69-18,368

ROSEN, Steven Charles, 1941-
INTERSENSORY ELECTROCORTICAL
CONDITIONING OF STEADY POTENTIAL
SHIFTS IN NORMAL AND EPILEPTIC
MONKEYS.

The City University of New York, Ph.D., 1969
Psychology, experimental
University Microfilms, Inc., Ann Arbor, Michigan

INTERSENSORY ELECTROCORTICAL CONDITIONING OF STEADY POTENTIAL SHIFTS IN NORMAL AND EPILEPTIC MONKEYS

by

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A dissertation submitted to the
Graduate Faculty in Psychology
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy,
The City University of New York.

1969

This manuscript has been read and accepted for the University Committee in Psychology as satisfying the dissertation requirement for the degree of Doctor of Philosophy.

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Acknowledgements

The author wishes to express his gratitude to Dr. John S. Stamm for seemingly unending support and encouragement. Both the conception and execution of this research are in large part the result of a long and fruitful personal association with Dr. Stamm. An equal measure of gratitude goes to Dr. William S. Battersby for his invaluable suggestions and contributions to this author's understanding of the significance and relevance of bioelectric potentials. The author should also like to thank Dr. Philip Ziegler for his services on the dissertation committee and acknowledge his special indebtedness to Mr. Howard Teich for his competent technical assistance. The research was made possible by funds provided by the National Science Foundation Grant GB 2693 and the City University of New York. The author would like to thank these institutions for their support.

Abstract

The relations of bioelectric steady potentials (SPs) to processes of cortical excitability were examined in experiments involving normal and epileptic monkeys. Recordings of electrocortical responses to acoustic (clicks) and photic stimuli (flicker), made with DC amplifiers and transcortical nonpolarizable electrodes chronically implanted in visual and auditory cortex, showed surface negative SP shifts in areas of sensory cortex corresponding to the modality of the stimulus. Further, it was found that in normal monkeys pairings of clicks (CS) with flicker (US) resulted in conditioned electrical responses (CRs) to clicks alone. CRs consisted of negative SP shifts in visual cortex and positive shifts in auditory cortex. The significance of conditioned shifts was then evaluated in a second conditioning experiment involving epileptic monkeys. Recordings of focal epileptic discharges (spikes), induced by alumina cream implants in either visual or auditory cortex, showed an increased incidence of spikes associated with the acquisition of conditioned negative SP shifts in visual cortex and a decreased incidence of spikes with positive shifts in auditory cortex. Conditioned shifts were interpreted as reflecting processes of cortical excitability with negative shifts indicative of increased, and positive shifts decreased, excitability states. This hypothesis was

further supported by the findings of supplementary experiments in which the magnitude of the SP response in visual cortex was found to be a function of (1) flicker frequency and intensity and (2) the interval between successive stimuli when positive steady afterpotentials were in various states of growth and decay. A final supplementary experiment demonstrated the temporal conditioning of epileptic spike activity.

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INTERSENSORY ELECTROCORTICAL CONDITIONING OF STEADY POTENTIAL SHIFTS IN NORMAL AND EPILEPTIC MONKEYS

Introduction

Definition of steady potentials

Steady potentials (SPs) may best be defined as a class of bioelectric potential differences between pairs of recording electrodes. Since direct-coupled (DC) amplifier (as well as nonpolarizable electrodes) are essential for obtaining undistorted records of SPs, the terms "DC potential" and "D.C. potential" (sometimes meaning direct current potentials) have enjoyed popular usuage. As Rowland (1967) points out, these terms are best left as electrical circuit notations since they imply characteristics which are of uncertain biological significance. Other terms which have been commonly used are "steady state potentials," "standing potentials," and "slow potentials." With regard to "slow potentials," reference is usually made to oscillatory potentials with periods so long as to require DC amplification. The inherent rhythmicity of these potentials distinguishes them from SPs, as defined above, but they undoubtedly relate to neural mechanisms underlying SPs and should be considered.

Steady potentials can literally be recorded with electrodes placed anywhere in the brain. Those recorded with electrodes across the cerebral cortex (from surface to depth) however, give the most promise of relating to problems of brain function. The term "cortical SP" shall be used to refer to SPs obtained with at least one recording electrode placed on the cortical surface and the second, or reference electrode, placed on either a cut surface of the cortex on the same gyrus or in the underlying subcortical white matter. Ventricular references have also been used and are generally regarded as relevant in the discussion of cortical SPs, however, reference electrodes on bone surfaces or in bone sinuses, which are in common use, are of questionable value in direct SP measurements since absolute SP measures will largely be determined by interelectrode differences. Perhaps the most significant feature of the cortical SP is that changes or shifts can be affected by changing physiological and behavioral conditions. The term "SP shift" introduced by O'Leary and Goldring (1964) shall be used herein to refer to alterations of the SP over time.

Historical background

Steady potentials (SPs) are the earliest known bioelectric potentials to be recorded directly from the cerebral cortex of the living brain. In 1875, Richard Caton, and English physiologist, applied nonpolarizable electrodes to the exposed cortex of an unanesthetized rabbit and recorded the current flow (resulting from a difference in electrical potential) between one electrode placed on the external surface of the cortex and another electrode on an adjacent cut surface (Brazier, 1963). By observing the needle deflection of his Thompson galvanometer, Caton found the external surface to be electropositive with reference to cut surface and moreover, that light stimulating the eye of the rabbit (supplied by an oxy-hydrogen lamp) caused negative variations of the maintained positive deflection. The negative variations were interpreted as having a relation to the function of the cortex. Caton also found small incessant fluctuations of his galvanometer needle in the absence of all stimulation. Within the historical perspective of the twentieth century, Brazier (1963) and Rowland (1968) point to his finding as nothing less than the discovery of the electroencephalogram (EEG).

Some fifteen years later similar discoveries were independently reported by a young Polish physiology student named Adolf Beck (Brazier, 1963). As did Caton before him, Beck used a galvanometer and Zinc-Zinc sulfide nonpolarizable electrodes to measure electrical potentials directly from the cortex of experimental animals. Beck found that light could evoke negative potential swings of his galvanometer needle when at least one electrode was on occipital cortex. In addition, he found that negative swings could sometimes be evoked in temporal cortex by acoustic stimuli such as shouting by the experimenter. Even more surprisingly, Beck reported that the small, appearently spontaneous, oscillations of his galvanometer needle seen in the absence of stimulation (the EEG) disappeared when light was used to stimulate the eye. Thus Beck was first to describe the "blocking" or "suppressing" activity of peripheral stimuli on the EEG and its association with SP variations.

Both Caton and Beck may be credited with the discovery of the cortical SP, the EEG, and phenomena such as EEG blocking. In additon, they were first to recognize that negative SP variations or shifts reflect excitation of the cerebral cortex relating to brain function. Further, Beck's data suggest relations of SP phenomena to EEG activity and, questions posed by Caton concerning the relatively widespread nature of shifts he observed in response to photic stimulation, show, according to Rowland (1968), that he aniticipated the concepts of specific and nonspecific cortical systems. However, it should be noted that, in spite of these early advances in the recording of bioelectric potentials, little more has been added to our knowledge of cortical SPs since that time. The advent of electronic amplifiers coupled with Berger's (1929) discovery of the EEG in humans has diverted attention exclusively to the smaller amplitude electrical potential oscillations and other bioelectric phenomena (i.e. single unit discharges) at the high end of the frequency spectrum of electrocortical events associated with brain function.

Subsequent interest in cortical SPs was evoked by the suggestion of Gerard (1936) that "d-c fields" may exert a synchronizing influence of the activity of cortical neurons and by subsequent findings (Gerard, and Libet, 1940) which pointed to a polarization of electric charge across the cortex with the superifical dendritic layers electronegative with respect to underlying axonal layers of the same neuron populations. The concept of "d-c fields" across the cortex was unquestionably attractive to Kohler who was seeking electrocortical "isomorphs" of "perceptual fields."

Kohler and his colleagues subsequently confirmed the findings of Caton and Beck, as did Libet and Kahn some years earlier (1946), by recording SP shifts over visual and auditory cortex of the cat in response to photic and acoustic stimuli, respectively (Kohler and O'Connell, 1957; Kohler, Neff, and Wegener, 1955). Kohler et al. extended these findings to man by recording "quasi steady currents" from the scalp over visual and auditory cortex (Kohler and Held, 1949; Kohler and Wegener, 1955). Kohler was able to show that the focus of excitation in the occiput could be made to shift in a direction opposite to that of a light moving across the visual field and that with repetivitve stimulation, responses were subject to rapid habituation thus, providing strong evidence for the functional significance of cortical SP shifts. More recent investigations by Gumnit (1960 and 1961), Gumnit and Grossman (1961) and Lickey and Fox (1966) have established the limits to which cortical SP shifts may be localized in sensory areas and have specified local and diffuse components which may relate to specific and nonspecific cortical systems.

Another current area of investigation into cortical SPs involves the recording of SP shifts in free-moving experimental animals with chronically implanted nonpolarizing electrodes. Caspers (1961 and 1963) reported relatively long enduring surface negative SP shifts accompanying the transition from sleep to wakefulness in rats. The initiation of movement typically results in SP shifts in the fully awake rat which can be shown to be independent of electrical recording artifacts. Similar findings have been reported by Rowland and Goldstone (1963) and Rowland (1968) for the cat. Consummatory behavior in particular has been associated with large SP shifts as well as other rewarding stimuli, including electrical stimulation of the hypothalamus (Wurtz, 1965; Rowland, 1968).

Perhaps the most significiant feature of chronically recorded SPs is their susceptibility to modification by conditioning procedures. SP shifts have been reported as concomitants of classical conditioning in behavioral studies conducted by Rusinov (1960). Rusinov observed the acquisition of 1.0 mV baseline shifts to a conditioned stimulus (light) when paired with an aversive unconditioned stimulus (shock). Similar findings cited by Rusinov (1960) were reported by Shvets in 1958. However, most of the studies involving modification of cortical SPs reported in the literature have involved so called "electrocortical conditioning" as opposed to more traditional "classical" or "instrumental" conditioning. Following the early lead of Durup and Fessard (1935), and Jasper and Cruickshank (1937), among others, the classical conditioning paradigm has been applied in such a way that the response to be conditioned (CR) is the cortical potential itself (EEG activation patterns in the case of the earlier investigators), rather than the more conventional response of muscles or glands. In acute experiments, for example Morrell (1960), has shown that the somatosensory response to electrical stimulation of the centre median nucleus in the rabbit can be conditioned to an auditory stimulus following stimulus pairings. No behavioral responses were observed. Similarly, SP responses to electroshock of the radial nerve were reported to be conditioned to clicks in flaxedilized cats (Rowland, 1960). Reversal of the polarity of the conditioned SP shifts was seen with extinction procedures. The most extensive study of conditioned SP shifts involving chronic preparations thus far, is that of Rowland and Goldstone (1963), who found that widespread SP shifts associated with the presentation of food could be conditioned to clicks or light flashes. The magnitude of the shifts were subject to variation by experimental manipulations of drive states. The conditioned shifts were interpreted as reflecting generalized arousal or orienting responses. No study thus far with chronic preparations has involved intersensory electrocortical conditioning in which the sensory SP response of the cortex to stimuli of one modality is conditioned to stimuli of a second stimulus modality, as has been shown for conditioned activation patterns in the EEG by Morrell and Jasper (1960). Demonstration of such phenomena may provide new information concerning the mechanisms underlying electrocortical conditioning.

It should be pointed out at this point that although electrocortical conditioning studies involving SP shifts may provide important data concerning a number of cortical processes, in addition to being of interest in their own right, little if any data bear on the direct relation of conditioned SP shifts to neural mechanisms subserving learning. Indeed, studies involving other components of conditioned electrocortical activity (i.e., frequency specific activity) have shown an essential independence of conditioned cortical events and elicited (conditioned) behavioral responses (Chow *et al.*, 1957; Schuckman and Battersby, 1965). Though SP shifts will be conditioned in the following experiments no attempt as such will be made to relate the findings to learning. Conditioned shifts will be elicited in monkeys by inter-sensory conditioning procedures primarily as a matter of convenience so that their effects on ongoing epileptic activity and their relation to general processes of cortical excitability can be examined.

Interpretation and significance of cortical steady potentials

The literature concerning the signifcance of cortical SP shifts has been extensively reviewed by Brazier (1963) and O'Leary and Goldring (1964). The latter authors discuss at length the broad spectrum of physiological and behavioral conditions which give rise to SP shifts including; hypoglycemia, anoxia, and pH changes, as well as thermal, chemical, and electrical stimulation. Included also are the effects of peripheral (sensory) stimulation and changes in behavioral states. O'Leary and Goldring conclude that the concept of the cortical steady potential is inexact in that the measurements of the SP "must include a composite of factors: including membrane potentials exteriorly recorded en masse, as well as a variable content of injury, oxygen diffusion, pH, and perhaps glial potentials." However, these authors go on to state that "the concept that each cortical pyramid cell presents a resting potential gradient along the sometimes extensive length which intervenes between its subsurface dendritic plexus and its soma situated in one of the deeper layers, gives promise of gathering all the electrical phenomena of the neuron under a single aegis."

Their statement implies that, in spite of the inexactitude of SP measures, much of the available electrophysiological data supports the concept of cortical origin reflecting the activity of cortical neurons. According to O'Leary and Goldring, surface negative shifts reflect relative changes in the positive SP (recorded in mammalian cortex) with activation of cortical cells. Increased surface negativity is seen as the result of the propagation of current (excitation) to the superficial dendritic layers of the cortex from pyramidal cells in the middle or lower layers of the cortex, consequent to their discharge (firing). The spread of excitation is thought to be mediated by ascending apical dendrites which are predominantly oriented perpendicular to the cortical surface. The cortical surface is estimated to be several millivolts positive with respect to the depth and SP shifts under conditions of "normal" excitation are generally less than 1.0 mV. (Shifts accompanying spreading depression often exceed several mV.) Therefore, a negative SP shift in fact reflects a relative diminution of the positive SP.

