Table 3. Sedimentation of "gut-factor" from the supernatant of a homogenate centrifuged at 50,000 *g*-min. Starting material was 64 rectums. All centrifugations were for 10 minutes.

Centrifugation (No. of *g*)	Rectum equivalents in sediments	Cumulative recovery (%)
18,000	9.1	14.2
36,000	9.8	29.5
72,000	8.5	42.8
127,000	5.4	51.3
127,000	0	51.3

ments) in the heavy fraction. Differential centrifugation of the supernatant of a homogenate centrifuged at 50,000 *g*-min (Table 3) demonstrates that a centrifugal force in excess of 720,000 *g*-min is required for complete sedimentation of the light fraction. This fraction has been resolved further by means of density gradient centrifugation but has not yet been identified.

The active factor was dissociated from its particulate fraction with hypotonic solutions. Under these conditions, the "gut-factor" lost from the sedimentable fraction could not be detected in the soluble fraction and, presumably, had been inactivated. Other lines of evidence indicate that this inactivation proceeds enzymatically. Quantitative recovery of the "gut-factor" from the roach viscera was achieved only with extraction media that cause denaturation of enzymes (for example, acidic ethanol, 5 percent trichloroacetic acid, 1*N* acetic acid). Two attempts to extract foreguts with 0.1*N* acetic acid gave yields of 10 to 20 percent of the normal amount; water extractions recovered less than 10 percent of the activity. Presumably for the same reason (that is, enzymatic degradation) the yield of "gut-factor" from foreguts dissected from previously frozen roaches was only some 25 percent of normal. If such an enzyme exists and has physiological significance, then it represents a further analogy to the well-known cholinergic system.

On the basis of this preliminary evidence, it is proposed that the active factor functions as an excitatory neuromuscular transmitter substance in the longitudinal muscles of the proctodeum and probably in other visceral muscles of the cockroach.

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8. *Tenbroeck* type tissue grinder, clearance .010 to .015 cm, manufactured by Kontes Glass Company.
9. I thank J. A. Sholdice for technical assistance and Dr. W. Chefurka for criticism of the manuscript.
10. Contribution Number 328, Research Institute, Canada Agriculture, London.

3 October 1966

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Neural Basis of the Sense of Flutter-Vibration

Abstract. *Comparison of human detection thresholds for oscillatory movement of the skin of the hand with response properties of first-order myelinated mechanoreceptive afferents from the monkey's hand, activated in an identical stimulus pattern, indicates that flutter-vibration is a dual form of mechanical sensibility, served peripherally by two different sets of fibers.*

One approach to the study of the neural mechanisms in sensation is to combine two experimental designs which developed separately and which differ in aim and scope. Electrophysiological studies provide measures of the neural encoding of sensory stimuli as trains of impulses in first-order nerve fibers, and of the successive relay and transformation of this input across subcortical sensory nuclei, and through the first stage of processing in the cerebral cortex. They have so far revealed little of those central neural mechanisms thought to lead to subjective sensory experience. Psychophysics seeks lawful relations between those experiences and certain physical aspects of the stimuli which evoke them. These laws establish: (i) the dynamic range required of the input side of the system to account for its output; (ii) the information about the stimulus which must be preserved in the initial encoding to account for that in the output; and (iii) a basis for determining which of the codes available to the pulse-operated input system may be of functional significance for the intact, behaving organism. It is thought that a continued updated comparison of findings of the two types—psychophysical and neurophysiological—will allow the design of experiments aimed at elucidating the *terra incognita* of central mechanisms.

In such a combined study we have measured the threshold for the perception of oscillatory movement of the skin of human finger pads. In monkeys we recorded the nerve impulses set up by identical stimuli in first-order myelinated mechanoreceptive afferents innervating the skin and deep tissues of the hand. The results indicate that: (i) the sense of flutter-vibration can be accounted for only on the assumption that at least two quite different sets of afferents are responsible, each for a different limb of the human frequency function; (ii) the perception of oscillatory movement requires that a periodic signal appear in the input impulse trains, with probabilities in the range 0.7 to 0.9; and (iii) the differential recognition of frequency requires a central nervous system mechanism for the measurement of the length of the period.

