FUNCTIONAL CAPACITIES OF TACTILE AFFERENT FIBRES IN NEONATAL KITTENS

By D. G. FERRINGTON and MARK J. ROWE

From the School of Physiology and Pharmacology, University of New South Wales, Sydney, Australia, 2033

SUMMARY

1. Responses were recorded from individual tactile afferent fibres isolated by microdissection from the median nerve of pentobarbitone-anaesthetized neonatal kittens (1–5 days post-natal age). Experiments were also conducted on adult cats to permit precise comparisons between neonatal and adult fibres.

2. Neonatal fibres with receptive fields on the glabrous skin of the foot pads were classified into two broad groups, a slowly adapting class (40 %) which responded throughout a 1 sec period of steady indentation and a rapidly adapting or dynamically sensitive class comprising 60 % of units. Fibres in these two groups had overlapping conduction velocities in the range 4·3 to 7·5 m/sec and were believed to be the developing Group II afferents of the adult.

3. Neonatal slowly adapting fibres qualitatively resembled their adult counterparts. They displayed graded stimulus–response relations which, over the steepest segment of the curves, had mean slopes of 15·7 impulses/100 μm of indentation. Plateau levels of response were often reached at amplitudes of skin indentation of < 0·5–0·7 mm.

4. Dynamically sensitive fibres with receptive fields on the glabrous skin were studied using sinusoidal cutaneous vibration which in the adult enables them to be divided into two distinct classes. However, in the neonate, they formed a continuum whether criteria of sensitivity or responsiveness were used.

5. In response to vibration neonatal fibres differed from adult ones according to the following quantitative indices: (i) *sensitivity* as measured by both absolute thresholds and thresholds for a 1 : 1 pattern of response, both of which were higher in the neonate than in the adult at all frequencies > 50 Hz and differed by an order of magnitude at frequencies $\geqslant$ 200 Hz; (ii) *responsiveness* based on the mean impulse rate evoked at a fixed amplitude of cutaneous vibration; (iii) *band width* of vibratory sensitivity which in the neonate was confined to approximately 5–300 Hz whereas in the two classes of adult units it covered the range 5–800 Hz; (iv) *capacity for coding information about vibration frequency*. Impulse activity of neonatal fibres was less tightly phase-locked to the vibratory stimulus and showed a poorer reflection of the periodic nature of the vibratory stimulus than impulse patterns of adult units.

6. The results reveal that tactile receptors and afferent fibres in the neonate are functionally immature. Their restricted coding capacities suggest that peripheral tactile sensory mechanisms impose limits on the ability of the new-born animal to derive information about its tactile environment.

INTRODUCTION

In a variety of adult animals, including the cat and monkey, three distinct functional classes of large myelinated afferent fibres have been identified in association with tactile receptors in the distal glabrous skin of the limbs (Jänig, Schmidt & Zimmermann, 1968; Talbot, Darian-Smith, Kornhuber & Mountcastle, 1968; Jänig, 1971; Iggo & Ogawa, 1977). One class consists of slowly adapting tactile afferents which appear to be associated with Merkel cell receptor endings in the epidermal pegs (Jänig, 1971; Munger, Pubols & Pubols, 1971; Pubols, Pubols & Munger, 1972). The remaining fibres which are sensitive only to the dynamic components of tactile stimuli can be divided into two distinct classes according to their sensitivity and responsiveness to cutaneous vibration. One class, comprising units which are most sensitive to vibration at 20–50 Hz, appears from correlative morphological and functional studies to be associated with intradermal, encapsulated receptors known as the Krause corpuscles in non-primates and Meissner corpuscles in primates (Talbot, Darian-Smith, Kornhuber & Mountcastle, 1968; Jänig, 1971; Munger & Pubols, 1972; Iggo & Ogawa, 1977), these two corpuscles being considered variants of a single broad group (Halata, 1975). Units in the other class are exquisitely sensitive to cutaneous vibration at 200–400 Hz and are presumably associated with Pacinian corpuscles (Hunt & McIntyre, 1960; Hunt, 1961; Sato, 1961; Lindblom & Lund, 1966; Jänig, Schmidt & Zimmerman, 1968; Talbot *et al.* 1968; Lynn, 1969).

Although the tactile receptors, located in the cat's foot pads are reported to be functionally mature at birth (Gibson, Beitel & Welker, 1975; Beitel, Gibson & Welker, 1977) their morphological maturation is not complete for some months subsequent to birth (Malinovský; 1970; Malinovský & Sommerová, 1972; Zelená, 1978). Intradermal corpuscles are first apparent at 7–10 days post-natally and their mature form is found only after the age of 2–4 months (Malinovský, 1970). The Pacinian corpuscles are first mature at 3 months of age, their post-natal maturation involving development of the inner core, the formation of numerous enveloping lamellae and an approximately fourfold increase in the length and breadth dimensions of the corpuscle (Malinovský & Sommerová, 1972). Furthermore, Merkel endings, at least in the Haarscheibe, or tactile pad receptors, of the kitten hairy skin also appear to be morphologically immature at birth (Kasprzak, Tapper & Craig, 1970).

As part of a series of studies on the functional maturation of tactile sensory pathways we have examined the properties of tactile afferent fibres in neonatal kittens (1–5 days of age) and made quantitative comparisons of their coding capacities with those of the adult cat. Our results do not support previous conclusions, first, that mechanoreceptors are functionally mature at birth and, second, that the post-natal maturation of somatosensory-based behavioural repertoires is therefore dependent *only* on the maturation of central neural mechanisms (Beitel, Gibson & Welker, 1977).

METHODS

Experiments were performed on neonatal kittens (1st to 5th post-natal day) whose mean weight was 124 g ± 32 (s.d.). They were anaesthetized initially with sodium pentobarbitone (25–30 mg/kg, I.P.) and maintained with supplementary doses of 5 mg/kg, I.P. Five experiments were also conducted on adult cats anaesthetized with pentobarbitone (35 mg/kg, I.P. with 5 mg/kg supplements).

Once the animal was anaesthetized, e.c.g. electrodes were attached, a rectal thermistor inserted and in most animals the trachea cannulated. After shaving the distal forelimb it was fixed, pads uppermost, in a mould of paraffin wax to stabilize the limb and permit accurate positioning of the mechanical stimulator. The median or ulnar nerve was exposed in the forearm, freed from surrounding tissue and cut at the proximal end of its exposed portion. It was then covered with physiological saline while the perineural sheath was opened to free the component bundles of nerve fibres. Saline was replaced with liquid paraffin which was retained in a pool formed by the skin flaps. A black dissecting plate was placed beneath the nerve and a pair of stainless-steel recording electrodes positioned above the plate. Rectal temperature was held at 38 ± 1 °C using an infra-red lamp directed over the whole animal. At the termination of the experiment an overdose of pentobarbitone was given.

Recording and stimulating procedures

Impulse activity of single afferent fibres was recorded from thin nerve strands isolated by microdissection from larger nerve bundles. The strands were draped over one electrode, the second electrode being in contact with nearby muscle tissue. Recorded signals were fed to a preamplifier thence to an audio amplifier and speaker, and to an oscilloscope display. They could also be passed through a filter unit before going to a magnetic tape recorder and to a differential amplitude discriminator from which constant output pulses could be relayed to a counter unit and a laboratory computer (PDP-8).