The above interpretation of cortical SPs suggests a neuronal model which can account for negative shifts with increased cortical excitation. A second interpretation of SP shifts offered by O'Leary and Goldring, similar to that originally suggested by Gerard (1940), is that SP shifts also reflect excitability changes within the cortex with surface shifts reflecting other cortical processes serving to "gate" or "modulate" subsequent neuronal activity. Studies of the effects of applied polarization on single unit discharges by Creutzfeld _et al._ (1962), and the work of Von Euler and Green (1960) on the effects of local cat-and an-electrotonus applied to the single cell, are cited to support this view. However, estimations of the current density required to excite either the neuron or the isolated membrane are less than that would be expected extracellularly under normal conditions of cortical excitation (Creutzfeld _et al._, 1962; Lickey and Fox, 1966). In view of findings of SP shifts leading to seizure discharges reviewed by Goldring and O'Leary (1963) and an earlier report by Strumwasser and Rosenthal (1960) that current levels in the nanoampere range can effect the excitability of single frog cerebellar cells, some question of the validity of this hypothesis still remains open to question.

Hypotheses and plan of experiments

Based on the foregoing analysis, essentially two hypotheses may be advanced concerning the significance and relevance of cortical SP shifts to problems of brain function; (1) that SP shifts reflect processes of cortical excitation, with negative shifts indicative of increased, and positive shifts decreased, excitation levels, and (2) that SP shifts reflect related processes which serve to "gate" or "modulate" subsequent cortical activity. The latter hypothesis implies a relation of SP shifts to processes of cortical excitability. The objective of the present research was to provide data to further evaluate each of these two hypotheses in turn.

In order to test the first hypothesis an intersensory electrocortical conditioning study, involving chronic preparations, was planned to determine first, if SP shifts can be evoked in sensory cortex of the monkey corresponding to the modality of the stimulus, and second, if SP shifts in response to stimuli in one modality can be conditioned to stimuli in another modality. This procedure was selected because it (1) affords an opportunity to assess the relative degree to which conditioned shifts can be localized within the cortex and (2) it conveniently allowed for the use of intermittent stimuli with specificable frequency characteristics which result in, among other things, conditioned frequency specific activity (Chow _et al._, 1957; Morrell and Jasper, 1956; Yoshii and Hockaday, 1958). Thus the experiment was planned to permit an examination of the relation of conditioned SP shifts to a second dimension of conditioned electrocortical activity usually associated with cortical excitation. The procedure briefly outlined, was as follows.

Four monkeys were prepared with pairs of miniature nonpolarizable electrodes chronically implanted in sensory cortex. One electrode of each pair was placed on the surface of the cortex and the other was inserted into adjacent subcortical white matter so that cortical SPs could thus be recorded directly. Intermittent acoustic (clicks) and photic stimuli (flicker) served as the conditioned (CS) and unconditioned stimulus (US), respectively. Following seven daily sessions of Adaptation procedures in which responses to the conditioning stimuli were habituated stimulus pairings were introduced. The Conditioning procedure in which flicker followed clicks by three seconds (CS-US interval equal to three seconds) was continued for seven sessions of 30 pairings each so that relatively long term changes in conditioned responses could be evaluated. Following Conditioning, Extinction procedures were carried out in which the CS was presented alone.

In order to evaluate the second hypothesis concerning the relation of SP shifts to processes of cortical excitability the foregoing intersensory electrocortical conditioning procedure was repeated with four epileptic monkeys. Preliminary findings with normal monkeys (Rosen and Stamm, 1966) indicated that conditioning resulted in negative SP shifts in occipital cortex and positive shifts in temporal cortex during the CS-US interval. If conditioned shifts are indicative of processes of cortical excitability, with negative shifts indicative of increased, and positive decreased, then one might expect that conditioned negative SP shifts in occipital cortex would be associated with enhanced rates of epileptic spiking for occipital lobe epileptics, and conversely, that conditioned positive shifts in temporal cortex, resulting from the same procedure, would be associated with suppressed rates of spiking in temporal lobe epileptics. Accordingly, two groups of epileptic monkeys, one with epileptogenic implants in temporal cortex and another with similar implants in occipital cortex, were prepared. Both groups had electrodes implanted around the implants and conditioning was carried out as with normal monkeys.

In a series of three supplementary experiments each of the hypotheses was subject to further examination. The first hypothesis was examined in the study which attempted to relate the magnitude of the SP shift in visual cortex to the parameters of the photic stimulus. The second hypothesis was studied in an experiment in which the interval between successive stimuli was varied. The magnitude of the SP response to photic stimuli was then related to positive steady afterpotentials resulting from prior stimulation. In a final experiment the conditioning of epileptic spikes was demonstrated by temporal conditioning procedures with reference to SP shifts.

EXPERIMENT 1

INTERSENSORY ELECTROCORTICAL CONDITIONING OF STEADY POTENTIAL SHIFTS IN NORMAL MONKEYS

Introduction

In a number of experimental studies in which direct-coupled (DC) amplifiers have been used to record steady potentials from the cerebral cortex, surface negative steady potential (SP) shifts have been associated with increased cortical excitation. Sensory stimuli, for example, have been found to evoke surface negative SP shifts in areas of sensory cortex corresponding to the modality of the stimulus (Gumnit, 1961; Kohler, *et al*., 1955; Lickey and Fox, 1966; Rowland, 1963). Other studies have shown that sensory stimuli which are not modality specific may also elicit SP shifts in an area of sensory cortex, particularly if these stimuli are paired contiguously with stimuli which are modality specific for that area. Thus, it has been possible to demonstrate the intersensory electrocortical conditioning of SP shifts, a process of considerable interest in the understanding of the neural basis of learning (Morrell, 1960; Rowland, 1961; Rowland and Goldstone, 1963; Rusinov, 1960).

It should be pointed out, however, that most of the conditioning studies reported thus far have involved acute preparations in which it was difficult, if not impossible, to observe long term changes in the conditioned

SP responses and, in the one study involving chronic preparations (Rowland and Goldstone, 1963), in which sensory stimuli were paired with the presentation of food, conditioned SP shifts were relatively widespread and were associated with orienting and arousal phenomena. Therefore, in order to specify further the nature of intersensory electrocortical conditioning of SP shifts in chronic preparations the following experiment was conducted.

SP shifts were conditioned in monkeys with chronically implanted nonpolarizable electrodes in visual and auditory cortex. Intermittent acoustic (clicks) and photic (flicker) stimuli were selected as conditioned (CS) and unconditioned (US) stimuli respectively, because these stimuli, in addition to evoking reliable SP responses in sensory cortex (Gumnit and Grossman, 1961; Kohler, et al., 1955; Rowland and Goldstone, 1963), have been shown in electrocortical conditioning studies to result in conditioned frequency specific activity (Morrell and Jasper, 1955). It was, therefore, possible in the present experiment to investigate the relation of conditioned SP shifts to another dimension of conditioned electrocortical activity.

Method

Miniature silver-silver chloride (Ag-AgCl) nonpolarizable electrodes were chronically implanted in each of four normal monkeys (Macaca speciosa) of 3 to 5 kgs bodyweight. The electrodes were constructed of small glass tubes (10 mm long and 8 mm in diameter) drawn at one end into a fine capillary shaft approximately 1 to 2 mm in diameter. The tubes were filled with a saline-agar gel into which was immersed a coil of fine silver wire (.010 gauge) coated with a thin layer of silver chloride. The tubes were sealed at both ends with dental cement and coated with a layer of vinyl insulation. From the wide end of the tube emerged a silver wire lead continuous with the chlorided silver wire coil. At the time of surgery the capillary end was cut to the desired length and the resulting electrode tip (recording surface) consisted of a circular section of exposed saline-agar (1 to 2 mm in diameter). Pairs of these electrodes were implanted at a given cortical site. One electrode of a pair was usually placed upon the pial surface of the brain and the other was inserted approximately 10 mm into the adjacent underlying white matter. The distances between tips ranged between 1 to 2 cm. The D.C. impedence between the electrodes, measured in normal saline, was 30 to 50 Kohms.

In a given subject electrodes were implanted unilaterally in frontal, temporal, parietal, and occipital cortex. The frontal electrodes were situated on the superior bank of the principal sulcus approximately 1 cm anterior to the arcuate sulcus. The temporal electrodes were situated in the superior temporal gyrus so that the cortical electrode was placed on the supra temporal plane of the superior temporal gyrus and the depth electrode was inserted into the gyrus. Posterior parietal electrodes were placed 1 cm anterior to the lunate sulcus and 1 to 2 cm from the midline. Finally, occipital electrodes were placed on the lateral aspect of the occipital lobe 1 to 2 cm posterior to the lunate and several cms from the occipital pole. An additional electrode was placed at the frontal pole and served as an occasional reference. All electrodes were fixed to the skull by means of stainless steel screws and dental cement. The silver wire leads from each electrode were soldered to points on a nine point female Amphenol connector. The connector in turn was cemented to the skull. Fascia and skin were sutured above the resulting cement mound.

Apparatus

For electrographic recordings the monkeys were seated in a restraining chair which was placed in an electrically shielded soundproof chamber. Inputs were led via an

Amphenol connector and low noise cables to four low level, chopper stabilized DC preamplifiers (Grass model 5P1) of a six channel Grass Polygraph. The input impedance of the preamplifiers was 1 megohm DC and the bandpass was 0 to 60 cps (down 3 db at 60 cps). Ink write-outs were usually obtained and responses were periodically monitored on a CRO (Tektronix model 502A).

The recording chamber used in the experiment contained a Grass Photo-Stimulator lamp and a 12 inch loudspeaker. The lamp was situated 30 cms from the eyes of the subject and the center of the speaker was 40 cms below the lamp. The speaker was driven by a square wave generator and amplifier, and the lamp was activated by a Grass Photo-Stimulator (model PS-2). A semi-automatic timing circuit was used to trigger the sequence and duration of intermittent acoustic and photic stimuli selected for the conditioning procedure. The onset and termination of stimuli was noted on the tracings by means of an electrical coding device. The conditioning chamber was generally dark and no other acoustic stimuli were provided except for those generated by the monkeys themselves.

Procedure

Simple conditioning.--The experimental sequence consisted of seven daily sessions each of: Adaptation, Conditioning, and Extinction procedures. The stimuli presented were a six second train of 6/sec acoustic clicks (CS) and a three second train of 8/sec flicker (US). During each session five minutes of baseline ECGs were obtained before and after stimulus presentations. Stimuli were presented at random intervals with an average inter-stimulus interval of one minute. The Adaptation sessions consisted of random presentations of single stimuli, 30 CS and 30 US presentations per session. During Conditioning sessions 30 paired stimuli were presented with the acoustic CS preceding the photic US by three seconds (CS-US interval being equal to three seconds), and both stimuli terminating simultaneously. In addition, ten single CSs per session were randomly interspersed among conditioning trials as controls. During Extinction sessions, 30 single CSs were presented. The US was never presented.

Differential conditioning.--The differential conditioning procedure conducted with two monkeys consisted of 10 sessions each of Adaptation, Conditioning, and Extinction. During an Adaptation session each of the following stimuli was presented 20 times in random order: the CS+ (CS followed by the US), the CS- (CS not followed by the US), and the US. For one monkey the CS+ was a six second train of 6/sec clicks and the CS- was a six second train of 14/sec clicks, while for the other monkey the reverse click frequencies were presented. The US was always a three second train of 8/sec flicker. During the Conditioning session there were random presentations of pairings of the CS+ and the US and 20 single CS- presentations. The Extinction phase which followed was similar to the Adaptation phase, except that the US presentations were ommitted. For all experimental sessions the intertrial intervals averaged one minute in duration and five minute baseline recordings taken at the start and end of each session.

Data analysis.--The magnitude of SP shifts following sensory stimulation were measured with reference to pre-stimulus SP baselines. The SP baseline was established by fitting a straight line to a six second period of the pre-stimulus ECG trace, such that the sum of areas enclosed by the ECG trace above and below the baseline were equal. All response amplitude and duration measures following stimulus presentation were made with reference to this baseline. Measures of conditioned SP shifts were obtained by measuring the peak amplitude and duration of surface negative potentials during the CS-US interval, and by planimeter measures of the area enclosed by negative waves and the SP baseline. These area measures are considered as integrated response measures reflecting the total energy output of the response. For each session the mean response measure, expressed in V-sec, was computed for each subject.

Results

Response to sensory stimulation

The presentation of a single stimulus, either a click or a light flash, resulted in an evoked response which was recorded from the primary sensory cortical area corresponding to the modality of the stimulus. Responses in other cortical areas were also seen, but these were subject to rapid habituation. The primary response generally consisted of 25 to 100 μV fast positive and negative waves, lasting 100 to 200 msec, and slow negative and positive afterpotentials, of similar amplitude, lasting several seconds. Low voltage fast activity in the ECG usually accompanied the slow negative components of the response. Both the amplitude and duration measures of various response components showed considerable variability. More consistent response patterns were obtained however, when intermittent stimuli were presented.