The mechanical stimulator is described elsewhere (*1*); the stimulus pattern for both monkey and human experiments is shown in Fig. 1. The depth of skin indentation was held constant in any given series by monitoring and adjusting the point of contact. Stimulus rates were 6 per minute in human and 12 per minute in monkey experiments. Human observers were seated at a table, right arm mounted in padding, hand impressed palm upward in plasticine. The stimulator was movable in all dimensions; it was normal to the surface of the distal pad of the forefinger or middle finger. Contact point with skin was determined microscopically (16 ×). Thresholds for the perception of oscillatory movement were determined by the method of limits; they were sharp and reproducible. A white noise masked the faint hum of the oscillating probe.

Monkeys were anesthetized with sodium pentobarbital, their body and skin temperatures were regulated, and their arms were arranged for stimulation of the skin of the palm, as described for humans, and for microdissection of single myelinated nerve fibers from the median nerve (*2*). Sixteen or 32 stimulus trials were run for each frequency at each intensity tested. Nerve impulse records were occasionally stored on magnetic tape. Alternatively, inter-event time intervals were measured online (resolution, 125 μsec) by a LINC computer. Programs allowed production of a variety of graphic and statistical displays: more than 250 fibers were studied; for 87 of these the frequency-intensity relation was determined (from 2 to 300 cycles/sec). This was done in two ways. First, the probability that a period, equal to stimulus cycle-length, appeared in the population of impulse intervals was calculated for each intensity (for errors from 1 to 15 percent), and the resulting probability functions were plotted. Second, a storage oscilloscope was used to determine that intensity at which a nerve impulse, locked in phase, was thought by the observer to be produced by each cycle of the oscillating stimulus. Analysis showed that this visual criterion corresponded to p's = 0.7 to 0.9 that each impulse interval occurring during the oscillating stimulus would be within ± 5 percent of the stimulus cycle period. This level of certainty was accepted as the threshold for frequency following, and a number of "tuning curves" were determined by visual inspection.