When a single fibre was isolated electrophysiologically its receptive field was delineated by gentle tapping with a fine probe. Fibres selected for study had tactile receptive fields on the glabrous skin of the forelimb foot pads. Precise and reproducible mechanical stimuli were derived from a servo-controlled mechanical stimulator used in previous studies from this laboratory (Darian-Smith, Rowe & Sessle, 1968; Carmody & Rowe, 1974; Bystrzycka, Nail & Rowe, 1977; Ferrington, Nail & Rowe, 1977; Douglas, Ferrington & Rowe, 1978; Bennett, Ferrington & Rowe, 1980; Ferrington & Rowe, 1980). Stimuli were applied to the point of maximum sensitivity within the receptive field of the fibre using a circular probe, 1 mm in diameter for kitten studies and 2 mm for the adults. They were repeated at rates no faster than 1 per 10 sec to allow recovery of skin position (Beitel *et al.* 1977). Skin recovery was assessed indirectly in terms of whether responses were reduced on successive repetitions of a fixed stimulus.

All fibres studied were tested initially for their responsiveness to a steady indentation of rectangular form lasting 1–1·5 sec (rise time 30 msec; amplitude $\leqslant 1$ mm). They could be classified into two broad groups, the slowly adapting units which responded throughout the steady stimulus and the rapidly adapting or dynamically sensitive fibres which responded only to the transient components of the step indentation. For studying dynamically sensitive fibres a train of sinusoidal vibration lasting 1 sec was superimposed on, and started 300 msec after the onset of a rectangular indentation whose amplitude was usually 270 μm for the kitten studies and 670 μm for the adult. The different amplitudes for this step indentation and the different stimulator probe sizes used in the kitten and adult studies were chosen to take account of differences between the neonatal kitten and adult cat in the size of their foot pads. Furthermore, the smaller amplitude of the step indentation was needed in kitten studies as larger amplitudes (0·5–1 mm) sometimes appeared to produce tissue damage.

Each dynamically sensitive tactile afferent fibre was tested at a series of different vibratory frequencies to determine the vibration amplitude at which one impulse was evoked on each cycle of vibration. This was termed the 1 : 1 threshold or tuning-point for that vibration frequency (Jänig *et al.* 1968; Talbot *et al.* 1968; Iggo & Ogawa, 1977). Further analyses included the determination of absolute response thresholds to vibration, the construction of stimulus–response curves and computer-based evaluation of phase-locking and periodicity in the impulse patterns. In any one analysis the initial movement in the vibratory displacement always occurred from the null position in a fixed direction. The constancy of this initial displacement was crucial for analyses of the phase-locking of impulse activity to the vibratory stimulus waveform.

Computer analyses of impulse data

A laboratory computer was programmed to produce post-stimulus (or peri-stimulus) time histograms, cycle histograms or time interval histograms. The post- or peri-stimulus time histograms use a pulse at the start of, or before, the mechanical stimulus as a timing marker and

therefore show the probability of impulse occurrence during the time of the mechanical stimulus. The cycle histograms use a pulse associated with the onset of each vibratory cycle as the stimulus marker and display the probability of an impulse occurring throughout the period of the vibratory cycle. Histograms of this type have been constructed in previous studies on the phase-locking of neural activity to oscillatory stimuli in both the somatosensory (Talbot *et al.* 1968; Mountcastle, Talbot, Sakata & Hyvärinen, 1969; Hyvärinen, Poranen & Jokinen, 1974; Bystrzycka *et al.* 1977; Douglas *et al.* 1978; Bennett, Ferrington & Rowe, 1980; Ferrington & Rowe, 1980) and the auditory pathways (Rose, Brugge, Anderson & Hind, 1967). Some cycle histograms were rotated to approximately centre the peak of activity in the distribution. This does not interfere with the present analysis in which the actual phase relation is not important. Usually twenty to thirty repetitions of the vibratory stimulus train were used to construct the post-stimulus time histograms and, depending on the vibration frequency, 200–3000 cycles of vibration, delivered in 1 sec trains were used for constructing the cycle histograms.

The time interval histograms display the distribution of interspike intervals during responses to cutaneous vibration and were usually constructed from 500 to 2000 intervals. Histograms were normally constructed at the time the neurone was under study, although in some cases they were constructed from data recorded along with appropriate time markers on magnetic tape. These analyses permitted evaluation of the extent to which the impulse activity evoked by vibratory stimulation was phase-locked or entrained to the vibratory stimulus wave form and whether, in the case of the time interval histograms the impulse-activity displayed a periodicity matching that of the vibratory stimulus train.

The laboratory computer also performed a numerical analysis which gave an index of dispersion, the minimum standard deviation, for the distribution of impulse activity in the cycle histogram (Mountcastle *et al.* 1969; Bystrzycka *et al.* 1977; Ferrington & Rowe, 1980). This provided a quantitative evaluation of the extent to which impulse activity was phase-locked to the vibration wave form.

RESULTS

Classification of tactile afferent fibres

Seventy-four responsive fibres were isolated in neonatal animals. Their tactile receptive fields were confined to part of the central pad or individual toe pads when tested with gentle stimulation using a fine hand-held probe. For most units the fields were spot-like and in absolute area, appeared smaller than those of adult units examined both in this study and previously (Jänig *et al.* 1968; Talbot *et al.* 1968; Iggo & Ogawa, 1977). However, as precisely quantified procedures were usually not used for assessing receptive fields we are unable to make rigorous comparisons. In response to a step indentation of the skin, twenty-nine (40 %) neonatal units showed a maintained response (Fig. 1*A*, *B*) and were classified as slowly adapting units. The remaining fibres responded only to the transient components of the step indentation (Fig. 2*A*, *B*) and were classified as rapidly adapting or dynamically sensitive units. There was rarely any doubt about the classification of units into these two broad groups with the usual testing indentation of 270 μm. However, any uncertainty over whether a maintained response was elicited could be resolved by using a higher amplitude of the step indentation.

Conduction velocities determined for a small sample of neonatal afferent fibres including both slowly adapting and dynamically sensitive units ranged from 4·3 to 7·5 m/sec which corresponded with previous values reported for neonatal tactile afferents believed to be the developing Group II units of the adult (Beitel *et al.* 1977).

Slowly adapting tactile afferent fibres

The responses of neonatal slowly adapting units to a step indentation qualitatively resembled those of adult units (Adrian & Zottermann, 1926; Werner & Mountcastle, 1965; Mountcastle, Talbot & Kornhuber, 1966; Knibestöl, 1975; Pubols & Pubols,
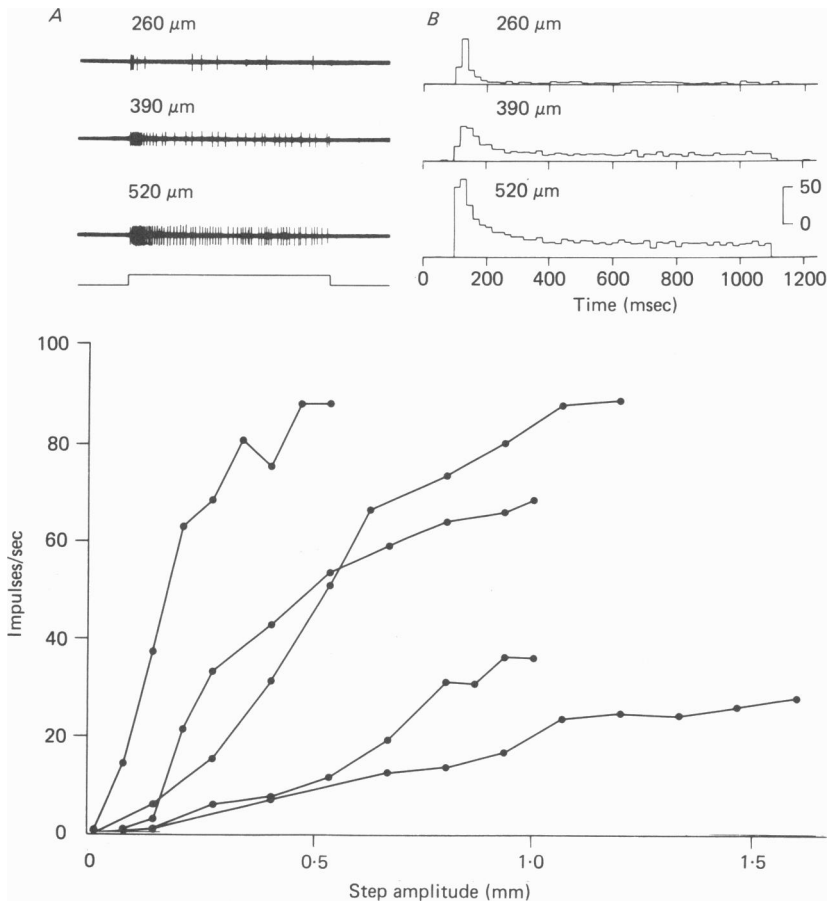

