is nonspecific and that it occurs as a result of inhalation of very small particles.

The extent to which this biological response is reversible will be an important consideration in determining the potential hazard from long-term exposure. In addition, the development of a dose-response relationship at some lower levels and duration of exposure will serve to verify the public health significance of this study. Further work is needed to settle these questions.

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Operant Controlled Neural Event: Formal and Systematic Approach to Electrical Coding of Behavior in Brain

Abstract. *Traditional studies of electrophysiological correlates of behavior contain inherent high variability resulting from the arbitrary choice of behaviors, brain locations, and wave parameters. The operant control of neural events is a formal and systematic approach to the study of prespecified parameters and components of brain activity as they encode behaviors. Two studies in which the electrical activity of brain was the criterion for reinforcement demonstrate the acquisition, under such operant control, of two mutually exclusive behaviors or states which selectively alter evoked potential components.*

The purpose of this report is to raise some reservations regarding traditional approaches to the study of neural correlates of behavior and to offer a strategy for investigating behaviorally relevant bioelectrical activity of brain. Electrical activity of the brain has been of continuous interest in studies of neural correlates of behavior, especially learning (*1*, *2*), in which bioelectrical activity of brain in the form of cortical evoked potentials, single cell discharge, spontaneous electroencephalogram, d-c shifts, impedance changes, and the like has been related to changes in behavioral state accompanying learning or differential performance.

Although a variety of such changes has been described, replicability has been a recurrent problem, and the variable, transient, unreliable, and arbitrary character of such responses is well recognized (*1*); this character is consistent with any arbitrarily selected collateral response system (heart rate changes, galvanic skin response, and other).

We are concerned with the apparent simplicity of these studies and feel that reexamination of such correlative paradigms leads to the conclusion that the understanding and control in such experiments may be less than is believed, for a number of reasons as follows.

1) The inherent variability of spontaneous behavior contributes substantially to the variability of the bioelectric response to a so-called "neutral" stimulus, resulting in less than complete confidence in the knowledge of the conditioned stimulus.

2) The choice of a given behavior is arbitrary in reference to a chosen brain location. The fact that electric responses to stimuli can be recorded from many widely separated areas of the brain has encouraged the placement of large arrays of electrodes to compensate for the lack of specific information by force of number of placements in locating brain responses that may be relevant to the specific behavioral paradigm.

3) Transient responses may be expected, and the process of establishing a particular functional connection may at some specific time in training be considered complete. Continuation of the behavioral task may depend upon processes not necessarily occurring at the original location. Thus, the component nature of complex behavior may be both multiple and sequentially represented in brain.

4) The arbitrary choice of behavior also takes into account in acquisition or performance only one or a few endpoint responses, and not the infinitely complex and unknown (possibly not parallel in time) set of collateral responses that are occurring in the conditioning paradigm (*3*) and that have unknown individual and conjoint influences on the bioelectric response. Most important is that behavior is rarely described accurately on a time base comparable to the base used to describe the momentary fluctuations in excitability as reflected in the bioelectric response. Correlation of momentary and discrete bioelectric events, therefore, with multiply determined molar behaviors may be in error by one or more orders of magnitude (days compared to milliseconds).

5) Bioelectric response parameters for evaluation is often not prespecified and awaits the empirical outcome of an experiment. Parameter specification for correlation with molar behavior is necessarily arbitrary and may be unrelated to the major response system under conditioning control by the animal.

Therefore, knowledge of the effective stimulus, of the actual response being conditioned, of the relevant recording site, and of relevant parameters of the dependent variable probably contributes substantially to the variability of results in studies of bioelectric correlates of behavior.

A modified approach to the study of the behaviorally significant bioelectric events is described here. This approach provides for the formal, sequential, and systematic study of bioelectric response parameters which either separately or conjointly are relevant to or encode learned behavior. Available techniques (*4*, *5*) are used and these methods are applied to functional bioelectric coding.

