# XXVII. COMMUNICATIONS BIOPHYSICS*

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## RESEARCH OBJECTIVES AND SUMMARY OF RESEARCH

The principal activities of the Communications Biophysics Group tend to combine electrophysiological and behavioral experiments with machine data processing and analytical methods. Our major objective is to obtain a better understanding of sensory communication – in particular, of hearing. But in addition to our major research interests, we have found it profitable to apply our tools and methods selectively to other organisms and other systems as suggested below.

The group continues close cooperation with the Eaton-Peabody Laboratory of Auditory Physiology at the Massachusetts Eye and Ear Infirmary. This laboratory is operated cooperatively by M. I. T. and the Eye and Ear Infirmary. The cooperative arrangements include joint appointments of scientific staff. Some of the projects described below (Sec. A) will actually be carried out at the Eaton-Peabody Laboratory by staff members of the Communications Biophysics Group.

Our research program can be divided into five or six major areas. The programs for each area will be discussed individually.

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## A. Signal Transmission in the Auditory System

Research on signal transmission in the auditory system (mainly of cats) will be continued in cooperation with the Eaton-Peabody Laboratory at the Massachusetts Eye and Ear Infirmary.

### 1. Mechanical Signals in the Middle and Inner Ear

During the past year we have tested a system for measuring small velocities using the Mossbauer effect. We are now in the process of improving this system; we expect to use it for extending our measurements on the middle ear, and we will test its applicability to measurements in the cochlea. We also intend to attempt measurements of sound pressure in the cochlear fluids.

### 2. Transducer Mechanisms in the Cochlea

a. Electric responses to acoustic stimuli have been measured with microelectrodes inside the cochlea. Our results are not consistent with previously published measurements with respect to the way in which the cochlear-microphonic potential changes from one region (scala) to another. Our determination of the location of the microelectrode from histological examination of the tissue is not precise enough to associate recorded electric events with a particular kind of cell. We have begun experiments using microelectrodes filled with dye, which can be ejected electrophoretically, with the hope that individual cells in the organ of Corti can be marked after we have recorded from them. We intend to try this technique on the cat cochlea and also in the basilar papilla of a lizard (Gerronhotus multicarinatus), whose sensory organ is quite simple and accessible. Dr. Michael Mulroy, a postdoctoral fellow at the Massachusetts Eye and Ear Infirmary, who has studied the anatomy of reptile inner ear, is working with us.

b. A series of measurements of responses of ears in cats that have been poisoned with kanamycin has confirmed the anatomical observation that this drug (and related ones) destroy the transduction mechanism (and the hair cells); the auditory nerve fibers remain responsive to electrical stimulation. Since this drug seems to destroy portions of the organ of Corti while other parts (along the cochlea) remain operational, we intend to make measurements on animals that have been treated with kanamycin, with the aim of learning something about the way activity in one region of the cochlea influences responses in other regions.

c. Responses of auditory nerve fibers to electrical stimulation of the cochlea have a component that has many of the properties of responses to acoustic stimuli. This component will be studied with the aim of determining how the (presumably) electromechanical coupling takes place and what possible implications this has for the normal transduction process.

d. Spike responses from two fibers in the auditory nerve can be recorded simultaneously. Analysis of this kind of data is being undertaken with the aim of determining the correlation between the stochastic processes that influence individual fibers.

e. From our measurements of responses of auditory nerve fibers to stimulation by two tones, we conclude that cochlear filtering is nonlinear even at low stimulus levels. To provide some insight into the kinds of processing that might occur, we are examining time-frequency relations in the responses from single auditory-nerve fibers. These studies include (i) the dependence of the phase of neural discharge upon intensity and frequency of a simple tonal stimulus, (ii) the relation between tuning curves and time properties of click responses, and (iii) the relation between amplitude and phase of the cancellation tone in combination-tone experiments.

3. Cochlear Nucleus

The fibers of the auditory nerve terminate in the brain stem in the cochlear nucleus. With the cooperation of Dr. R. K. Morest, of the Department of Anatomy, Harvard Medical School, combined anatomical and physiological studies of the cochlear nucleus will be aimed at relating specific cell response patterns to synaptic and cell morphology.

4. Superior Olivary Complex

Some of the nerve fibers from the cochlear nucleus terminate in the superior olivary complex. During the past year, we have completed a survey of the electric responses of neural units in this region. We have (i) divided the units into classes based on their firing patterns in response to sounds, (ii) localized each unit, and (iii) found correlations between the classes and their anatomical locations. We are now attempting to record intracellularly from one of these classes and to mark these cells by injecting dye into them so that we can determine the anatomically defined group that corresponds to our physiologically defined group.

5. Measurements of the effects of electrical stimulation of the efferent olivo-cochlear bundle on responses of the auditory nerve will be extended to include the effects of the uncrossed olivo-cochlear bundle.

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B. Neuroelectric Correlates of Behavior

The study of eyelid responses in the rat continues to be the focal point of our research on the neurophysiology of conditioning. The long-range goal is to trace the central influences on the motoneurons that mediate these relatively simple movements during conditioning. During the past year, electrophysiological methods were used to determine the organization of the facial nucleus and, in particular, the location of cell bodies of motoneurons that innervate the eyelid muscles. In general, the results of this electrophysiological mapping were in agreement with the available anatomical data, and motoneurons serving the eyelids were found in a relatively discrete column of cells situated dorsally in the nucleus. This work was described in a Master's thesis submitted to the Department of Electrical Engineering, M. I. T., in June 1968, by Lee Danisch. On the behavioral side of this research a satisfactory method has finally been obtained for measuring the position and movements of the eyelids in the behaving rat. A major effort during the coming year will be made to determine how readily the control of eyelid responses can be achieved through classical and operant conditioning procedures. Habituation of the corneal reflex will also be studied in the normal animal; this will augment work now in progress on the activity of single motoneurons during habituation of the same reflex in the curarized rat. Work will also be initiated to determine the location and response properties of second-order afferents in the trigeminal nuclei that are activated by stimulation of the cornea or other tissues in the vicinity of the eye.

Much of our effort during the past year was spent in finishing a series of studies of acoustically evoked potentials in the rat. In particular, changes in click-evoked potentials in auditory cortex and several subcortical structures during sleep and pharmacologically induced "state" changes have been described in considerable detail. Computer techniques developed for the sleep and drug work were also employed in laminar studies

of cortical evoked potentials. Studies of acoustically evoked potentials will not continue during the coming year.

R. D. Hall

C. Other Neurophysiological Research

As well as the programmatic research described above, work in our laboratory has also included and will continue to include a number of other studies of the nervous system. These are often of a kind that we feel will profit from our general interest in analytical and computer techniques, and they serve to enrich our intellectual environment by exposing both students and senior personnel to a variety of problems and techniques in the study of the nervous system. Current projects, some of which will continue, include the following.

1. The study of neural circuitry in the proprioceptive control system of the crayfish abdomen has shown that a feedback pathway involving the stretch receptors and related tail extensor muscles can be modulated by neural input from skeletal hairs on several parts of the body. Quantitative aspects of this modulation are being studied, and the search for other neural inputs that can modulate the SR muscle feedback loop continues.

2. During the past year, a doctoral thesis on steady-state membrane noise current of a voltage-clamped lobster axon was completed. It was shown that the power density spectrum of the membrane noise current could be fitted by the following expression:

$$S(f) = \frac{A + k|I_k|^m}{f},$$

where $I_k$ is the mean steady-state potassium current.

During the past year, a new project using the sucrose-gap, voltage-clamp technique on lobster axons was initiated. The purpose of this project is to investigate relatively long-term phenomena exhibited by nerve fibers. It has been found that some nerve fibers exhibit a repetitive sequence of action potentials in response to a constant current applied to the fiber. Some fibers will respond to the same stimulus with only one action potential. The question is, Can these rather different response characteristics be accounted for by the present accepted models for the initiation of action potentials?

3. A program of electrophysiology and anatomy is proposed to study the mechanism of transduction in hair cells, particularly in those of lateral-line organs found in fish and amphibians. The lateral-line system is closely related embryologically and developmentally to the auditory and vestibular systems, and the transcucer cells in these systems are very similar morphologically and probably share many physiological properties. The advantages of studying these cells in the lateral line as opposed to the labyrinth of the inner ear are (i) greater accessibility (the lateral-line organs on which this work will be done are "free-standing" organs, occurring at the skin surface); (ii) consequent possibility of direct visual identification and observation of the hair cells for the purpose of electrode penetration; (iii) the large size of lateral-line hair cells found in certain amphibian species increases the likelihood of prolonged recording from them with minimum disruption of their function; (iv) the relative simplicity of organization of the lateral-line organs, compared with labyrinthine organs, with respect to number of hair cells per organ, coupling to the environment, and innervation, may permit a more direct analysis and interpretation of the experimental results.

The problems to which the initial effort will be directed concern (i) the relation between mechanical displacement of the cupula above the hair cell and the receptor potential (the existence of the latter having been recently demonstrated by Harris and Frishkopf in the hair cells of the mudpuppy lateral-line organ); (ii) testing of the

hypothesis that this receptor potential is an essential step in the processes leading to nerve excitation; and (iii) studies of correlates between the physiological characteristics of the hair cells and their anatomy.

To approach these problems requires a method capable of stimulating individual organs while recording simultaneously from within hair cells and from the nerve fibers innervating them; anatomical techniques to permit identification of recording sites are also required. These methods exist and have been applied to this system by Harris and Frishkopf.

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## D. Psychophysics

Most of our psychophysical research, like our physiological research, is concerned with the sense of hearing. In some cases, the research goal is a detailed understanding of the auditory system. In other cases, our interest is focused more on problems of sensory psychology and perception, and on man's ability to make use of information in auditory displays.

During the past few years, the principal research projects have been concerned with the processes by which the auditory system combines the signals received at the two ears (binaural interaction), the limitations imposed on auditory performance by the random nature of the transduction from acoustic waveforms to neural firing patterns on the auditory nerve (limitations imposed by the random peripheral transduction), and the response variability in experiments that require subjects to respond differentially to stimuli of different intensities ( intensity resolution).

During the coming year, in addition to continuing work in these areas, we intend to begin studying periodicity pitch and abnormal hearing. The research on abnormal hearing will constitute a major new project, and initial efforts will be devoted to the development of an appropriate research program. More detailed comments on our principal research projects follow.

### 1. Binaural Hearing

During the past two years, our interest in binaural interaction and in limitations imposed by the random peripheral transduction have led to the development of a new model of binaural hearing. The preliminary work on this project consisted in computing the performance that would be expected if there existed a central processor that received the auditory-nerve fibers from the two ears as inputs, and operated ideally except for the absence of an absolute time reference. In conjunction with this theoretical work, experiments were performed on interaural discrimination of time and amplitude for tone stimuli. The results of this research suggest that the central processor, in addition to lacking an absolute time reference, is incapable of making optimum use of the timing information in the neural firings. For example, it has been shown that if the timing information were used optimally, the just-noticeable difference in interaural time delay for low-frequency tones would be approximately 1 μsec, rather than the 10 μsec actually achieved. (The conclusion that the timing information in the auditory nerve firings is not used optimally is also supported by studies of monaural frequency discrimination.) Theoretical work now in progress indicates that if the central processor is assumed to be incapable of comparing firing times that occur more than a fraction of a millisecond apart, then computed performance and actual performance are the same for a fairly wide variety of binaural tasks.

In future research, we intend to continue our theoretical modeling of the peripheral transformation and the central processor, to perform further psychophysical

experiments on interaural discrimination of tones, to extend our experimental results on interaural discrimination to other types of stimuli (such as clicks and noise), to examine further the detection of tones and narrow-band noise signals in backgrounds of narrow-band and wideband noise, and to study the effects on detection performance of contralateral cueing. Also, as the model for the central processor is further developed, we shall need to investigate the extent to which the functions performed by this processor can be identified with functions performed by central neurophysiological elements.[1]

2. Intensity Resolution

For many years, psychophysicists have attempted to characterize the manner in which we perceive relations among stimuli differing only in intensity, by studying performance in detection, discrimination, identification, and scaling experiments. Despite these efforts, our understanding of intensity perception is extremely limited. Not only are there many unsolved problems within each narrowly defined area, but little is known about how the results in the different areas are related. One of the goals of our present research program is to develop a unified theory that will enable us to determine and understand these relations. Our prelimanary work on this project has focused on the problem of intensity resolution and has led to a theory composed of two parts: a decision model, and an internal-noise model. The decision model is similar to that used in detection theory and relates the experimental data to sensitivity and judgmental bias. The internal-noise model includes limitations imposed by imperfect sensors and imperfect memory, and relates the sensitivity to the stimulus set. In the model for memory, the assumption is made that two distinct operating modes exist: a "sensory trace rehearsal mode" (in which the subject attempts to remember the sound itself), and a "verbal context-coding mode" (in which the subject attempts to compare the sound with the general context of sounds in the experiment and to remember a verbal representation of this comparison). Experiments to test this theory have been performed on detection, discrimination, identification, category scaling, magnitude estimation, and short-term memory. On the whole, the data obtained from these experiments tend to support the theory. One result of this work is a clarification of the classical discrepancy between the human's excellent ability to discriminate intensity and his exceedingly poor ability to identify intensity (the so-called "7±2" phenomenon).

