focal regions of these RFs apparent for the PC-related neurons (Fig. 2). Many were close to the margins of the forelimb toe pads where Pacinian corpuscles are known to be concentrated (Kumamoto et al. 1993; Lynn 1969; Malinovsky 1966) while other fields, on more proximal limb locations, may represent sites from which stimuli may have spread to activate PC receptors in regions such as the interosseous membranes or the joints.

**Vibration-sensitive DCN neurons activated by HFA sources**

The vibration-sensitive neurons with lowest vibrotactile thresholds at frequencies of ≤ 50 Hz appeared to be activated selectively by HFA inputs and displayed a graded responsiveness as a function of changes in vibration intensity. The impulse traces of Fig. 3 show the range of responsiveness for one HFA-neuron and its gradation of output as a function of amplitude increases at vibration frequencies of 10-100 Hz. Responses occur sporadically on some cycles at low amplitude, become more regular, and, at low vibration frequencies (≤20 Hz), give way to pairs or bursts of spikes on individual cycles at the higher amplitudes, such that the firing rates usually exceed the vibration frequency in this low range of stimulus frequencies. However, at higher vibration frequencies (50 and 100 Hz) there are fewer instances of these paired or burst responses on individual cycles. Quantification of the response (in impulses s⁻¹) as a function of the vibration amplitude permitted construction of stimulus-response relations (Fig. 4A) which, for a different, but representative neuron of this HFA-related type, show that thresholds are lowest (5-20 µm) in the frequency range 5-50 Hz, with a higher value at 100 Hz, and little
evidence of sensitivity at 200 Hz. Furthermore, the graded relations apparent at 5-100 Hz in Fig. 4A for this particular neuron, and, at 20 Hz, for seven different HFA-related neurons in Fig. 4B, ensure that, at these low frequencies, individual neurons of this class can contribute a sensitive signal of the changing intensity of vibrotactile perturbations in the hairy skin.

**DCN neurons activated by high-frequency vibration of hairy skin**

The second class of purely dynamically-sensitive neurons activated from the hairy skin displayed a broader bandwidth of vibrotactile sensitivity than the HFA-related neurons, and displayed peak sensitivity at frequencies of ≥100 Hz (Fig. 5). These attributes together with their RF characteristics, described above, imply an input from Pacinian corpuscle receptors even though these are known to be absent or rare in association with the hairy skin itself (Brown and Iggo 1967; Tuckett et al. 1978). However, the high vibrotactile sensitivity of these receptors, whether in the vicinity of footpads or in deeper locations, such as interosseous membranes or in the regions of joints, enables these receptors to be activated by stimuli applied to the hairy skin itself (Merzenich and Harrington 1969). Figure 5 shows, for three putative PC-related neurons, the high sensitivity (threshold <2 µm) and responsiveness at high vibration frequencies (100-300 Hz) and, from the stimulus-response relations of Fig. 5B, the relative-insensitivity of the PC-related neurons at low vibration frequencies (≤ 50 Hz). The relations in Fig. 5B reveal a graded responsiveness as a function of amplitude over a very narrow amplitude range, <10-20 µm, at 200 and 300 Hz before reaching a plateau level of response but, at lower frequencies, had a broader dynamic range of responsiveness, as reflected in the graded nature of the stimulus-response relations at these frequencies. This behaviour is evident in the impulse traces of Fig. 5C for a third PC-related cuneate neuron that shows graded response levels as a function of amplitude increases at 50 and 100 Hz, but an abrupt increase to high levels of responsiveness at both 200 and 300 Hz.

Signalling of intensive changes beyond the point where plateau levels of responsiveness are reached in individual neurons may depend upon the recruitment of additional afferent fibers
and, in turn, additional cuneate neurons, allowing the information to be coded by gradations in the total impulse traffic in the population of responding neurons (Johnson 1974). There was no evidence of a 1:1 plateau in the stimulus-response relations of Figs. 4 and 5 at impulse rates corresponding to one impulse/cycle of vibration as is often observed in vibration-sensitive primary afferent fibers, whether of the RA or PC classes that supply glabrous skin, or the HFA afferents themselves in the hairy skin (Ferrington et al. 1984; Ferrington and Rowe 1980a; Johnson 1974; Merzenich and Harrington 1969; Talbot et al. 1968; Zachariah et al. 2001). In the afferent fibers, 1:1 plateaux are often reached at low vibration amplitudes and are maintained over a broad range of amplitudes before a second steep rise in response occurs to a second plateau corresponding to a 2:1 pattern of response (i.e., 2 impulses/cycle), giving rise to marked discontinuities in the stimulus-response relations of the individual afferent fibers.