Several findings have been important for our renewed belief that the sequen-

tial stimulus information conveyed to the central nervous system may be equally well evaluated by a rigorous analysis of the evoked potential waveform or by analysis of single cell activity. We have demonstrated (*6*) that the evoked potential or spontaneous waveform recorded from the brain is closely related to the sequential, pulsatile activity of a single cell. The curve of probability of firing of a single cell either continuously or at intervals after a stimulus is duplicated accurately by the waveform of the evoked potential or of spontaneous slow activity. Thus the evoked potential represents, as does sequential single cell firing probability, the spontaneous or stimulus-initiated pulse code of the nervous system. Our study, therefore, is a first step in the development of an approach by which parameters of the sequential components of the evoked potential waveform which are relevant to the coding of learned behaviors may be formally specified.

The approach consists of the following phases. Instead of being made a dependent variable, the evoked potential is made the criterion for reinforcement or the independent variable, and the location and parameters of components of the evoked responses may be specified in advance. By allowing the animals a free range of behavior and by making reinforcement contingent upon the prespecified neural response, the animals are allowed to use whatever behavior is available in generating the specified response. A neural response of relatively low probability is chosen from an animal's repertoire. Reinforcement is expected to increase the probability of the occurrence of the response if it is relevant to behavior, that is, under control of reinforcement.

Our general procedure for operant conditioning is as follows. Initially, measures are taken of the mean and variance of an evoked response, from an arbitrarily chosen brain location. On this basis, a criterion response is established, and the probability of its occurrence without reinforcement is determined. This probability is determined anew each day before the training session. During training, reinforcement is presented immediately after the occurrence of a criterion response. The pattern of reinforcements generated by the experimental animal serves as the basis for reinforcement for a yoked-control animal—which in every respect is treated as the experimental animal—except that reinforcement is not contingent upon its own response, but was determined by tapes produced by the performance of the experimental animals on the same day.

Specific to these studies, four cats were implanted with (i) long-term cortical and subcortical electrodes led to a multiconnector plug fastened to the skull, and (ii) a plastic tube anchored at one end near the plug and at the other to an upper tooth, for the rapid delivery of milk reinforcement, leading respectively to the appropriate amplifiers and milk reservoir. The animals were placed in an empty shielded box with a frosted glass window and were allowed a free range of activity, except for the physical limitations of the cable and box. The entire conditioning program was under the on-line, real-time control of a computer system (PDP-8) located in another room.

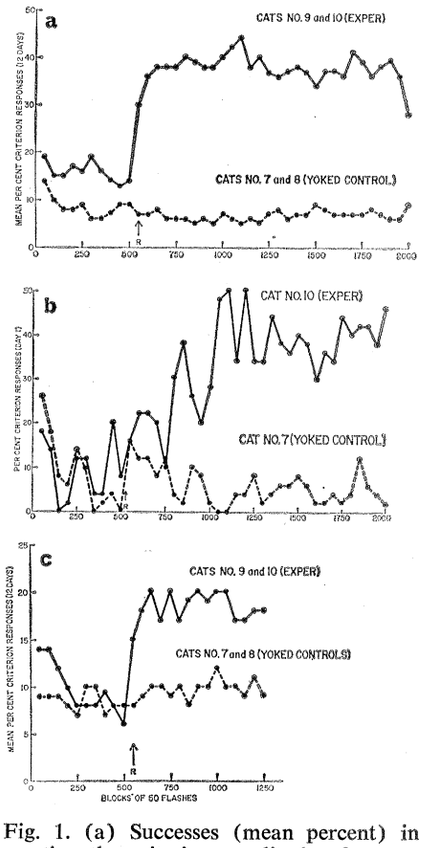

Fig. 1. (a) Successes (mean percent) in meeting the criterion amplitude of a prespecified evoked potential component over 12 days. The letter *R* with the arrow indicates the end of the extinction period and beginning of reinforcement each day. Animals are reinforced for increased negativity at 170 to 193 msec after the light flash. (b) Acquisition (percentage of successes) in meeting the criterion response for the first day of extinction and training. (c) Performance (percentage of successes) in meeting the criterion amplitude of the same prespecified evoked potential components as in (a), but animals are reinforced for decreased negativity.