Our plans for the future include the following projects. First, we intend to fill in a number of weak points in our experimental data. During the past year, we have performed a wide variety of experiments and mapped out general contours. In certain cases, it will be necessary to obtain more extensive data. Second, there are some experimental results that suggest that the theory requires refinement. For example, the data indicate (and the model does not predict) that sensitivity in identification experiments is slightly worse in the middle of the intensity range than at the extremes (even after the effects of judgmental bias are removed). Similarly, the data obtained in experiments on short-term memory suggest that our hypothesized division of the memory process into two distinct modes (the "sensory trace mode" and the "verbal context-coding mode") is somewhat too severe. Third, we intend to extend our work (both theoretical and experimental) to configurations in which the subject is presented with a standard intensity on each trial to serve as an anchor point. In most of our work thus far, the problem of how sensitivity is affected by such standards has been ignored. Further research is also required in connection with the effects of variations in stimulus duration, intertrial duration, and order of stimulus presentation. Finally, we hope to apply our theory to the results of matching experiments (including cross-modality matching) and examine its applicability to other sensory continua.[2]

3. Periodicity Pitch

The project on periodicity pitch is just now being initiated. Previous research on this topic has shown that the pitch of periodic stimuli can be closely correlated with the

fundamental frequency, even though the stimulus contains no energy at that frequency. It has also been shown that the correlation between pitch and fundamental frequency cannot be attributed to auditory nonlinearities that generate energy at this frequency. Thus, it has been hypothesized that the pitch of the periodic stimulus arises from temporal encoding of the stimulus period.[3] Under some conditions, however, the hypothesized temporal encoding fails to operate as expected. For example, in certain cases, there does not exist any simple tone that can be satisfactorily matched in pitch to a periodic stimulus lacking energy at the fundamental frequency. Also, the pitch of any periodic stimulus consisting of only high-order harmonics (i.e., harmonic numbers greater than approximately ten) is ill-defined compared with that perceived with lower harmonic numbers. In view of difficulties such as these, an alternative hypothesis has been proposed in which it is assumed that higher centers in the auditory system learn to associate the spectral pattern of a stimulus that lacks the fundamental with that of a stimulus that contains the fundamental.[4] While it is impossible to determine from psychophysical data the identity of the physical mechanism that mediates the stimulus-response transformation, with appropriate psychophysical data one can examine the problem of whether simple hypotheses of temporal processing provide a clear correlation between behavioral data and physiological data (e.g., from the auditory nerve, and possibly from the cochlear nucleus). We have started exploratory psychophysical experiments to investigate conditions (such as those previously mentioned) under which temporal stimulus information fails to produce clear pitch perception. We plan to study systematically various conditions that facilitate or inhibit pitch matches between simple tones and periodic stimuli, and to study the effect of harmonic number upon a subject's ability to discriminate pitch differences between periodic (and nearly periodic) stimuli. These experiments should provide us with a clearer knowledge of the stimulus conditions required for an important class of pitch perceptions, as well as a basis for comparisons between behavioral and physiological data.

4. Abnormal Hearing

We plan to study abnormal hearing in order to increase our general understanding of the auditory system, to contribute to the development of more efficient audiometry, and to help develop improved prosthetic devices. We believe that there is a substantial gap between what is now scientifically and technologically feasible and the practices and applications that are currently in use; and the immediate resources at M.I.T. in the areas of psychophysics, auditory physiology, speech communication, and engineering, combined with the opportunities for becoming associated with first-rate medical and clinical groups in the Boston area, place us in a uniquely advantageous position for carrying on this work. During the comming year, we intend to familiarize ourselves with previous research, establish contacts with appropriate medical and clinical groups, and organize our research program.

N. I. Durlach, J. L. Goldstein,
W. A. Rosenblith, W. M. Siebert

References

1. Previous work on this project is described in H. S. Colburn, "New Model of Binaural Hearing," J. Acoust. Soc. Am. 44, 369 (1968); H. S. Colburn, "Preliminary Work on a New Model of Binaural Hearing," Quarterly Progress Report No. 87, Research Laboratory of Electronics, M.I.T., October 15, 1967, pp. 170-177; R. M. Hershkowitz, "Discrimination of Interaural Time and Intensity Off the Midline," Quarterly Progress Report No. 87, op. cit., pp. 177-181; R. H. Domnitz, "Auditory Discrimination of Interaural Time," S.M. Thesis, Department of Electrical Engineering, M.I.T., 1968.

2. Preliminary results of the research on this project are described in N. I. Durlach and L. D. Braida, "Perception of Intensity Relations," J. Acoust. Soc. Am. 44, 388 (1968); L. D. Braida, "An Equivalence between Magnitude Estimation and Absolute Identification for Sound Intensity," J. Acoust. Soc. Am. 44, 384 (1968); R. M. Farrell, C. T. Pynn, and L. D. Braida, "Determination of Intensity Discrimination by Absolute Identification Procedures," J. Acoust. Soc. Am. 44, 389 (1968); J. E. Berliner and N. I. Durlach, "Short-Term Memory in Intensity Discrimination," J. Acoust. Soc. Am. 44, 389 (1968); N. I. Durlach and L. D. Braida, "Psychophysics of Intensity Resolution," Quarterly Progress Report No. 91, Research Laboratory of Electronics, M. I. T., October 15, 1968, pp. 240-249; J. E. Berliner, "Short-Term Memory in Intensity Discrimination of Tones," S. M. Thesis, Department of Electrical Engineering, M. I. T., 1968.

3. J. F. Schouten, R. J. Ritzma, and B. L. Cardozo, "Pitch of the Residue," J. Acoust. Soc. Am. 34, 1418-1424 (1962).

4. W. R. Thurlow, "Perception of Low Auditory Pitch: A Multicue Mediation Theory," Psychol. Rev. 70, 461-470 (1963).

## E. Methods of Processing Electrophysiological Data

1. The relationship of the recovery cycles of visual evoked responses in man to changes in subject state over long periods of time and under conditions of task-induced attention will be analyzed with the aid of special computers and displays. The recovery function to paired flashes is of particular interest as a sensitive measure of cortical excitability and temporal resolution, but in the usual way in which it is derived from averaged evoked responses it is assumed that the function is insensitive to changes in excitatory state over the averaging period. The efficiency of various criteria of subject state (background EEG, response to single flashes, visual reaction time) in predicting the recovery function will be examined.

R. W. Lansing

2. During the past two years we have developed several techniques for summarizing large amounts of neuroelectric data.[1-3] In the coming year we plan to continue this program with specific emphasis on the following projects.

a. The analysis techniques already developed will be applied to a variety of data recorded from both animal and human subjects during a wide range of behavioral conditions. The results of these analyses will be compared with the data reduction procedures usually employed for neuroelectric data. If the commonly employed analysis involves time averaging, the validity of the stationarity assumption will be considered.

b. Our first efforts have been focused almost entirely on characterizing the temporal qualities of neuroelectric activity. Other kinds of data of physiological origin are amenable to this sort of analysis. In particular, we intend to investigate the possibilities of applying the display techniques that we have developed to the electrocardiogram.

c. In some cases, analysis involves the computation of a representation of the data in the form of a sequence of short-term statistics. We propose to develop appropriate electronic circuitry to compute these representations locally, in order to reduce the information-handling capacity of the channel required to transmit this information to a central processor. The initial research, of course, may involve simulation of the local system on a central programmable digital computer.

S. K. Burns

References

1. S. K. Burns, "Methods for the Description of the Temporal Structure of the Human Electroencephalogram," Ph. D. Thesis, Department of Electrical Engineering, M. I. T., May 1967.
2. S. K. Burns, A. A. Borbély, and R. D. Hall, "Evoked Potentials: Three-Dimensional Display," Science 157, 457-459 (July 28, 1967).
3. S. K. Burns, "Cumulative Behavior of Averaged Evoked Potentials," Quarterly Progress Report No. 89, Research Laboratory of Electronics, M. I. T. April 15, 1968, pp. 253-258.

## F. Cardiovascular System Studies

The mammalian blood pressure is regulated by a complex physiological feedback mechanism. One loop in this mechanism controls heart rate in such a manner that blood pressure is kept constant under a variety of conditions. Our work is concerned with the quantitative characterization of the components in this feedback loop.

In the past, we have concentrated on the description of the aggregate input-output characteristics of the blood-pressure receptor nerves, using multiple fiber recordings, but now we are in a position to describe the detailed firing pattern of single nerve fibers. Successful characterization of the firing patterns would be a significant aid in understanding the functioning of these biological transducers.

We have recently developed a linear model to describe changes in heart rate that are caused by variations in the frequency of impulses on the cardiac vagal efferent nerves. This model is valid only over a limited range, since at very slow heart rates significant nonlinearities are present in the system. At present, we are investigating the physiological origin of these nonlinearities.

P. G. Katona

## SELECTED PUBLICATIONS

D. Albe-Fessard, J. Massion, R. D. Hall, and W. A. Rosenblith, "Average Evoked Responses to Somatic Stimulation in the Cat," Progr. Brain Res., Vol. 22, Brain Reflexes, E. A. Asratyan (ed.) (Elsevier Publishing Company, Amsterdam, 1968), pp. 206-216.

J. S. Barlow, "Computer Analyses of Clinical Electroencephalographic Ink Tracings with the Aid of a High-Speed Automatic Curve Reader," IEEE Trans., Vol. BME-15, pp. 54-61, 1968.

J. S. Barlow and R. A. DiPerna, "Continuous Multichannel Automatic Curve Reader," IEEE Trans., Vol. BME-15, pp. 46-53, 1968.

J. E. Berliner and N. I. Durlach, "Short-Term Memory in Intensity Discrimination," J. Acoust. Soc. Am. 44, 389(A) (1968).

L. D. Braida, "An Equivalence between Magnitude Estimation and Absolute Identification for Sound Intensity," J. Acoust. Soc. Am. 44, 384(A) (1968).

H. S. Colburn, "New Model of Binaural Hearing," J. Acoust. Soc. Am. 44, 369(A) (1968).

N. I. Durlach and L. D. Braida, "Perception of Intensity Relations," J. Acoust. Soc. Am. 44, 388(A) (1968).

R. M. Farrell, C. T. Pynn, and L. D. Braida, "Determination of Intensity Discrimination and Detection Capabilities by Absolute Identification Procedures," J. Acoust. Soc. Am. 44, 389(A) (1968).

J. L. Goldstein and N. Y. S. Kiang, "Neural Correlates of the Aural Combination Tone $2f_1-f_2$," Proc. IEEE 56, 981-992 (1968).

J. L. Goldstein and N. Y. S. Kiang, "Neural Correlates of the Aural Combination Tone $2f_1-f_2$," J. Acoust. Soc. 44, 362(A) (1968).

R. D. Hall, "Habituation of Evoked Potentials in the Rat under Conditions of Behavioral Control," Electroenceph. clin. Neurophysiol. 24, 155-165 (1968).

A. J. M. Houtsma, "Discrimination of Frequency Ratios," J. Acoust. Soc. Am. 44, 383(A) (1968).

P. G. Katona, J. W. Poitras, N. Pantelakia, E. W. Jensen, and G. O. Barnett, "Deterministic Nature of Baroreceptor Firing," 215, 1-7 (1968).

P. G. Katona and G. O. Barnett, "Central Origin of Asymmetry in the Carotid Sinus Reflex," Ann. N. Y. Acad. Sci. (in press).