Bandwidth of vibrotactile sensitivity in HFA- and PC-related neurons of the DCN

The bandwidth of frequencies over which the HFA-related DCN neurons can signal vibrotactile information may be represented in terms of the threshold profiles of Fig. 6A, where the individual values have been derived from stimulus-response relations of the type illustrated in Figs. 4 and 5. The threshold measures for HFA-neurons in Fig. 6A represent estimates of the minimum vibration amplitude at which a discernible response increment occurred within a given stimulus-response relation. The majority of HFA-related neurons displayed minimum thresholds at ≤ 50 Hz and a rise in threshold above ~50 Hz, that may, in fact, be underestimated in the graphs of Fig. 6A as some neurons show only a transient response at 100 and 200 Hz to the start of the vibration train where the onset component of the first cycle is not a pure sinusoid and therefore has a complex frequency composition. The declining sensitivity, expressed as the increase in thresholds in Fig. 6A and C, of HFA-related neurons at frequencies above ~50 Hz establishes that the operating range, or bandwidth, of vibrotactile sensitivity for HFA-related cuneate neurons is largely confined to frequencies below 50-100 Hz, in contrast to the broader bandwidth of sensitivity of the PC-neurons (Fig. 6B,C). The considerable variation in threshold
values from neuron-to-neuron, whether for the HFA- or PC-related class (Fig. 6A and B), presumably reflects not only the sensitivity differences among the sensory nerve endings but also the proximity of the endings to the stimulation site, in particular, in the case of PC-related units. The plots of mean vibration thresholds in Fig. 6C emphasize the differential bandwidths of sensitivity for the HFA- and PC-related classes and indicate the switchover that occurs from the HFA to the PC class in peak vibrotactile sensitivity around 20-50 Hz.

**Coding for the frequency of vibrotactile disturbances in hairy skin**

There is much evidence that the coding of vibrotactile frequency information derived from the glabrous skin of the primate hand or cat footpads depends upon phaselocking and temporal patterning of impulse activity within the relevant classes of afferent fibers and central neurons (Douglas et al. 1978; Ferrington et al. 1984, 1987a,b,c; Ferrington and Rowe 1980a,b; Mountcastle et al. 1969; Talbot et al. 1968). In order to investigate vibrotactile frequency coding for DCN neurons activated from the hairy skin we have examined the phaselocking and patterning of activity in HFA- and PC-related neurons activated by controlled vibrotactile stimulation in hairy skin.

**Impulse patterning in cuneate responses to vibrotactile stimulation in the hairy skin**

Although the impulse traces of Fig. 3 show some suggestion of phaselocking in the responses at the lowest vibration frequencies of 10 and 20 Hz, it is not clear, on the time scales illustrated in this figure, and in Fig. 5, whether these responses and, in particular, those to higher vibration frequencies, are phaselocked and retain a patterning of activity that might reflect the periodicity inherent in the vibration stimulus. In order to overcome this limitation, the spike train was expanded in Fig.7 to illustrate the responses of an HFA-related neuron to the first 5 to 10 cycles of vibration at 5-100 Hz. Phaselocking of this neuron's response was retained at frequencies ranging up to 75 Hz, but at higher frequencies the failure rate increased on individual cycles, in particular, at vibration frequencies above 30 Hz.
Although failures of response on some vibration cycles become more frequent beyond the initial 10 cycles displayed in Fig. 7, responsiveness throughout the 1s trains of vibration was well maintained even at frequencies up to 100 Hz as demonstrated in the upper PSTHs of Fig. 8A-F. These paired PSTHs in Fig. 8A-F were constructed for both the entire stimulation period (upper histogram) and for an expanded view of the initial 5-10 cycles (lower histogram). Although the upper histograms show the overall response profile throughout and beyond the vibration stimulus, the analysis time and temporal resolution obscure any temporal patterning of the responses except at 5 and 10 Hz. However, the lower histogram within each set provides evidence of phaselocking at all frequencies from 5 to at least 75 Hz, reflected in the preferential impulse groupings approximating the cycle period.