The presentation of trains of auditory clicks or flicker resulted in primary sensory responses consisting of two major components: a 100 to 200μV surface negative SP component and a frequency specific component. The electrocortical responses to six second trains of stimulation, seen in one monkey, are shown in Figure 1 for acoustic, and in Figure 2 for photic stimulation. The responses recorded in temporal and occipital cortex, respectively, show the frequency specific ECG component waves superimposed on surface negative SP shifts. Both components of the response generally persisted for the duration of the stimulus. Termination of the stimulus resulted in a cessation of frequency specific activity and a return of the negative SP shift to its prestimulus baseline. Small positive afterpotentials were also recorded which lasted as long as one minute. In addition, intermittent acoustic and photic stimuli frequently elicited responses in frontal cortex (Figures 1 and 2) which consisted of fast (100 to 200 msec) positive and negative waves at the onset of stimulation, and subsequent slow negative SP shifts which lasted 2 to 4 seconds. This response pattern appeared independent of the stimulus modality. In some subjects, flicker also evoked responses

Figure 1.-- Electrocorticogram obtained with nonpolarizing electrodes and DC amplifiers showing responses of frontal, temporal, occiptial, and pairetal cortex to 8/sec flicker. Traces represent bipolar recordings between surface and depth electrode placements in frontal (Fs-Fs), temporal (Ts-Td), occipital (Os-Od), and parietal (Ps-Pd). Surface negativity is upward. Bottom trace indicates presentation of 6/sec clicks. Calibration as shown.. Note sensory response in temporal cortex consisting of 6/sec frequency specific component superimposed on 100 μV surface negative SP shift.

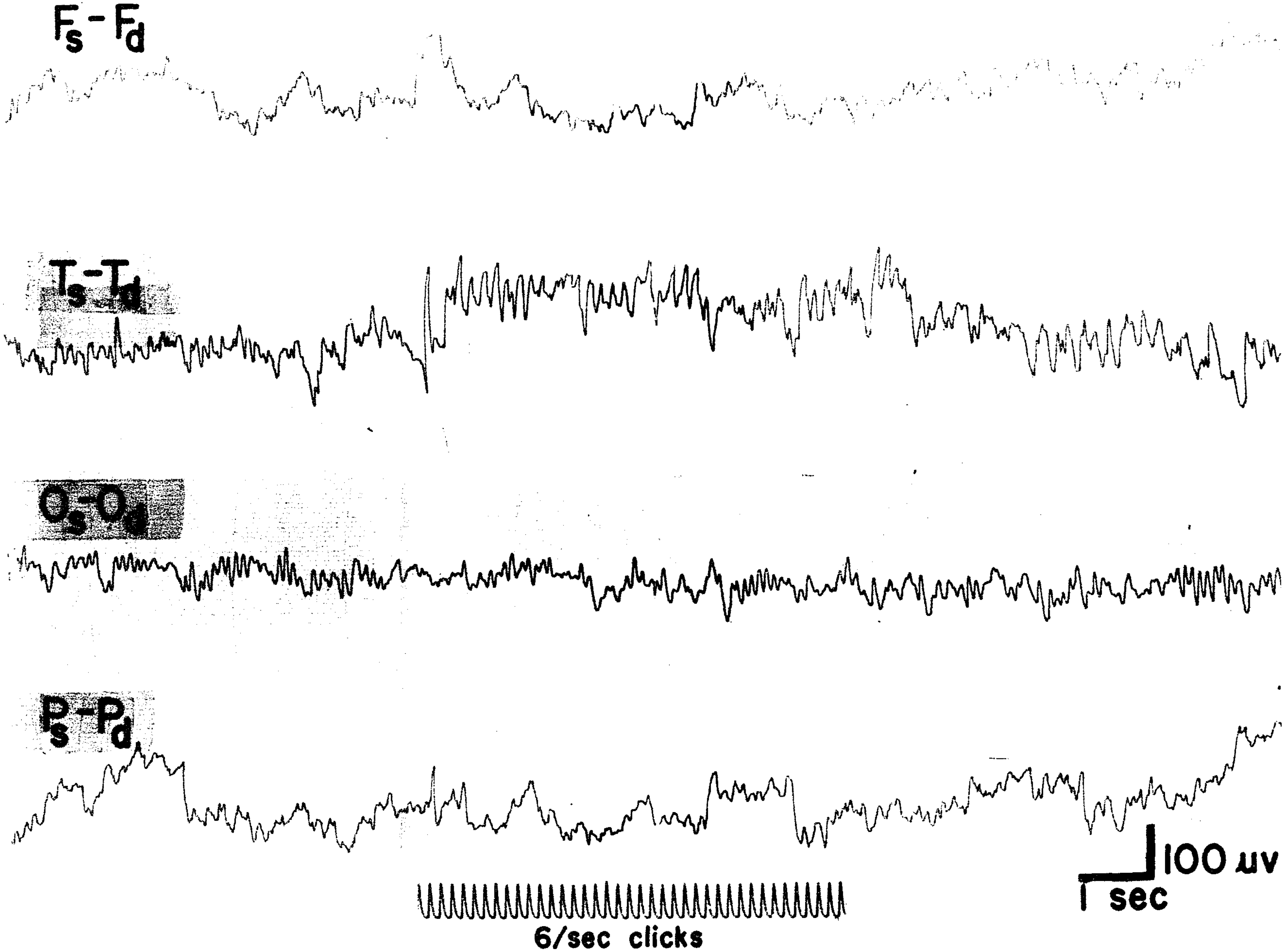
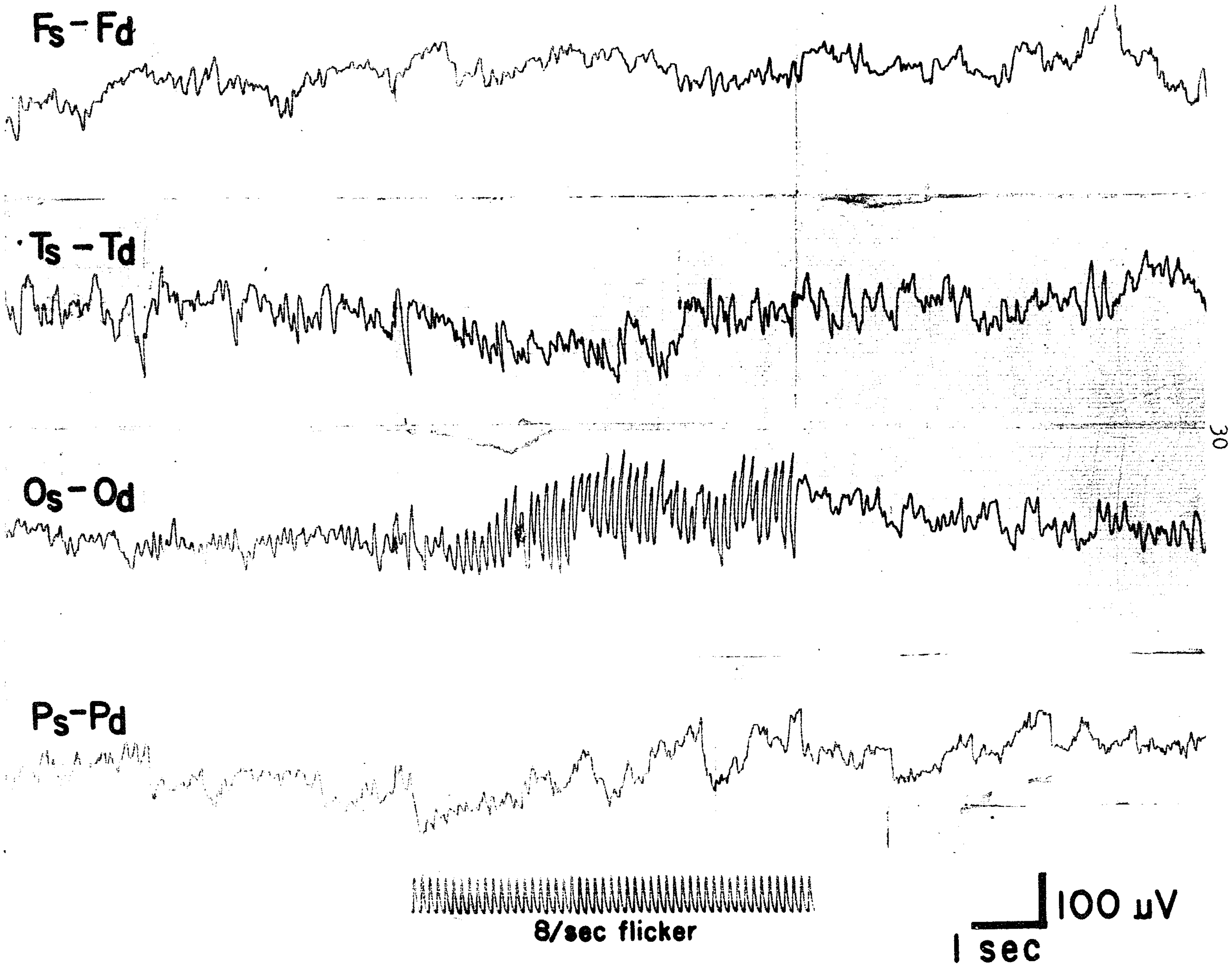

Fs - Fd
Ts - Td
Os - Od
Ps - Pd
100 uv
1 sec
6/sec clicks

Figure 2.--Electrocorticogram obtained with nonpolarizing electrodes and DC amplifiers showing responses of frontal, temporal, occipital, and parietal cortex to 8/sec flicker. Note (1) sensory response in occipital cortex consisting of 8/sec frequency specific component superimposed on 100 to 200 μV surface negative SP shift, and (2) surface positive SP shift in temporal cortex.

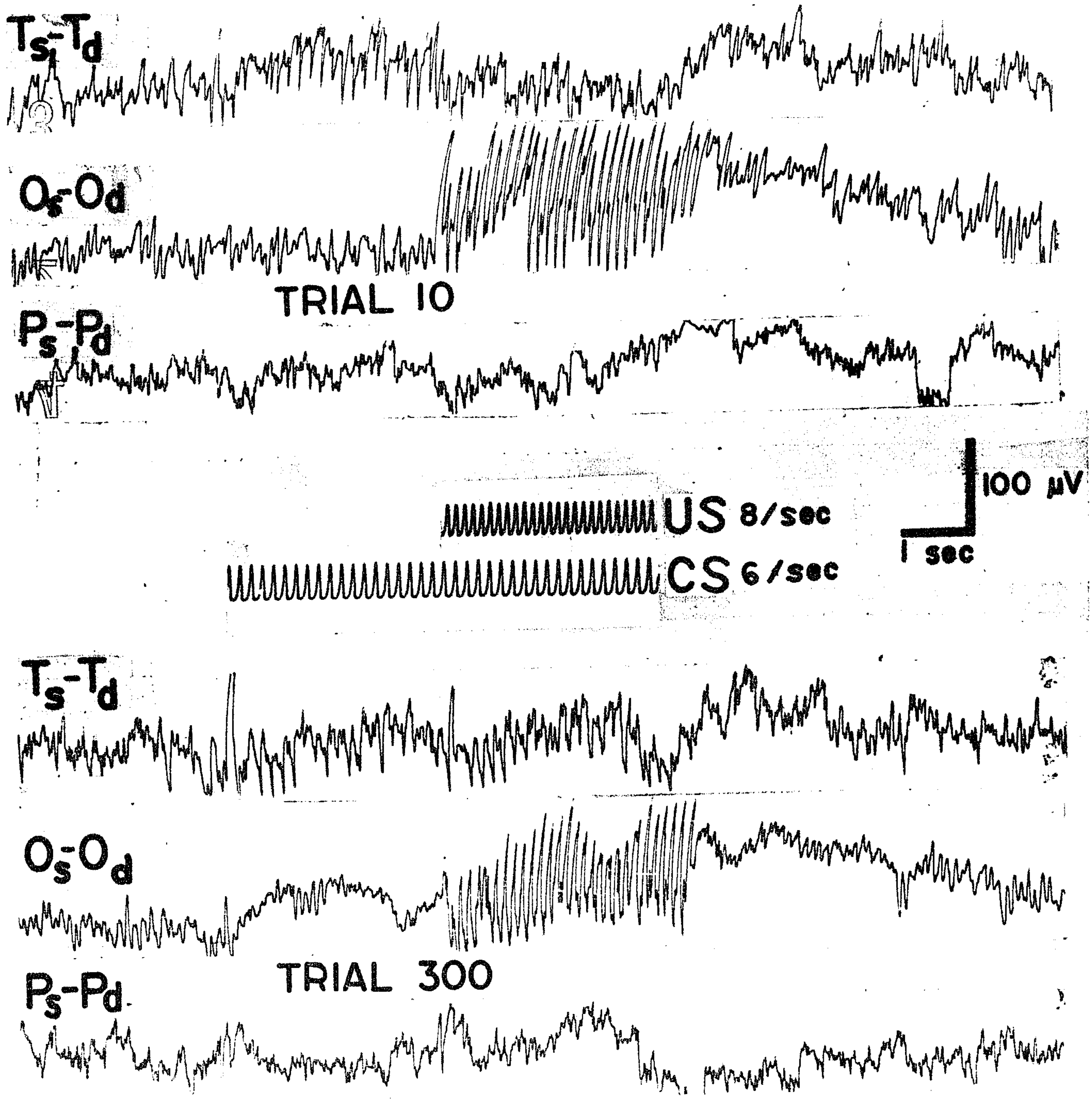

Fs - Fd
Ts - Td
30
Os - Od
Ps-Pd
8/sec flicker
100 µV
1 sec

in parietal and temporal cortex. The response in parietal cortex consisted of either low amplitude frequency-specific activity, or slow-rising surface negative SP shifts. The response in temporal cortex, as seen in Figure 2, consisted of a surface positive SP shift of 25 to 50μV. This response was seen most clearly in those subjects who had prominent responses to acoustic stimulation.

