Reports

Auditory Information from Subcortical Electrical Stimulation in Cats

Abstract. Animals trained to respond to sound stimuli were found to perform the learned response when they were electrically stimulated through electrodes chronically implanted in subcortical structures of the auditory pathway. Other animals trained to respond to electrical stimulation of subcortical auditory structures showed differential transfer effects depending on the positions of the stimulating electrodes.

It has long been known that auditory sensations may be produced in human subjects by direct electrical stimulation in or near auditory areas of the cerebral cortex (*1*). The sensory effects produced—buzzing, knocking, booming, and so on—have been crude, perhaps because of the difficulty of entering a functional system at the level of its greatest organizational complexity. Some evidence has also come from conditioning studies on animals, in which direct electrical stimulation of areas of the cerebral cortex has been successfully used as the conditioned stimulus (*2*). Surprisingly enough, no attempt has been made up to the present (*3*) to inject behaviorally meaningful information into lower, presumably less complex, levels of the auditory system where there might be more promise of controlling the qualities of the sensations produced.

As a first step in the investigation of this problem, ten cats were prepared with bipolar electrodes chronically implanted at different levels of the central auditory pathway. The animals were divided into two groups. Group 1 consisted of four animals which were trained to avoid mild shock to the foot by flexing the left hind leg when presented with an auditory warning signal. After training, these animals were tested to see whether the conditioned response could be elicited by direct stimulation of the inferior colliculus or the cochlear nucleus. Group 2 was made up of six animals which were conditioned to respond to direct stimulation at different subcortical levels of the auditory or visual pathways. The electrodes were placed in the region of the inferior colliculus in three animals, in the medial geniculate body of one, in the auditory radiations in one, and in the optic tract in one. The first three animals were trained to make a leg flexion response; the other three were trained to avoid shock by crossing from one compartment to the other of a double-grill box when given a warning burst of direct stimulation. After initial training, the animals of group 2 were tested for transfer of the learned habit to stimulation of leads other than those used in conditioning and to presentation of different sounds over a loudspeaker. Electrode positions were confirmed electrophysiologically and anatomically.

All conditioning and test procedures were carried out with the experimental animal isolated in a sound-treated room. Electrical stimuli were delivered from a Grass S-IV stimulator and consisted of trains of monophasic or biphasic square waves. Stimulation current flow to the animal was monitored oscillographically.

During tests of transfer of the learned responses to novel stimuli, animals were never punished for failure to respond to the new stimuli.

The results obtained with group 1 were unequivocal. After being trained to respond to sound stimuli, all animals of this group immediately gave the conditioned response when electrically stimulated in the region of the inferior colliculus. The ratios of correct responses to numbers of test trials for these animals were 42/56, 33/53, 12/34, and 7/12. In contrast to these results, stimulation of the cochlear nucleus was much less effective: two of the animals never responded at all to such stimulation and the other two showed signs of early extinction of the response. At all but the lowest stimulation currents given at the cochlear nucleus, unconditioned motor responses were elicited, perhaps through inadvertent stimulation of vestibular fibers. These unconditioned responses seemed to interfere with expression of the conditioned response.

All animals of group 2 were successfully trained to respond to direct stimulation of selected electrodes positioned in subcortical levels of either the auditory or visual system. However, transfer results for these animals showed important differences. Of the three animals trained with inferior colliculus stimulation, only one responded significantly to test sound stimuli, giving 15 conditioned responses (CR's) to 25 test trials. The electrodes used for stimulating this animal during conditioning were located dorsolaterally in the colliculus and were across brachium fibers; there was no transfer of the response when stimulation was applied through electrodes centrally placed in the main nucleus of the inferior colliculus or in the tegmentum. All electrodes of the other two animals trained with colliculus stimulation were in the main body of the nucleus, and stimulation through any electrode was effective in eliciting the learned response.

The animal trained to respond to stimulation of the auditory radiations showed transfer of the response to stimulation of auditory cortex (5 CR's in six trials) and to stimulation with single acoustic clicks (8 CR's in 11 trials) but did not respond to acoustic stimuli with tonal quality (0 CR's in five trials).

One animal of group 2, after training with medial geniculate stimulation, showed a very low response level to both sound stimulation (1 CR in nine trials) and lateral geniculate stimulation (1 CR in nine trials).

The final animal of group 2, after being trained to respond to direct stimulation of the optic tract, did not show transfer of the response to direct stimulation of the medial geniculate body (0 CR's in eight trials). It also failed to respond to sound stimuli (0 CR's in three trials).

Bilateral ablation of auditory areas of the cortex produced a severe postoperative deficit in three of the four animals tested; two were trained to direct stimulation and one to sound.

The evidence presented here strongly supports the conclusion that auditory sensations may be produced by direct electrical stimulation at subcortical levels of the central acoustic pathway. The results suggest that greater control of the quality of the sensations pro-

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

duced may be obtained by subcortical than by cortical stimulation. Especially suggestive are the data showing behavioral differentiation of stimulation sites in mesencephalic auditory system structures, particularly as there is some electrophysiological evidence for tonotopic organization within the inferior colliculus (*4*). It is hoped that the combined behavioral and stimulation techniques of this preliminary study will prove to be powerful tools for the investigation of theories of the central code of the auditory system (*5*) and perhaps of the visual system as well (*6, 7*).