Fig. 1. Responses of slowly adapting tactile afferent fibres in the neonate to step indentations of the skin. *A*: impulse traces of responses to three different amplitudes of the step indentation, the analogue of which is shown below the impulse traces. *B*: peristimulus time histograms constructed from twenty successive responses of the same fibre to the 1 sec step indentation at the three indicated amplitudes; stimulus onset occurred 100 msec after the start of the analysis period. The vertical scale in the lowest histogram indicates 50 impulse counts. Stimulus site, the pad of toe 4. *C*: stimulus–response relations for 5 representative neonatal slowly adapting fibres. Each point represents the mean of at least five stimulus deliveries. All fibres had receptive fields on the foot pads.

1976) in consisting of an initial peak of activity to the onset phase of the indentation followed by a decline to a lower level of activity which was maintained throughout the steady phase of the indentation (Fig. 1*A*, *B*). Because of the initial peak in the response the mean impulse frequency obtained at a given stimulus intensity will vary

with the duration of the step indentation. This was therefore fixed at 1 sec in constructing stimulus–response relations such as those in Fig. 1C which cover the range from the most sensitive to the most insensitive of the slowly adapting fibres we have studied in the neonate. Commonly these relations display a slow initial rise, and in the later part of the curve a plateau level of discharge giving the curves a sigmoidal form (Fig. 1C). Over the steepest linear portion, connecting at least three or four points on the curve, the slope ranged from 4·6 to 37·0 impulses/100 μm of indentation ($n = 10$) with a mean of 15·7 impulses/100 μm. The dynamic range, defined as the range of stimulus indentation over which the unit exhibits a graded responsiveness (Carmody & Rowe, 1974; Bystrzycka *et al.* 1977) was often $< 0·5$–0·7 mm (Fig. 1C). Variability in responses (S.D. about mean) was relatively constant over the range of the stimulus–response curves.

Dynamically sensitive tactile afferent fibres

Rapidly adapting or dynamically sensitive tactile afferent fibres were examined using cutaneous vibratory stimulation which in the adult permits the corresponding broad group to be divided into two distinct functional classes. In studying the responses of both neonatal and adult fibres to cutaneous vibration we have examined response parameters important for the coding of information which determines first, the subjective capacity for the detection and recognition of cutaneous vibration, secondly, the range of frequencies over which vibration can be sensed and third, the subjective capacity for making discriminative judgements about its frequency.

Response characteristics and classification of dynamically sensitive neonatal units

In an attempt to establish the identity of their associated receptors forty dynamically sensitive neonatal units were tested for their sensitivity and responsiveness to sinusoidal vibration applied to the glabrous skin of the foot pad. In contrast to adult fibres there was no clear segregation into two distinct classes of vibration sensitive units. Some were most sensitive to vibration at frequencies of 10–30 Hz, the responses of one such fibre to 20 Hz vibration being shown in Fig. 2A. It displays a weak response at vibration amplitudes < 75 μm but with increasing amplitudes its response becomes entrained to a pattern of one impulse per cycle of vibration. This 1 : 1 response level prevails over a range of vibration amplitudes as is also observed for adult units. The responses of a second neonatal unit which was most sensitive at frequencies of 80–200 Hz is seen in Fig. 2B. An entrained, 1 : 1 level of response was achieved in this unit at vibration amplitudes above 30 μm.

In order to quantify the sensitivity and responsiveness of neonatal units stimulus–response relations were constructed at a series of different frequencies by plotting the mean response (impulses/sec) against vibration amplitude (Fig. 3). The relations in Fig. 3A, constructed for a unit with lowest 1 : 1 threshold at $\leqslant 30$ Hz resemble those of adult fibres sensitive to low frequency vibration (Talbot *et al.* 1968; Iggo & Ogawa, 1977) and may therefore be derived from a fibre associated with developing Krause corpuscles. Other neonatal fibres showed some resemblance to adult Pacinian afferent fibres in responding well to cutaneous vibration at frequencies > 100 Hz (Fig. 3B). However, many units were intermediate in their properties between those whose stimulus–response relations are shown in Fig. 3, precluding a division into two clear groups.
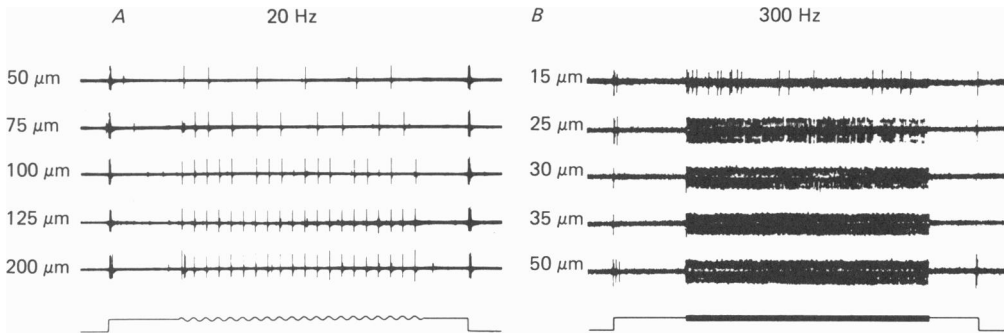
Fig. 2. Responses of two neonatal dynamically sensitive tactile afferents to different amplitudes of cutaneous vibration at 20 Hz in *A*, for a unit most sensitive to low frequencies, 10–30 Hz, and at 300 Hz in *B*, for a unit most sensitive at high frequencies, > 80 Hz. In each case the 1 sec vibration train started 300 msec after the onset of a 1·5 sec step indentation as shown by the stimulus wave forms beneath *A* and *B*. The amplitude of vibration used is indicated to the left of each trace. Stimulus site for both units was the central foot pad.
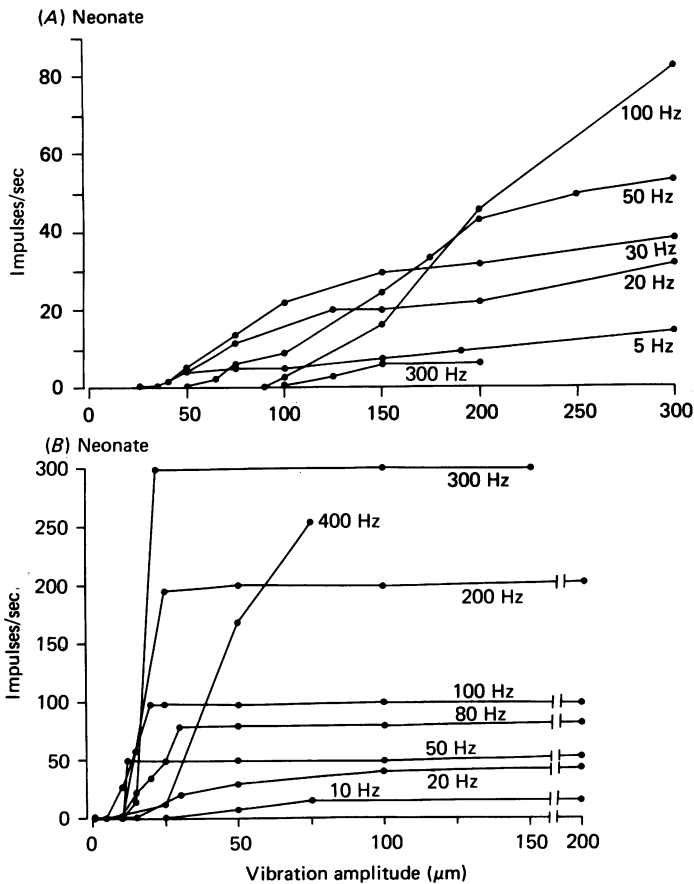