Intersensory conditioning (conditioned electrographic responses)

Figure 3 shows samples of ECGs obtained for one monkey during two phases of the conditioning procedure with a 6 second train of clicks as the CS and a 3 second train of flicker as the US. During the early phase of conditioning (Trial 10), the following responses were recorded: (1) the acoustic stimulus (CS) evoked 50 to 100μV surface negative SP shifts and concomitant 6/sec frequency specific activity in temporal cortex; (2) the photic stimulus (US) evoked 100 to 200μV surface negative SP shifts and concomitant 8/sec frequency specific activity in occipital cortex; (3) the photic stimulus also resulted in return of the temporal lobe negative responses to baseline and its subsequent shift to slight positivity. Neither stimulus resulted in consistent responses in parietal cortex, although a slight negative going SP

Figure 3.--Electrocorticograms obtained during two stages of intersensory electrocortical conditioning. Traces represent surface to depth recordings with electrodes in temporal (Ts-Td), occipital (Os-Od), and parietal (Ps-Pd) cortical areas. Negativity is upward. Presentation of CS (6/sec train of clicks) and US (8/sec flicker) are indicated for both Trial 10 (top three traces) and Trial 300 (bottom three traces). Note "conditioned" electrical response in occipital cortex (Trial 300) consisting of a burst of 8/sec frequency specific activity at peak of 50μV negative SP shift.



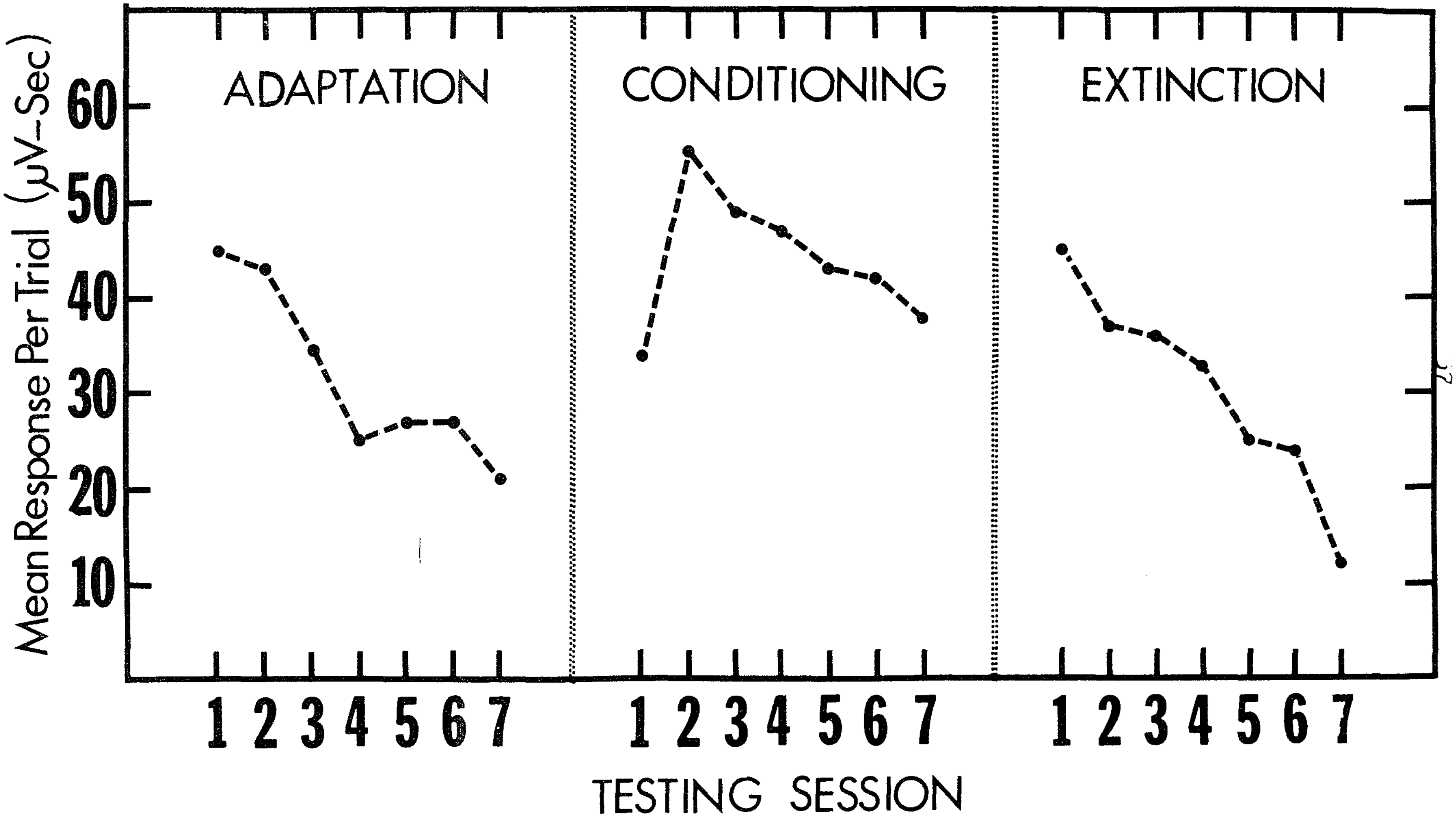
T_s-T_d
O_s-O_d
TRIAL 10
P_s-P_d
US 8/sec
CS 6/sec
100 µV
1 sec
T_s-T_d
O_s-O_d
TRIAL 300
P_s-P_d

shift appeared with the presentation of flicker. During a later phase of conditioning (Figure 3, Trial 100) the following additional phenomena comprising the CR were recorded: (1) a 25 to 50μV surface negative SP shift elicited by the CS in occipital cortex, and (2) 8/sec frequency specific activity superimposed upon the peak of the conditioned negative shift. Conditioned frequency specific activity was seen in three of the four monkeys. It appeared at the peak of the SP shift during the first few conditioning sessions but during subsequent sessions it occurred independent of the development of SP shifts in the CS-US interval.

During the course of the experimental session several test trials were given where only the CS was presented. During these trials conditioned negative SP shifts were again elicited in occipital cortex. In auditory cortex the primary negative SP shift to clicks was followed by a surface positive component which started about three seconds after the onset of the CS, when the US had been presented during conditioning trials. This observation strongly suggests that the conditioning procedure resulted not only in conditioned negative SP shifts in occipital cortex, but also in conditioned positive SP shifts in temporal cortex. A preliminary analysis of this data for one monkey appears in Figure 29 of Appendix B.

Conditioned negative SP shifts were recorded from occipital cortex in each of the four Ss during the CS-US interval.. The mean response magnitude per trial for all Ss in a given session is shown in Figure 4 for Adaptation and Extinction control session, as well as for the Conditioning sessions. Figure 4 indicates that clicks elicited relatively large surface negative SP shifts in occipital cortex during the first few Adaptation sessions. It is not unlikely that these responses reflect generalized arousal phenomena, associated with the novelty of the test situation and the stimuli. Indeed, during the early sessions each of the conditioning stimuli elicited widespread surface negative SP shifts from all implanted electrode sites and these responses diminished markedly during the subsequent Adaptation sessions. Stimulus pairing during the Conditioning phase resulted in an increase in the magnitude of surface negative SP shifts in occipital cortex elicited by clicks. The magnitude of SP shifts for Conditioning sessions 2 through 4 was above that of any of the Adaptation or Extinction sessions. The Extinction procedure again resulted in a gradual diminution of negative SP shifts to clicks. The response magnitudes for the Extinction sessions are similar to those

Figure 4.--Mean CR magnitude per trial (expressed in μV-sec) for four monkeys during successive Adaptation, Conditioning, and Extinction sessions. For Conditioning each point represents the mean response over the 3 second CS-US interval for thirty trials of each session. For the Adaptation and Extinction sessions the response during the first 3 seconds of the CS were measured.


Mean Response Per Trial (μV–Sec)
60
50
40
30
20
10
ADAPTATION
CONDITIONING
EXTINCTION
1 2 3 4 5 6 7
1 2 3 4 5 6 7
1 2 3 4 5 6 7
TESTING SESSION

obtained during later Adaptation sessions.

Table 1 shows the mean SP shift per trial (with its corresponding standard deviation) for individual subjects for the first and last three testing sessions of the Adaptation, Conditioning, and Extinction phases of the experiment. The table indicates that for each of the subjects the mean response for the Condtioning sessions is greater than either the last three Adaptation or the last three Extinction sessions. The table also shows that responses in the later Adaptation and Extinction sessions tend to be less than corresponding responses in the early sessions, whereas, the difference between late and early Conditioning sessions, as seen in three subjects, is of somewhat less magnitude. The relatively large standard deviations indicate considerable variability of SP shift magnitudes observed from trial to trial in these chronic preparations.

Differential condtioning

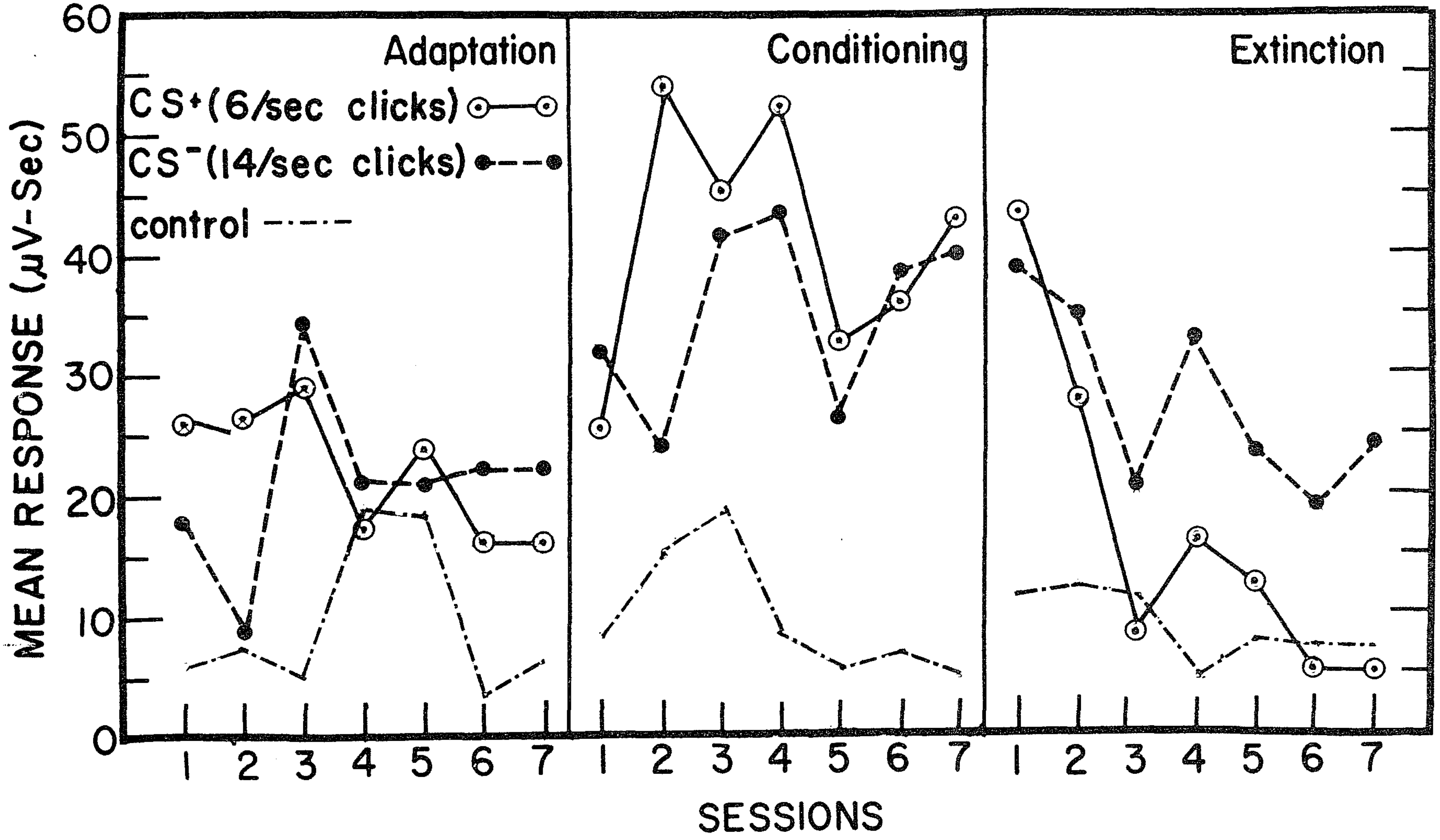
Figure 5 presents the results for the differential conditioning procedure for one subject when trains of 6/sec clicks (CS+) were paired with trains of flicker (US), while 14/sec clicks (CS-) were not paired with flicker. This figure also presents the magnitudes of negative SP shifts in occipital cortex during 3 second

TABLE 1

MEAN AND STANDARD DEVIATION OF SP SHIFT MAGNITUDE (V.SEC.) PER TRIAL FOR BLOCKS OF THREE SESSIONS

	Experimental Procedure											
	Adaptation				Conditioning				Extinction			
	1-3		5-7		1-3		5-7		1-3		5-7	
Monkey	X	sd	X	sd	X	sd	X	sd	X	sd	X	sd
114	35	22	25	19	33	32	26	26	23	23	16	17
118	39	45	26	32	48	37	45	28	39	31	26	25
148	59	49	36	32	70	39	63	21	72	20	33	18
150	27	25	14	18	34	27	40	24	23	18	16	22

Figure 5.--CR magnitudes for one monkey during differential conditioning to CS+ (paired with US) and CS- (no US). Each point represents the mean response over the first 3 seconds of CS presentations for the 20 trials of each session. The "control" data was obtained for 3 second periods when no stimuli were presented.