For the PC-related neurons, there were marked variations, from neuron-to-neuron, in the capacity for displaying phaselocked responses to vibratory stimuli applied to the hairy skin. However, the PC-neurons in contrast to their HFA-related counterparts, could display tight phaselocking at frequencies up to 200-300 Hz, as is apparent even in the expanded impulse traces of Fig. 9A which show responses to the first 20 cycles of vibration of five frequencies, from 20 Hz to 300 Hz, and is confirmed in the PSTHs plotted in Fig. 9B from responses accumulated at these same vibration frequencies.

**Quantification of phaselocking in responses to vibrotactile inputs from hairy skin**

Quantification of the tightness of phaselocking in the responses of DCN neurons to vibrotactile inputs from hairy skin was based upon the construction of cycle histograms (CHs; Figs. 10), whose analysis times (represented by the abscissa time scale in each CH) correspond to the *cycle period* of the vibration. The CH distributions (see Methods) have a rectangular distribution in the absence of phaselocking, but display a preferential aggregation of impulse counts within a restricted segment of the histogram when the response is phaselocked. A relatively tight grouping of the impulse activity is apparent in the CHs of Fig. 10A for the responses of an HFA-neuron to vibration frequencies of 10-50 Hz in particular, and is reflected
in the high values for both quantitative measures of phaselocking, the *percentage entrainment* and the *Resultant*, R (see Methods). Values for R in Fig. 10 exceeded 0.8 at frequencies up to 50 Hz and declined, but remained significant, at 75 and 100 Hz with values of 0.67 and 0.38 respectively. For neurons in the PC-related class however, the quantitative measures of phaselocking remained high even up to frequencies of 300 Hz as indicated in Fig. 10B where values for both measures were highest between 50 and 200 Hz.

Although these measures show that the responses of cuneate neurons were clearly phaselocked to the vibration, the individual neuron’s capacity to signal the periodicity, or frequency parameter of the vibration depends, not only upon the impulse activity being phaselocked, but also upon whether the response level is sufficient to reflect the cycle-by-cycle pattern of the vibration. This is revealed to some extent by qualitative inspection of the impulse traces (Figs.3, 7 and 9) but more precisely, by constructing time interval histograms (TIHs), or interspike interval histograms (ISIHs) as they are also known, that display the distribution of interspike intervals in the response and the extent to which the impulse activity displays a patterning that matches the periodicity in the vibratory stimulus. In Fig. 10B the TIHs show, for a representative HFA-neuron, an initial peak at the short interval of <5-10 ms reflecting the discharge of pairs or triplets of closely spaced spikes on individual cycles of the vibration, but then a large grouping of intervals around the vibration cycle period (for example, at ~100 ms at 10 Hz, ~33 ms at 30 Hz, ~20 ms at 50 Hz, and ~13 ms at 75 Hz) reflecting the high incidence of response on successive cycles of the vibration train. Even at 50 and 75 Hz a substantial proportion (~80% and 75%) of interspike intervals approximate the cycle period. However, at these frequencies, smaller peaks emerge in the distributions at sub-harmonic intervals, that is, at interspike intervals 2 or 3 times the cycle period, indicating that the neuron failed to respond on some vibration cycles, and that the impulse pattern did not provide a continuous reflection of the vibration periodicity throughout the duration of the 1s vibration train. At 100 Hz there are no
peaks of response at the cycle period of 10 ms or at any sub-harmonic, indicating little or no phase-locking for this HFA-neuron at this frequency.