At intervals of 4 seconds, the computer triggered a Grass PS2 photostimulator, delivering a 10-msec light flash to the animal through the window of the recording box. Analog brain voltage from the amplifiers, 150 msec before and 350 msec after the light flash, was digitized for the computer's operation. The digital values were displayed on a storage oscilloscope. To establish a training criterion before conditioning, the mean and standard deviation of 2000 responses from the left visual cortex (VII) were computed. In the first study, the criterion was established by requiring that the mean voltage of a selected portion of the evoked response (between 170 and 193 msec after the stimulus) be 1.2 standard deviations more negative than the mean before conditioning. In the second study, it was required that this same voltage be 1.2 standard deviations more positive than the mean before conditioning.

During conditioning, flashes were presented as above, but after each flash the incoming value of the selected points was compared with the criterion value and reinforcement was delivered if the criterion was met. Thus, the program provided reinforcement of behavioral states of the animals which altered by a criterion amount the selected parameters (voltage in this case) of the flash-evoked potential of visual cortex.

A food-deprived animal was expected to maximize those responses which led to reinforcement. However, unless the amplitude of the evoked potential at the points selected was in some way related to either behavioral or neural information, it should be impossible for the animal to generate them with a probability higher than that determined during base-line measurements. It is possible that amplitude at these points might be increased by some other means. For example, the conditioning program might be construed as classical conditioning with partial reinforcement, rather than operant conditioning, in that some part of the time a reinforcement is immediately preceded by a light flash. This and other possibilities are excluded by the yoked-control animals.

For training of behaviors related to increased negativity in the first study, 2000 trials (flashes) per day were presented to the animals for 12 days, with no reinforcement for the first 500 trials so that we could determine the base rate of responding. A response 1.2 standard deviations above the mean would be expected to occur by chance

approximately 12 percent of the time. The observed mean rate of criterion responding during the 12 nonreinforcement sessions was 8.7 percent for the yoked-control animals and 16.9 percent for experimental animals. This slightly higher preconditioning rate of criterion responding found in experimental animals was anticipated since, once the behavior was conditioned, the trials on which the determinations were made for the experimental animals also functioned as extinction sessions.

The results of this initial study demonstrate the acquisition, by animals reinforced in this way for increased negativity, of responses which alter parameters (amplitude) of specific components of the evoked potential. Figure 1a contrasts the mean daily performance (12 days) of the experimental animals with their respective yoked-control animals during periods of nonreinforcement and periods of reinforcement. The sudden increase in criterion responses to a high level (37.9 percent) by the experimental animals at the onset of reinforcement is in sharp contrast to the continued, stable, low performance (6.2 percent) of the controls. Further, there was no overlap between experimentals and controls for any 50 flash block on any day for the reinforcement period.

An animal performing as high as 70 percent criterion responses for any block of 50 flashes or for any day and showing no gross motor behavior which could be related to the performance sat quite still in a variety of positions in the recording box. No differences between experimental and control animals were observed. The underlying behavioral or other events, therefore, which produce the observed alteration in brain activity are obscure.