Adaptation
Conditioning
Extinction
CS+(6/sec clicks)
CS⁻(14/sec clicks)
control
MEAN RESPONSE (μV-Sec)
0
10
20
30
40
50
60
1
2
3
4
5
6
7
SESSIONS

"control" periods, when no stimuli were presented. During the Adaptation phase no consistent differences in mean response magnitudes were obtained between CS+ and CS- presentations. Differential conditioning resulted in greater CRs for both CS+ and CS- presentations than those elicited during Adaptation, with the highest magnitudes of CS+ occuring during sessions 2 to 4. The differences between pairs of mean responses were found not to be statistically significant. During the Extinction phase both conditioned responses decreased again to their Adaptation levels. The responses during the Control periods, which were measured for three second intervals before presentation of the CS, suggest that conditioned negativity may occur spontaneously during the intertrial intervals. The present results indicate that the magnitude of the conditioned negative SP shift may be affected by differential conditioning procedures, but that it is difficult to obtain appreciable and significant response differences when clicks of differing frequencies are used for paired and unpaired stimuli. Similar findings were obtained with a second monkey in which the frequencies of the CS+ and CS- were reversed. (See Figure 30 of Appendix B.)

Discussion

The results of the present experiment clearly indicate that intermittent acoustic and photic stimuli evoke surface negative SP shifts in areas of sensory cortex of the monkey corresponding to the modality of the stimulus. These findings are consistent with the results of both acute experiments (Gumnit, 1960; Gumnit and Grossman, 1961; Libet and Kahn; 1946; Lickey and Fox, 1966) and chronic studies (Gumnit, 1963; Rowland and Goldstone, 1963) of SP shifts in cerebral cortex of the cat. Collectively, the body of experimental evidence which has accumulated in the past few years strongly suggests that surface negative SP shifts arise from the cortical substrate and reflect dendritic potentials in the superficial cortical layers. Recordings in the present experiment of surface negative SP shifts with surface-to-depth bipolar electrodes lends particular support to this view.

The data further indicate that surface negative SP shifts evoked by stimuli in one sensory modality can be conditioned to stimuli in another modality following stimulus pairings with no conventional reinforcement. The findings that SP shifts in visual cortex could be conditioned to clicks are consistent with results of both acute (Morrell, 1960; Rowland, 1960; Rusinov, 1960) and chronic studies (Rowland and Goldstone, 1963) of conditioned SP shifts in the cat. In general the results of conditioning studies indicate that SP shifts may arise as a result of other than specific thalamic inputs to primary sensory cortical areas. A number of investigations indicate that nonspecific and diffuse thalamic inputs may give rise to surface negative SP shifts (Arduini, 1958; Brookhart et al., 1958; Goldring and O'Leary, 1957). In this respect, Lickey and Fox (1966) have described both local and diffuse SP components of primary sensory cortical responses. Presumably, the local component results from direct inputs via specific sensory nuclei in the thalamus (terminating in the lower cortical layers), and the diffuse component results from diffuse inputs via nonspecific nuclei (termination in the upper cortical layers). It is, therefore, likely that conditioned cortical SP shifts in the present experiment resulted in part from nonspecific, diffusely projecting inputs from the thalamus particularly since these shifts were frequently accompanied by negative SP shifts in frontal and parietal cortex, and because they were subject to relatively rapid diminution or habituation after several daily testing sessions.

In addition to the conditioned surface negative SP shifts in occipital cortex, other components of conditioned electrocorticographic responses were obtained, namely discharge in occipital cortex at the frequency of the US and positive SP shifts in temporal cortex associated with presentation of the US. The occurrence of frequency specific discharges during the course of intersensory conditioning has previously been described by Morrell and Jasper (1955) who, since they recorded cortical potentials with metallic electrodes and capacitor coupled (AC) amplifiers, could not observe concomitant SP shifts. The present findings that frequency specific discharges begin only when conditioned SP shifts approach their negative peaks,, during the early phases of conditioning, suggest a possible causal relation between these two components of the electrocortical activity of the brain. Conditioned positive shifts in sensory cortex corresponding to the modality of the CS have not been (to this author's knowledge) hitherto reported.

The present findings of conditioned negative SP shifts in occipital and positive SP shifts in temporal cortex, suggest that these shifts reflect processes related to cortical excitability. This view is supported by reports that surface negative SPs are concomitants of the onset of seizure discharges in normal and epileptogenic cortex. The experimental findings led to Goldring and O'Leary's (1963) conclusion that SP potential shifts "precede" or "usher in" seizure discharges. A direct investigation of this hypothesis might be attempted by application of the present conditioning procedure to monkeys with focal epileptogenic implants. The results of such an experiment are presented in the subsequent section.

Summary

In each of the four stumptail monkeys pairs of miniature Ag-AgCl nonpolarizable electrodes were chronically implanted in frontal, temporal, parietal, and occipital cortex with one electrode of each pair on the pial surface of the brain and the other in adjacent subcortical white matter. Electrocorticograms (ECGs) recorded with DC amplifiers showed surface negative steady potential (SP) shifts in response to intermittent acoustic (clicks) and photic (flicker) stimuli. Negative SP shifts were recorded primarily from sensory areas corresponding to the modality of the stimulus. Intersensory electrocortical conditioning procedures in which clicks (CS) were paired with flicker (US) resulted in 25 to 50 μV "conditioned" negative SP shifts in occipital cortex (CR) and "conditioned" positive SP shifts in auditory cortex. In addition, "conditioned" frequency responses, specific to the US frequency, were recorded in occipital cortex. These responses were most frequently superimposed upon the peak conditioned negative SP waves. Conditioned SP shifts were interpreted as reflecting changes in cortical excitation, with negative shifts indicative of increased, and positive shifts decreased, excitability states.

EXPERIMENT 2

INTERSENSORY ELECTROCORTICAL CONDITIONING OF STEADY POTENTIAL SHIFTS IN EPILEPTIC MONKEYS

Introduction

Cortical steady potential (SP) shifts have been "conditioned" in sensory cortex of monkeys with chronically implanted nonpolarizable electrodes (Rosen and Stamm, 1966). The intersensory conditioning procedure in which auditory clicks (CS) were paired with flicker (US) resulted in "conditioned" surface negative SP shifts in visual cortex and "conditioned" positive shifts in auditory cortex. These shifts have been interpreted as reflecting changes in cortical excitation, with negative shifts indicative of increased, and positive shifts, decreased excitation levels.

With regard to the significance of cortical SP shifts two hypotheses may be advanced; (1) that SP shifts are merely concomitants of neuronal activity with in the cortex and, therefore, simply reflect changes in cortical excitation, and (2) that in addition to reflecting the activity of cortical neurons, SP shifts reflect processes which serve to "gate" or "modulate" subsequent neuronal activity. The latter hypothesis has been supported by findings which indicate that the application of surface positive (depth negative) polarizing currents to the cortex results in: (1) augmentation of negative components of evoked responses (Bishop and O'Leary, 1950); (2) increases in the rate of firing of single cortical units (Bindman, Lippold, and Redrearn, 1962; Creutzfeld, Fromm, and Kapp, 1962); and (3) initiation of seizure discharges (Goldring and O'Leary, 1951). Surface negative polarizing currents appear to have opposite effects. The relevance of these findings to the hypothesis is restricted however, in that the results were obtained with acute preparations subject to special experimental conditions. Consequently, the generality of the hypothesis would be strengthened by an investigation of the relation of SP shifts to ongoing neuronal activity with chronic preparations.

In the present study the relationships of "conditioned" SP shifts to one form of neuronal activity, namely epileptic discharges, was examined. SPs were conditioned in monkeys with experimentally induced epileptogenic implants in either visual or auditory cortex. The effects of conditioned negative and positive SP shifts resulting from an intersensory electrocortical conditioning procedure were then evaluated with respect to ongoing epileptic activity. If SP shifts do indeed reflect cortical processes related to excitability, then one would expect that conditioned negative SP shifts would be associated with enhanced epileptic activity, and conversely, that positive shifts would be associated with a suppression of epileptic activity.

Method

Miniature Ag-AgCl nonpolarizable electrodes (of similar construction to those described in Experiment 1) were chronically implanted in four monkeys (Macaca speciosa) with experimentally induced epileptogenic lesions. In two of the epileptic monkeys a single teflon cup (8 mm in diameter) packed with alumina cream (commercial Amphojel boiled to the consistency of a thick paste) had been surgically implanted in occipital cortex. The disk had been placed beneath the dura on the lateral aspect of the left occipital lobe, approximately 2 cm from the pole and 3 cm from the midline.. In two other epileptic monkeys a single cup had been implanted in left temporal cortex. The disk was placed on the supratemporal plane of the superior temporal gyrus (approximating primary auditory cortex). Six weeks after surgery scalp EEGs were recorded to determine the locus and extent of EEG abnormalities.

Following the appearance of focal epileptic signs in the EEGs of all subjects, the electrodes were implanted in frontal, temporal, parietal, and occipital cortex. One electrode of a pair was placed on the pial surface of the brain and the other was inserted approximately 1 cm into the adjacent subcortical white matter. The surface electrodes in epileptogenic sensory cortex were usually placed within 6 to 8 mm of the perimeter of the implanted teflon cup. The DC impedance between electrodes measured in normal saline was 30 to 50 kohms. The silver wire leads from each electrode were soldered to points on an Amphenol connector and the connector in turn was permanently fixed to the subjects skull by means of stainless steel screws and dental cement.

Apparatus.--For intersensory electrocortical conditioning the monkeys were seated in a restraining chair which was placed in an electrically shielded soundproof conditioning chamber described in Experiment 1. As in the preceding experiment, recordings were obtained with low level, chopper stabilized DC amplifiers (Grass model 5P1). The mode of stimulus presentations was also similar to that described previously.

Procedure

The experimental sequence consisted of seven daily sessions each of Adaptation, Conditioning, and Extinction procedures, outlined in Experiment 1 for simple conditioning. The stimuli presented were the same as in the preceding experiment except that the US was a six second, not a three second, train of 8/sec flicker.

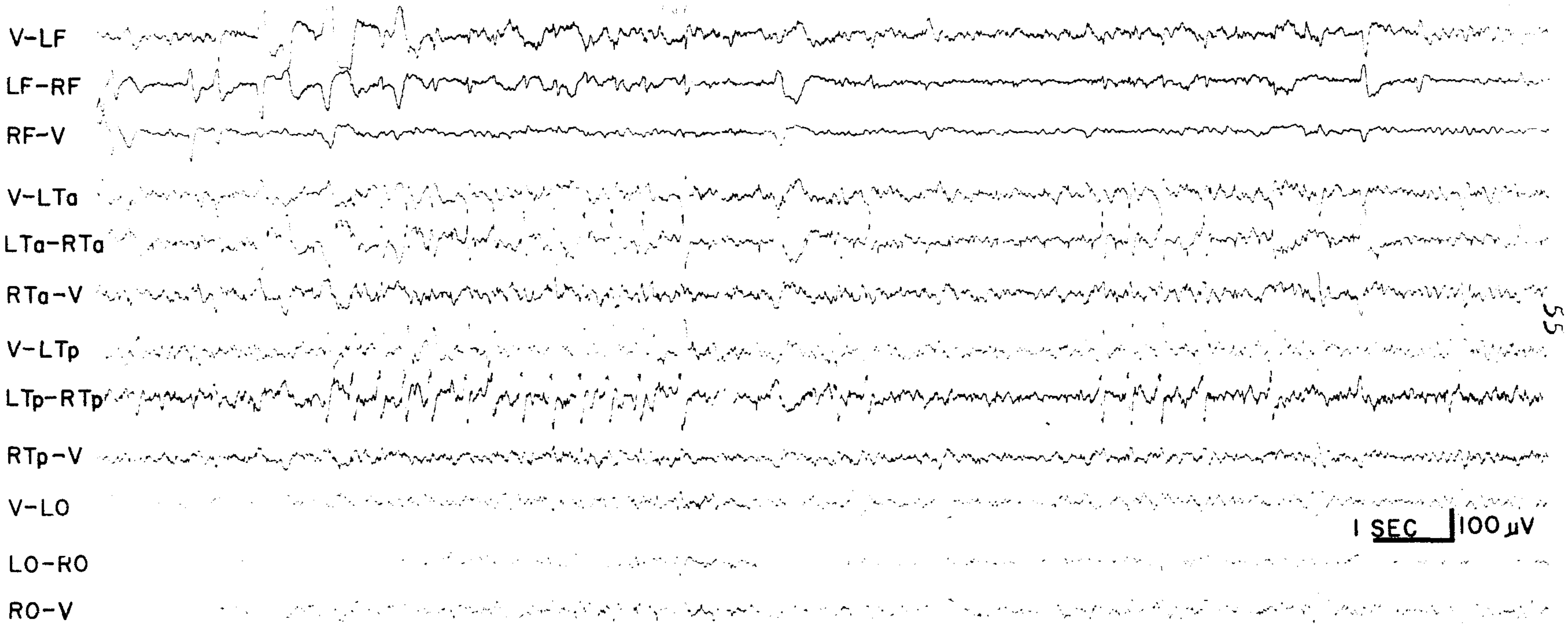
Data analysis.--The magnitude of SP shifts following sensory stimulation were measured with reference to prestimulus SP baselines in accordance with the method described in Experiment 1. In the present experiment epileptic activity was analyzed in the following manner: epileptic "spikes" were defined as ECG waves whose peak to peak amplitudes exceeded 200μV and whose base widths (duration) was less than 200 msec. The incidence of such waves during the CS-US interval as well as during comparable intervals during the Adaptation and Extinction sessions were computed for each subject. By dividing the number of spikes in the CS-US interval for a given session by the number of CS presentations, a measure of the mean spikes per trial was obtained.

