diffraction pattern, indicating that it is an amorphous substance; its mineralogical composition remains an enigma.

The calcium oxalate hydrate, weddellite, is a common constituent of urinary calculi in man and other mammals and has been with question identified in waste products of plants (*5*). Bannister and Hey (*6*) also identified weddellite crystals in sediments of the Weddell Sea and concluded from the perfectly developed form of the crystals and their lack of mechanical abrasion that they were an authigenic mineral constituent of the sediments. The bottom deposits containing the weddellite crystals were stored in containers originally used for provisions on shipboard and mostly were allowed to dry before being analyzed for their mineral content. Hence, questions have been raised concerning the origin of the crystals (*7*). Recovery of the weddellite crystals from bottom sediments geographically close to the Antarctic stations where the original samples were found, and the fact that this mineral has not been reported from marine deposits elsewhere, favor the original interpretations that it is an authigenic mineral of the deepwater sediments in the Weddell Sea.

The data presented here extend the range of weddellite precipitation in biologic systems from the mammals to an invertebrate species. The localization of the mineral in discrete microarchitectural units, at homologous sites in the gizzard plates of the gastropod, indicates that for the first time we are dealing with a normal biochemical precipitate. This is in contrast to the occurrence of weddellite in renal calculi of mammals, which are pathologic mineral secretions. The weddellite crystals of *Scaphander cylindrellus* are initially enclosed in an organic matrix. Mechanical wear of the weddellite-bearing surface of the plates brings the crystals in direct contact with the gizzard contents. The gizzard plates of the *Scaphander* species serve as mechanical crushers of the exoskeletons of invertebrate prey, which consist of calcite and aragonite. The hardness of the weddellite is about 4 (*8*); whereas that of the mineral phases of the carbonate found in the skeletons of the prey is 3 to 3.5. Hence, the tipping of the crushing surface of the gizzard plates in *S. cylindrellus* with weddellite is of mechanical advantage in its functional use by the organism.

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4. The sediment sample containing the weddellite crystals was preserved in a solution of 70 percent alcohol.
5. The crystals are uniaxial positive, $\omega = 1.5237 \pm 0.0006$, $\epsilon = 1.5434 \pm 0.0012$. The *d*-spacings and line intensities of the powder diffraction patterns for the crystals match those listed for the mineral weddellite in the American Society for Testing Materials catalog.
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6 August 1968; revised 30 September 1968

Neuronal Coding by Cortical Cells of the Frequency of Oscillating Peripheral Stimuli

Abstract. *One class of neurons in the somatic sensory cortex of unanesthetized monkeys is rhythmically entrained by sinusoidal mechanical stimulation of the skin of the hand at low frequencies. A second class, which is linked to Pacinian afferents, increases its rate of discharge in response to high-frequency peripheral stimuli but is not entrained. The vibratory sense is served by two distinct classes of cortical cells. The code for the group sensitive to low-frequency stimuli is the temporal order of impulses; for the high-frequency group the code is the labeled line.*

One objective in the study of central neural mechanisms in sensation and perception is the elucidation of the patterns of activity of central neurons in relation to peripheral stimuli that vary with time; another is to learn how certain properties of the latter are coded in the discharge patterns of the neurons. In somesthesis this correlation is based on the neural activity evoked throughout the somatic afferent system by sine-wave oscillatory stimulation of the skin. The idea is that the perception of vibration must be related to neural mechanisms at a high level of the nervous system which provide cues to the location, frequency, and intensity of the vibratory stimuli. Identical vibratory stimuli have been applied to the hands of humans for psychophysical studies and to those of monkeys for the study of activity in single peripheral nerve fibers (*1*). Two sets of fibers signal with precision the frequency of vibratory stimuli: the quickly adapting afferents which terminate in the dermal ridges of the glabrous skin and those which originate in the subcutaneous Pacinian corpuscles. At intensities comparable to human thresholds, low-frequency stimuli (5 to 50 hz) drive the cutaneous movement detectors, and high-frequency stimuli (50 to 400 hz) drive the Pacinian afferents. At the threshold tuning points the frequencies of the stimuli are replicated by periodic discharges in the first-order afferent fibers.