P. G. Katona, J. W. Poitras, and G. O. Barnett, "Asymmetry in the Carotid Sinus Reflex," Introduction to Biocybernetics (John Wiley and Sons, Inc., New York, in press).

N. Y. S. Kiang, "A Survey of Recent Developments in the Study of Auditory Physiology," Ann. Otol. Rhinol. Laryngol. 77, 656-675 (August 1968).

P. J. Metz, G. von Bismarck, and N. I. Durlach, "Further Results on Binaural Unmasking and the EC Model," J. Acoust. Soc. Am. 43, 1085-1091 (1968).

M. Nomoto, "Computer Simulation of Sharpening Effects on Response Areas of Single Auditory Units," J. Acoust. Soc. Am. 44, 362(A) (1968).

W. A. Rosenblith, "Brain Function and Human Progress," Symposium on Brain Research and Human Behavior, organized by UNESCO in cooperation with the International Brain Research Organization (IBRO), Unesco House, Paris, March 11-15, 1968.

W. A. Rosenblith, "Communication Engineering Views the Brain Anno 1967," Bioengineering – An Engineering View, Proc. Symposium on Engineering Significance of the Biological Sciences, George Bugliarello (ed.) (San Francisco Press, San Francisco, California, 1968), pp. 257-268.

W. A. Rosenblith, "Introductory Remarks," Social Requirements for Urban Design, Science, Engineering, and the City, Publication 1498, National Academy of Sciences, Washington, D. C., pp. 7-10, 1968.

M. B. Sachs and N. Y. S. Kiang, "Two-Tone Inhibition in Auditory-Nerve Fibers," J. Acoust. Soc. Am. 43, 1120-1128 (1968).

W. M. Siebert, "Stimulus Transformations in the Peripheral Auditory System," Recognizing Patterns: Studies in Living and Automatic Systems (P. A. Kolers and M. Eden, eds.) (The M. I. T. Press, Cambridge, Mass., 1968), pp. 104-133.

T. F. Weiss and W. A. Rosenblith, "Studies of Neural Elements and Systems: Some Prefatory Editorial Remarks," Proc. IEEE 56, 891-894 (1968).

M. L. Wiederhold and N. Y. S. Kiang, "Electrical Stimulation of the Crossed Olivocochlear Bundle: Its Effect on Spontaneous Firings of Auditory-Nerve Fibers," J. Acoust. Soc. Am. 44, 362(A) (1968).

## THESES 1968

1. David H. Altmann, "Drilling and Localizing One-Hundred Micron Holes in the Cat's Cochlea," S. B. Thesis, Department of Electrical Engineering, M. I. T.
2. John H. Arnold, Jr., "A Heart Monitor Utilizing An Average Interval Comparator," S. B. Thesis, Department of Electrical Engineering, M. I. T.
3. Jeffrey E. Berliner, "Short-Term Memory in Intensity Discrimination of Tones," S. M. Thesis, Department of Electrical Engineering, M. I. T.
4. Ronald C. Cerrato, "A Demonstration of Pulse Communication Systems," S. M. Thesis, Department of Electrical Engineering, M. I. T.
5. Steven C. Chamberlain, "Efferent Inhibition of Electrically Stimulated Response in Cat's Auditory Nerve," S. B. Thesis, Department of Electrical Engineering, M. I. T.
6. Stanley Chang, "Tactile Gating of a Crayfish Stretch Receptor Reflex," S. B. Thesis, Department of Electrical Engineering, M. I. T.
7. Richard Coppola, "Click-Evoked Potentials in the Paralyzed Rat during Conditioning," S. B. Thesis, Department of Electrical Engineering, M. I. T.
8. Allen W. Currano, "Average Responses to Clicks Recorded on the Scalp of Man," S. B. Thesis, Department of Electrical Engineering, M. I. T.
9. Lee A. Danisch, "Organization of the Motor Nucleus of the Facial Nerve in Rats," S. M. Thesis, Department of Electrical Engineering, M. I. T.
10. P. Demko Jr., "Hybrid Simulation of the H-H Model for Nerve Membrane," S. M. Thesis, Department of Electrical Engineering, M. I. T.
11. Robert H. Domnitz, "Auditory Discrimination of Interaural Time," S. M. Thesis, Department of Electrical Engineering, M. I. T.
12. Ellis E. Eves II, "Interference Effects in Short-Term Auditory Memory," S. B. Thesis, Department of Electrical Engineering, M. I. T.
13. Daniel M. Green, "A Television Character Generation System," S. B. Thesis, Department of Electrical Engineering, M. I. T.
14. John J. Guinan, Jr., "Firing Patterns and Locations of Single Auditory Neurons in the Brainstem (Superior Olivary Complex) of Anesthetized Cats," Ph. D. Thesis, Department of Electrical Engineering, M. I. T.
15. John D. Hild, "Interaural Time JND as a Function of Interaural Time Delay," S. B. Thesis, Department of Electrical Engineering, M. I. T.
16. Martin S. Kohn, "Characterization of Ectopic Heartbeats," S. M. Thesis, Department of Electrical Engineering M. I. T.
17. Robert Y. S. Li, "Correlation between the Vagus Nerve Firings and the Aortic Pressures," S. B. Thesis, Department of Electrical Engineering, M. I. T.
18. Philip F. May, "A Portable Cardiac Monitor with Audio Output," S. B. Thesis, Department of Electrical Engineering, M. I. T.
19. Eugene G. Merrill, "Observations on the Activity of the Respiratory Neurons in Cat," Ph. D. Thesis, Department of Biology, M. I. T.
20. David C. Mountain, Jr., "A Quantitative Model of Inhibition in the Crayfish Stretch Receptor," S. B. Thesis, Department of Electrical Engineering, M. I. T.
21. Charles Mpagazehe, "Interaural Time JND as a Function of Amplitude Difference," S. B. Thesis, Department of Electrical Engineering, M. I. T.
22. Maximo Nanita, "Ventricular Extrasystole Detector," S. M. Thesis, Department of Electrical Engineering, M. I. T.

23. John B. Patterson, "Incoherent Sampling Systems: A Quantitative Comparison," S. M. Thesis, Department of Electrical Engineering, M. I. T.

24. Denis J-M. Poussart, "Current-Noise in the Nerve Membrane: Measurements under Voltage-Clamp," Ph. D. Thesis, Department of Electrical Engineering, M. I. T.

25. Craig T. Pynn, "Identification and Discrimination for Sound Intensities," S. B. Thesis, Department of Electrical Engineering, M. I. T.

26. Irving H. Thomae, "Chromatic Adaptation in Single Units of the Optic Nerve of the Ground Squirrel," Ph. D. Thesis, Department of Electrical Engineering, M. I. T.

27. Bruce A. Twickler, "Measurement of the Transfer Characteristics of the Middle Ear Using the Mössbauer Effect," S. M. Thesis, Department of Electrical Engineering, M. I. T.

## A. MEASUREMENT OF VELOCITIES IN THE MIDDLE EAR USING THE MÖSSBAUER EFFECT

Knowledge of the behavior of mechanical signals in the middle and external ear has been limited by the difficulties involved in measuring the small displacements involved.[1] It has recently been shown[2, 3] that the Mössbauer effect can be used to make sensitive

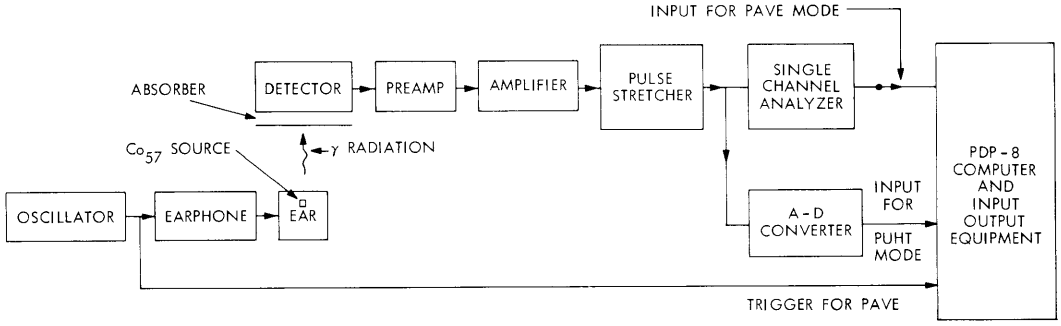


Fig. XXVII-1. Block diagram of the system.

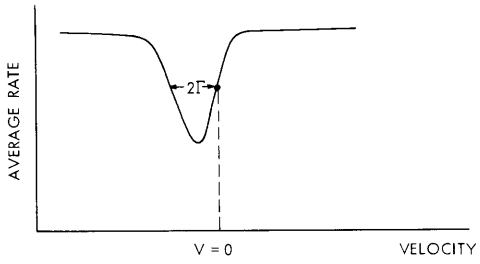


Fig. XXVII-2.
Mössbauer resonance curve. For our system, with a $CO_{57}$ in palladium as a source and a stainless-steel absorber, $\Gamma \cong 0.2$ mm/sec.

velocity measurements in the ear. Our preliminary testing of a Mössbauer system on the cat middle ear is reported here.[4]

Figure XXVII-1 is a diagram of the system. The gamma rays emitted from the source pass through an absorber and are detected in a gas tube (proportional counter.) The height of the pulse from the detector is proportional to the energy of the gamma ray. After the pulse is amplified and stretched, the a-d converter samples the pulse height and feeds the digital height into the computer.

In the PUHT (PUlse HeighT) mode the computer displays a histogram of pulse heights. The single-channel analyzer is then adjusted so as to pass only pulses within the peak of 14.4 keV.

The gamma radiation source and absorber were chosen so as to give an isomer shift approximately equal to the half-width ($\Gamma$) of the Mössbauer resonance. The average rate of the 14.4-keV gamma rays then depends on velocity, as shown in Fig. XXVII-2.

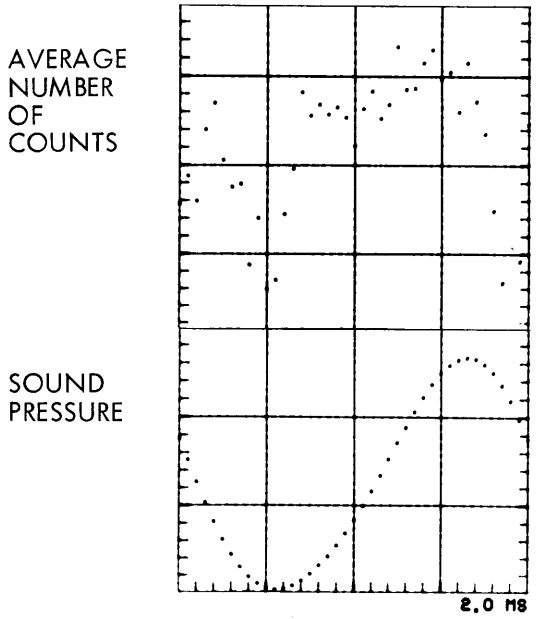


Fig. XXVII-3.

Waveforms of one cycle of count rate and sound pressure. Frequency, 500 Hz; sound pressure level, 100 dB SPL. The stimulus period is divided into 40 intervals, each 50 μsec long. Two minima in the count rate occur in each cycle (in this case) because the peak velocity is large enough (>0.2 mm/sec) to go past the minimum in the curve of Fig. XXVII-2. Counts were averaged for 26 minutes to obtain the upper waveform.

With the radioactive source moving with a small sinusoidal velocity, the velocity waveform can be determined from the number of counts occurring in different time intervals during the period of the sinusoid. In the PAVE (Pulse AVErage) mode the computer divides a period into equal time segments and averages the counts over many periods of the stimulus. A sample measurement made with a source (measuring approximately 1/2 × 1/4 mm) on the stapes is shown in Fig. XXVII-3. Our measurements (on one cat) do not differ significantly from those made by other methods. This method is considerably more convenient and sensitive than our earlier method.[1]

Improvements in the system are planned to reduce the averaging time required to obtain an acceptable signal-to-noise ratio.

B. A. Twickler, W. T. Peake

References

1. J. J. Guinan, Jr. and W. T. Peake, "Middle-Ear Characteristics of Anesthetized Cats," J. Acoust. Soc. Am. 41, 1237-1261 (1967).

2. P. Gilad, S. Shtrikman, P. Hillman, M. Rubinstein, and A. Eviatar, "Application of the Mössbauer Method to Ear Vibrations," J. Acoust. Soc. Am. 41, 1232-1236 (1967).