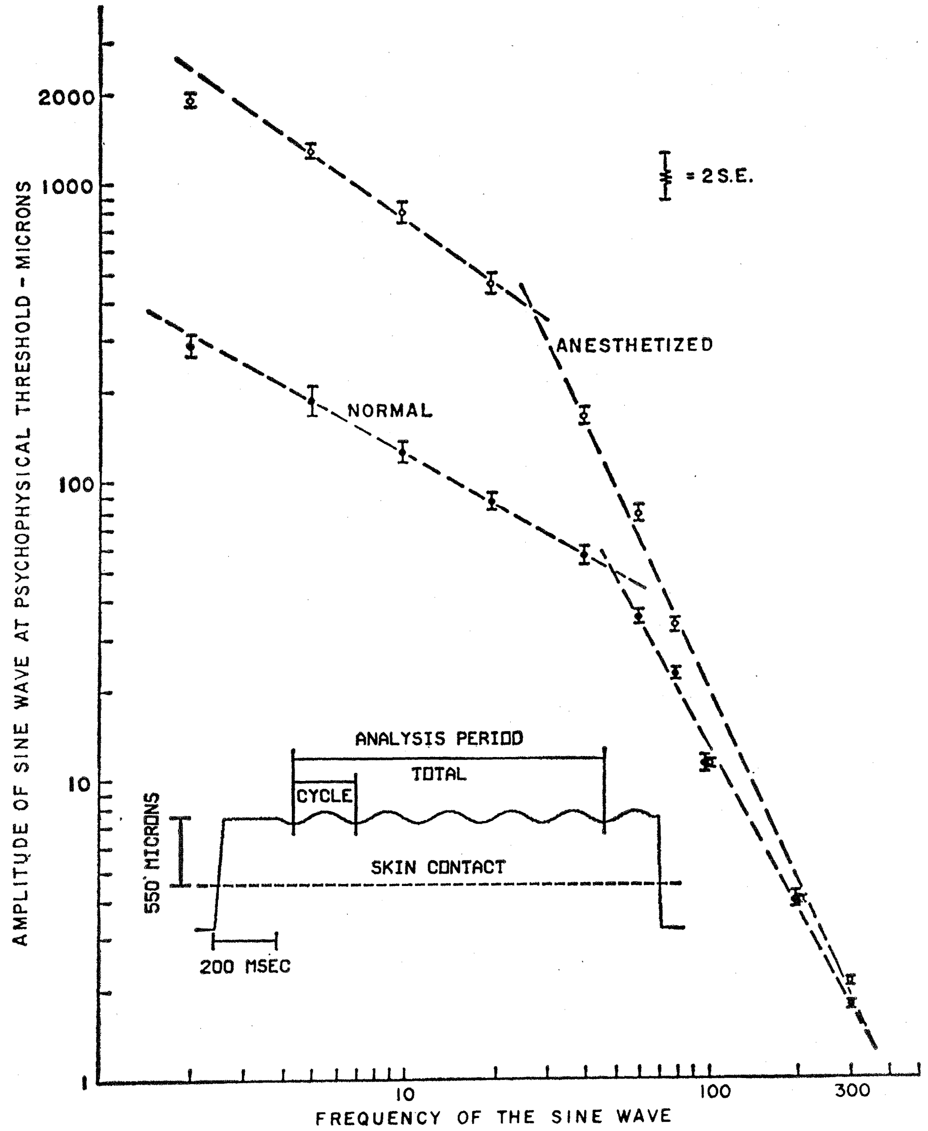

Fig. 1. The relation between the amplitude of a sine wave oscillatory movement of the skin, delivered as indicated in the inset, and the human threshold for the perception of movement, at a series of different frequencies. Points are means for ten adult observers. Local anesthesia of the stimulated site, on the thenar eminence of the hand, was accomplished by cocaine iontophoresis. Anesthesia elevated remarkably the threshold for perception in the low-frequency range (2 to 40 cycles/sec); the sensitivity which remains may be due to stimulus spread at high testing intensities. Sensitivity to high-frequency oscillation is little affected by skin anesthesia, which at the time of testing was complete for all cutaneous modalities. (Inset) Computer plot of the digitalized output of the displacement transducer measuring the position of the probe used for stimulating the skin. In its rest position the stimulus probe is more than 200 μ away from the skin. At the beginning of the stimulus pattern the probe is moved rapidly to a new position (rise-time 30 msec) which indents the skin approximately 570 μ. After a delay of 200 msec, sinusoidal stimulation begins with initial phase a withdrawal of the probe from the steady-state position. The duration of the sinusoidal stimulation is 930 msec (– 0, + 1 sine wave period). Total duration of the stimulus pattern is 1.4 seconds. The detailed analysis of the nerve impulse response patterns has been confined to the period shown. The frequency and amplitude of the sine wave can be varied independently.

For each fiber the 2-mm probe tip was oriented to cover symmetrically the cutaneous receptive field, which for most such fields was less than 2 mm in diameter. For Pacinian afferents the skin site was chosen at which a high-frequency stimulus entrained the discharge of the fiber, at lowest intensity.

The threshold curve for the perception of vibratory motion delivered to the human hand is the lower one of Fig. 1. The function is known to rise again for frequencies above 300 cycles/sec (*3*). The mean threshold values are best fitted by two straight lines, in log-log coordinates, intersecting at about 40 to 50 cycles/sec. When the skin is locally anesthetized by cocaine iontophoresis (*4*) there is a manyfold elevation in threshold for frequencies of 5 to 40 cycles/sec, while those for higher frequencies are scarcely changed (see upper curve, Fig. 1). This suggests that the perception of oscillatory movement depends upon two sets of primary afferents, one innervating the skin and the other the deep tissues of the hand, accounting for the low- and high-frequency limbs of the human function, respectively. Furthermore, our subjects reported that at low frequencies the sensation evoked by a suprathreshold stimulus resembled a faint flutter localized to the skin at the site stimulated. In contrast, as the stimulus frequency increased beyond 60 cycles/sec, at the same strengths relative to threshold, the sensation changed to one of vibratory hum, moved deep within the hand, and spread so that it could not be localized accurately.