Fig. 3. Stimulus–response relations for dynamically sensitive neonatal fibres responsive to cutaneous vibration. In *A* the unit was most sensitive to low frequency vibration (≤ 30 Hz) while the data in *B* come from a unit most sensitive to higher frequencies (50–200 Hz). At each frequency the points represent the mean of five to ten successive responses to 1 sec trains of vibration at the indicated amplitudes(abscissa). Stimulus sites were the central pad (*A*) and the pad of toe 3 (*B*).

Tuning curves based on 1 : 1 *thresholds and absolute thresholds for responses to cutaneous vibration*

Tuning curves in which the threshold for the 1 : 1 response was plotted against vibration frequency were constructed (Fig. 4) in order to illustrate first, the continuum found in the distribution of vibration sensitivity for neonatal units, and secondly,
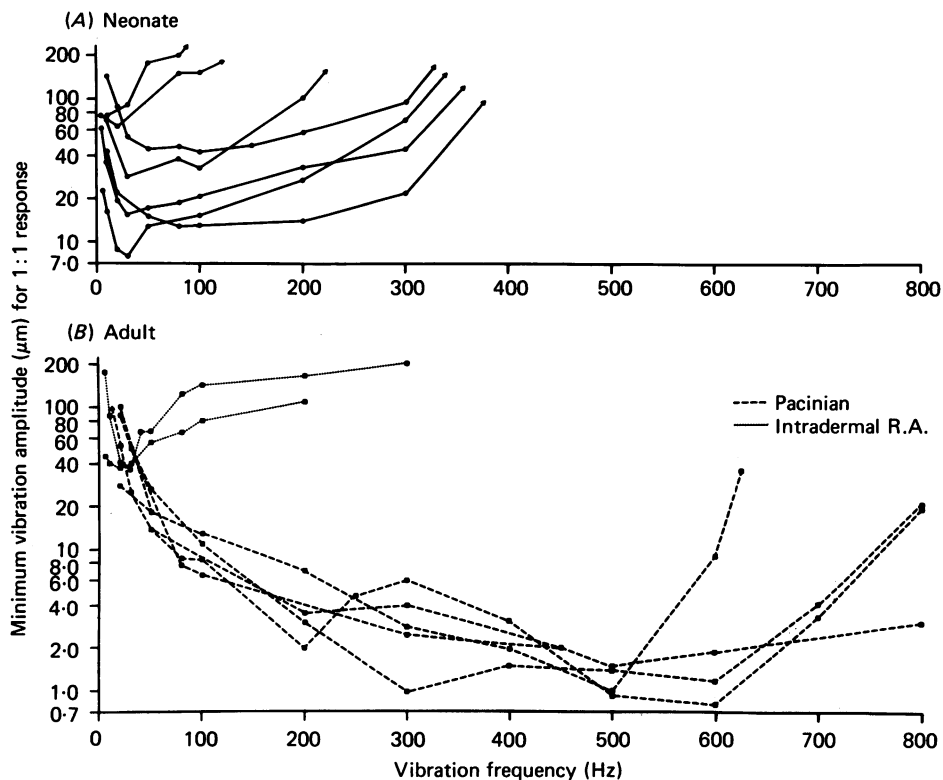

Fig. 4. Tuning curves showing the 1 : 1 thresholds and bandwidths of vibration sensitivity for seven neonatal (*A*) and seven adult (*B*) dynamically sensitive tactile afferent fibres. The curves were constructed from the stimulus–response data by plotting for each frequency the lowest vibration amplitude at which a 1 : 1 pattern of response was attained. Note the different range of amplitudes for the ordinates (log scales) in *A* and *B*. Arrows at the right hand end of the curves for neonatal units indicate estimated values as the 1 : 1 thresholds exceeded the vibration amplitudes available for testing at these higher frequencies. Whereas adult units fall into two discrete classes the seven representative neonatal units form a continuum.

the bandwidth of vibratory sensitivity for these units. The lowest 1 : 1 thresholds were distributed from 10 to 100 Hz (Fig. 4) whereas the tuning curves for seven adult fibres in Fig. 4 *B* reveal a clear segregation into two classes, one with lowest 1 : 1 thresholds at 300–600 Hz, the other at 20–30 Hz.

At their 'best' frequency the most sensitive neonatal units had 1 : 1 thresholds of approximately 8–50 μm (Fig. 4*A*) in comparison with values of 0·8–2 μm for the most sensitive adult units, those of the Pacinian type (Fig. 4*B*). If shorter vibration

trains, containing a maximum of 50 cycles of vibration were used instead of the standard 1 sec duration train there was little change in the 1 : 1 thresholds for neonatal fibres at frequencies < 200 Hz. At 200 and 300 Hz thresholds were somewhat lower but still an order of magnitude greater than those for adult Pacinian fibres.
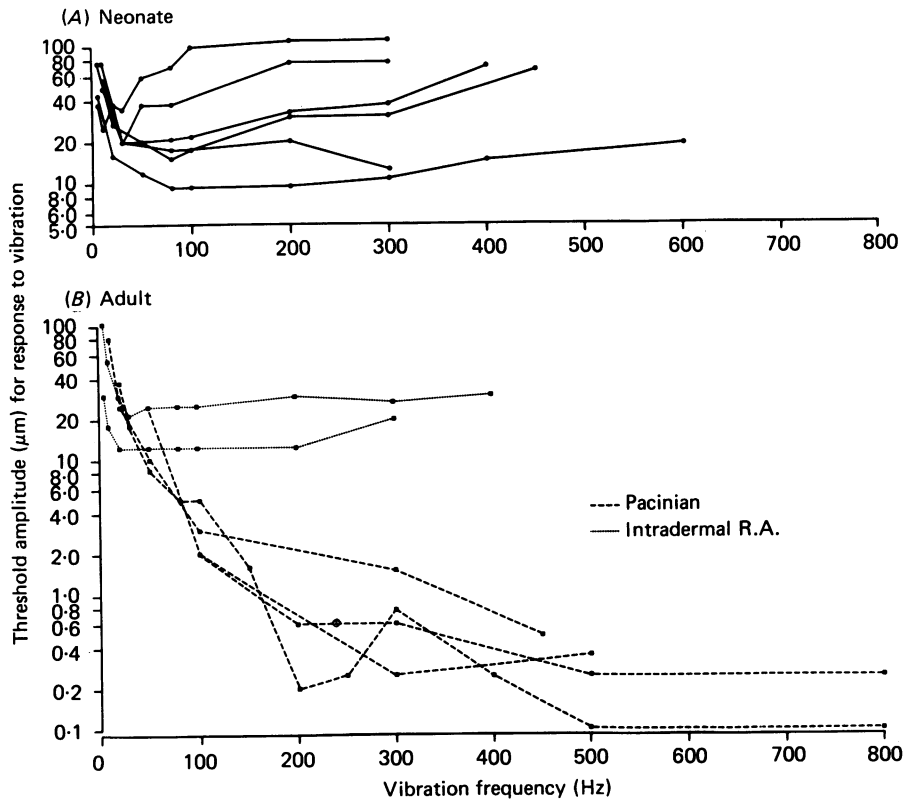

Fig. 5. Sensitivity curves based on absolute thresholds for responses to cutaneous vibration for six neonatal (*A*) and six adult (*B*) dynamically sensitive tactile afferent fibres. Values were derived from stimulus–response curves of the type seen in Fig. 3. Note the different range of amplitudes for the ordinates (log scales) in *A* and *B*. Adult units form two discrete classes as in Fig. 4 but neonatal units form a continuum.