3. B. M. Johnstone, A. J. F. Boyle, "Basilar Membrane Vibration Examined with the Mössbauer Technique," Science, 158, 389-390 (1967).

4. B. A. Twickler, "Measurement of the Transfer Characteristics of the Middle Ear Using the Mössbauer Effect," S.M. Thesis, Department of Electrical Engineering M. I. T., September 1968.

## B. MICROELECTRODE AND STIMULATION STUDIES OF SPONTANEOUS SIGNALS FROM BRAIN TISSUE CULTURES*

Signal production from brain tissue in vitro can be evoked or spontaneous. Spontaneous signals have been reported for three hours in vitro from preparations of fish,[2] insect,[3] and amphibian brain[29, 30] and for weeks in cultures of brain tissue from chick embryo,[12] rabbit,[4] human adult,[13] and mouse.[10] Spontaneous signals have been detected in cultures of most areas of the brain.[14-18, 22] Spontaneous neuronal spike potentials and slow signals (10 msec-1 sec) occur for periods of days in explants of brain tissue.[17, 22, 24] In explants of chick embryo telencephalon, the slow signals occur in patterned groups (sequences) each containing approximately the same number of signals. These recur rhythmically at regular intervals for days. Spontaneous slow signals respond to environmental changes,[21, 22] and to administration of anesthetics,[19] strychnine,[20] and barbiturates[22] in a manner indicating an association with vital CNS activities.

Signals have been evoked by various stimuli from cultures of rabbit,[4] rat,[8] embryo chick,[9] mouse,[10] cat,[34] and human[42] brain and in adult (12-20 weeks old) chicks[1] from the Wülst area of the telencephalon adjacent to the part used in the studies reported here. Electrically evoked responses directly from glial cells have been described[33] and later reported to be nonphysiological.[43] Changes in intraglial potentials do occur, however, in response to evoked neuronal activity.[36-38]

This report describes (i) histological features of the explants, including interneuronal connections, (ii) some further aspects of the behavior of the spontaneous signals, and (iii) the effect of electrical stimulation on the spontaneous activity. For the first time, evidence is presented for the hypotheses (i) that spontaneous slow signals are formed in glial cells in contact with a neuronal network in an explant, in response to the ionic fluxes associated with neuronal firings and synaptic activations, and (ii) that the network of neurons in the explant acts in a coordinated fashion to cause the characteristic grouping and long-term behavior of slow signals. The simple technique used in this study has important potentialities in investigations of coordinated activities of neurons and glial-neuronal interplay in warm-blooded species.

### 1. Materials and Methods

For microelectrode studies the chamber was a flattened hollow glass cylinder, 18 mm in diameter and 4.3 mm high, with a 4-mm gap in the side wall for the introduction of

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microelectrodes.[39] It lay on one flat end and half of the chamber opposite the microelectrode port was filled by a piece of fritted glass (frit) of coarse porosity saturated with nutrient fluid (0.25% chick serum protein in a balanced salt solution[12]). Three 90-μ (gross) platinum wire electrodes, insulated to within 1 mm of the tip by a fine coat of teflon, were sealed into the chamber. Two were reference electrodes (for microelectrode and recording gross electrodes, respectively) lying between the frit and the cover glass forming the floor. The recording gross electrode lay between the frit and the cover glass forming the roof and protruded ~1 mm beyond the frit in contact with the underside of the roof.

The culture was a 1-5 $mm^3$ thin piece of tissue, convex on one aspect and flat on the other, obtained by a single cut through the curved surface of the superficial corticoid area (the right posterior pole) of 14-day chick embryo telencephalon used during its second day in culture. It lay on the recording electrode in the angle between frit and chamber roof with its only free surface (the underbelly) facing the airspace. The chamber was kept at 35°C in an incubator on the stage of a Zeiss photomicroscope, and the humidity was kept as high as possible without clouding the chamber roof and interfering with the phase-contrast visualization of the explant.

The microelectrodes were glass micropipettes with tip diameters of less than 1μ, filled with 3 M KCl and with impedances of 10-52 MΩ. They were moved by Narashigi micromanipulators. Products of autolysis of the deep nonsurviving parts of the explants made the surface layers fragile, and tearing of the surface was prevented by using the first DC shift between reference electrode and microelectrode to detect contact between the microelectrode and the culture.

Culture chamber for the stimulation studies. This chamber was a disposable plastic Petri dish, 35 mm in diameter and 10 mm high, containing a pad of cellulose sponge, 10 mm square and 4 mm high, on which lay a 5-mm square piece of filter paper. A reference electrode, of platinum wire, 90 μ in diameter, was tied through one of the lower corners of the sponge. A similar recording electrode insulated to within 1 mm of its tip by a fine coat of teflon penetrated the sponge and filter paper to end under the explant as it lay on the filter paper. The sponge and paper were saturated with nutrient fluid and the explant (as for the microelectrode study) was placed on the electrode in the center of the filter paper. During stimulations the lid of the Petri dish was replaced by a cover glass, 50 mm in diameter, with a small hole near its center through which stimulating electrodes could be passed. During movement of the stimulating electrodes, the cover glass moved freely over the open end of the Petri dish, kept it covered, and minimized evaporation. The chamber lay in a plastic incubator on a microscope stage and was maintained at 35°C by radiant heat controlled by a thermistor within the incubator.

Stimulating electrodes. These electrodes were paired glass micropipettes (tip

diameters 5-10μ) filled with nutrient fluid and with impedances of 2-10 MΩ per pair measured in nutrient fluid. They were moved into position by using Narashigi micromanipulators. The tips were kept as close together as possible.

The stimuli were 0.1-msec square waves generated by a Tektronix 161 pulse generator and Tektronix 162 waveform generator. They were applied to the platinum lead wires of the stimulating electrodes through an Argonaut LIT 069 isolation transformer. Currents up to 125 μA, depending on electrode impedance, could be applied to the explants but 10 μA or less was usually sufficient. These currents were calculated from voltage and impedance and not measured directly.

The stimulus artefact was minimal and did not obscure the responses to stimulation because (a) the high cutoff level of the recording system was less than the principal frequency of the stimulus, (b) the amplitude of the stimulus was small, and (c) the interval between stimulus and response was long enough to permit any stimulus artefact to subside before the onset of a response.

Amplifiers for microelectrode signals were Medistor A-35B negative capacity electrometers and Dana 3400 DC amplifiers. Signals from gross electrodes in both microelectrode and stimulation studies were amplified by using Grass P511R AC amplifiers.

Recording on magnetic tape was done at 15 in./sec on a Hewlett-Packard Sanborn 3907B Tape System. Signals were visualized with the use of Tektronix 502-A oscilloscopes or T.I. oscilloriters.

Computer studies were performed on a PDP-4 computer system with analog-to-digital conversion and display features.

Histochemical techniques: Direct phase contrast studies were performed by carefully teasing the culture in a drop of nutrient fluid on a glass slide and then placing a cover glass over it and compressing with a 500-g weight for several minutes. The cover glass was then sealed with Diatex (Allen Svenson, Malmö, Sweden). This preparation lasted for 2-3 hours. Dendritic and axonal endings were more readily discernible if the teased culture was placed in an equal mixture of 1% gold chloride and saturated aqueous mercuric chloride diluted 40 times with nutrient fluid and incubated at room temperature for 15 minutes before placing it under the cover glass.

One-half micron sections of the cultures were prepared by fixing the culture in paraformaldehyde-glutaraldehyde-phosphate fixative at pH 7.2.[35] After 3-12 hours fixation the cultures were dehydrated in ethanol and embedded in Epon. The sections were cut on a Porter-Blum MT1 Microtome and stained with toluidine blue by the hot-plate technique.[41]

2. Results and Interpretation

Histological study of the area of brain used for explants confirmed the presence of a multilamellar structure described[7, 26] as a possible homologue of mammalian cortex.

Active explants showed intact glial cells, neurons, and histiocytes. Ependymal cells were seen in only a few of our explants. The neurons were interconnected by axonal and dendritic processes involving structures that satisfied the light microscopy criteria for synapses (Fig. 1). 0.5% methylene blue in the nutrient fluid stains (the granules of) neurons without suppressing their electrical activity[23] so that they can be located, even in thicker parts of explants. By using this technique, good slow spontaneous signals were detected when the microelectrode tip was not in neurons or in the immediate extra-neuronal space. The methylene blue technique was not used for the other studies reported here.

Computer studies. The computer was programmed to give frequency distribution histograms for the duration of, and intervals between, thousands of slow spontaneous AC amplified signals (Fig. 2). The duration histograms have a striking mutual similarity, as do the histograms, for the intersignal intervals. Thus the duration and intersignal parameters of the slow signals from many different cultures are sufficiently similar to suggest that they are formed in the same way in each culture.

Microelectrode studies. Many simultaneous gross and microelectrode studies on telencephalic explants have shown that, contrary to our previous description of three types of signals (spike potentials, intermediate and long-duration signals), there are only two basic types of signal: 1-10 msec, 10-50 mV, "short duration" (spike potentials), and 5 msec-1 sec, 5 μV-5 mV slow signals. The slow signals last longer than the inter-signal intervals so that they overlap and fuse to form what was previously described as the long-duration signal. The last signal in the sequence in Fig. 3 B gives an impression of the appearance of an individual slow signal.

By using a gross electrode, it is possible to show that short-duration and slow signals occur simultaneously in the same explant, but they have not been detected simultaneously from a single microelectrode site.

Microelectrode studies confirm that activity is confined to small discrete foci in an explant. Two electrodes touching the surface of the explant less than 100 μ apart may not both detect on-going activity. Spontaneous sequences detected by microelectrodes are composed of monophasic slow signals equivalent to those detected by the gross electrode but simpler and of greater magnitude. The smaller magnitude in gross-electrode recordings is probably due to losses by shunting in the gross electrode and frit. It can be partly overcome by inserting vertical insulating baffles into the frit, which causes a fivefold increase in signal magnitude. Also, if only the tip of the recording gross electrode is in contact with the explant surface, signal magnitude approximates that obtained from microelectrodes.

Microelectrode exploration of the surface of explants. Only the free edge and the underbelly of the explant are accessible for exploration in the microelectrode chamber. Recordings with the microelectrode tip placed just within the surface at successive

positions 100-200 μ apart in a line across the underbelly of an explant starting at the free-edge site of maximum activity (Fig. 4 A) often show the reversal phenomenon. As the microelectrode tip is moved across the explant there is an abrupt reversal in polarity of a few of the signals in the sequence (the same few signals, usually the first few and the last few, in each sequence change from positive-going to negative-going (Fig. 4 C)). Intermediate stages in the polarity change between the initial positive-going form and the final negative-going form of any one signal have not been seen in the seven experiments done thus far. As the microelectrode tip is moved farther across the explant more of the signals in the sequence reverse polarity until all are affected (Fig. 4 D). The exact order of the reversal and the amount of movement of the electrode tip needed to accomplish it varies from culture to culture. If the microelectrode is taken back across the underbelly in similar steps, the reverse changes occur and the original polarities are regained. A stationary gross electrode implanted under the same explant detects no change in form or polarity of signals at the time the reversal phenomenon is being detected by the microelectrode (Fig. 4). The changes in signal polarity must therefore be associated with the changes in position of the microelectrode tip. Each change in position moves the microelectrode over a distance greater than any CNS cell and its processes (save for the axon which, if involved, would have given significantly different signals) into contact with another cell or group of cells.

The inverted signals do not differ significantly in number or (save for the inversion) in form from the equivalent signals in preceding noninverted sequences. The magnitude of the DC change from the baseline associated with the inverted signals is usually less than that for the equivalent positive-going sequence. Some variations on this general description of the phenomenon will be mentioned in the discussion.

The reversal phenomenon may occasionally be detected along the free edge and not across the underbelly; the exact location of the linear zone showing this phenomenon probably depends on the positioning of the explant on the sintered glass. A stationary implanted gross electrode may detect the same partially inverted form of each sequence during the whole life of the explant. Since partially inverted forms of the sequence can be detected from only minute areas of the active focus when using microelectrodes, the area of pick-up by the gross electrode must sometimes be very small.