The acquisition of such operant controlled neural events may be extremely rapid, as short as a single training session. Figure 1b shows the performance of an experimental animal and his yoked control on the first training day. Comparison of Fig. 1, a and b, indicates that the performance of these animals on this first day is quite representative of that on the next 11 days. We suspect, however, that acquisition rate depends on the complexity of the formal questions asked concerning the functional utilization of specific parameters of the neural events under investigation. The use of criteria involving second-order or derivative measures, or combinations of parameters, or combinations of locations in time on the bioelectric

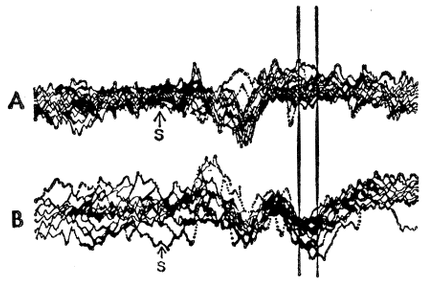

Fig. 2. Oscilloscope traces from animal reinforced for behaviors producing decreased negativity. (A) Fifteen traces taken from an extinction period; (B) 15 traces taken from the reinforcement period immediately following. Eight of the responses in (B) resulted in reinforcement. The criterion component of the evoked response lies between the two parallel vertical lines. The letter *S* with the arrow indicates the light flash stimulus. There are specificity and localization of the change. There is also some increased variability before the stimulus in (B).

signal will effect such acquisition functions.

A second study was conducted to indicate the lability and generality of the operant controlled neural events and to further demonstrate that the results of the first experiment were not specific to the particular response (increased negativity) chosen. States which resulted in decreased negativity at the same location on the evoked potential as previously were reinforced. The criterion responses for the first and second studies were mutually exclusive and represented a difference between them of 2.4 standard deviations.

The second study indicates that, as before, animals can generate a behavior or state which results in such neural changes. Figure 1c shows that mean daily performance for experimental animals (18.9 percent) differs dramatically during the reinforcement period from that of the yoked controls (10.4 percent), while performance during the 12 nonreinforced sessions again was comparable (9.5 percent for the experimental animals and 8.9 percent for the controls). Again, performance of the experimental animals was somewhat higher during nonreinforcement, attributable to effects of repetitive training and extinction. This conditioned decrease in negativity of the chosen evoked potential component immediately followed the training which resulted in increased negativity in the same animals. Again, no related behaviors were observed. In all cases, after the two experiments, yoked-control animals, reinforced for the same changes in bioelectric activity as experimental animals, showed similar conditioning, thus eliminating the possibility of any inherent differences between the two groups.

The specificity of changes should also be emphasized. The definition of the criterion response restricted only the amplitude of the response between 170 and 193 msec, but did not specify the manner in which the decreased negativity was to occur nor the extent to which correlated changes in other components of the evoked potential might occur. Nevertheless, it was observed that an animal may generate highly localized and specific responses which effect only the designated components of the evoked response.

The discrete nature of the change in response is suggested by the raw data (oscilloscope traces) of Fig. 2, which shows 15 evoked responses from an experimental animal during a nonreinforcement period and 15 responses during reinforcement. The evoked potentials generated during reinforcement represent 8 out of 15 successes. For all of the reinforced sweeps the potential differs from the nonreinforced condition. More important, is the specificity of the alteration and its restriction to the designated component (area between the parallel vertical lines).

The response of this animal, represents a specific and local change in the direction of the potential and not simply an alteration of overall voltage, although such specificity was not required by the experimental paradigm. Minimally, the evoked potential in this case is not a single functional event, coding a single message throughout its extent. Such specificity of functional control suggests the relative functional independence of the individual components of the evoked potential, and implies considerable specificity, complexity, and relative independence of component behaviors.

Operant control of neural events as an approach to functional coding in bioelectric events allows systematic and formal study of prespecified parameters of bioelectric activity, singly or together as they functionally relate to behaviors. In these studies the operant controlled neural event has already indicated the relative independence of specific wave components. The approach, therefore, affords all the information concerning the functional aspects of bioelectric events available from traditional correlative methods in which some behavior is observed, but with the addi-

tional power of parameter selection and rapid resolution of sequential experimental questions. The operant controlled neural event, however, provides no more and possibly less information regarding the behaviors being coded than do correlative methods. The same problems of multiple determination and multiple representation of behaviors by brain events exist in the present system, with the exception that there is greater assurance that the relevant behaviors are in a steady state. The elucidation of specific behaviors or classes of behaviors related to specific neural events must await measurement of behaviors and brain events on the same time base with the same zero time and with the same resolution.