We here describe the way in which neurons of the somatic sensory area (the postcentral gyrus) of the cerebral cortex respond to vibratory stimuli in the unanesthetized monkey (*Macaca mulatta*). With the animal under pentobarbital anesthesia the skull was opened, and a Lucite chamber was fixed above the target area. The scalp wound was sutured, and the animal was allowed to recover overnight in a primate chair. The next morning gallamine triethiodide was injected intraperitoneally, and respiration was maintained by means of a face mask. The tissues about the trachea were infiltrated with a permanent local anesthetic, Efocain. An endotracheal tube was inserted and connected to a positive-pressure respirator. Expired CO_2 was kept at about 4 percent. The head was fixed by gripping the Lucite chamber; the animals rested on soft padding. They appeared to be free of pain, for they alternated between sleep and wakefulness, as indicated by pupillary and electroencephalographic signs. The dura beneath the skull opening within the chamber was excised, and the area of the postcentral gyrus associated with the hand was detailed by mapping of the evoked potential. Tungsten microelectrodes were inserted into the cortex by way of the hydraulically sealed chamber (*2*). An electronically controlled skin-displacement stimulator delivered sine-wave stimuli, of various frequencies and amplitudes, superimposed on a step indentation of the skin of 560 μm. A LINC computer was used for stimulation, data collection, and data analysis (*1*).

More than 1000 neurons in Brodmann's areas 3 and 1 were studied,

comprising several classes; two classes are described here. Those of one class (600 neurons) adapt quickly to steady skin indentations and are readily driven by low-frequency sinusoids (5 to 50 hz) at intensities comparable to the human perception of flutter. These cells are thought to receive afferent input largely restricted to that of the rapidly adapting peripheral fibers originating in the glabrous skin of the hand. The other class of relatively uncommon neurons (40 neurons) is activated only by peripheral stimuli containing high-frequency components (50 to 400 hz) but at very low intensities. These neurons must receive input originating in Pacinian afferents (*1*). In contrast to the peripheral fibers, central neurons display no sharply defined tuning points, as the intensities of sinusoids increase, although significant changes in neuronal discharge patterns do occur at intensities comparable to human thresholds. Moreover, the coding mechanisms appear to differ for the two sets of central neurons.

For the quickly adapting cortical neurons that are sensitive to low frequency, overall rates of discharge increase gradually as the amplitudes of stimulating sinusoids increase; the patterns of growth and the final rates differ little for stimulating frequencies over the range of 10 to 50 hz. However, within this range humans discriminate most precisely between frequencies; for example, the discriminable increment, ΔF, is less than 5 hz at a base of 25 hz. It therefore appeared useful to explore the possibility that the cortical signal of the stimulating frequency is contained in the temporal order of impulses, and that this order might differ significantly between trains of impulses whose overall rates differ very little.

Initial representatives of the responses of a typical cortical neuron of this type to a 40-hz sinusoid, at each of several amplitudes, are given in Fig. 1. Cyclic entrainment occurs even at 12 μm, the weakest intensity which affected the neuron. This entrainment is more narrowly restricted in time with stronger stimuli. Using one of a number of arbitrary criteria (for example, 80 percent of the discharges occurring in 50 percent of the cycle), one may establish tuning curves for cortical neurons. Those for the five most sensitive neurons of the 20 studied are plotted in Fig. 2, superimposed on the human threshold function for the sense of flutter-vibration. We infer that a cyclic entrainment of about this degree is required in some liminal but unknown number of cortical

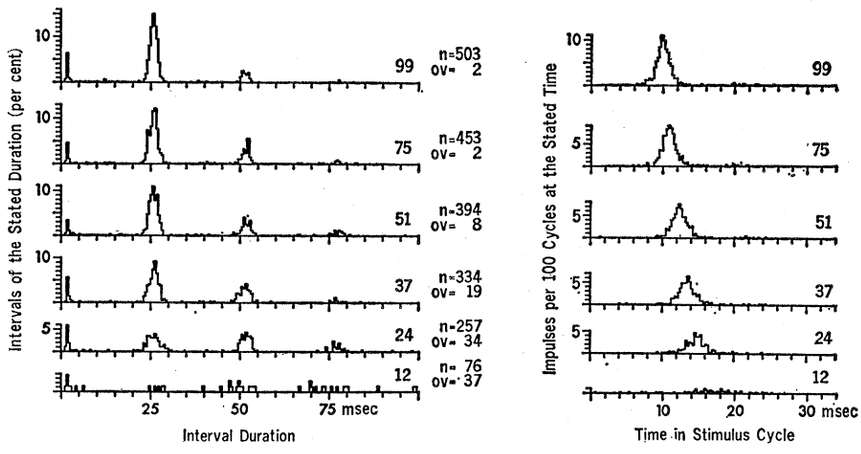

Fig. 1. Interval (left) and cycle (right) histograms for responses of a quickly adapting cortical neuron to low-frequency stimulation delivered to the glabrous skin of the hand at 40 hz (sine wave period of 25 msec). Neuron is located in area 3 of contralateral postcentral gyrus of unanesthetized monkey. The amplitude of the sine wave in micrometers is noted at the right of each histogram. The interval histograms present the distribution of the intervals between successive nerve impulses; the cycle histograms present the distribution of time intervals between the onset of each sine-wave cycle and nerve impulses occurring during the cycle. Each histogram includes data collected during 16 separate periods (1 second) of sinusoidal stimulation. Bin size is 0.5 msec for interval and 0.25 msec for cycle histograms. The value *n* is the number of intervals analyzed; *ov* (overflow) is the number of intervals longer than 100 msec.