Signals from very small active foci. The smallest active foci often show relatively simple, nearly equidistant slow signals, instead of the usual patterned sequences. Microelectrode exploration at various sites around one such very small focus detected a series of 10-msec signals at one site, a series of similar 20-msec signals at another site, and a series of approximately 60-msec signals at a third site. Most of these were simple rounded peaks with slow decay, others were more complex with second or even third subsequent less prominent peaks. Superimposition of many simple signals of equal duration revealed several distinct levels of magnitude, thereby suggesting that the larger

signals may be the result of summing of simultaneous lesser components. Superimposition of complex signals showed that the secondary components occurred at fixed times after the primary component (Fig. 5 A). With an initial component lasting 10 msec, secondary peaks also lasted 10 msec, and occurred at multiples of 2 1/2 msec until 30 msec after the initial component, and then more irregularly. Similar superimposition of signals from gross-electrode recordings (Fig. 5 B) showed that both the intervals between and the durations of the secondary components bore simple numerical relationships to the duration of the original component. Thus complex slow signals are combinations of basic components that sum when they are simultaneous or occur at regular intervals after the initial component.

Other sites in and near the very small focus showed isolated positive and negative-going signals ranging in duration from 5 msec to 60 msec.

3. Results of Stimulation

Each of the 12 cultures that were used had previously shown the typical spontaneous repeated patterned sequences of slow signals characteristic of the unstimulated activity of the explants.[22] Short-duration signals were also evident as part of the sequence in some explants. The effects of the stimulation were as follows.

a. Application of Single Stimuli

1. Spike potentials and slow signals can be evoked as parts of sequences usually after a delay of 200-700 msec (Fig. 6).

2. The position of the stimulating electrodes is critical to within 100 μ for successful stimulation.

3. There is a threshold stimulus for successful stimulation at a given site of the electrodes. Higher stimulus amplitudes then cause shorter sequences with fewer signals.

4. When stimuli are delivered near the end of a normal intersequence interval there is a high proportion of successful evocations of sequences. The chances of successful evocation become progressively less as the stimulus is delivered earlier in the intersequence interval, and stimulation is seldom successful immediately after a sequence (Fig. 7 B and C). Sequences can be evoked, however, at intervals as short as 1/10 of those between spontaneous sequences.

5. Evoked sequences are similar to, but shorter than, the spontaneous ones (Fig. 7 A).

6. Stimuli applied during a sequence are effective only if they coincide with a naturally occurring signal (Fig. 7 B).

b. Barrage of Stimuli

A barrage is more effective than a single stimulus in evoking a response, but is often followed by temporary or permanent cessation of activity.

c. Application of a Series of Single Stimuli at Regular Time Intervals (Pacing) (Fig. 7).

1. Sequences are evoked which are shorter than spontaneous sequences or sequences evoked by an isolated single stimulus (Fig. 8).

2. Sequences evoked by pacing occur at intervals that are shorter than those between spontaneous sequences, but not as short as those that can be obtained between a spontaneous sequence and one evoked subsequently by a single stimulus (Fig. 8).

3. Progressively decreasing interstimulus intervals from 10-sec sequences cause longer evoked sequences and longer intersequence intervals. This effect is maximal when the interstimulus interval approximates the intersignal interval for spontaneous sequences (Fig. 8). Pacing at interstimulus intervals shorter than this causes shorter evoked sequences with greater intersequence intervals.

4. The first one or two stimuli following an evoked sequence may cause only one or two approximately 100-msec rounded low-amplitude signals (Fig. 6). Similar signals may also precede evoked sequences, but have neither been seen to precede spontaneous sequences nor to follow unsuccessful stimuli (that is, they are not a stimulus artefact).

5. Prolonged pacing causes relatively uniform sequence duration, sequence form, and intersequence intervals, but occasionally spontaneous sequences occur in the interstimulus intervals – the phenomenon of "escape."

6. After prolonged experiments involving stimulation there may be failure to respond to pacing stimuli even when sequences can be evoked by isolated stimuli.

7. Increased magnitude of pacing stimuli causes shorter intersequence intervals and shorter sequences containing rounded smoothed signals.

d. Repeated stimulation at noneffective sites over long periods of time does not alter ongoing spontaneous activity or subsequent responses to effective stimulation.

e. After long periods of intermittent stimulation, a previously successful stimulus may fail to evoke a response; however, a stimulus of increased magnitude may then be successful.

f. Long periods of intermittent stimulation (i.e., a few hours) usually cause longer intersequence intervals and simpler sequences containing fewer signals (Fig. 8). A similar change takes place in spontaneous activity after an unstimulated explant has been active for some days.

4. Discussion

The similarity in the patterned arrangement of the slow signals and of the parameters of the slow signals from many different explants (Fig. 2) makes it likely that these signals are a true physiological entity, and all arise in the same kind of cell.

Since neuronal spike potentials and slow signals have never been detected at the same microelectrode site and slow signals are detected when the microelectrode tip is not in

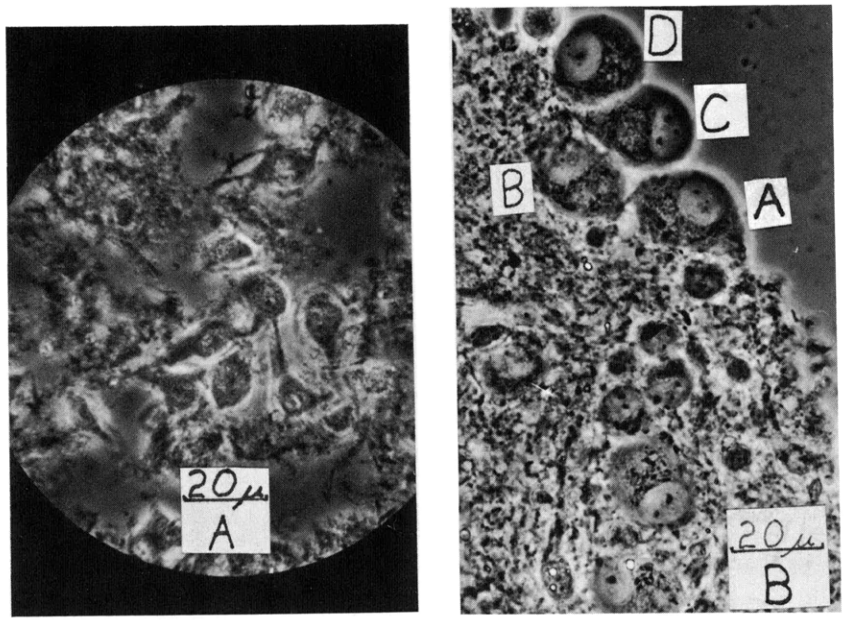


Fig. 1. A: Gold chloride preparation of a 1-day culture of 14-day chick telencephalon showing the connections (probably synaptic) of the axons of two neurons to the soma of a third small neuron. Phase contrast 620X. B: Fresh teased preparation of a 1-day culture of 14-day chick telencephalon showing the dendrite of A ending in a probably synaptic bouton on the soma of B which sends one dendrite to the hillock and one dendrite to the soma of C. Possible dendritic connections of C to D are seen in right and left corners. Phase contrast 670X.

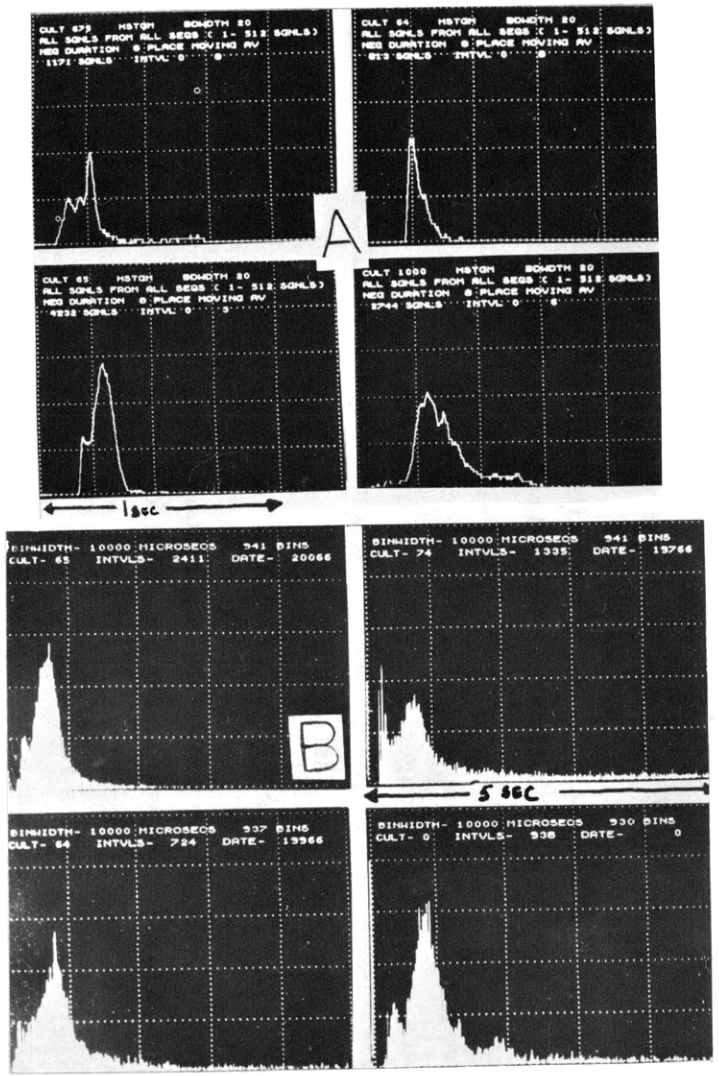


Fig. 2. A: Frequency distribution histograms of durations of AC amplified slow signals from four different cultures of 14-day chick embryo telencephalon. B: Frequency distribution histograms for intervals between AC amplified slow signals from four different cultures of 14-day chick embryo telencephalon. The similarities within each set of four histograms are obvious.

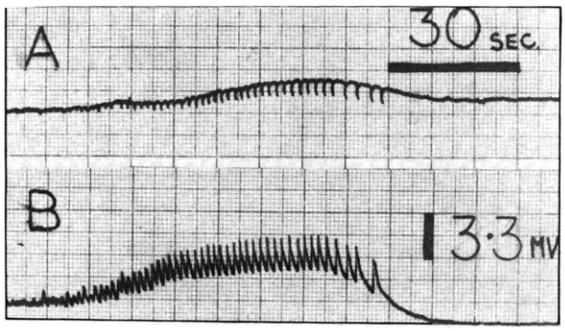


Fig. 3. Inverted form (A) of the sequence of signals detected by a microelectrode as a result of the 'reversal phenomenon.' B is the original noninverted form of the sequence. DC amplification. The fast components of the signals have inverted but not the fused slowest components.

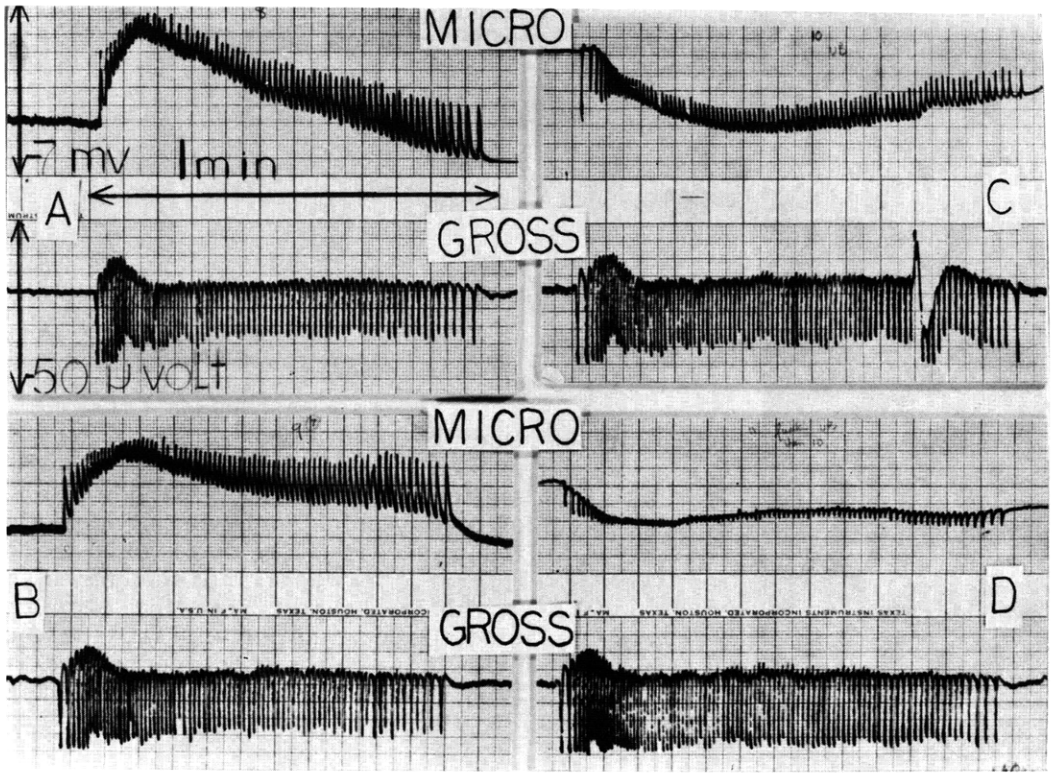


Fig. 4. Reversal of direction of polarity of slow signals during surface exploration of explant of 14-day chick embryo telencephalon by microelectrodes. Pairs of sequences of signals recorded simultaneously from microelectrode (marked micro) with DC amplifiers and from stationary implanted gross electrodes (marked gross) with AC amplifiers. A and B pairs of traces are from two neighboring sites at the point of maximum intensity. C and D pairs of traces are from two consecutive positions of the microelectrode tip on the underbelly of the explant 100-200 μ apart and in line with position B. The distortion at the end of record C is an artefact. The similarity between all the gross records shows that there was no change in the basic activity of the focus, while a progressive change in polarity of some of the signals in each sequence was being recorded by the microelectrode in positions C and D.