The band width of vibratory frequencies over which individual units achieve their 1 : 1 pattern of response obviously depends on the vibration amplitude chosen as the criterion level. From the tuning curves of Fig. 4 it can be seen that at amplitudes of 25–50 μm the Pacinian units in the adult have bandwidths of sensitivity extending from a lower limit of approximately 30–40 Hz up to 600–800 Hz, whereas all neonatal units had very much narrower band widths even at vibration amplitudes as high as 100 and 200 μm. For the four most broadly tuned units the upper limits of their band widths at the 50 μm amplitude were 340, 265, 165 and 140 Hz (Fig. 4*A*). However, the lower limits of their ranges extended to frequencies of 10–20 Hz, values which were lower than those for adult Pacinian units.

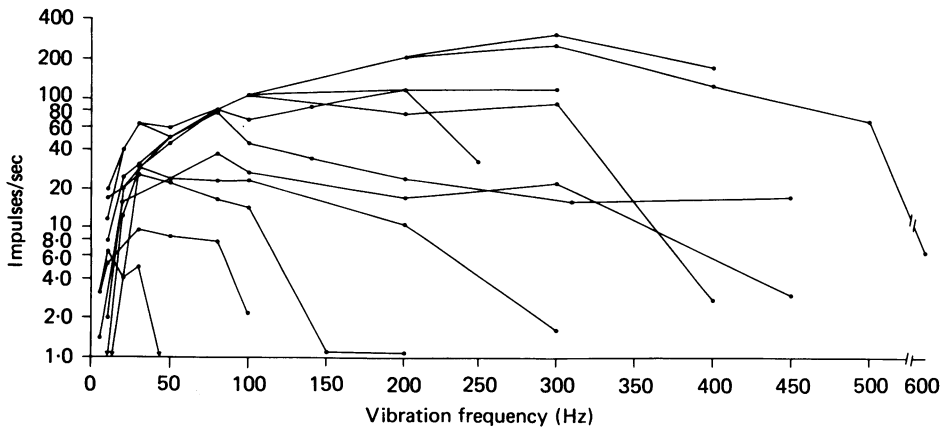Comparison of the absolute thresholds for responses to cutaneous vibration again

Fig. 6. Bandwidths of responsiveness to cutaneous vibration of neonatal dynamically sensitive tactile afferent fibres. Each point, derived from stimulus–response data, represents the mean of five to ten successive responses to cutaneous vibration, in all cases at an amplitude of 50 μm at the indicated frequency (abscissa). The eleven representative neonatal units display a continuum of responsiveness. Arrows at the extremity of some curves denote response values below those indicated on the ordinate.
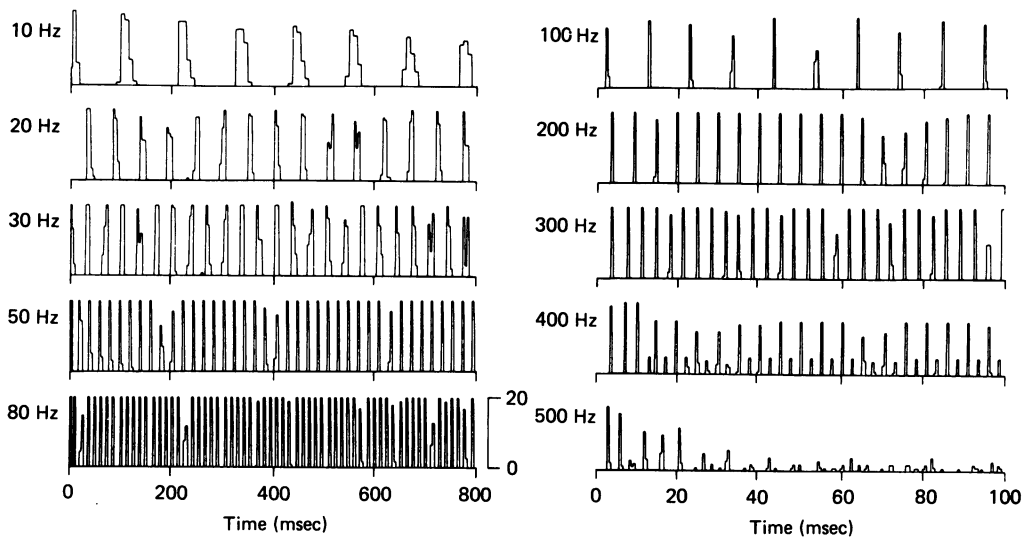

Fig. 7. Post-stimulus time histograms constructed from responses of a neonatal afferent fibre to cutaneous vibration at ten different frequencies. Each histogram, based on twenty successive responses shows the distribution of impulse activity during either the first 800 msec segment (10–80 Hz) or the first 100 msec segment (100–500 Hz) of the 1 sec vibration train. In each case the vibration was 50 μm in amplitude and was superimposed on a 130 μm step indentation. The height of each histogram column represents the number of impulses accumulated in each epoch of the analysis. The time scale below each set applies to all histograms of that group. The calibration bar to the right hand side of the 80 Hz histogram represents the height of a histogram column containing 20 impulse counts and applies to all histograms in the Figure. The stimulus site was on the central foot pad.

revealed striking differences between neonatal and adult units, in particular at high vibration frequencies (Fig. 5). The absolute thresholds at 300 Hz exceeded 10 μm (Fig. 5A) for all neonatal units sampled whereas they ranged from 0·25 to 1·5 μm for the four adult Pacinian units tested (Fig. 5B). Only at frequencies below 50 Hz were the absolute thresholds in approximately the same range for the two age groups.

Measures of responsiveness of neonatal fibres to cutaneous vibration

As different conclusions about a unit's 'best' frequency may be derived depending upon whether its properties are assessed according to criteria of *sensitivity* or *responsiveness* (Erulkar, 1975) we have also examined the *responsiveness* of neonatal fibres to cutaneous vibration using this as an additional criterion for comparison with adult fibres. When the responses (impulses/sec) of eleven representative neonatal fibres were plotted against vibration frequency (amplitude 50 μm) in Fig. 6 a continuous distribution is again obtained with no suggestion of two discrete functional classes. Response maxima occur for four units at $\leqslant$ 30 Hz and at higher frequencies for the remaining units, four units being maximally responsive at $\geqslant$ 200 Hz. In comparison with the adult Pacinian units all neonatal units display a lower level of responsiveness at the higher vibration frequencies.