Mean values for the quantitative measures of phaselocking derived from the CH distributions have been plotted in Fig. 11 as a function of vibration frequency for the two classes of DCN neuron involved in processing vibrotactile information from the hairy skin. With both measures, the percentage entrainment (Fig. 11A), and the vector strength or Resultant (Fig. 11B), the HFA neurons appear to have tighter phaselocking at low frequencies (<50Hz) than do the PC neurons and are therefore able to provide the better signal of vibrotactile frequency over this range than is the PC-related class of neuron. However, a switch-over occurs around 50 Hz, with values for HFA neurons falling below those for PC neurons at the higher frequencies. The differences appear less marked with percentage entrainment measures (Fig. 11A), as this is a less sensitive measure than the Resultant because percentage entrainment values have the maximum value of 100% for any distribution in which the responses are within half the vibration cycle period, whether they are scattered across the whole of that half-cycle period or confined tightly within just a narrow segment of it. However, on both measures, the PC neurons display a peak in phaselocking at 50-100 Hz and retain significant phaselocking up to frequencies of ~300 Hz.

DISCUSSION

The natural tactile stimuli most frequently encountered on the hairy skin of the arms and legs are likely to arise from objects brushing across the skin surface or its projecting hairs (e.g. see Discussion in Gynther et al. 1995). These moving stimuli will set up various forms of complex vibrational disturbance in the skin that will generate spatio-temporal patterns of sensory inputs that we interpret as the nature and texture of the contacted object. Because of the difficulty of standardizing and quantifying such stimuli we have chosen in this study to apply controlled and reproducible stimuli directly to the shaved skin surface. We have also chosen to do this using stimuli identical to those used for earlier quantitative analysis of the neurons.
activated from the glabrous skin of the limb extremities (Connor et al. 1984; Douglas et al. 1978; Ferrington et al. 1987a, 1988) and identical to those employed in parallel human psychophysical studies of vibrotactile frequency discrimination in the hairy region of skin (Mahns et al. 2005). The use of such reproducible stimuli has permitted a quantitative analysis of DCN coding capacities for hairy skin inputs to be carried out and has revealed, in summary, that individual DCN neurons are capable of encoding, with a high degree of fidelity, information about vibrotactile disturbances in hairy skin. The results are consistent with these neurons having a role in signalling and coding such information for behavioral performance (Mahns et al. 2005), and establish that their capacities for signalling such information are far superior to those of individual neurons in the parallel ascending SCT pathway (Sahai et al. 2005).

**Classification of DCN neurons responsive to tactile input from hairy skin**

In the present study we have identified three principal classes of DCN neurons driven by tactile inputs from the hairy skin. These comprise an SA class responsive to static skin displacement, and two purely dynamically-sensitive classes of neurons distinguished principally according to their differential sensitivity and bandwidth of responsiveness to vibrotactile stimuli. One dynamically-sensitive class was most sensitive to vibration frequencies below 50-80 Hz, had receptive fields within the hairy skin itself, and appeared to derive its peripheral input from the HFA class of afferent fibers. The second dynamically-sensitive class of hairy-skin related DCN neurons displayed a broader vibrotactile bandwidth that extended to frequencies above 200-300 Hz and appeared to derive its input, in part at least, from PC receptors often remote from the skin surface itself. For some neurons of this class, in particular, those whose sensitivity extended down to vibration frequencies of <30 Hz, there may have been some convergent input from HFA sources as well as from the PC afferent input. This present classification of hairy skin-related DCN neurons into the three broad classes conforms closely to the classification arrived at many years ago by Amassian and de Vito (1957), Gordon and Jukes (1964) and Perl et al. (1962) and is reminiscent of a similar breakdown into three principal classes of DCN tactile
neurons activated by glabrous skin input (Bystrzycka et al. 1977; Connor et al. 1984; Douglas et al. 1978; Dykes et al. 1982; Ferrington et al. 1987a, 1988; Gordon and Jukes 1964). Each of these classes is driven principally by one or other of the three major classes of tactile sensory fibers (the SA, RA and PC fibers respectively) that supply this region of glabrous skin in the cat and in primates (Ferrington et al. 1984; Ferrington and Rowe 1980a; Iggo and Ogawa 1977; Jänig 1971; Jänig et al. 1968; Johansson and Vallbo 1979; Talbot et al. 1968; Vallbo and Johansson 1984). While there is widespread agreement about this three-way breakdown for glabrous skin-related DCN neurons there is a little more discordance over the hairy skin-related classification. For example, the classification by Golovchinsky (1980) included many more subclasses, although he acknowledged that the separation among these DCN classes was not necessarily clear cut. Furthermore, his sample may have included primary afferent fibers as he reported first, that inhibition was rare in contrast to the high incidence observed in many other studies on DCN neurons (Bystrzycka et al. 1977; Gordon and Jukes 1964; Gordon and Paine 1960; Jänig et al. 1977; McComas 1963; Perl et al. 1962). Second, he reported that the functional properties and receptive field characteristics were, in the great majority of units, similar to first order afferents, and third, he found the vibration-sensitive units were, in many cases, entrained to discharge in a metronomic, one-impulse-per-cycle manner (Figs.6 and 9C, in Golovchinsky 1980) at frequencies up to and even beyond 400-500Hz, behavior considered by others to be confined to the primary afferent fibers (Connor et al. 1984; Douglas et al. 1978; Ferrington et al. 1987a,b,c; Gynther et al. 1995; Perl et al. 1962; Rowe 2002; Vickery et al. 1994; Zachariah et al. 2001).