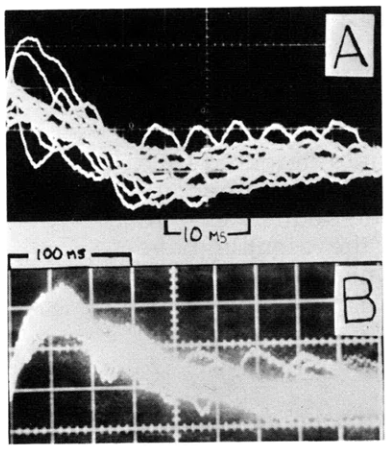


Fig. 5. A: Superimposition of 8 successive slow signals (the oscilloscope sweep being triggered by the beginning of the signal) detected by a microelectrode near a very small focus. AC amplification has been used to accentuate the secondary peaks of these complex signals. The relationship of the primary component of the signal to the secondary components and the times of their occurrence is obvious. B: Similar superimposition from an AC recording of a gross electrode recording of another explant.

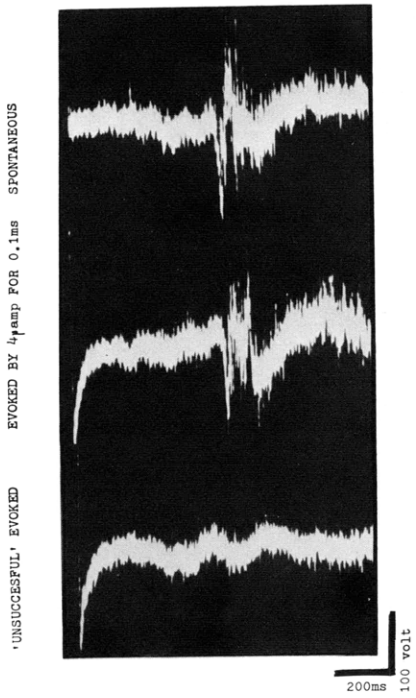


Fig. 6. Oscilloscopic traces of spontaneous and evoked signals detected by a 90-μ platinum stationary implanted electrode in an explant of 14-day chick embryo telencephalon after 1 day in vitro. The two evoked traces were triggered by the stimuli (0.1 msec, 12.5-μA) at the left edge. Note the similarity (including combination of fast and slow signals) between the spontaneous and successfully evoked signals. The lower trace shows the 100-msec rounded signal described in the text.

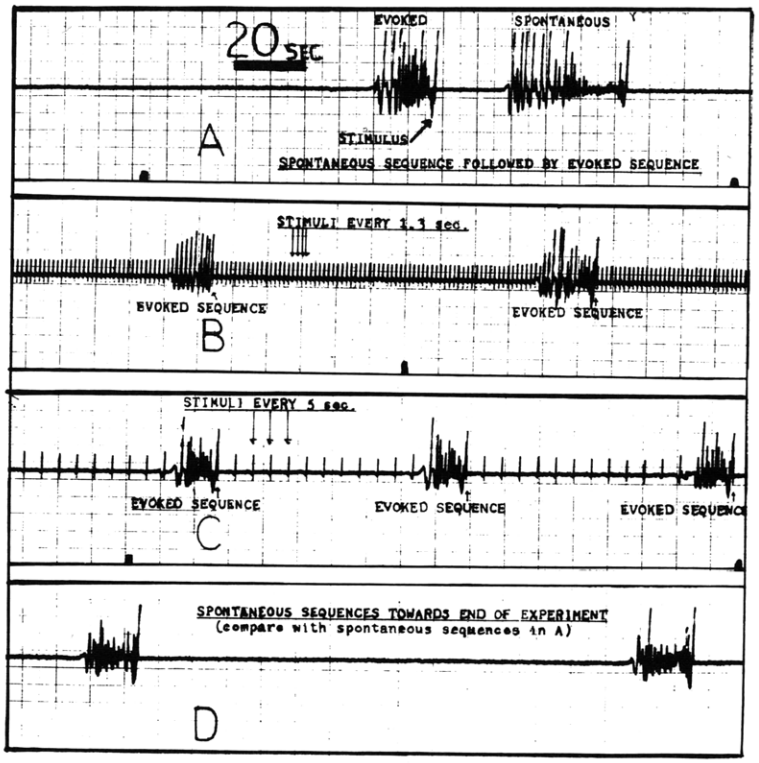


Fig. 7. Effects of stimulation on signal production detected by a 90-μ platinum stationary implanted electrode in an explant of 14-day chick embryo telencephalon after 1 day in culture. AC amplification (hence slow terminal part of signals and DC shift not seen). The time marker in A applies to all. A: Spontaneous sequence (on right) during the early part of the experiment followed by a shorter sequence evoked by a 0.1-msec, 12.5-μA stimulus after less than a quarter of a normal intersequence interval. B: Sequences of slow signals evoked by a series of 0.1-msec, 6-μA stimuli at 1.3-sec intervals. C: Sequences of slow signals evoked by a series of 0.1-msec, 6-μA stimuli at 5-sec intervals. The sequences and intersequence intervals are shorter than in B. D: Two spontaneous sequences late in the experiment for comparison with spontaneous sequences early in experiment (A).

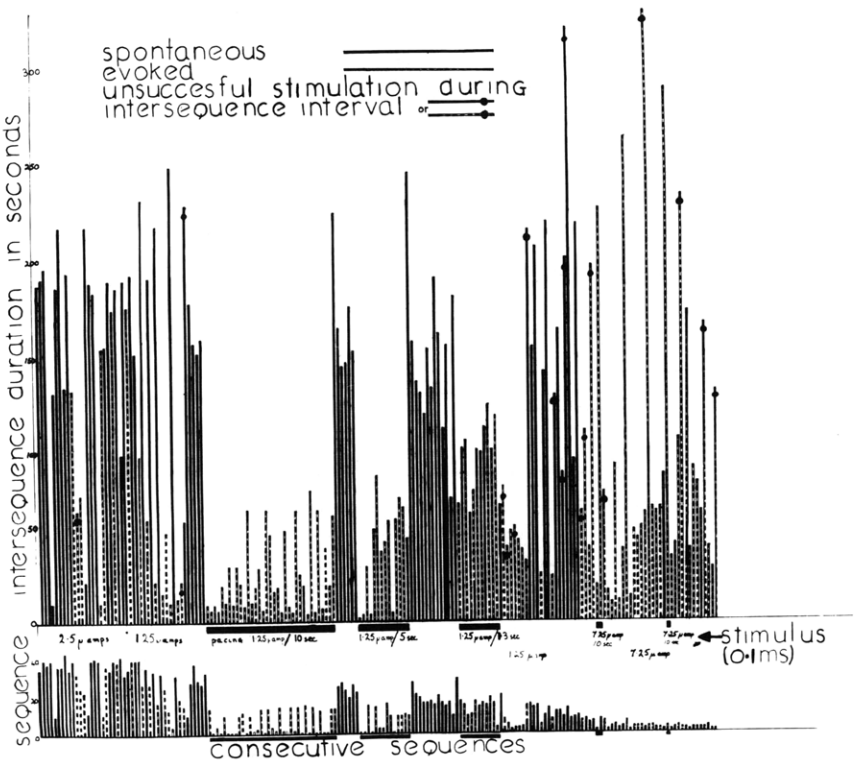


Fig. 8. Duration histogram of sequence durations (lower diagram) and intersequence intervals (upper diagram) during the spontaneous (continuous lines) and evoked (broken lines) activity of an explant of 14-day chick embryo telencephalon after 1 day in vitro. The length of each line in the lower diagram represents the duration of a sequence of signals, and the length of the line immediately above it represents the duration of the subsequent intersequence interval. A dark disc on a line indicates that unsuccessful efforts had been make to evoke a response during the intersequence interval. A thick horizontal line indicates that a continuous series of stimuli were being applied at the regular intervals noted below the line (pacing). The actual activity from this explant is shown in Fig. 1.

or just outside a neuron, the slow signals do not arise in the interior of the neuron.

Nearly all contacts with the tissue in an active focus detect slow signals. The microelectrode tip is very unlikely to attain the same relative position to specialized areas of the neuron (such as dendrites) as often as this, so that dendrites on any other specialized external part of a neuron cannot be the source of these signals. The signals must therefore originate in the non-neuronal part of the explant.

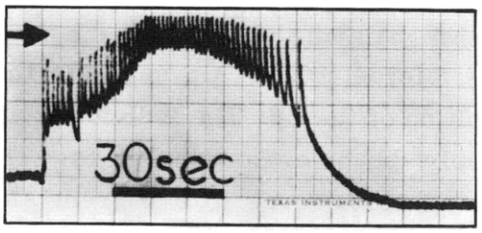


Fig. 9. Single sequence from a DC amplified microelectrode (20 MΩ, <1 μ tip) recording of spontaneous slow signals from explant telencephalon from 14-day chick embryo after 1 day in culture. Positive up.

The sequences of slow signals (Figs. 3, 4, and 9) have a striking resemblance in form and time course to intraglial signals evoked by physiological and electrical stimuli applied to neurons or axons adjacent to glial cells in preparations of Leech central nervous system[36] and Necturus optic nerve.[37]

Glial cells are the only cells that constitute a sufficient proportion of the non-neuronal population of an explant to be contacted by each random insertion of a microelectrode into an active focus. Thus the slow signals detected on practically every such insertion probably arise from glial cells.

The lack of any trace of spike potentials within the slow signals is evidence of the absence of direct electrical coupling of neurons and generator cells and is typical of signals generated within glial cells.[34, 36, 37, 43] There is a functional association, however, between spike potentials from neuronal firings and slow signals because the slow signals respond to changes in environment and to the administration of drugs in a manner indicating that they are associated with vital CNS activities believed to be resident in neuronal activity. By using gross electrodes, short-duration and slow signals can be seen to occur simultaneously in response to stimulation and during spontaneous activity. This occurrence and the presence of a minimal threshold level of stimulus, which is needed for effective evocation of slow signals, are consistent with a causal relationship between neuronal activity and the slow signals.

Glial cells apparently do not have any primary electrical activity of their own, nor do they respond directly to electrical stimulation, but merely reflect the activity of adjacent neurons,[36-38] so that the slow waves in response to stimulation must result from simultaneous short-duration signals. Except in areas showing the reversal phenomenon, the form of sequences varies little between neighboring (100-200 μ apart)

sites of the microelectrode tip. Rapid attenuation of signals with distance in the tissue and the nearly uniform amplitude of signals within sequences indicate that the generator of each signal in the sequence, if separate, would have to be almost equidistant from the microelectrode tip. Combinations of a few signals from each different cell in contact with the microelectrode at each successful penetration would be very unlikely to give similar sequences at each different microelectrode site. This could occur only if the signal generators (glial cells) at the site of each successful penetration were individually capable of generating the whole sequence of signals.