Coding of information about cutaneous vibratory frequency by afferent fibres in the neonate

In response to cutaneous vibration neonatal fibres displayed a phase-locked, periodic pattern of impulse activity over a more restricted bandwidth of frequencies than did adult fibres. The post-stimulus time histograms in Fig. 7 were constructed from the responses of a neonatal unit whose 1 : 1 thresholds to vibration were lowest at 20–100 Hz. At frequencies up to 300 Hz the separation of major peaks in the histograms reflects the sine wave period for each of these vibratory frequencies and indicates that impulses are spaced at intervals corresponding to the vibratory stimulus period or to subharmonics of these intervals. At the higher frequencies, 400 and 500 Hz, this unit was weakly responsive and impulse activity was poorly phase-locked to the vibratory stimulus.

Quantitative measurements on phase-locked responses of neonatal units to vibration

In order to make precise comparisons between neonatal and adult units in their capacity for frequency coding it was necessary to derive quantitative measures of the extent to which impulse activity was phase-locked to the vibration wave form. Such measures were obtained from cycle histograms (see Methods) which use an analysis period corresponding to the vibratory cycle period. In the absence of phase-locked activity the cycle histograms display a rectangular shape, whereas the occurrence of impulses at a preferred segment of the vibratory wave form, i.e. phase-locked activity, produces a distribution in which the impulse counts are confined to a restricted segment of the histogram. The comparison of cycle histograms for representative adult and neonatal fibres in Fig. 8 shows that at high frequencies neonatal fibres display a broader distribution of impulse occurrences within the cycle period indicating a less tightly phase-locked pattern of impulse activity.

In order to quantify more precisely the extent of phase-locking in responses to

vibration we computed an index of dispersion, the minimum standard deviation in msec, for the cycle histogram distribution (Mountcastle *et al.* 1969; Ferrington & Rowe, 1980). The ratio, standard deviation divided by the duration of the cycle period, provided a normalized coefficient of variability for the time of impulse occurrence in the vibratory cycle. When plotted against vibration frequency for
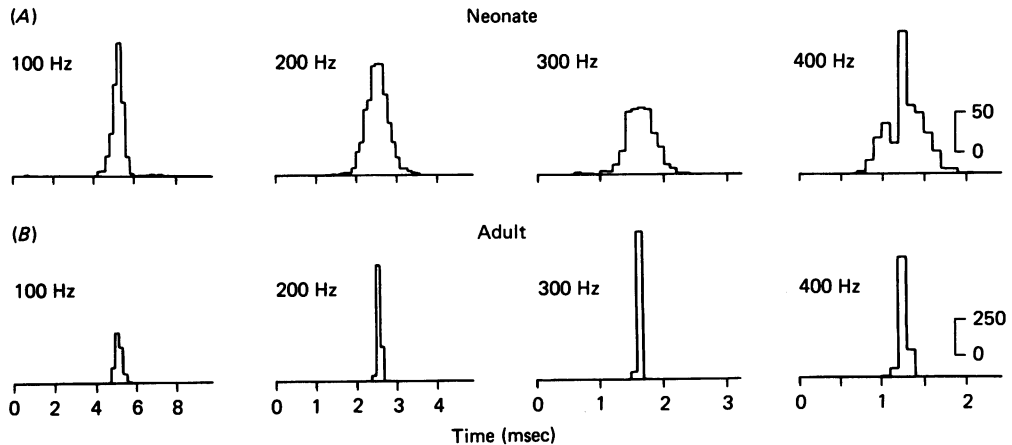

Fig. 8. Cycle histograms showing the distribution of impulse activity within the vibratory stimulus cycle for the responses of representative neonatal (*A*) and adult (*B*) afferent fibres at 100–400 Hz. The histograms were constructed from impulse activity occurring in up to 2000 cycles of vibration, depending on frequency, delivered in 1 sec trains to the skin of the foot pads. The analysis period for each histogram corresponded to or was fractionally less than the sine wave period for the frequency indicated to the left of the histogram. Vibration amplitude was 25 μm in *A* and *B* with the exception of 400 Hz in *A* where it was 50 μm, the response of the neonatal unit to 400 Hz (25 μm) being very weak. Calibration bars on the right hand side of each row of histograms indicate the height of a histogram cloumn containing the indicated number of impulse counts.

representative neonatal and adult fibres the graphs demonstrate (Fig. 9) that neonatal fibres in contrast to adult fibres are not divided into two groups in terms of their capacity to provide a reliable signal of vibration frequency. Furthermore, values for the coefficient of variability in the neonate exceeded values for adult Pacinian fibres at all frequencies above 100 Hz. For all units in Fig. 9 the index of dispersion was determined at a vibration amplitude which was nearest the 1 : 1 response threshold of the fibre; however, the relatively poor phase-locking of impulse activity for neonatal units was found over a broad range of vibration amplitudes.

Measures of periodicity in response patterns of neonatal fibres to cutaneous vibration

While post-stimulus time histogram and cycle histogram analyses establish that impulse activity is phase-locked to the vibratory stimulus they do not reveal the extent to which impulses occur at intervals corresponding to the vibratory cycle period or to sub-harmonics of that period. Time interval histograms were constructed to obtain a measure of the extent to which impulse patterns reflect the periodicity of the vibratory stimulus. The probability of inter-impulse intervals approximating the

sine wave period ($\pm 10\%$) equalled 1 at very low vibration amplitudes ($< 5\,\mu$m) for adult Pacinian fibres (Fig. 10 B and C). However, in the impulse patterns of neonatal fibres the probability is low until vibration amplitudes exceed at least 30 μm (Fig. 10 A and C). In Fig. 10 A the neonatal unit is essentially unresponsive to 300 Hz at
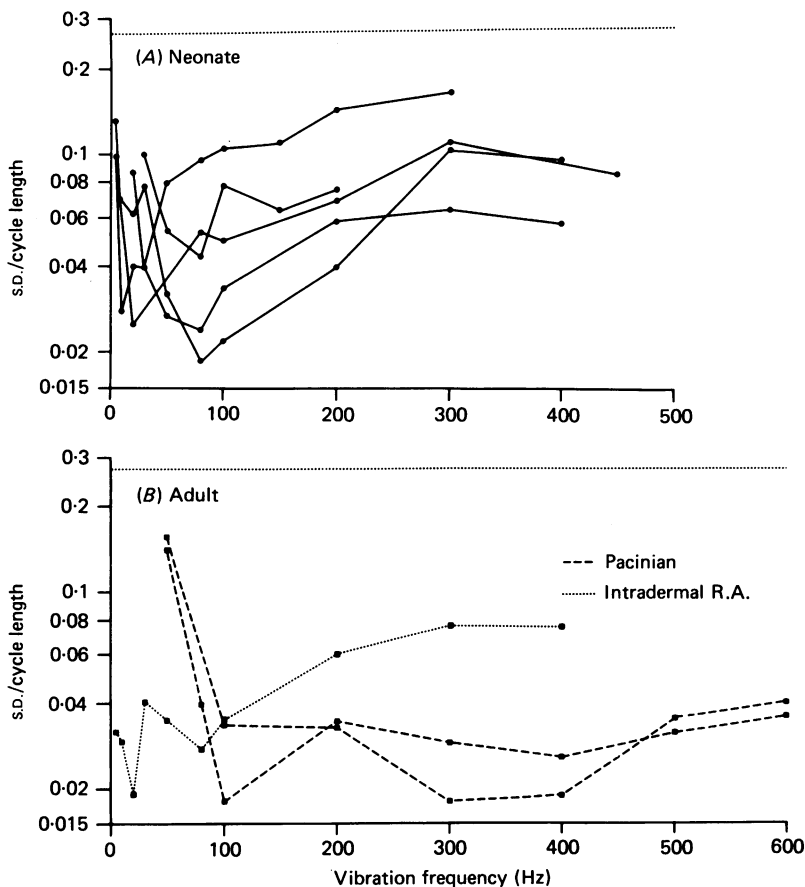