Of the myelinated afferents innervating the hand, there are three that might signal oscillatory movement of the skin surface. The first terminates in the dermal ridges. It responds to a step indentation of the skin with a high frequency discharge that declines within 100 to 200 msec to a more or less steady rate, which persists for stimulus durations of at least several seconds. This rate of discharge is a linear function of the degree of skin indentation (*2*). When a sine wave oscillation of low freqeuncy is added to the step indentation, the steady discharge is rhythmically entrained by the stimulus. However, it does so at low intensities only for frequencies below 10 cycles/sec. At higher frequencies the threshold for frequency modulation rises steeply above the human thresholds. For this reason we conclude that this afferent does not contribute to the human sense of flutter-vibration.

The second mechanoreceptive afferent to be considered also innervates the dermal ridges. At low intensities frequency following may be irregular, or locked in submultiples of the stimulus frequency. Slight intensity increments produce one-to-one phase locking. This point has been determined over the sensitive frequency range to construct a tuning curve; such curves have been determined for 67 cutaneous movement detectors. Although 32 of these show minima below 30 μ, at their best frequencies, the remaining 35 are distributed continuously as regards minimal tuning points over the intensive continuum, with minima up to 100 μ, or more. Eight of the more sensitive group are shown in Fig. 2. Their low-frequency limbs blanket the low-frequency end of the human threshold function. Thus this class of afferents can account for the human sensitivity in the range of 2 to 40 cycles/sec, and no other afferent innervating the skin of the hand has been discovered which could do so.

Further increases in intensity above threshold will in many fibers produce a disruption of the periodic pattern of discharge. These changes in the internal structure of the neural impulse trains are thought to account for the observation of von Bekesy (*5*) that for low frequencies the human perception of the pitch of skin vibrations first rises and then falls as intensity increases above threshold (*6*).

The third mechanoreceptive myelinated afferent innervating the hand, thought to play a role in the sense of flutter-vibration, differs remarkably from that just described. Its terminals lie in the deep tissue. Although it does not innervate the skin, it is so sensitive to mechanical deformation that it is discharged by minute puffs of air delivered to the skin surface. Indeed, at optimal frequencies of 150 to 250 cycles/sec fibers of this type may follow stimulus frequency perfectly when the oscillatory movement is only a few microns in amplitude. It is thought that these afferents terminate peripherally in Pacinian corpuscles, for their properties resemble those of afferents positively identified as terminating in Pacinian corpuscles and studied by Hunt and McIntyre, Sato, and other investigators (*7*). As stimulus intensity is increased, fibers of this type may initially discharge irregularly, or at some submultiple of the stimulus frequency, as do the cutaneous movement detectors described above. With slight increments, however, the point of phase-locking is quickly reached, as shown by the records of Fig. 3. Further increases in intensity produce double firing for some stimulus cycles, and irregularities of following throughout the train. This appears to account for the observation (*5*) that at high frequencies the human perception of vibratory pitch, produced by a given frequency of skin oscillation, first rises slightly and then falls precipitously, as intensity increases through and beyond the threshold for the perception of movement.

Tuning points were measured for 20 Pacinian afferents. These are given in Fig. 2, which illustrates the fact that they