In the reversal phenomenon, the all-or-nothing change in the polarity of the same few signals in each sequence after a movement of the microelectrode tip suggests that there is a discrete but similar generator at each position of the tip. The individual glial cells in contact with the tip at each site are presumably the discrete generators. The change in polarity cannot be due to movement from a current source to a current sink with reference to any individual cell because the movement of the tip of the microelectrode is greater than the extent of any single glial cell. In any case, the partial nature of change of polarity of each sequence could not be explained by this.

The intervals between the commencement of slow signals in sequences are longer than can be explained on the basis of simple conduction, refractory, or synaptic delays between the neurons responsible for the slow signals in the approximately 1 $mm^3$ of active tissue in our explants. These intervals can be explained if the neurons do not act as individuals, but in the coordinated manner of members of a neuronal net. This type of network activity starts when few neurons at the edge of the net fire and supply synaptic input to fire adjacent neurons which, in turn, activate the next adjacent neurons so that a wave of firing crosses the net. As each wave approaches a neuron in the net, it causes progressive excitation culminating in a burst of activity. The neurons that were activated originally then recover responsiveness, receive synaptic input, and fire to initiate a second wave of excitation. Such waves will continue to cross the net at intervals until some neurons remain unresponsive and block propagation.

Computer models of neuronal nets have been studied.[28] The results show that only nets with short interneuronal connections display activity similar to that obtained from the explants. The neurons in the explants have been shown to be interconnected. They form a neuronal net that probably has predominantly short connections, the long ones having been cut during explantation or made ineffective because one of their associated neurons lies outside the 100-200-$\mu$ effective range of oxygen diffusion from the surface of the explant. A computer net of this type whose neurons have nonresponsive periods of 10-20 msec has peaks of over-all neuronal firings at intervals of approximately 100 msec, and significantly longer intervals between arrival of successive waves of excitation at a given neuron. If the nonresponsive period of the network neurons is increased, the intervals between peaks of over-all network activity become proportionately longer.

The administration of strychnine to explants[20] causes a decrease of intersignal intervals similar to that which it causes in spinal-cord neurons by reducing Renshaw cell inhibition.[5] This suggests that each neuron in the explant has an inhibitory feedback from an associated inhibitory cell. The presence of these inhibitory synapses is also suggested by the zone of negative-going slow signals (seen in the reversal phenomenon) whose activity is reduced or abolished by the application of strychnine. The nonresponsive periods of neurons in the explant which have such feedback inhibition are likely to be longer than the 20 msec mentioned above. If the neuronal net in the explant behaves as the computer net does, the interval between successive waves of excitation of a given neuron and the slow signals derived from it would be likely to be close to that actually observed (200-800 msec).

Introduction of long interneuronal connections into a model net[28] converts the wave-like activity into diffuse, more random activations of the neurons. A similar change can be observed in explants made at right angles to the usual directions (i.e., at right angles to the surface of the telencephalon) so as to preserve some of the long interneuronal connections. Relatively few explants taken in this way are active and these tend to show a lack of uniformity of sequence. This is in contrast to the nearly 100% yield of active cultures showing characteristic sequences with the usual explantation technique used. The necessity for a predominance of short interneuronal connections in the net in the production of our type of spontaneous activity may explain the absence of this type of activity in vivo where there are many long interneuronal connections, and in long-term cultures[10] where there are probably insufficient interneuronal connections.

Many features of slow signal production are explained by the peculiarities of network activity. Each burst of firing in a neuron causes a slow signal in each adjacent glial cell as in Leech[36] and Necturus glial cells.[37] Intersignal intervals are the times between arrivals of successive firing fronts at this neuron. The number of signals in a sequence is the number of waves of activity that reach the neuron before firing ceases or subsides to a level insufficient for propagation through the net. The complexity of a microelectrode signal depends on the number of firings in each burst and the number of neurons surveyed by the glial cell (Fig. 4 A). The form of a signal detected by a gross electrode is the combination of the signals from glial cells and neurons within its pickup range (Fig. 4 B).

An active focus thus is envisioned as a net of interconnected neurons each with a shell of glial cells individually capable of generating sequences of slow signals. A secondary shell of glial cells may show attenuated versions of the sequences through their tight junctions,[31, 36, 37] with glial cells actually generating slow signals.

The intraglial signals previously reported[36-38] were responses to changes in extracellular potassium resulting from neuronal firings and synaptic activations.[27] The signals in our explants probably arise in the same way, the occurrence of negative, as

well as positive, slow signals being evidence for synaptic participation in their formation. The ionic changes associated with inhibitory synaptic activity in general have not been identified, but they are likely to be the electrochemical opposite of those associated with excitatory synaptic activity and cause a negative-going change in DC level within the glial cell.

Synapses occupy nearly 40% of the neuronal surface[32] with a frequency of activation that is much greater than the firing rate of the neurons on which they lie, so they contribute significantly to the extracellular ionic changes. Electron microscopic studies[25, 31, 40] show the intimate nature of the contact, since the glial cells invest and often protrude into synaptic clefts. Excitatory synaptic action will cause brief increases in potassium ions and positive-going change in the intraglial DC level. This will give rise to the early fast part of any associated glial signal (inhibitory synaptic events are likely to cause equivalent negative-going change in the intraglial DC level). The neuronal firing subsequent to synaptic activation will cause an approximate 100-msec increase of potassium ions (the potassium current),[6] starting near the end of the electrical event and resulting in a relatively long positive-going DC change in adjacent glial cells. It is probably largely responsible for the terminal slow part of slow signals.

The reversal phenomenon permits an estimate of the part played by synaptic activity in the formation of the slow signals. The polarity change during the reversal phenomenon usually affects the whole slow signal so that its fast initial part and the terminal slower components become negative-going. The inhibitory synaptic contribution is, however, sometimes not enough to counteract the potassium-current contribution and, although the whole signal is less positive-going than before, the initial fast part of the signal may become negative-going while the remainder is still more positive than the resting baseline (Fig. 3). The mutual cancellation of somatic and inhibitory synaptic contributions during the reversal phenomenon would also explain the lower amplitude of inverted signals (i.e, excursion from the resting baseline), as compared with the equivalent positive-going ones in preceding sequences. Thus, in addition to the factors already listed, the form of a slow signal will also depend on the number and type of synapses and the area of neuronal soma that contribute to the ionic fluxes around the glial cell involved.

Slow signals are not equally spaced in a sequence, and there is an inverse relationship between duration of intersignal interval and the DC shift from the baseline at the same time (Fig. 9) during spontaneous activity. This can be explained as follows: The intervals between slow signals in a sequence are insufficient for dissipation of the ionic flux that caused the signals. Potassium ions accumulate and move the neuronal membrane potential towards the depolarization point. Fewer synaptic events are then needed to cause firings during the next wave of excitation which, therefore, moves more rapidly over the net. In this way, intersignal intervals (and the

nonresponsive periods) are shortened progressively during this part of the sequence. The reduction in the synaptic input necessary for neuronal firing is reflected in the relatively smaller magnitude of the initial faster part of the slow signals during this part of the sequence. Toward the end of the sequence, the intersignal intervals become longer and the fast component of the slow signal becomes more dominant. This is in accord with the idea that a greater synaptic input is needed to fire neurons. Since the extracellular potassium must still be high, it is possible that there is some temporary intraneuronal metabolic deficiency during this part of the sequence.

In the absence of any external signal input into the net, the number of neuronal firings determines the number of subsequent synaptic events. If more synaptic events are needed to produce a neuronal firing, there will be fewer firings and fewer subsequent synaptic events and then fewer neuronal firings. In this way, the waves of excitation (and the slow signals derived from them) will move more slowly across the net, so that the intersignal interval increases, and eventually neuronal activity and slow-signal production will stop, at least temporarily.

Since the reversal phenomenon is seen only in movements of the microelectrode in one direction across a linear zone on the surface, it is probably related to the histological architecture of the explant. A diagrammatic representation of an arrangement

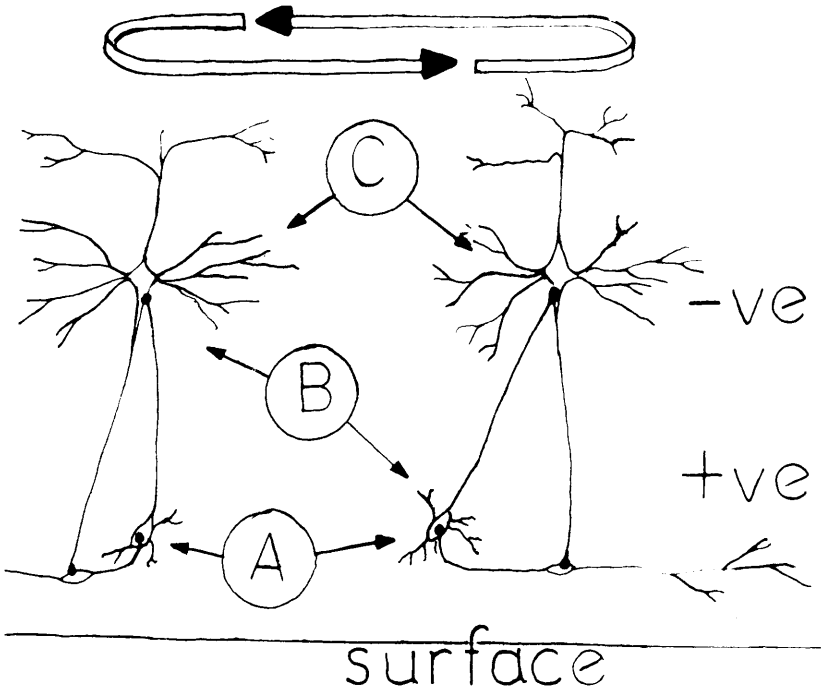


Fig. 10. A graphic representation of two cortical neuronal complexes showing the excitatory (+) and inhibitory (-) synapses.

of a few neurons from the whole net (and their monitoring glial cells) which could explain the reversal phenomenon is shown in Fig. 10. Two neuronal complexes typical of many cortices are seen, each with a pyramidal cell synapsing with a transversely oriented cell connected to a small neuron which, in turn, has a 'feedback' inhibitory synapse on the original pyramidal cell. Glial cells A, B and C survey the indicated synapses and neurons. Since the path of the firing front through a neural net probably varies slightly during each sequence, it is supposed in this case that the path of the firing front moves from left to right pyramidal cell and back during the sequence. During the first part of the sequence, the ionic fluxes causing the slow signals are the result of activity of the left neuron and the synapses on it. During the next part of the sequence, the path of the wave of activity involves the right neuron and the synapses on it, and returns to involve the left neuron and the synapses on it during the last part of the sequence. Thus the initial, central, and last parts of sequences generated in glial cell A will all be composed of positive-going signals. If the microelectrode tip is moved to record from glial cell B, the first part of the sequence will be negative-going, the central part will be positive-going, and the last part negative-going. If the tip is moved again to record from glial cell C, all three parts of the sequence will be negative-going.

The zones of relative positivity and negativity experienced during microelectrode penetrations of the brain *in vivo* (including the Wülst area on adult chicks)[1] and by us and others[10] *in vitro* may be due to an orientation of neuronal subsystems in a manner such as that in Fig. 5, the excitatory synapses being at one level and inhibitory synapses at another level.

Glial membrane potentials reported[33, 34, 36-38, 43] were recorded from relatively isolated cells in culture or dissected preparations. We have not observed glial membrane potentials in our pieces of tissue which retain some of their cellular interrelationships, nor have the majority of neurophysiologists when using whole animals. If membrane potentials could be detected for each glial cell traversed by each microelectrode insertion *in vivo*, they would be a very frequent and dramatic part of every experimental record. Thus there is the possibility that typical membrane potentials are not usually detectable, so the fact that glial membrane potentials have not been detected in the explants does not exclude an origin of the signals in glial cells. The average ionic composition of the intercellular fluid over the whole cell varies little between adjacent glial cells. Thus their membrane potentials will be similar and the microelectrode moving directly from one glial cell to another will detect little change. The only changes likely to be seen would occur when the microelectrode tip moved from the extracellular space into a glial cell and vice versa. Even if cell membranes from adjacent cells did not stretch ahead of a moving microelectrode tip and rupture together, the tip would spend an insignificant time in the 200 Å width of the intercellular cleft as compared with the 10 μ diameter of a glial cell, and there is likely to be little in the way of membrane

potentials during penetrations of explants.