Fig. 9. Quantitative measures of the extent to which impulse activity of neonatal (A) and adult (B) fibres is phase-locked to the vibratory stimulus waveform. The measure of variability, minimum standard deviation divided by the cycle period, is plotted against vibration frequency for five neonatal fibres in A and three adult fibres in B. For each fibre values derived at a given frequency were obtained from cycle histograms constructed from responses at the 1 : 1 threshold, or at vibration amplitudes just below this in the case of neonatal units at $\geqslant$ 300 Hz. The interrupted horizontal lines at values of 0·28 in A and B represent the coefficient of variability obtained from cycle histograms which have perfectly rectangular distributions and which therefore display no phase-locked pattern of impulse activity.

an amplitude of 15 μm, while at 25 μm most inter-impulse intervals are grouped at twice the cycle period of 3·3 msec. At higher amplitudes, 30–100 μm, more intervals approximate the cycle period and therefore the impulse trains more fully reflect the periodic nature of the vibratory stimulus. Even when all or most intervals occur near the sine wave period the scatter in the distribution appears somewhat greater
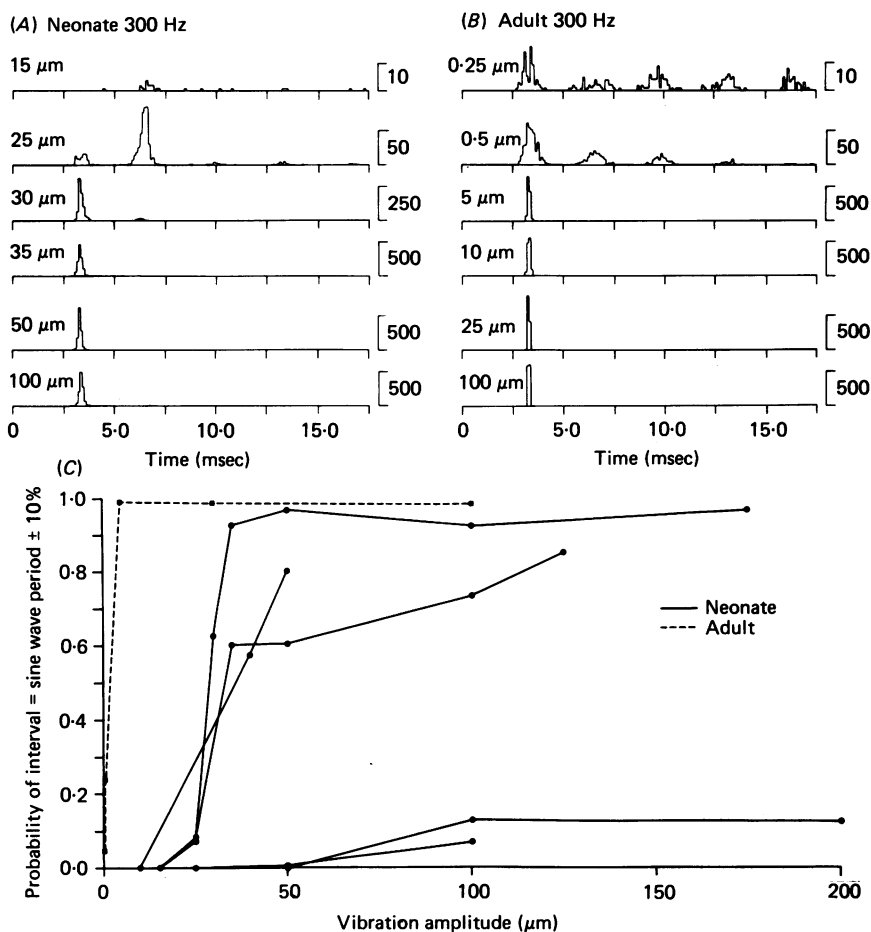
Fig. 10. Time interval histograms displaying the distribution of inter-impulse intervals in responses of neonatal (A) and adult (B) afferent fibres to six different amplitudes of cutaneous vibration at 300 Hz. Each histogram was constructed from impulse activity accumulated in 1200 cycles of vibration delivered in 1 sec trains. Inter-impulse intervals were tightly grouped around the sine wave period (3·3 msec) at much lower vibration amplitudes for the adult fibre in B than for the neonatal fibre (A) although the latter represented the neonatal unit whose impulse patterns most accurately reflected the vibratory periodicity. The calibration bars on the right hand side of each histogram indicate the height of a histogram column containing the indicated number of inter-impulse intervals. A quantitative measure, the probability of inter-impulse intervals occurring within ± 10 % of the sine wave period, was derived from the time interval histograms as an index of the extent to which the impulse patterns reflected the periodicity of the vibratory stimulus wave form. This is plotted in C against vibration amplitude for the adult unit whose time interval histograms are shown in B and for five neonatal fibres including the unit from A.

than that for adult units (Fig. 10). The graphs of interval probabilities in Fig. 10C include, among the 5 neonatal units, the three which were most responsive at 300 Hz, and two units which did not reach a 1 : 1 pattern of response at 300 Hz. The uppermost neonatal curve was for the unit whose time interval histograms are plotted in Fig. 10A.

DISCUSSION

Classification of neonatal tactile afferent fibres

The proportions in the neonate of slowly and rapidly adapting units among tactile afferents innervating the glabrous skin of the foot pads correspond closely with those reported for the adult cat (Iggo & Ogawa, 1977). A lower proportion may have been observed had longer step indentations been employed as responses from slowly adapting units in the hairy skin adapt over a period of seconds in the neonate compared with a period of several minutes in the adult (Ekholm, 1967). However, using our criterion of responsiveness over a one second period of indentation there was rarely any uncertainty about the classification, in particular if several different amplitudes of the step were used. Although Gibson *et al.* (1975) observed that some neonatal units adapted rapidly to a small indentation and displayed slowly adapting responses to larger indentations their suggestion that such units may constitute a special transitional class between slow adaptors and rapid adaptors is probably no more justified than in the adult where somewhat lower thresholds for the transient onset response than for the maintained response have also been observed for slowly adapting units (Knibestöl, 1975). Our observations suggest that the broad functional division of tactile units into two groups according to adaptation rate appears to be fixed by the time of birth.

Functional properties of slowly adapting tactile afferent fibres in the neonate

Both sensitivity, as measured by the absolute threshold for a response, and responsiveness of slowly adapting units varied widely from unit to unit as observed by Gibson *et al.* (1975). However, in both these respects adult slowly adapting units also display considerable variability (Mountcastle *et al.* 1966; Knibestöl, 1975; Pubols & Pubols, 1976). The slope of the stimulus-response relations appeared steeper than for adult units (Mountcastle *et al.* 1966; Knibestöl, 1975; Darian-Smith *et al.* 1968) probably reflecting the greater compressional force achieved at a given indentation in the small neonatal foot pads. Presumably as a further consequence of the smaller size of the neonatal foot pads many units reached a plateau, or saturation level of response over a narrower range of indentation than is seen in the adult (Mountcastle *et al.* 1966; Knibestöl, 1975). The form of stimulus–response relations showed considerable variability from unit to unit supporting the notion that there is no universal best-fitting function for these relations (Kruger & Kenton, 1973; Pubols & Pubols, 1976; Douglas *et al.* 1978). Nevertheless, the graded nature of the stimulus–response relations and relatively constant variability in responses over the response range imply that slowly adapting fibres in the neonate can convey discriminative information about the magnitude of steady skin indentation.