Results

Epileptic foci

Serial scalp EEG recordings from monkeys with alumina cream implants revealed localized epileptic signs six to eight weeks after implantation. In all monkeys the focus of epileptic discharges corresponded to the site of implantation. Recordings from one monkey with an epileptogenic implant in left temporal cortex, as shown by Figure 6, revealed episodes of 100 to 200μV spike discharges from left anterior and posterior temporal electrode locations. Epileptiform EEG patterns were not recorded from contralateral or other electrode locations. Similar recordings were obtained from monkeys with epileptogenic implants in occipital cortex. Spike discharges were

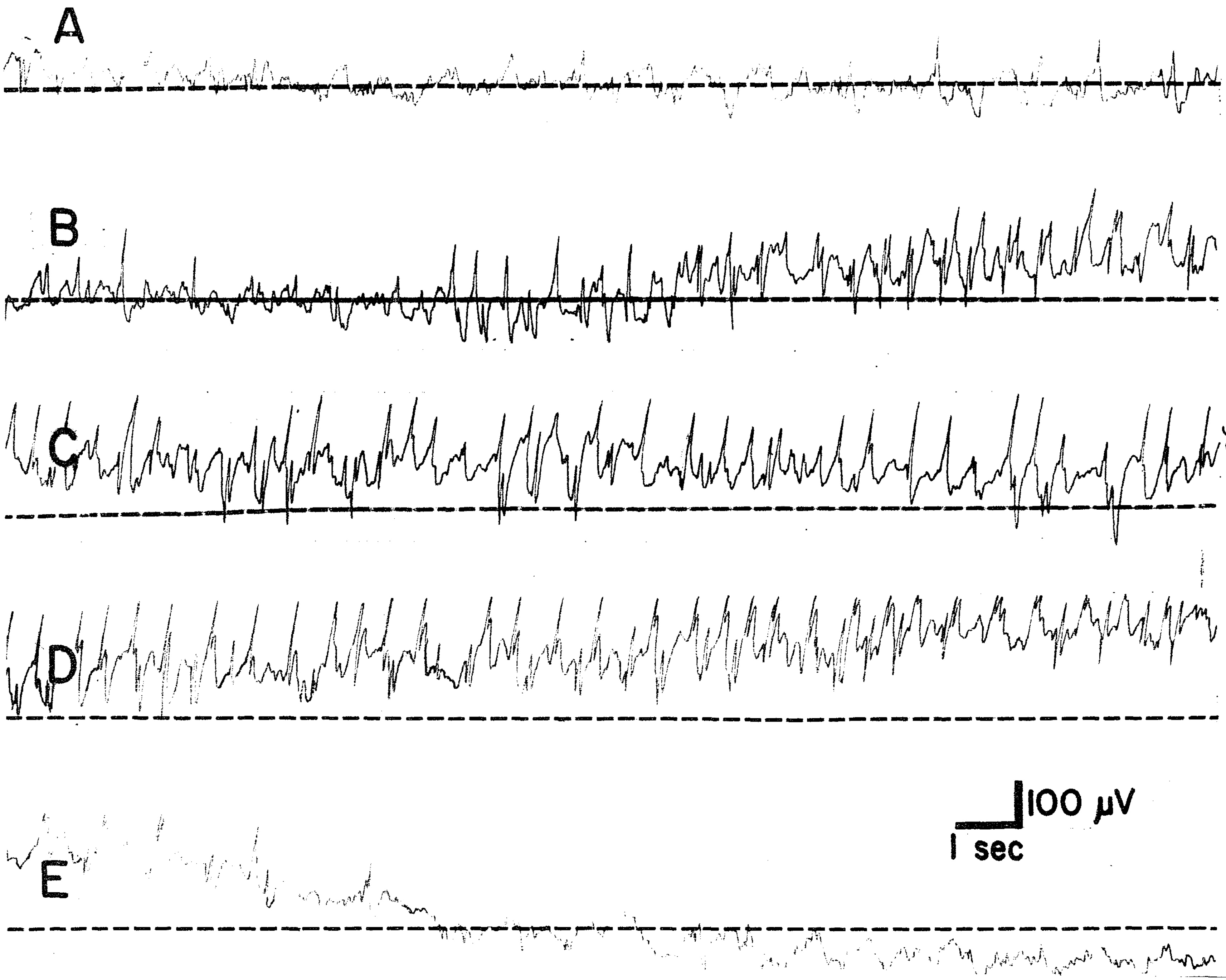
Figure 6.--Scalp EEG of a monkey with an alumina cream implant in left superior temporal cortex. Bipolar recordings between electrode placements as indicated:
V = vertex; F = frontal; Ta = anterior temporal;
Tp = posterior temporal; O = occipital; L = left;
R = right.


V-LF
LF-RF
RF-V
V-LTa
LTa-RTa
RTa-V
V-LTp
LTp-RTp
RTp-V
V-LO
LO-RO
RO-V
1 SEC
100 µV

localized in occipital cortex of the implanted hemisphere.

Cortical recordings taken with nonpolarizable elctrodes also showed localized epileptiform patterns. In bipolar recordings, with nonpolarizable electrode placements on the cortical surface near the alumina cream implant and in the underlying white matter, temporal lobe epileptic monkeys showed trains of surface negative fast spikes, of 100 to 200 µV, lasting several seconds. In one monkey (no. 154) spike discharges propagated to the frontal, but not to parietal or occipital electrode placements. In monkeys with occipital epileptogenic inplants, epileptiform activity, consisting either of trains of 100 to 200 µV surface negative fast spikes or of higher voltage positive spikes of 200 to 500 msec duration, were recorded from occipital, but not from frontal, temporal, or parietal electrode locations. The focal epileptic discharges were accompanied by slow surface negative SP shifts in two of the four epileptic monkeys. As seen by Figure 7, after the onset of a seizure episode, the SP baseline would gradually shift to 100 to 200 µV negativity, return to the pre-ictal level as the rate of epileptic discharges decreased, and become slightly positive during the post-ictal period. This finding is consistent with reports of DC or steady potential shifts accompanying seizure activity

Figure 7.--Continuous DC recordimg (A to F) across temporal cortex, with surface electrode near the epileptogenic implant, during a seizure episode. The dashed lines indicate the preictal SP baseline. Negativity is upwards. Calibrations as indicated.


A
B
C
D
E
100 µV
1 sec

(Goldring and O'Leary, 1963).

Responses to sensory stimuli

The presentations of intermittent photic stimuli or acoustic clicks to epileptic subjects elicited cortical evoked responses and SP shifts similar to those obtained with normal monkeys (Experiment 1, Figures 1 and 2). In addition, the sensory stimuli occasionally initiated focal epileptic spiking. In temporal epileptic monkeys both clicks and flicker could elicit focal spiking, whereas epileptic discharges in occipital epileptic subjects were only initiated by flicker. Temporal lobe spiking usually occurred only during and shortly after stimulus presentations. By contrast the occipital epileptic discharges continued for 20 to 40 seconds after the termination of the photic stimulus, at which time surface positive SP afterpotentials returned to prestimulus baseline levels.

Intersensory conditioning

Conditioning of the electrocortical responses to flicker (US) was attempted in all epileptic monkeys by pairings of the US with trains of auditory clicks (CS). Conditioned surface negative SP shifts in occipital cortex were observed in all epileptic subjects. In the two monkeys with epileptogenic implants in temporal cortex, the conditioning procedure resulted in conditioned shifts similar to those described for normal monkeys (Experiment 1). As shown by Figure 8 for the 70th trial, initiation of the CS resulted in (1) 50 to 100 μV conditioned surface negative SP shifts and, (2) conditioned 8/sec frequency specific activity superimposed upon the peaks of the conditioned negative waves. These components of conditioned electrocortical activity were conspicuously absent during early phases of the conditioning procedure, as seen by Figure 6, Trial 7. The incidence of focal epileptic discharges in temporal cortex had no effect on conditioned occipital lobe activity. As can further be seen by Figure 8, Trial 70, conditioned shifts and frequency specific activity could be elicited by the CS even though a seizure episode with high voltage negative spiking from the temporal electrodes, and propagation to frontal cortex, began several seconds prior to the conditioning trial. Of particular interest is the observation that the rate of temporal lobe spiking is reduced during the CS-US interval.

Intersensory conditioning in occipital lobe epileptics also resulted in conditioned negative SP shifts in occipital cortex. Typical data for a severely epileptic occipital lobe monkey (no. 156) are shown in Figure 9.

Figure 8.--Electrocorticograms obtained with nonpolarizable electrodes and DC amplifiers during the course of intersensory electrocortical conditioning in a temporal epileptic monkey. Traces are recorded between surface and depth electrodes placed in frontal (Fs-Fd), temporal (Ts-Td), parietal (Ps-Pd), and occipital (Os-Od) cortex. Top four traces were taken during early phase and bottom four traces during later phase of conditioning. Presentations of CS (6 second train of 6/sec clicks) and of US (6 second train of 8/sec flicker) are indicated, respectively, by solid and dashed lines. Calibrations as shown.

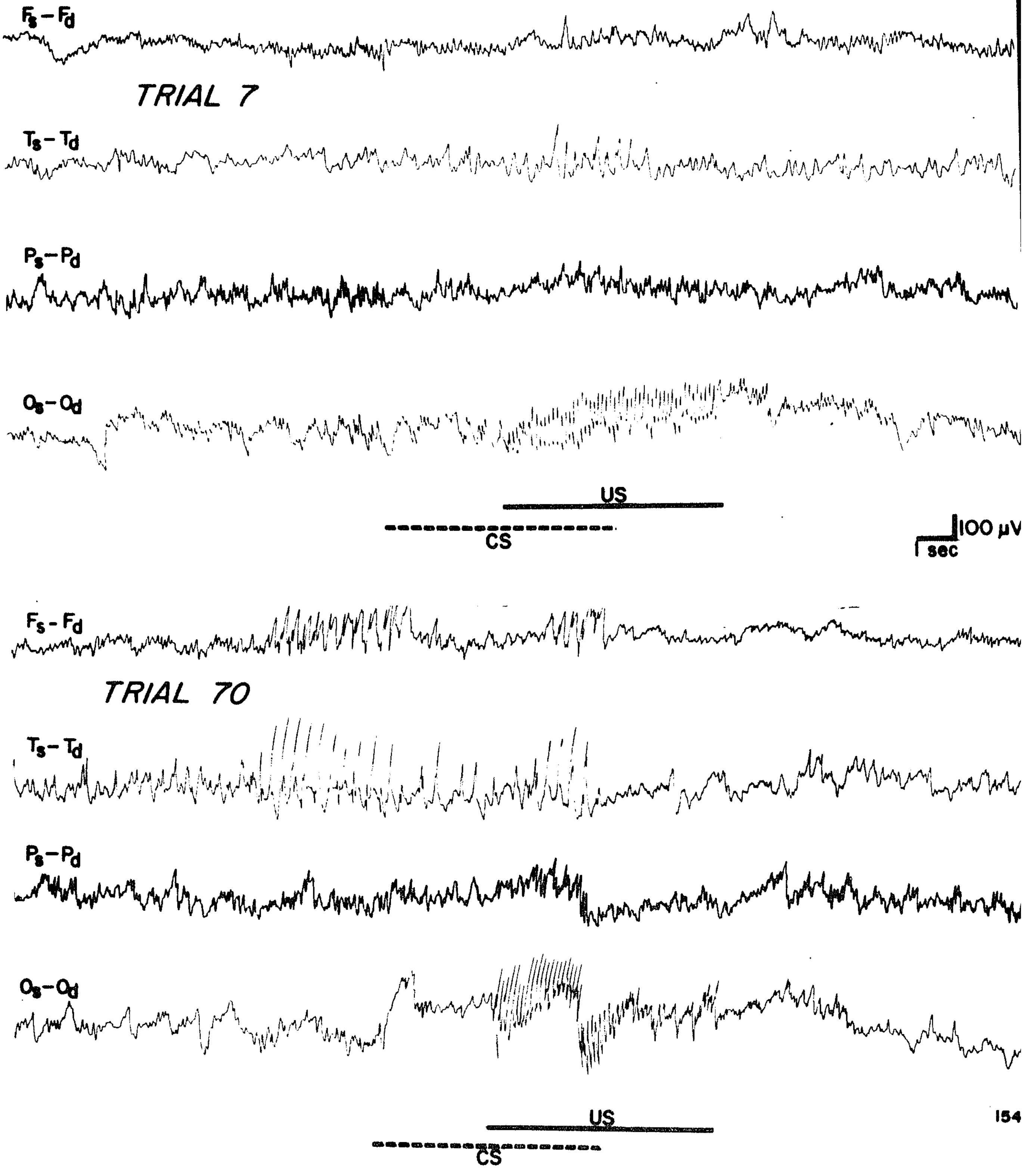
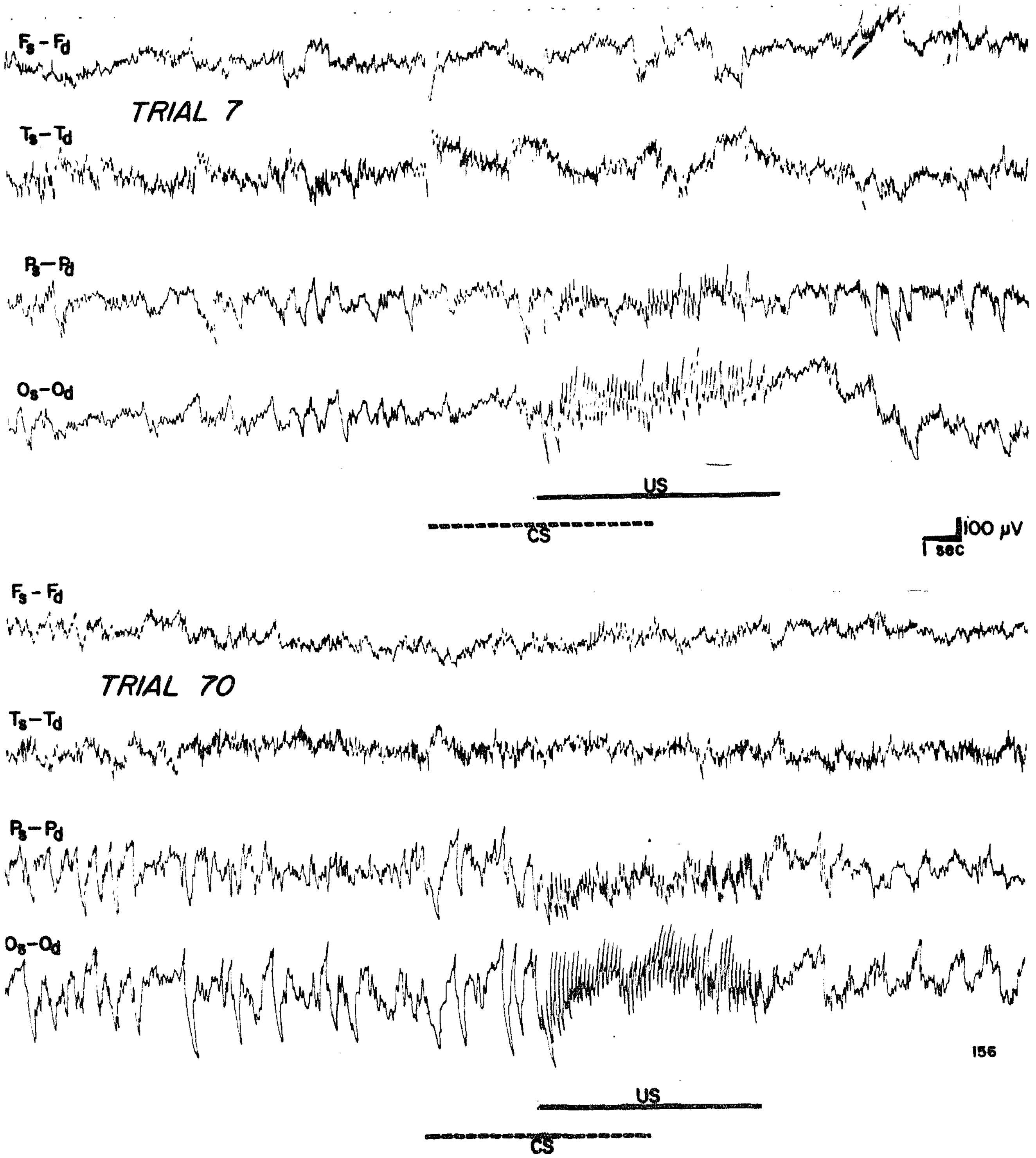