neurons before a sinusoidal stimulus is recognized by a human subject as oscillating rather than steady, and before its frequency is identified.

The histograms to the left in Fig. 1 represent the distribution of the time intervals between impulses in the same responses used to construct the adjacent cycle histograms. Even when cyclic entrainment is very strong, time intervals of one, two, and three times the cycle length (here 25 msec) occur. The appearance of some very short intervals indicates that on some cycles more than one impulse was discharged. In contrast to the related peripheral nerve fibers (*1*), gradually increasing strength of stimulus did not abruptly (at a tuning point) produce perfect entrainment with one impulse phase-locked to each cycle of the stimulus. What position along the gradient of change matches the peripheral tuning point, which correlates so closely with the human threshold (*1*)? One approach to this problem is to determine the likelihood that the cycle length, within some arbitrarily chosen limits of error (for example, $\pm$ 20 percent) will be represented in the temporal order of neuronal impulses. We have determined this probability for cortical neurons that adapt quickly from histograms of the first-order intervals; the averaged results for 13 neurons are given in Fig. 3. Neurons of this class are tuned differentially by stimuli in the low-frequency range, with an average best frequency of 40 hz. The curve at

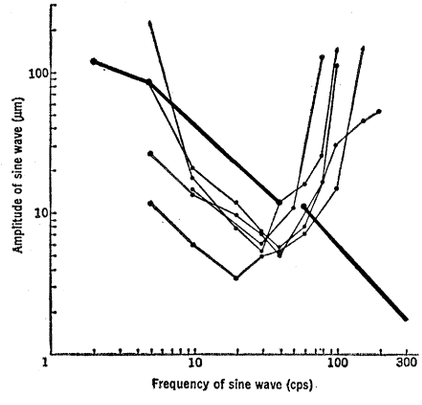

Fig. 2 (left). The heavy line plots the human threshold function for the sense of flutter-vibration (*1*). The amplitudes of sinusoidal mechanical stimuli at which human subjects recognize them as oscillating rather than as steady are plotted in micrometers on the ordinate as a function of stimulus frequency on the abscissa. The function is known to turn upward again for frequencies above 300 hz. The thinner lines are tuning curves for cortical neurons of the postcentral gyrus studied in unanesthetized monkeys. Each point is the amplitude of the stimulating sinusoid, delivered to the skin of the monkey's hand, that is required to produce cyclic entrainment at an arbitrarily chosen level; in this case the requirement was that 80 percent of the impulses occur in 50 percent of the stimulus cycle.