**Coding of vibrotactile frequency information from hairy skin by cuneate neurons**

The cuneate neurons driven selectively by the HFA class of peripheral afferents were most tightly phaselocked in response to vibratory disturbances in the hairy skin at frequencies up to 50 Hz. Furthermore, their impulse levels enabled them to respond at these frequencies in a cycle-by-cycle manner, thus replicating in their impulse pattern the periodicity inherent in the
vibration stimulus. However, at higher frequencies (in particular, at ≥100 Hz) their tightness of phaselocking declined, together with their responsiveness, which imposes constraints at these frequencies on their ability to signal information in an impulse pattern code about the frequency or ‘pitch’ parameter of these higher frequency vibrotactile stimuli.

The HFA-related class of cuneate neuron appeared to have a similar capacity for signalling vibrotactile frequency information to that of the RA class of neurons associated with low-frequency vibrotactile inputs from the glabrous skin (Connor et al. 1984; Douglas et al. 1978; Ferrington et al. 1987a, 1988), as percentage entrainment measures for responses to different vibration frequencies up to 50 Hz had average values in the range, 87-93%, for HFA neurons (Fig. 11) in comparison with values of about 85-97% for RA neurons examined in an earlier study from our laboratory (Fig. 11B in Douglas et al. 1978). At higher frequencies, they were also similar, with the glabrous skin RA class having mean percentage entrainment values of >80% at 80 and 100 Hz and >65% at 200 Hz (Fig. 11B, Douglas et al. 1978), and the HFA-related class, values of 78% at 100Hz and ~75% at 200 Hz (Fig. 11, present paper).

The capacity of HFA-related cuneate neurons to reliably signal information about low-frequency vibrotactile events reinforces our earlier paired-recording studies demonstrating that the synaptic connection between single HFA fibers and cuneate neurons can display high transmission security with the capacity to reliably retain temporal information about vibrotactile events. In the present experiments, the vibrotactile stimuli would have recruited an indeterminate number of HFA fibers with differences in both conduction velocities and phase relations for their vibration-induced impulse activity. However, this resulting convergence of several recruited HFA fibers upon individual cuneate neurons led to little degradation in the phaselocking of cuneate responses compared with the circumstance in which the input was selectively derived from a single HFA fiber (Zachariah et al. 2001). The explanation for this may be that the discrepancies in conduction velocity and phase relations of the convergent HFA fibers are minor, or that the response phase of the target cuneate neuron is dominated by just one
of its convergent input fibers as we have found previously in the case of convergent PC fiber inputs to DCN neurons (Ferrington et al. 1987c; Rowe 1990).