$F_s - F_d$
TRIAL 7
$T_s - T_d$
$P_s - P_d$
$O_s - O_d$
US
CS
100 µV
1 sec
$F_s - F_d$
TRIAL 70
$T_s - T_d$
$P_s - P_d$
$O_s - O_d$
US
CS
154

Figure 9.--Electrocorticograms obtained during the course of intersensory electrocortical conditioning in an occipital epileptic monkey. Traces are recorded between surface and depth electrodes placed in frontal (Fs-Fd), temporal (Ts-Td), parietal (Ps-Pd), and occipital (Os-Od) cortex. Top four traces were taken during early phase and bottom four traces during later phase of conditioning. Presentations of CS (6 second trains of 6/sec clicks) and of US (6 second trains of 8/sec flicker) are indicated, respectively, by solid and dashed lines. Calibrations as shown.


$F_s - F_d$
TRIAL 7
$T_s - T_d$
$P_s - P_d$
$O_s - O_d$
US
CS
100 µV
1 sec
$F_s - F_d$
TRIAL 70
$T_s - T_d$
$P_s - P_d$
$O_s - O_d$
156
US
CS

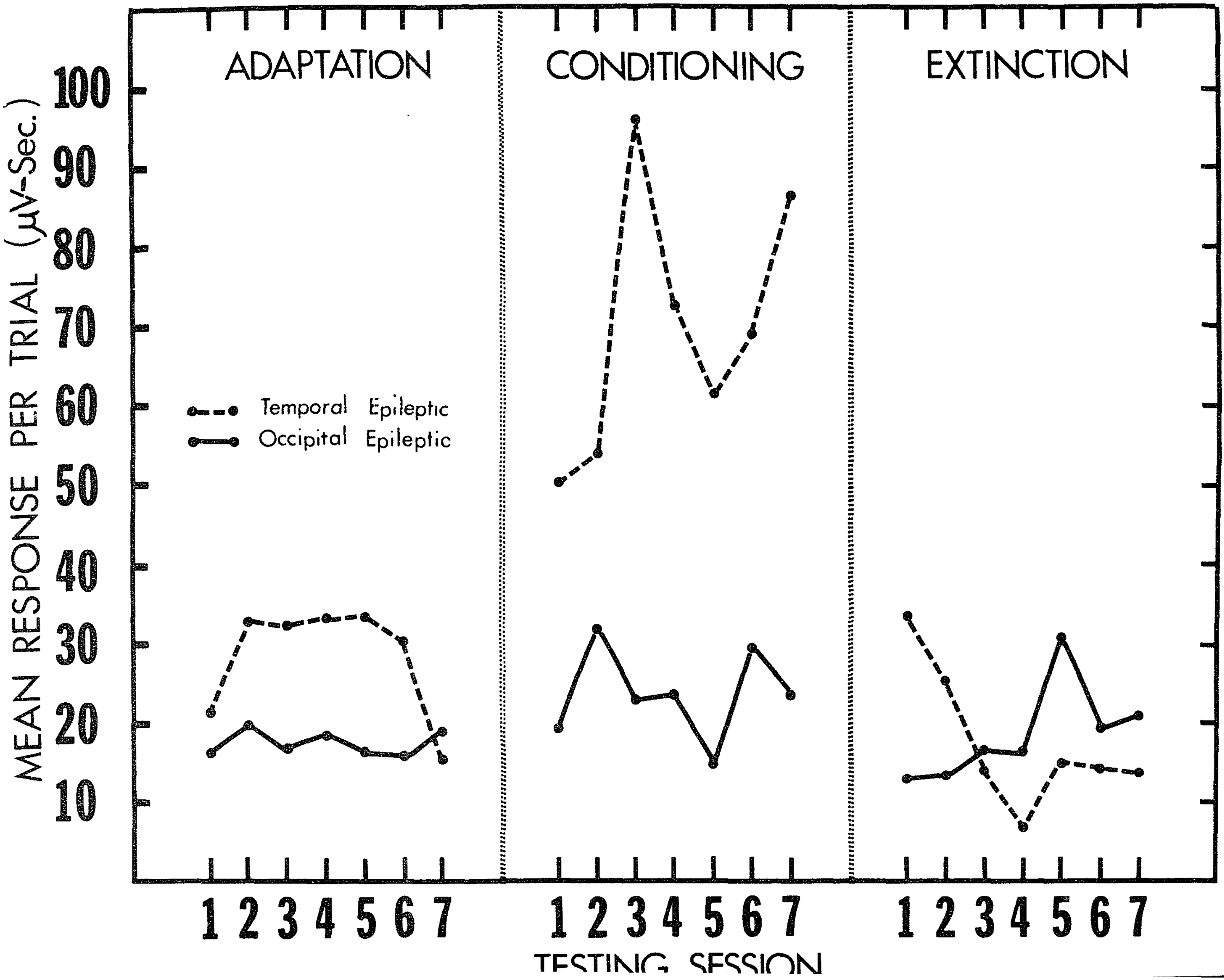
As can be seen for Trial 7, 25 to 50 μV conditioned negative shifts were present early in the condtioning procedure. With subsequent stimulus pairings conditioned shifts of increasing magnitude were observed but these were frequenctly obscured by large positive (200-300 μV) spikes occuring in the CS-US interval. As seen in Trial 70 of Figure 9, positive epileptic spikes in the CS-US interval were usually superimposed on conditioned negative SP waves with the effect of ten times reducing measures of conditioned response magnitude. Comparison of the acquisition curve for monkey no. 156 with that of a temporal lobe epileptic (no. 154) who exhibited a comparable rate of epileptic spiking, reveals what appears to be conditioning deficits (Figure 10). The magnitude of the mean SP shift per trial for the temporal lobe epileptic, computed for individual Conditioning sessions is consistently twice as large as that seen for the occipital lobe epileptic, whereas mean shifts per trial for Adaptation and Extinction sessions are similar for both monkeys. However, when the data for each monkey are pooled so that mean shifts per trial are computed on the basis of blocks of three consecutive testing sessions, as in Table 2, evidence for conditioning in each of the epileptic monkeys is clearly seen. Table 2 indicates that mean shifts per

TABLE 2

MEAN AND STANDARD DEVIATION OF SP SHIFT MAGNITUDE (V.SEC.) PER TRIAL FOR BLOCKS OF THREE SESSIONS

		Experimental Procedure											
		Adaptation				Conditioning				Extinction			
		1-3		5-7		1-3		5-7		1-3		5-7	
Monkey	Implant	X	sd	X	sd	X	sd	X	sd	X	sd	X	sd
154	Temp.	30	43	27	34	68	49	73	54	24	49	16	26
160	Temp.	28	29	16	24	32	31	40	28	22	24	19	34
149	Occ.	11	15	10	10	30	18	26	19	20	32	15	28
156	Occ.	18	43	17	26	24	28	22	61	14	25	23	28

Figure 10.--Mean CR magnitude per trial (expressed in μV-sec) during successive Adaptation, Conditioning, and Extinction sessions for one temporal lobe and one occipital lobe epileptic monkey. For Conditioning each point represents the mean response over the 3 second CS-US interval for thirty trials of each session. For the Adaptation and Extinction sessions the response during the first 3 seconds of the CS were measured.


ADAPTATION
CONDITIONING
EXTINCTION
MEAN RESPONSE PER TRIAL (μV-Sec.)
100
90
80
70
60
50
40
30
20
10
Temporal Epileptic
Occipital Epileptic
1 2 3 4 5 6 7
1 2 3 4 5 6 7
1 2 3 4 5 6 7
TESTING SESSION

trial for blocks of Conditioning sessions are consistently greater than mean shifts for blocks of Adaptation and Extinction sessions. This result is seen for each monkey. Significant differences between means for individual monkeys were not obtained in light of the large standard deviations associated with each mean in Table 2.

The results of conditioning in occipital lobe epileptics also showed evidence of positive shifts occuring in temporal cortex during the CS-US interval as described in Experiment 1. Data shown in the temporal lobe trace of Figure 9, Trial 7, indicate a diminution of the negative shift resulting from acoustic (CS) presentations. The diminution of the negative shift (relative positive shift) occurred during the CS-US interval and again during subsequent presentations of the photic US. The significance of this positive shift will be discussed in the subsequent section. Also worthy of note was that conditioned frequency specific activity was rarely seen in occipital lobe epileptic monkeys.

Analysis of epileptic activity

An analysis of the incidence of criterion epileptic spikes in the CS-US interval of conditioning trials, and in control intervals of Adaptation and Extinction trials, revealed surprising differences between temporal lobe and occipital lobe epileptic monkeys. During the Adaptation phase of the experiment all monkeys initially exhibited relatively high incidences of spikes which, with subsequent Adaptation, diminished to fairly low levels. These findings are summarized in Table 3 where the mean number of spikes per trial based on blocks of three consecutive testing sessions is computed for each monkey. Conditioning, on the other hand, resulted in group differences where the incidence of spikes increased markedly for occipital lobe epileptics and decreased for the temporal lobe epileptics. This finding was true particularly for the first three Conditioning sessions seen in Table 3. Further conditioning resulted in a subsequent decrease in occipital lobe spiking and a marked increase in the incidence of temporal lobe spiking seen in one monkey. The incidence of spikes in the second temporal lobe epileptic remained at a low level, which generally characterized the epileptic activity of this monkey, throughout the entire testing sequence. During the Extinction phase of the experiment the incidences of epileptic spikes for temporal lobe and occipital lobe epileptics differed once again. Extinction procedures resulted in reduced spiking in the occipital lobe epileptics and increased spiking in the temporal lobe epileptics.