Dynamically sensitive tactile afferent fibres

In contrast to adult units it was not possible, using cutaneous vibration, to distinguish two classes of dynamically sensitive tactile afferents in the neonate. The neonatal fibres displayed a continuum of responses whether criteria of sensitivity or responsiveness were employed (see Figs. 4–6). The functional dichotomy in the adult

is attributable to two different receptor classes contributing to vibratory responsiveness, one class, believed to be associated with the intradermal Krause or Meissner corpuscles, being most sensitive to vibration at 20–50 Hz and the other, associated with Pacinian corpuscles, being most sensitive at 80–600 Hz (Hunt & McIntyre, 1960; Hunt, 1961; Sato, 1961; Lindblom & Lund, 1966; Jänig *et al.* 1968; Talbot *et al.* 1968; Lynn, 1969; Jänig, 1971; Iggo & Ogawa, 1977). As the sensitivity and responsiveness of neonatal units did not correspond exclusively with one or other of the adult classes we believe that afferent fibres from both these receptor classes contributed to our neonatal sample. Furthermore, as no conduction velocity differential exists for the two classes in the adult it seems improbable that a marked bias should have been introduced by our sampling procedures in the neonate. A further criterion, receptive field size, which in the adult aids in the differentiation of these two dynamically sensitive classes, was not diagnostic for the two groups as all units in our sample had small, circumscribed receptive fields. Assuming that Pacinian afferent fibres were included in the sample their smaller receptive fields compared with their adult counterparts may reflect their relative insensitivity to high frequency mechanical disturbances (> 100 Hz) for which the mechanical impedance of the tissues is least (Sherrick, 1953; Békésy, 1940). As fibres associated with the intradermal class of corpuscles in the adult are usually most sensitive to vibration at frequencies < 40 Hz (Talbot *et al.* 1968; Iggo & Ogawa, 1977) we believe that those neonatal units most sensitive to cutaneous vibration at frequencies of ≥ 80 Hz are almost certainly associated with developing Pacinian corpuscles. Furthermore, this view is reinforced by some preliminary observations on older (10–15 day old) animals in which two distinct classes of dynamically sensitive unit are identifiable, the Pacinian class displaying higher thresholds, lower 'best' frequencies (100–200 Hz) and a curtailed bandwidth of vibratory sensitivity in comparison with their adult counterparts

The curtailment of responsiveness at high vibration frequencies in neonatal units is not unexpected as afferent nerve fibres in the new-born kitten have a poorer capacity to discharge impulses at high frequency than do adult fibres (Ekholm, 1967). This restriction, because of a longer refractory period in the fibres (Beitel *et al.* 1977), would predictably curtail the band width of vibratory responsiveness for neonatal Pacinian units. However, as absolute thresholds for vibratory responsiveness were considerably higher in the neonatal fibres at all vibration frequencies above 50 Hz the receptors themselves are probably also functionally immature. This may also be reflected in the higher thresholds for 1 : 1 responses in the neonate and in the more limited capacity of neonatal units to respond above 80 Hz with a tightly phase-locked pattern of activity to vibration. These aspects of functional immaturity may be related to the morphological immaturity of the Pacinian corpuscles at the time of birth (Malinovský & Sommerová, 1972). The corpuscles have a poorly organized inner core and their outer capsule lacks both the full complement of lamellae and the distinct interlamella spaces. These structural features may account for their very much lower sensitivity to vibratory disturbances than adult Pacinian units. As very few neonatal units were most sensitive at vibratory frequencies of ≥ 80 Hz we suspect that even those most sensitive at lower frequencies may constitute a mixture of less mature Pacinian units as well as developing Krause units. Because of this it is difficult to determine whether neonatal Krause units are functionally mature in terms

of sensitivity and band width of responsiveness. Considerable variability in their 1 : 1 thresholds is apparent at 20–30 Hz, however, even in the adult animal great variability in thresholds is also apparent for identified units of the intradermal class (Talbot *et al.* 1968; Iggo & Ogawa, 1977).

Coding capacities of neonatal tactile afferents and behavioural implications for the new-born animal

Presumably tactile sensation associated with the distal glabrous skin depends in the neonate as in the adult (Jänig *et al.* 1968; Talbot *et al.* 1968; Iggo & Ogawa, 1977), on the three tactile receptor types in this region. The present demonstration that their functional capacities are more restricted than those of adult units implies that the receptors and afferent fibres impose constraints on the sensory capacities of the neonatal animal. These constraints apply to the different aspects of *detection*, *recognition* and *discrimination* of tactile stimuli. First, the subjective *detection* of a vibratory disturbance on the skin, which is sensed as a feeling of 'roughness', depends on the absolute thresholds of the dynamically sensitive tactile afferents (Talbot *et al.* 1968). As the absolute thresholds for responses of neonatal units to cutaneous vibration exceeded those of adult units at > 50 Hz and involved a discrepancy of an order of magnitude at ⩾ 200 Hz, the neonate must have a poorer capacity than the adult for detecting cutaneous vibratory disturbances, in particular those of a high frequency nature. Secondly, the ability to recognize the regular periodic nature of the vibratory stimulus which seems to depend on the appearance of a 1 : 1 pattern of impulse activity in the afferent fibres (Talbot *et al.* 1968) must also be deficient in the neonate because of the disparity between neonatal and adult units in the threshold vibration amplitude at which a 1 : 1 pattern of response is evoked. A further constraint for the neonate in its recognition of cutaneous vibratory disturbances is imposed by the narrow band width of vibration sensitivity of its afferent fibres. Even at high vibration amplitudes the upper limit of their vibratory sensitivity was restricted to approximately 300 Hz in contrast to adult afferent fibres which are sensitive up to ⩾ 800 Hz.

Additional restrictions on the tactile sensory capacity of the neonate are suggested by the quantitative analyses of impulse patterns evoked in response to vibration. The impulse activity is less tighly phase-locked to the vibratory stimulus than in adult units and shows a poorer reflection of the periodic nature of the vibratory stimulus. As a phase-locked, periodic pattern of impulse activity appears to be a requirement for vibratory frequency discrimination (Talbot *et al.* 1968; Mountcastle *et al.* 1969; LaMotte & Mountcastle, 1975; Bystrzycka *et al.* 1977; Rowe & Ferrington, 1977; Douglas *et al.* 1978; Bennett *et al.* 1980; Ferrington & Rowe, 1980) it appears that neonatal afferents encode less reliable information about vibratory frequency than their adult counterparts.

The restrictions revealed in the coding capacities of neonatal afferent fibres imply that the ability of the new-born animal to derive information about its tactile environment is limited by its peripheral tactile sensory mechanisms quite apart from any limitations which might be imposed at the central level. The stimulus form used in our studies, sinusoidal vibration, simulates in a controlled way the vibratory patterns set up in the skin in association with cutaneous texture and pattern recognition.

Tactile judgements about textures are ordinarily made by moving the sensing surface of the skin over the textured surface thereby translating a pattern of spatial periodicity in the surface to one of temporal periodicity in the afferent neural discharge arising from receptors at a given point on the skin. From the properties of the neonatal afferents it may be inferred that the new-born animal's ability to recognize finely textured surfaces and its ability to discriminate differences in the texture and patterns of surfaces would be more restricted than that of the adult. Our data therefore suggests, in contrast to earlier conclusions of Beitel *et al.* (1977), that the acquisition of behavioural skills based upon tactile inputs may, in part at least, be dependent upon the maturation of the peripheral sensory apparatus.

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