**Coding by cuneate neurons of high-frequency vibrotactile information from hairy skin**

At higher frequencies (>50 - 80 Hz), a more reliable signal of cutaneous vibrotactile events is provided by the separate class of dynamically-sensitive DCN neurons whose principal input appears to be derived from Pacinian corpuscle receptors. As these receptors and their associated PC sensory fibers are absent or infrequent in the hairy skin (Brown and Iggo 1967; Tuckett et al. 1978) their recruitment by vibrotactile stimuli applied to the hairy skin must occur by spread of the mechanical perturbation to the site of these receptors, around the margins of the toe and foot pads (Kumamoto et al. 1993; Lynn 1969; Malinovsky 1966) in the case of vibrotactile stimuli delivered to the hairy skin in distal regions of the limb, and perhaps to the deeper Pacinian corpuscles associated with the interosseous membrane or joints of the limb, in the case of vibrotactile stimuli applied to more proximal parts of the limb. This need for stimulus spread would account for the higher behavioral thresholds for detection in the hairy skin of the mid-forearm compared with the glabrous skin in human subjects (Mahns et al. 2005; Merzenich and Harrington 1969; Talbot et al. 1968). While absolute thresholds for hairy skin-related PC neurons in the present study (Fig. 6) were lower than the human behavioral detection thresholds, the neuronal receptive fields in the cat hairy skin were often in regions, such as the margins of the foot pads, where the need for stimulus spread to the PC receptors was less than is the case for the human mid-forearm.

Once the threshold was reached for the activation from the hairy skin of the PC-related DCN neurons, many of them displayed a responsiveness and phaselocking of their responses which is consistent with them signalling, in an impulse pattern code, information about the frequency parameter of vibrotactile stimuli that may account for subjective performance in the domain of vibrotactile frequency discrimination from hairy skin (Mahns et al. 2005). Quite marked variations were observed from neuron-to-neuron, with the mean values for percentage
Entrainment declining from ~90% at 100 Hz to ~85% at 200 Hz and <90% at 300 Hz (Fig. 11).

A similar neuron-to-neuron variability was observed in our earlier quantitative studies of phaselocking in cuneate responses to vibrotactile inputs from the glabrous skin, where mean percentage entrainment values at these high vibration frequencies of 100-300 Hz were also around 80% (see Fig. 11 in Douglas et al. 1978 and Fig. 4 in Ferrington et al. 1987a). The similarity of the values is consistent with there being little difference in behavioral performance for vibrotactile frequency discrimination between hairy and glabrous skin in human subjects (Mahns et al. 2005), even though detection thresholds are different (Mahns et al. 2005; Merzenich and Harrington 1969; Talbot et al. 1968).

The comparison of the quantitative measures of phaselocking for HFA- and PC-related neurons (Fig. 11) indicates that the HFA class is principally responsible for vibrotactile frequency coding at the low frequencies (≤ 50 Hz) in hairy skin, with a switch-over occurring at around 50 - 80 Hz to the PC-related neurons as the principal neural substrate in the cuneate nucleus for the coding of high-frequency vibrotactile information from the hairy skin. However, entrainment up to vibration frequencies of 738 Hz, as reported by Golovchinsky (1980), was never encountered in the responses of cuneate neurons sampled in the present study, although it must be said that the criteria for entrainment in the earlier study were unclear, as were the duration of the vibrotactile stimulus train and the criteria for distinguishing primary afferent fibers from cuneate neurons.