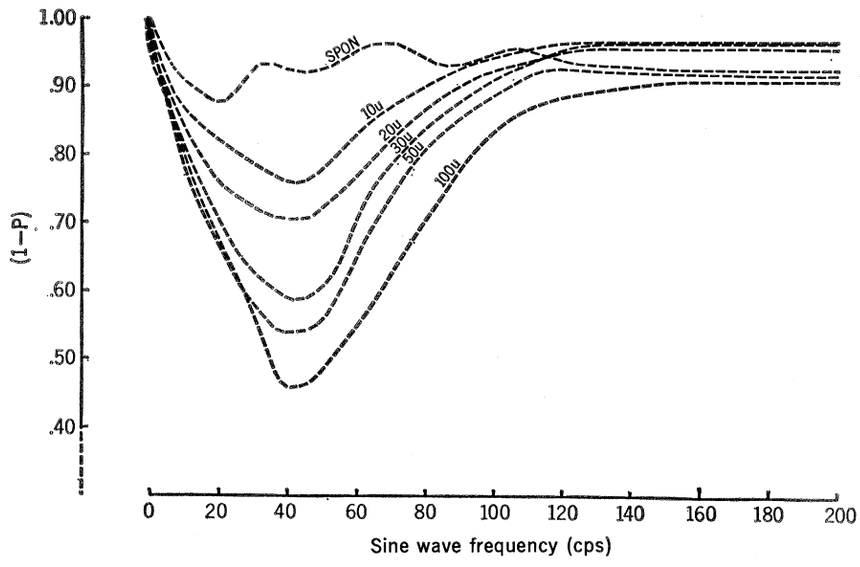

Fig. 3. Tuning curves for neurons in the postcentral gyrus of unanesthetized monkeys activated by sinusoidal mechanical stimulation of the glabrous skin of the contralateral hand. The curves are the averaged results for 13 neurons over a number of stimulus intensities. For the population of time intervals between impulses in the trains of responses to each stimulus (16 trials, each 1 sec in duration) the probability (P) was calculated that the stimulus cycle length, ± 20 percent, appeared in the interval distribution. These values were averaged for each point for the 13 neurons and plotted inversely. These cortical neurons are differentially sensitive to low frequencies of stimulation with a best frequency of about 40 hz. The curve for 10 μm shows that a significant signal of 40 hz appears in the impulse trains evoked by a 40-hz stimulus; 10 μm is approximately the human threshold for the detection of oscillatory movement of the skin of the hand at 40 hz.

a stimulus amplitude of 10 μm shows that a significant signal of stimulus frequency appears in the cortical neuronal discharge at 40 hz, at a strength equal to the human threshold for the perception of oscillatory movement at that frequency.

The cortical neurons thought to be related to peripheral Pacinian afferents behave quite differently. They are differentially sensitive to frequencies of 100 to 400 hz and may be driven to maximum and very high rates of discharge by such stimuli having an amplitude of only 1 to 5 μm, these intensity levels being comparable to human thresholds at these frequencies. Analysis of the temporal ordering of impulse discharges in these responses, however, reveals no significant signal of stimulus cycle length. Cyclic entrainment appears only at stimulus intensities two or more orders of magnitude above human thresholds. It seems possible to conclude that temporal ordering of impulses is not the cortical neuronal code for high-frequency vibration (*3*).

A combination of these results with earlier data on the human sense of flutter-vibration and the responses of peripheral nerve fibers to sinusoidal stimulation of the skin of the hand suggests that flutter-vibration is a dual sensibility, served by two distinct sets of peripheral fibers linked to two equally distinct sets of cortical neurons. Furthermore, low-frequency flutter is a derived form of tactile sensibility, depending for its unique character upon the temporal pattern of the stimuli and coded in the cortex by the temporal order of neuronal discharge. High-frequency vibration has the properties of a true modality, signaled by labeled lines both peripherally and centrally, regardless of the temporal order of discharge.

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27 September 1968

Erythropoiesis in the Rat: Differential Rates of DNA Synthesis and Cell Proliferation

Abstract. *Direct in vivo estimates of DNA synthesis time in early and late erythroblasts were obtained by using the H^3- and C^{14}-thymidine double-labeling technique. A double-emulsion autoradiographic procedure was used to resolve the two isotopes. Early erythroblasts were found to proliferate at a rate about five times that of late cells. This results primarily from a shorter mean DNA synthesis time in early cells (2.5 hours) than in late cells (6.5 hours).*

The mean duration of DNA synthesis time (T_s) is a basic component of any model of cell proliferation kinetics (*1*). Since little evidence is available to the contrary (*2*), the assumption is usually made that average T_s exhibits little variation within homogeneous cell populations. However, differences in the length of T_s have been suggested (*3*, *4*) for cell populations consisting of both less mature and more mature elements. Thus, the validity of assuming constancy of T_s throughout all maturational stages of a given cell lineage must be reexamined. This report presents results of a study of the proliferative characteristics of less mature and more mature rat erythroblasts, made by using double labeling–double emulsion autoradiography.

A more complete explanation of the techniques employed appears elsewhere (*5*, *6*). The double-labeling technique consists of administering in vivo two DNA pulse labels. The first label, H^3-thymidine (*7*), is followed 1 hour later by C^{14}-thymidine (*8*). Fifteen minutes after administering the second label the animals are killed. During the 1-hour time interval (T_a) between the administration of labels, a segment of cells in the DNA synthesis phase (S phase) leave this phase of the cell cycle labeled only with H^3 and are thus unable to incorporate the second label. Cells entering and remaining in the S phase incorporate the second label (C^{14}-thymidine) and are thereby distinguished from the first segment of H^3-labeled cells. The ratio of the number of cells labeled with H^3 alone (N_{H^3}) to all other labeled cells ($N_{C^{14}+H^3}$) yields an average estimate of T_s, since, $N_{H^3}/N_{C^{14}} = T_a/T_s$ (*9*). The labeling index can also be obtained from these preparations by determining the ratio of C^{14}-labeled cells to all cells in the population.