At present, it is difficult to think of behaviors which may be measured continuously with millisecond resolution in such an analog fashion. The major problem of brain-behavior relationship may now be in the measurement of behavior.

Finally, the operant controlled neural event has been demonstrated as being capable of experimentally separating functional implications of parameters and components of waves in brain. To the extent that electrophysiologists have developed hypotheses regarding microanatomical correlates of bioelectrogenesis in brain, in terms of cortical morphology or synaptic configuration or connectivity (*7*), such hypotheses should serve as a rational guide in the selection of parameters to be investigated as operant controlled neural events. To the extent that such microanatomical substrates are already understood in terms of parameters of electrical events or the reverse, the operant controlled neural event allows the determination of the relative independence of these as well as the separate and conjoint functional role of such anatomical systems in brain.

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Midbrain Single Units Correlating with Pupil Response to Light

Abstract. *The consensual response of the pupil in the cat was driven by means of a light flux impinging on the contralateral retina. Spike trains recorded extracellularly from single units in the midbrain show correlation with the concurrently recorded pupil area. The temporal dynamics found confirm two earlier studies of single-unit responses and quantitative nerve stimulation. Both of these indicate that most of the 200-millisecond transport delay resides in the neuromuscular apparatus. Neurons whose activity correlated either with constriction or with dilatation phases of change in the pupil area were observed.*

In reporting single-unit activity in the midbrain of the cat, Nisida and Okada (*1*) described an oculomotor unit with a spontaneous firing rate of 12 pulse/sec (*1*), and, Terdiman, Smith, and Stark (*2*) described a midbrain single unit with an average firing rate of 20 pulse/sec, the variations of which correlated with the dilatation phase of the pupil response to light.

In order to maintain a stable consensual (contralateral) pupil response to light the following techniques were used. Cats were first injected intraperitoneally with 20 mg per kilogram of body weight of sodium pentobarbital and were positioned in a stereotaxic frame (after the trachea and femoral vein had been cannulated). These animals were then placed in a shielded cage and were respired artificially while succinylcholine chloride (Anectine) was administered intravenously (20 mg/hour) in order to avoid any extraocular activity that might interfere with the actual recording of the pupil response. All experiments were performed with the on-line use of the IBM 1800 computer with a teletype to communicate with the computer from the remote laboratory (20 m). The desired time-varying voltages generated by the computer drove a linear light-function generator (glow modulator 1131C) (*3*). This light signal was conducted through fiber optics to the retina of the input eye where the incident light ranged from 0.001 to 10 mlu/m^2. The pupil of the input eye was completely dilated with cyclogyl (cyclopentolate hydrochloride); this resulted in an open-loop condition, that is, the iris in no way interfered with

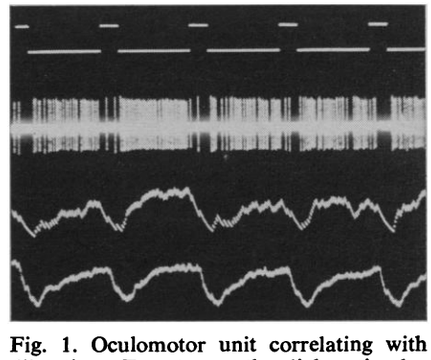

Fig. 1. Oculomotor unit correlating with dilatation. Top trace, the light stimulus changing from 0.01 to 5 mlu/m^2 for 800 msec every 4 seconds; second trace, light-off as well as spontaneous firing; third trace, these same pulses after passing through a simple model of a pupil that consists of only a low-pass filter (this allows one to predict roughly the expected response of pupil area); fourth trace, actual response of the pupil with the pupil changing (last response) from a dilated base line of 20 mm^2 to a constriction peak of 15 mm^2.