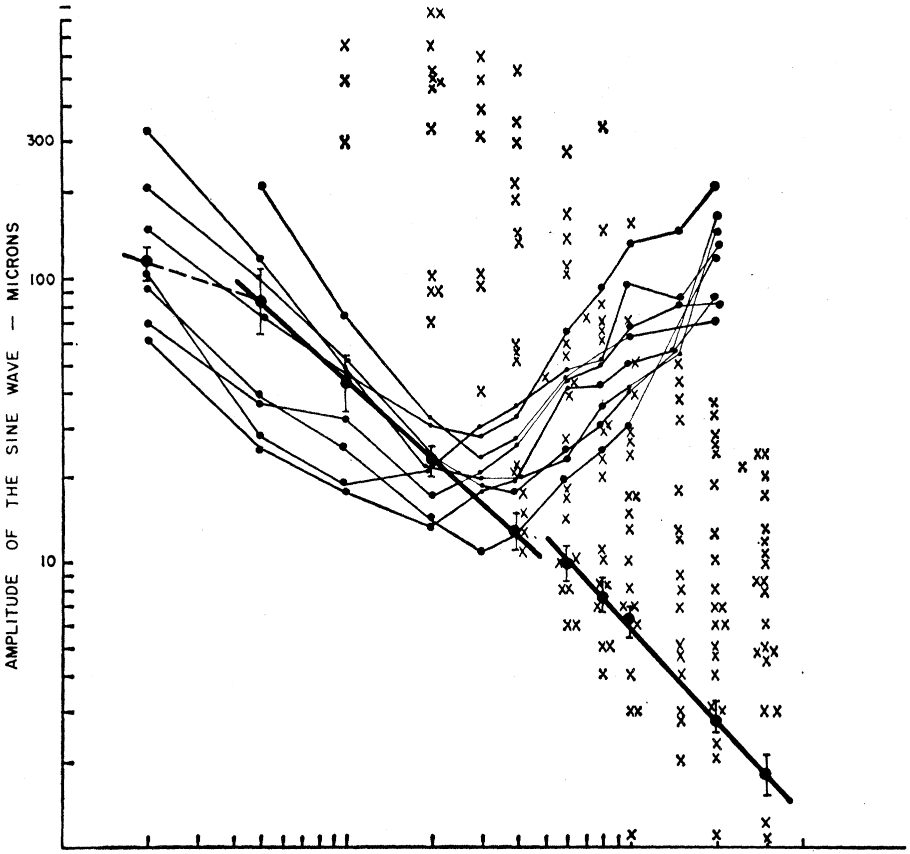

Fig. 2. The heavy lines plot the human threshold for the perception of sine wave oscillation of the skin when the stimulus is delivered to the volar surface of the distal phalanx of the forefinger, in the manner indicated in Fig. 1. Points are means for nine adult observers. The break between the two limbs of the threshold curve is much less acute than it is when stimuli are delivered to the thenar eminence, as shown by the lower curve of Fig. 3. Lighter lines plot the threshold tuning curves for a number of myelinated cutaneous afferent fibers of the median nerve which innervated the glabrous skin of the monkey hand. Tuning points on these latter curves were determined as described in the text. These cutaneous "movement detectors" are the only myelinated afferents from the skin of the hand which by their sensitivity to vibratory motion can account for the low-frequency limb of the human threshold curve. Crosses plot tuning points for a number of myelinated afferents ending peripherally in Pacinian corpuscles. When points for any one fiber are connected they form tuning curves approximately parallel to the high-frequency limb of the human tuning curve, for which only they can account.

Table 1. Percentages of the two populations of afferents which will be locked in phase (with a certainty described in the text) at stimulus intensities at which humans perceive flutter-vibration.

Afferent population	Frequency (cycles/sec)										
	2	5	10	20	40	60	80	100	150	200	300
Cutanous movement detectors ($n = 67$)	10	27	27	34	7	3	0	0	0	0	0
Pacinian corpuscles ($n = 20$)	0	0	0	0	10	30	40	25	20	15	10

overlap the high-frequency limb of the human threshold curve, but cannot account for its lower end, the range of flutter sensation produced by rhythmic activity in cutaneous movement detectors. No other mechanoreceptive afferent innervating the tissues of the hand has been discovered which can account for the sense of vibration at frequencies from 60 to 300 cycles/sec.

Thus the sense of flutter-vibration exhibits a dual nature, both in the subjective estimate of the nature of the sensation at different frequencies, and in the first-order afferents which by their dynamic properties might serve it. The coincidence of the two sets of tuning curves with the two limbs of the human threshold curve suggests that it is the presence of a *periodic* signal in the input trains of nerve impulses, at a certain probability, which evokes the perception of movement. Such a statement implies that the periodic signal is preserved through transmission across subcortical nuclei of the somatic afferent system, as indeed has been found by Perl *et al.* (*8*) to be the case for second-order cells of the dorsal column nuclei linked to Pacinian afferents. A further requirement is that the periodic signal appear in the activity of cortical neurons driven by oscillatory stimuli, a subject now under study. While it is possible that a sensation of oscillatory movement might be evoked in the attentive individual by such a periodic signal in a single afferent fiber, a more conservative position is that at least some fraction of the available population is active periodically, at the human threshold. What that fraction may be is indicated by Table 1.