The uniformity of the polarity and form of the signals obtained from microelectrode contacts over relatively large areas of active foci suggests that the microelectrode tip is in a similar situation on each. Glial cells are probably pushed aside and torn by the microelectrode tip as it slows down so that it will often come to rest in a damaged glial cell. Since previous work suggests that no activity can be detected by an electrode wholly outside single glial cells,[36, 37] it is possible that the tight junctions[31, 36, 37] of torn cells permit attenuated detection of the activity within adjacent glial cells. Thus, whichever situation prevails, the signals detected by a microelectrode in a glial milieu will still arise within glial cells. Investigations continue to provide direct evidence to replace indirect inferences by dye marking, etc., to establish histologically the recording sites involved.

Some of the other results of the stimulation studies need comment: The longest sequences and most regular sequential behavior during pacing occur when the interstimulus interval approximates the average intersignal interval for spontaneous sequences, probably because the stimuli are then in phase with the responsive part of the excitation-nonresponsive cycle. These sequences are shorter, however, than spontaneous ones because, as already described, each signal facilitates the next, decreasing the intersignal interval till the stimuli and the responsive period of the neurons move out of phase. The stimuli reach unresponsive neurons and the sequence ends after fewer signals than are seen in spontaneous sequences. The next one or two pacing stimuli will then produce only the previously described minor rounded signals which are followed by the usual nonresponsive periods. The higher the rate of pacing the more such unresponsive periods are likely to occur, and the longer will be the intervals between evoked sequences.

The less than 100 $\mu$ separation of effective and noneffective sites for stimulation in explants demonstrates the degree of delineation of active areas and restriction of conduction of the stimulus. The effectiveness of a barrage of stimuli in producing slow signals and the subsequent temporary or permanent cessation of activity are probably due to the barrage exciting enough neurons in the net so that they produce a slow signal and are then simultaneously nonresponsive and prevent the initiation of subsequent waves of excitation. A barrage is more effective than a single stimulus when given at a distance from the active focus because a single subthreshold stimulus fails to fire neurons, whereas a barrage of subthreshold stimuli is summed and does cause firing.

The minor rounded signals that may follow successful or partially successful stimulation (in the sense that it does not produce a sequence) have approximately 100-msec durations, which is consistent with glial responses to the potassium currents[6] following neuronal somatic firing. They are probably due to a stimulus activating a few neurons but failing to cause repeated propagation through the net.

The "escape" phenomenon described above has some resemblance to cardiac escape from (vagal) inhibition but we believe that it is related to metabolic recovery during pacing. Each partially successful stimulation creates a subsequent nonresponsive period and, to the extent that it uses up vital materials, delays metabolic recovery. Escape occurs when this metabolic recovery is more closely followed by a natural stimulus than by an electrical stimulus.

## 5. Conclusions

1. Neurons in an explant are interconnected in a net by axonal and dendritic processes probably involving synapses.

2. There are only two types of spontaneous signals detectable in the explants, i.e., spike potentials and slow signals (10 msec to several seconds duration) and the stimulation studies demonstrate a causal relationship between them.

3. The striking similarity in the signal duration and intersignal intervals of thousands of slow signals from each of many telencephalic explants indicates that these signals have a similar source and organization in all of these explants.

4. The slow signals arise in glial cells in response to ionic fluxes from the firing of adjacent neurons and the activation of the synapses on them.

5. The neurons in the explant do not act as individuals but in the coordinated manner of a neuronal net. The repetitive movement of wavelike zones of neuronal firings across this net cause the characteristic sequences of slow signals in glial cells adjacent to participating neurons.

6. The spacing of the slow signals within their natural groups and the form of DC change occurring during these groups of signals can be explained in terms of the signals being glial responses to the ionic fluxes associated with neuronal and synaptic activity.

7. There is a feedback inhibition of the neurons in the net which causes negative-going signals and is responsible for the relatively long intersignal intervals in the sequences.

8. The simple type of tissue preparation described in this report, particularly that used in the stimulation experiments, is a valuable research tool in investigations of the behavior of both neurons and glial cells and their inter-relations in all species. It could easily be used by students in classroom work.

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References


1. N. J. Adamo and R. L. King, "Evoked Responses in the Chicken Telencephalon to Auditory, Visual and Tactile Stimulation," Exptl. Neurol. 17, 498-504 (1967).
2. E. D. Adrian and F. J. J. Burtendijk, "Potential Changes in the Isolated Brainstem of Goldfish," J. Physiol. 71, 121-135 (1931).
3. E. D. Adrian, "Potential Changes in the Isolated Nervous Systems of Dysticus Marginales," J. Physiol. 72, 132-151 (1931).
4. A. Ames and B. S. Gurian, "Electrical Recording from Isolated Mammalian Retina Mounted as a Membrane," Arch. Ophthalmol. 70, 837-841 (1963).
5. K. Bradley, D. M. Easton, and J. C. Eccles, "An Investigation of Primary or Direct Inhibition," J. Physiol. (London) 122, 474-488 (1953).
6. L. G. Brock, J. S. Coombs, and J. C. Eccles, "The Recording of Potentials from Motoneurones with an Intracellular Electrode," J. Physiol. 117, 431-458 (1952).
7. E. H. Craigie, "Vascularity of the Cerebral Cortex in a Specimen of Apteryx – Additional Evidence of the Presence of a Homologue of Mammalian Neocortex," Anat. Rec. 43, 209-214 (1929).
8. S. M. Crain, "Development of Electrical Activity in the Cerebral Cortex of the Albino Rat," Proc. Soc. Exptl. Biol. Med. 81, 49-51 (1952).
9. S. M. Crain, "Resting and Action Potentials of Cultured Chick Embryo Spinal Ganglion Cells," J. Comp. Neurol. 104, 285-330 (1956).
10. S. M. Crain and M. B. Bornstein, "Bioelectric Activity of Neonatal Mouse Cerebral Cortex during Growth and Differentiation in Tissue Culture," Exptl. Neurol. 10, 425-450 (1964).
11. S. M. Crain, "Development of Bioelectric Activity during Growth of Neonatal Cortex in Tissue Culture," in Neurology and Electro-encephalographic Correlative Studies in Infancy (Grune and Stratton, New York, 1964), pp. 12-26.
12. A. W. B. Cunningham, M. Daugherty, and B. J. Rylander, "Spontaneous Potentials from Explants of Brain Tissue in Culture," Nature 186, 477 (1960).
13. A. W. B. Cunningham, "Spontaneous Potentials from Explants of Human Adult Cerebellum in Culture," Nature 190, 918 (1961).
14. A. W. B. Cunningham, "Spontaneous Potentials from Explants of Embryo Chick Thalamus (Mesencephalon) in Culture," Naturwiss. 48, 78-79 (1961).
15. A. W. B. Cunningham, "Spontaneous Potentials from Explants of Chick Embryo Spinal Cord in Tissue Culture," Naturwiss. 48, 719-720 (1961).
16. A. W. B. Cunningham, "Spontaneous Potentials from Explants of Chick Embryo Medulla Oblongata in Culture," Naturwiss. 48, 104-105 (1961).
17. A. W. B. Cunningham and B. J. Rylander, "Behavior of Spontaneous Potentials from Chick Cerebellar Explant during 120 hr in Culture," J. Neurophysiol. 24, 141-149 (1961).
18. A. W. B. Cunningham, "Spontaneous Potentials from Explants of Chick Embryo Pons in Tissue Culture," Experientia 17, 233 (1961).
19. A. W. B. Cunningham and S. G. Stephens, "The Qualitative Effect of Anesthetic Gases on Spontaneous Potentials from Brain Tissue in Culture," Experientia 17, 409-410 (1961).
20. A. W. B. Cunningham and S. G. Stephens, "Qualitative Effect of Strychnine and Brucine on Spontaneous Potentials from Explants of Telencephalon," Experientia 17, 569-575 (1961).

21. A. W. B. Cunningham and S. G. Stephens, "The Effect of Temperature Variation on Spontaneous Potentials Production from Explants of Brain Tissue in Culture," Experientia 18, 10-14 (1961).

22. A. W. B. Cunningham, "Qualitative Behavior of Spontaneous Potentials from Explants of 15-day Chick Embryo Telencephalon in vitro," J. Gen. Physiol. 45, 1065-1076 (1962).

23. A. W. B. Cunningham, R. R. Rojas-Corona, J. Freeman, and P. Levine, "Tissue Dynamics of Brain Tissue in vitro," Quarterly Progress Report No. 79, Research Laboratory of Electronics, M.I.T., October 15, 1965, pp. 247-261.

24. A. W. B. Cunningham, R. R. Rojas-Corona, and P. O'Lague, "Microelectrode Studies of Spontaneous Potentials from Chick Embryo Telencephalon in vitro," Experientia 22, 439-441 (1966).

25. E. de Robertis, Histophysiology of Synapses and Neurosecretion (Pergamon Press, Oxford, 1964).

26. A. Durward, "Observations on the Cell Masses in the Cerebral Hemispheres of the New Zealand Kiwi (Apteryx Australis)," J. Anat. 66, 437-466 (1932).

27. J. C. Eccles, The Physiology of Synapses (Springer-Verlag, Berlin, 1964).

28. B. G. Farley, "Neural Network Models and Slow Potentials of Electrophysiology," in Computers in Biomedical Research, edited by Waxman and Stacey (Academic Press, New York, 1965), Chap. 11.

29. R. W. Gerard and J. Z. Young, "Electrical Activity of the Central Nervous System of the Frog," Proc. Roy. Soc. (London) B122, 343-352 (1937).

30. R. Granit and L. A. Riddell, "The Electric Response of Light and Dark Adapted Frog's Eyes to Rhythmic and Controlled Stimuli," J. Physiol. 81, 1-28 (1934).

31. E. G. Gray, "Synapses of the Cerebral Cortex and Certain Specializations of Neuroglial Membranes," Electron Microscopy in Anatomy (Edward Arnold, London, 1961), p. 54.

32. R. A. Haggar and M. L. Barr, "Quantitative Data on the Size of Synaptic End-Bulbs in the Cat's Spinal Cord," J. Comp. Neurol. 93, 17-35 (1950).

33. W. Hild, I. Tasaki, and J. J. Chang, "Electrical Response of Astrocytic Glia from the Mammalian Central Nervous System Cultivated in vitro," Experientia 14, 220-221 (1958).

34. W. Hild and I. Tasaki, "Morphological and Physiological Properties of Neurones and Glial Cells in Tissue Culture," J. Neurophysiol. 25, 277-304 (1962).

35. M. J. Karnovsky, "A Formaldehyde-Glutaraldehyde Fixative of High Osmality for Use in Electron Microscopy," J. Cell. Biol. 27, 137 (1965).

36. S. W. Kuffler and D. D. Potter, "Glia in the Leech Central Nervous System: Physiological Properties and Neuron-Glia Relationship," J. Neurophysiol. 27, 290-320 (1964).

37. S. W. Kuffler, J. G. Nicholls, R. K. Orkand, "Physiological Properties of Glial Cells in the Central Nervous System of Amphibia," J. Neurophysiol. 29, 768-787 (1966).

38. S. W. Kuffler, "Neuroglial Cells, Physiological Properties and a Potassium Mediated Effect of Neuronal Activity on the Glial Membrane Potential," Proc. Roy. Soc. (London) B168, 1-20 (1967).

39. P. H. O'Lague, "Spontaneous Activity in 14-day Chick Embryo Telencephalon Tissue Culture," S.M. Thesis, Massachusetts Institute of Technology, 1965.

40. S. L. Palay, "The Morphology of the Synapse in the Central Nervous System," Exptl. Cell Research, Supp. 5, 275-293, 1958.

41. D. C. Pease, Histological Techniques for Electron Microscopy (Academic Press, New York, 2d edition, 1964), p. 260.

42. E. R. Peterson, S. M. Crain, and M. R. Murray, "Differentiation and Prolonged Maintenance of Bioelectrically Active Spinal Cord Cultures (Rat, Chick and Human)," Z. Zellforsch. 66, 130-154 (1965).

43. W. M. Wardell, "Electrical and Pharmacological Properties of Mammalian Neuroglial Cells in Tissue Culture," Proc. Roy. Soc. (London) B165, 326-361 (1966).