**Signalling of vibrotactile information at thalamocortical levels of the sensory pathway**

The capacity to encode information about the frequency parameter of vibrotactile events in the impulse patterns of individual neurons appears to be well-retained at the next level of the dorsal column-lemniscal pathway, in the ventralposterolateral (VPL) nucleus of the thalamus (Ghosh et al. 1992). From here, the information is conveyed over a parallel projection network to two principal cerebral cortical target regions, the primary and secondary somatosensory areas of the cortex (SI and SII respectively; Bennet et al. 1980; Ferrington and Rowe 1980; Fisher et
al. 1983; Mackie et al. 1996; Rowe et al. 1985; Turman et al. 1992, 1995; Zhang et al. 1996, 2001a,b). At the cortical level, a substantial decline is apparent in SI, in the tightness of phaselocking of individual responses, with phaselocking absent at vibration frequencies above ~100 Hz (Ferrington and Rowe 1980; Mountcastle et al. 1969), whereas within SII, individual neurons can retain phaselocked responses at vibration frequencies up to ~300 Hz (Ferrington and Rowe 1980b; Ghosh et al. 1992; Rowe et al. 1985; Rowe 1990). However, response levels in the individual SII neurons, for example, recorded over trains of vibration lasting 1s, are rarely above 60-80 impulses/s, and are therefore considerably lower than rates in DCN or even thalamic VPL neurons. This means that in response to vibration at 200-300 Hz, the individual SII neurons can discharge no more than one impulse per 3-4 cycles of vibration and are therefore unable to display a periodicity in their impulse activity matching that of the vibration cycle period, at least over these one-second long response segments. This breakdown of a cycle-by-cycle impulse patterning at the cortical level for higher-frequency vibrotactile disturbances may mean (if impulse patterning is the crucial neural substrate for frequency recognition) that frequency coding in the range above ~100 Hz may be dependent upon a concatenation of thalamo-cortical events that include the presence of patterned activity at the thalamic level (Ghosh et al. 1992; Rowe 1990). However, it should be emphasized that the overall decline apparent at a single neuron level in the tightness of phaselocking and the precision of impulse patterning, as one progresses from the primary afferent fiber level to the DCN, and thence the VPL thalamus and cortex, appears to be consistent with the steep increase in the discriminable increment, Δf, for subjective vibrotactile frequency discrimination, in particular, at frequencies above about 100 Hz (Bekesy 1962; Ghosh et al. 1992; Goff 1967; Mahns et al. 2005; Rothenberg et al. 1977).

Vibrotactile coding in different parallel ascending somatosensory pathways

It is clear, from both the earlier analyses of glabrous skin vibrotactile coding mechanisms in the DCN and from the present analysis for hairy skin, that individual neurons in this tactile sensory pathway, relaying through the gracile and cuneate nuclei have a much greater capacity
for signalling reliably the intensive and frequency parameters of vibrotactile stimuli than do their counterparts within the parallel spinocervical ascending system (Sahai et al. 2005). Indeed, this related analysis of the vibrotactile coding capacities of neurons in the spinocervical system (Sahai et al. 2005) suggests that they can contribute little more than an 'event-detector' role for vibrotactile sensibility. If these transmission characteristics through the spinal dorsal horn apply also for neurons of other major ascending somatosensory pathways that arise in the dorsal horn, such as the spinothalamic system, it is probable that these systems are also capable of providing only a crude account of tactile sensory events, an interpretation consistent with traditional views on the spinothalamic tract that have been reinforced by selective spinal lesion studies in experimental animals (Makous et al. 1996; Vierck 1998). A further argument for a limited role of the spinothalamic system in discriminative tactile signalling comes from the finding that some tactile sensory fiber classes, for example, the PC sensory fibers, appear to be only sparsely represented within this system (Douglas et al. 1978; Ferrington et al. 1986, 1987d; Surmeier et al. 1988; Willis et al. 1975).