The near coincidence of the minimal tuning points for the cutaneous movement detectors (30 ± S.E. = 1.4 cycles/sec) and the parallel courses of their tuning curves indicate that discrimination between different frequencies cannot be made in terms of which fibers are active at different frequencies, in contrast to the perception of auditory pitch, and this is true also for the high-frequency detectors thought to terminate in Pacinian corpuscles. Alternatively, freqency discrimination might depend upon the total number of impulses occurring over any short time of inspection of the stimulus. This seems altogether unlikely, however, for given the probabilities allowed, the total number of impulses may be identical for each of two frequencies between which discrimination can readily be made by human observers (*3*). A more attractive hypothesis now open for study is that frequency discrimination is made by a central neural mechanism capable of measuring the lengths of the dominant periodic intervals in the trains of impulses evoked by the two stimulus frequencies compared.

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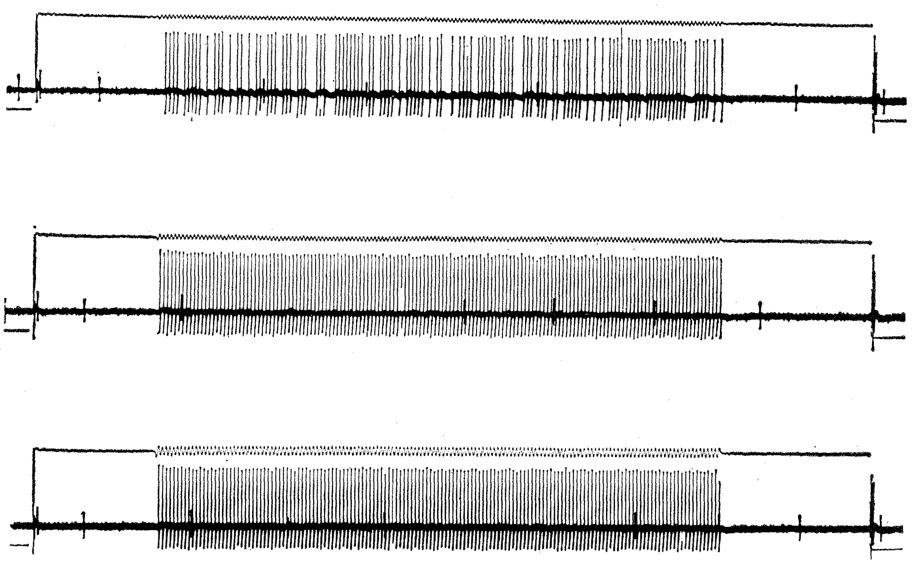

Fig. 3. Recordings of the electrical signs of nerve impulses in a myelinated afferent nerve fiber, isolated for study by microdissection from the median nerve of a monkey. The fiber terminated in the deep tissue of the thenar eminence, and by its dynamic properties was thought to terminate in a Pacinian corpuscle; it was not a muscle afferent. Stimulus probe placed at most sensitive spot on skin of thenar eminence. Stimulating arrangement as for Fig. 1. Frequency of the superimposed sine wave 150 cycles/sec, and its amplitude 16 μ, 19 μ, and 39 μ, for the upper, middle, and lower pairs of traces, respectively. Analysis by machine showed phase-locking of impulses to stimulus sinusoids, even for the upper trace where many sinusoids produce no impulse. Both phase-locking and frequency following are perfect for the middle and lower records; the one dropped spike in each is due to film emulsion defect, not failure of fiber to follow. Many afferents thought to be linked to Pacinian corpuscles of the deep tissues follow high-frequency oscillatory movement of the skin perfectly at much lower amplitudes than did the one illustrated. At best frequencies they frequently do so for amplitudes of the order of 1 μ.

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18 October 1966