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References and Notes

1. See W. Penfield and T. Rasmussen [*The Cerebral Cortex of Man* (Macmillan, New York, 1950)] for presentation of data on sensory effects from cortical stimulation in a long series of human subjects.
2. R. B. Loucks [*J. Comp. Psychol.* **25**, 315 (1938)] reported conditioning leg flexion or salivation in dogs with direct stimulation of "visual" cortex as the conditioned stimulus; R. W. Doty and L. T. Rutledge [*J. Neurophysiol.* **22**, 428 (1959)] found that cats trained in leg flexion with cortical direct stimulation as the cue were more easily trained to respond to photic or sound stimuli than naive animals, and vice versa; R. W. Doty and C. Giurgea [*The Physiologist* **1**, 17 (1958)] conditioned leg flexion in dogs by using direct stimulation of cortical points as the conditioned and unconditioned stimuli.
3. A preliminary report of this work was presented by W. D. Neff, P. C. Nieder, and R. E. Oesterreich [*Federation Proc.* **18**, 112 (1959)].
4. W. R. Thurlow, N. B. Gross, E. H. Kemp, K. Lowy, *J. Neurophysiol.* **14**, 289 (1951).
5. For a presentation of theoretical work on this subject see J. C. R. Licklider [in *Information Theory*, C. Cherry, Ed. (Butterworth, London, 1956)].
6. A suggestion for central coding of visual pattern information has been outlined by P. Nieder [*Science* **131**, 934 (1960)].
7. This research was supported in whole or in part by the U.S. Air Force under contracts No. AF 49(638)-925 and No. AF 19(604)-5526 monitored by the Air Force Office of Scientific Research and the Operational Applications Office of the Air Force Command and Control Development Division.
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Water and Myotonia in Goats

Our work on myotonia, in goats, was frustrated by the great variation in the gravity of the symptoms in one and the same animal. Our efforts to correlate these variations with meteorological changes, food, exercise, or other factors failed. Lately, we have found indication that the gravity of the symptoms depended on the water intake. To test this point, three myotonic goats were kept on mixed dry food, only very limited grazing being allowed. Water was withheld for periods of 3 to 7 days, these periods being followed by similar periods in which water was offered ad libitum. We found that on withholding water, the myotonic symptoms disappeared altogether within 3 days to return with full gravity within 2 to 3 days when water was given. These results are illustrated in Fig. 1. The abscissa shows days and the ordinate, the gravity of symptoms on an arbitrary scale (the intensity of stiffness was marked by one to five crosses, and the number of crosses was multiplied by the duration of stiffness in seconds). Upward arrows mean withholding water, downward arrows mean giving water (*1*).

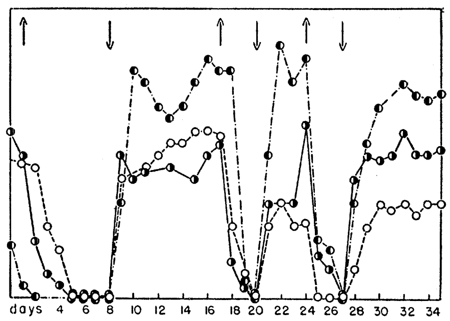

Fig. 1. Effect of water on myotonic symptoms in goats.

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Note

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Odontoblasts: Vacuoles and Inclusions

Abstract. Lipid granules have been found in the cytoplasm of odontoblasts and in the odontoblastic extensions within the dentinal tubules. It is suggested that these granules represent specific activity on the part of the normal cell as well as an increase in number after injury. While they are not limited to occurrence within the vacuoles in the cytoplasm of the odontoblasts, the relationship of the granules to the vacuoles suggests that the vacuoles are also a part of the physiologic activity of the adult odontoblasts.

This report is a preliminary account of the observation of lipid inclusions in the cell body of odontoblasts and in the cytoplasmic extension of the odontoblasts into the dentinal tubules. The granules were first observed during attempts to determine the contents of vacuoles which previously had been reported as occurring in odontoblasts. While other investigators have considered the vacuoles to be indicative of degenerative change (*1*) or inadequate fixation (*2*), or have mentioned them without attaching apparent significance to them (*3*), I have assumed them to be of physiologic significance.

Clinically healthy human teeth, both deciduous and permanent, were sectioned longitudinally within minutes after extraction. They were fixed in either neutral formalin or osmium tetraoxide as Flemming's strong fluid. Decalcification was achieved by using 0.5*M* sodium triethylenediamine tetraacetate buffered to *p*H 8.5 at 60°C. The specimens were embedded in paraffin after dehydration by graded ethyl alcohol or were washed and embedded immediately in polyethylene glycol. Paraffin sections were cut at 5 μ while the water wax sections were cut at 3 μ for observation by phase-contrast microscopy. Sections from each specimen were stained with hematoxylin and eosin, silver stains (*4*) and Sudan B. The material for phase microscopy was unstained. In addition to the sectioned material, fresh suspensions of odontoblasts scraped from the pulp chamber were studied and utilized as heat-fixed smears and as smears made from pulps prepared in the manner described above.

Vacuolization of the odontoblasts in the coronal portion of the pulps was found in each specimen; approximately every third to fifth cell was affected. The vacuoles varied from several microns in diameter to a proportion which appeared to engulf the nucleus. Cells in all levels of the palisade of odontoblasts were vacuolated. The shrinkage which was evident in the paraffin-embedded material distorted the vacuoles and produced spaces between the cell bodies of the odontoblasts. The vacuoles were observed in approximately the same frequency in fresh smears, heat-fixed smears, and smears made from fixed material.

Spherical bodies 1 to 2 μ in diameter were found in the cytoplasm of the odontoblasts fixed with osmic acid and in the formalin-fixed material, if this latter tissue was sectioned in water wax or smeared and if washing was minimized. In the material fixed with osmic acid, the granules could be preserved through alcohol dehydration and paraffin embedding and then could be demonstrated as silver-positive bodies. Bulk staining of the formalin-fixed material with Sudan B, with subsequent preparation of smears, also revealed the black to dark-green granules. Phase-contrast microscopy of fresh material, wet smears fixed with formalin or osmic acid, or water wax sectioned material