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Figure 1. Schematic representation of the mediolateral and rostrocaudal locations of DCN neurons activated by tactile inputs from the hairy skin. Neurons are identified according to the functional class they were assigned.
**Figure 2.** Figurines showing the distribution of receptive fields mapped by von Frey hairs of the indicated force (in mg.wt.), for cuneate neurons receiving input from the hairy skin on the dorsal (A) and ventral (B) aspects of the forelimb. C shows the receptive fields for the two gracile neurons receiving input from the lateral aspect of the hindlimb. Neurons whose fields are marked by diagonal shading were preferentially activated by HFA fibres, those with stippled fields appeared to be activated by PC inputs, and those indicated with cross-hatching by slowly adapting inputs.
Figure 3. Impulse traces for a representative HFA-related cuneate neuron showing responses to a range of vibrotactile frequencies and amplitudes. In each case the one-second long vibration train was superimposed upon a 1.5s background step (400 µm) that commenced 300ms before the vibration.
Figure 4. Stimulus-response relations for cuneate neurons responsive to vibrotactile inputs from hairy skin. The relations in A are for a representative HFA neuron at six different vibration frequencies and, in B, for 7 HFA-neurons at the fixed frequency of 20 Hz.
Figure 5. Vibrotactile sensitivity and responsiveness within the hairy skin for three PC-related cuneate neurons. A: impulse traces showing the low threshold (<2 µm) and graded responsiveness for one neuron at the fixed frequency of 300 Hz. B: stimulus-response relations plotting, for another neuron, the mean response (impulses/s) as a function of vibration amplitude at a range of frequencies. C: impulse traces showing responsiveness over a range of frequencies and amplitudes for a third PC neuron. In A and C, the waveforms represent the 1s-long vibration train superposed on the background step indentation.
**Figure 6.** Bandwidths of vibrotactile sensitivity for HFA-and PC-related cuneate neurons, plotted for different vibration frequencies (abscissa) as the amplitude (µm, on the ordinate) at which a discernible increase in spike output was apparent from the stimulus-response relations. A shows values for 12 HFA neurons; B, for 10 PC neurons, and C, for the mean threshold (± SEM) as a function of vibration frequency for the two classes.
Figure 7. Impulse patterning in the responses of an HFA-neuron to vibrotactile stimulation. The three impulse-trace replicas in each panel of A-F (in which the vertical deflections are standardized pulses generated by each neuronal spike occurrence) show the response pattern to the first 5 or 10 cycles of vibration at six different frequencies.
Figure 8. Peristimulus time histograms (PSTHs) constructed from the responses of an HFA-related cuneate neuron to six different vibration frequencies (5-100 Hz). The upper PSTHs in A-F show the overall response profile to the 1-s train of vibration superimposed on a 1.5s step indentation as indicated in the stimulus waveform below each PSTH. The lower PSTHs have been expanded in time to show the phaselocking in the responses to the first 5 or 10 cycles of the vibration train at each of the 6 frequencies. Vertical bars on the right hand side indicate the scale for accumulated impulse counts in each address of the PSTHs. Five responses were accumulated in constructing each of the histograms. The upper histogram in each pair was constructed from 70 bins each of 35.7 ms duration. The lower histograms were constructed from 100 bins whose widths differed with frequency and were 10 ms (A and B), 3.3 ms (C), 2ms (D), 1.3ms (E) and 1ms (F).
Figure 9. Phaselocked responses of a PC-related cuneate neuron to vibrotactile stimulation of hairy skin at five frequencies (20-300 Hz). In A, the impulse traces show the responses to the first 20 cycles of the vibration train, while the PSTHs constructed in B from five consecutive responses at each vibration frequency confirm the phaselocked pattern of response at each frequency. The vibration waveform for the 20-cycle segment at each frequency is shown beneath each impulse trace and each PSTH.
Figure 10. Phaselocking and impulse patterning in the responses of representative cuneate neurons of the HFA-related (A,B) and PC-related (C,D) classes across their respective bandwidths of response. The CHs in A and C show the probability of impulse occurrence within the vibration cycle period for responses of the HFA-neuron at 10-100 Hz and for the PC-neuron at frequencies of 20-300 Hz. The quantitative measures of phaselocking, based upon *percentage entrainment* (E) and the *Resultant* (R) are indicated above each CH. The time-interval histograms in B and D show the distribution of inter-spike-intervals for the same responses used to construct the CHs. Each histogram was constructed from 5 consecutive responses at the indicated vibration frequencies. Each CH in A was based on 100 bins with binwidths of 1, 0.3, 0.2, 0.13 and 0.1 ms respectively at 10, 30, 50, 75 and 100 Hz. In C the CHs were constructed from 50 bins with a width of 1, 0.4, 0.2, 0.1 and 0.06 ms respectively at 20, 50, 100, 200 and 300 Hz.
Figure 11. Quantitative evaluation of phaselocking in the responses of HFA- and PC-classes of cuneate neurons activated by vibrotactile stimulation of the hairy skin. In A, the mean (± SEM) percentage entrainment values are plotted for each class as a function of vibration frequency, while the mean vector strength, expressed as the resultant, (R), is plotted in B. In both A and B, the mean values for each frequency were obtained at a fixed vibration amplitude of 100 µm. The means for the HFA curves were derived from up to 16 neurons in A and 18 in B, and for the PC curves from up to 15 neurons in A and 12 in B.