TABLE 3

MEAN SPIKES PER TRIAL FOR BLOCKS OF THREE SESSIONS

		Experimental Procedure					
Monkey	Implant	Adaptation		Conditioning		Extinction	
		1-3	5-7	1-3	5-7	1-3	5-7
154	Temp.	6.3	2.3	1.3	5.3	5.0	20.3
160	Temp.	2.3	1.0	0.0	0.0	1.3	0.3
149	Occ.	2.0	2.3	3.0	1.6	2.3	0.3
156	Occ.	6.0	3.3	18.0	8.3	9.0	5.0

TABLE 4

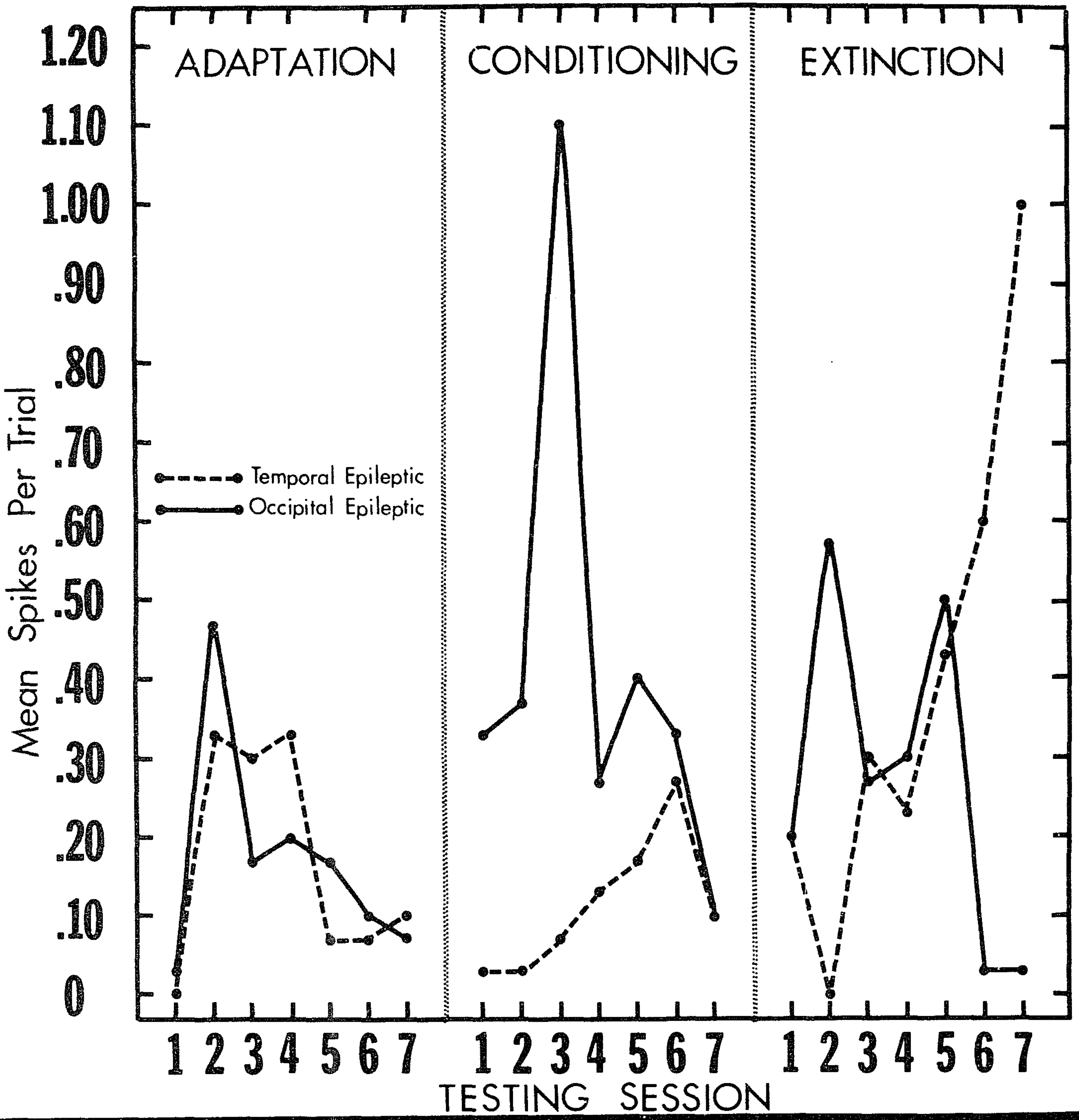
CORRELATION OF MEAN SP SHIFT MAGNITUDE PER TRIAL AND MEAN SPIKES PER TRIAL FOR INDIVIDUAL TESTING SESSIONS OF ADAPTATION, CONDITIONING, AND EXTINCTION PROCEDURES

Monkey	Implant	Adaptation	Conditioning	Extinction
154	Temp.	+.67*	+.26	-.35
160	Temp.	+.46	+.41	+.14
149	Occ.	+.46	+.61	+.31
156	Occ.	+.84**	-.02	+.14

* $p < .05$
** $p < .02$

The results summarized in Table 3 are interpreted as supporting the hypothesis that negative SP shifts are associated with enhancement of epileptic spiking, and conversely, that positive shifts are associated with a suppression of epileptic spiking. During the Adaptation procedure conditioning stimuli evoked widespread surface negative SP shifts (presumably related to diffuse, nonspecific, arousal systems) which were associated with increased epileptic spiking observed both in occipital lobe and temporal lobe monkeys alike. Habituation of the SP shifts during subsequent Adaptation sessions was notably associated with decreased epileptic spiking seen in all monkeys. Indeed, correlations, presented in Table 4, between mean negative SP shifts per trial and mean spikes per trial, computed for Adaptation sessions, revealed positive correlation coefficients (r) which were significant for two of the monkeys ($p < .05$). With Conditioning, increased negative SP shifts in occipital cortex were again associated with enhancement of spiking in occipital lobe epileptics. The reduced incidence of spiking in temporal lobe epileptics may be attributed to positive SP shifts in the CS-US interval conditioned in normal monkeys (Experiment 1) and in occipital lobe monkeys in the present experiment. Habituation of both the conditioned negative SP shifts in occipital cortex, and positive shifts in temporal cortex, again resulted in decreased occipital lobe and, conversely, increased temporal lobe spiking. Correlation coefficients computed for Conditioning sessions were not significant (Table 4) probably because conditioning and habituation processes interacted in a relatively complex fashion. Finally, during Extinction the same processes were operative. Diminution of conditioned negative SP shifts in occipital cortex was associated with decreased epileptic spiking in occipital lobe epileptics and diminution of the positive SP shifts in temporal cortex, with the reappearance of the primary negative SP shift to the acoustic CS, resulted in increased epileptic spiking in the temporal lobe epileptics. (A high negative correlation was obtained for monkey no. 154.) Illustrative spike data for one highly epileptic temporal lobe monkey (no. 154) are shown in Figure 11. Note the similarity of the mean spikes per trial during Adaptation sessions, the differences during the first few conditioning sessions and the differing trends during the extinction sessions. Similar though less clearcut observations were made of the less severely epileptic monkeys.

Figure 11.--Mean spikes per trial during successive Adaptation, Conditioning, and Extinction sessions for one temporal lobe and one occipital lobe epileptic monkey. For Conditioning each point represents the mean number of spikes recorded in the CS-US interval for thirty trials of each session. For Adaptation and Extinction sessions the spikes during the first 3 seconds of the CS were recorded.


ADAPTATION
CONDITIONING
EXTINCTION
Temporal Epileptic
Occipital Epileptic
Mean Spikes Per Trial
1.20
1.10
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
0
1 2 3 4 5 6 7
1 2 3 4 5 6 7
1 2 3 4 5 6 7
TESTING SESSION

Discussion

The results of the present investigation are consistent with the previous report of intersensory electrocortical conditioning of SP shifts in occipital cortex of normal monkeys (Experiment 1). Surface negative SP shifts and concomitant frequency specific activity, corresponding to the US frequency, were conditioned to clicks in monkeys with epileptogenic implants in superior temporal cortex. Unilateral epileptogenic lesions in the area of sensory cortex corresponding to the modality of the CS did not interfere with the conditioning process. The results of conditioning in monkeys with epileptogenic implants in occipital cortex however, revealed slight deficits in the acquisition of both conditioned shifts and conditioned frequency specific activity particularly, when data for a higly epileptic monkey was compared with that of normal and temporal lobe epileptic monkeys. Morrell, Roberts, and Jasper (1956) reported similar impairments in the acquisition of conditioned "alpha" blocking response in monkeys resulting from epileptogenic implants in sensory cortex corresponding to the CS modality. In order to account for the deficits observed in the present experiment two alternative explanations may be advanced: (1) that the propagation of abnormal electrical discharges generated by the epileptic focus disrupts the formation of neuronal connections necessary for conditioning to take place, and (2), that the epileptic focus, rather than interfering with the conditioning or "associative" processes per se, simply disrupts elaboration of the conditioned response. The latter explanation implies that conditioning does occur but that large positive epileptic spikes which accompany the CR obscure it from observation. This latter hypothesis is supported by the findings that (1) conditioned surface negative SP shifts were recorded from occipital cortex during the early phases of conditioning and, (2) that during subsequent phases, when the CR was well established both with respect to increased amplitude and duration, epileptic spikes were likely to be superimposed upon conditioned negative waves. The fact that the spikes were of high amplitude and often 500 msec in duration (similar to those reported by Gumnit and Takashashi in 1965 who also recorded epileptic activity with DC amplifiers and nonpolarizable electrodes) accounts for the reported lowered measures of conditioned SP response magnitude.

If we assume that conditioned negative SP shifts do occur in occipital cortex of occipital lobe epileptics during the conditioning phase of the experiment then the data support the hypothesis concerning the relation of SP shifts to processes of cortical excitability. The finding that the incidence of occipital lobe spikes in the CS-US interval of conditioning trials is greater than the incidence of spikes in control intervals of adaptation and extinction trials strongly suggests that negative shifts are associated with enhanced epileptic activity, and hence, increased cortical excitability. In addition, the present data support the converse of the aforementioned hypothesis namely, that positive SP shifts are associated with decreased cortical excitability. The finding that the incidence of temporal lobe spikes in the CS-US interval of conditioning trials, when positive shifts in temporal cortex were most evident (Experiment 1), was less than that of corresponding intervals of adaptation and extinction trials points strongly to the relation of positive SP shifts to decreased epileptic activity. This view is further supported by the fortuitous finding that conditioning stimuli presented while a spontaneous temporal lobe seizure was in progress resulted in suppressed rates of epileptic spiking in the CS-US interval but not in other intervals of the conditioning trial (see Figure 8, Trial 70).

In general, the data obtained with epileptic monkeys in the present study conforms with other reports of the relation of cortical SP shifts to epilepsy. A number of investigations have demonstrated that negative SP changes, of cortical origin, are reliable concomitants of the onset of seizure discharges in normal and epileptogenic cortex (Bates, 1963; Goldring and O'Leary, 1964; Mahnke and Ward, 1961; Morrell, 1960). Further, some investigators have suggested that the neuronal processes underlying SP shifts may be related to mechanisms involved in the initiation of seizure discharges. Evidence has been put forth by Goldring (1963), who has pointed to the fact that SP shifts "precede" or "usher in" seizure discharges induced in normal cortex by direct electrical stimulation of the cortex, midline thalamus, or lateral geniculate nucleus. Seizures induced by the adminstration of convulsant agents such as Metrazol, Picrotoxin, and methionine sulfoxamine, similarly, are preceded by SP shifts. Gumnit and Takahashi (1965) have also pointed out that the transition from interictal spiking to organized seizure activity occurs during a slow negative secondary potential associated with each spike at a punctate penicillin-induced epileptic focus. In the present experiment the findings that conditioned negative SP shifts also precede epileptic spiking with spikes superimposed on conditioned negative waves provides additional support for this view. Perhaps the most significant implication of the findings in the present study is that conditioned positive shifts are associated with suppression of epileptic discharges.. If SP shifts are related to mechanisms involved in the initiation of seizures then relatively simple intersensory conditioning procedures might be affective therapeutic tools for controlling or suppressing seizures in epileptic patients, particularly in patients with known lesions in sensory cortex. Further research is required to explore this conceivably fruitful apprach to our understanding and control of epilepsy.

Summary

After the initiation of focal epileptic discharges, produced by unilateral placement of alumina cream on either lateral occipital or superior temporal cortex in four stumptail monkeys, surface-to-depth bipolar Ag-AgCl nonpolarizable electrodes were chronically implanted in frontal, temporal, parietal, and occipital cortex. Recordings with DC amplifiers permitted examination of the interactions of surface negative SP shifts, evoked in auditory cortex to 6/sec and in visual cortex to 8/sec flicker, during the course of intersensory electrocortical conditioning. Conditioning to clicks (CS), with flicker

as in the US, resulted in: (1) 25 to 50 μV conditioned negative SP shifts in visual cortex, and (2) 20 to 40 μV conditioned positive SP shifts in auditory cortex. An analysis of the incidence of epileptic spiking in the CS-US interval of conditioning and control (adaptation and extinction) trials showed: (1) a higher incidence of occiptial spikes in occipital lobe epileptics for conditioning trials than for adaptation or extinction trials, and (2) a lower incidence of temporal spikes in temporal lobe epileptics for conditioning trials than for control trials. These data suggests that conditioned surface negative SP shifts are associated with an enhancement of epileptic activity and conversely, that surface positive SP shifts are associated with a suppression of epileptic activity. It would appear, therefore, that SP shifts reflect processes related to cortical excitability.

SUPPLEMENTARY EXPERIMENT 1

CORTICAL STEADY POTENTIAL RESPONSES AS FUNCTIONS OF PARAMETERS OF INTERMITTENT SENSORY STIMULATION

Introduction

In a number of experimental studies, in which direct-coupled (DC) amplifiers and nonpolarizable electrodes have been used to record steady potentials (SPs) from the cerebral cortex, sensory stimuli have been shown to evoke reliable surface negative SP shifts, particularly in areas of sensory cortex corresponding to the modality of the stimulus (Caspers, 1961; Gumnit, 1960; Kohler, et al., 1955; Lickey and Fox, 1966; Rowland, 1963). One interpretation of these findings is that SP shifts reflect neuronal processes associated with cortical excitation, and hence provide relevant data concerning possible neural mechanisms underlying sensation and perception (Kohler, et al., 1955; O'Leary and Goldring, 1964). However, quantitative relationships between parameters of the stimulus and characteristics of the SP response have as yet not been established. Clearly, the aforementioned hypothesis would be supported by demonstration of such relations. Therefore, the objective of the present experiment was to investigate the extent to which measures of SP responses in visual cortex are functions of the parameters of photic stimulation.

The major concern of the experiment was the characteristics of cortical SP shifts evoked in unanesthetized animals with chronically implanted electrodes. Preliminary findings indicated that the most reliable SP shifts were elicited by trains of intermittent photic stimuli (flicker), rather than by single light flashes, which for given intensities and durations resulted in varying responses, seen against the background of spontaneous electrocorticogram (ECG) activity. Thus, the parameters of the photic stimulus which were varied in the experiment were flicker frequency, flicker duration, and the intensity or brightness of the flicker. In all experiments the area of the projected stimulus was held constant.

Method

Data were collected from two normal monkeys which were previously subjects in Experiment 1. Nonpolarizable electrodes had been chronically implanted three to four months prior to testing in the present experiments.

Apparatus

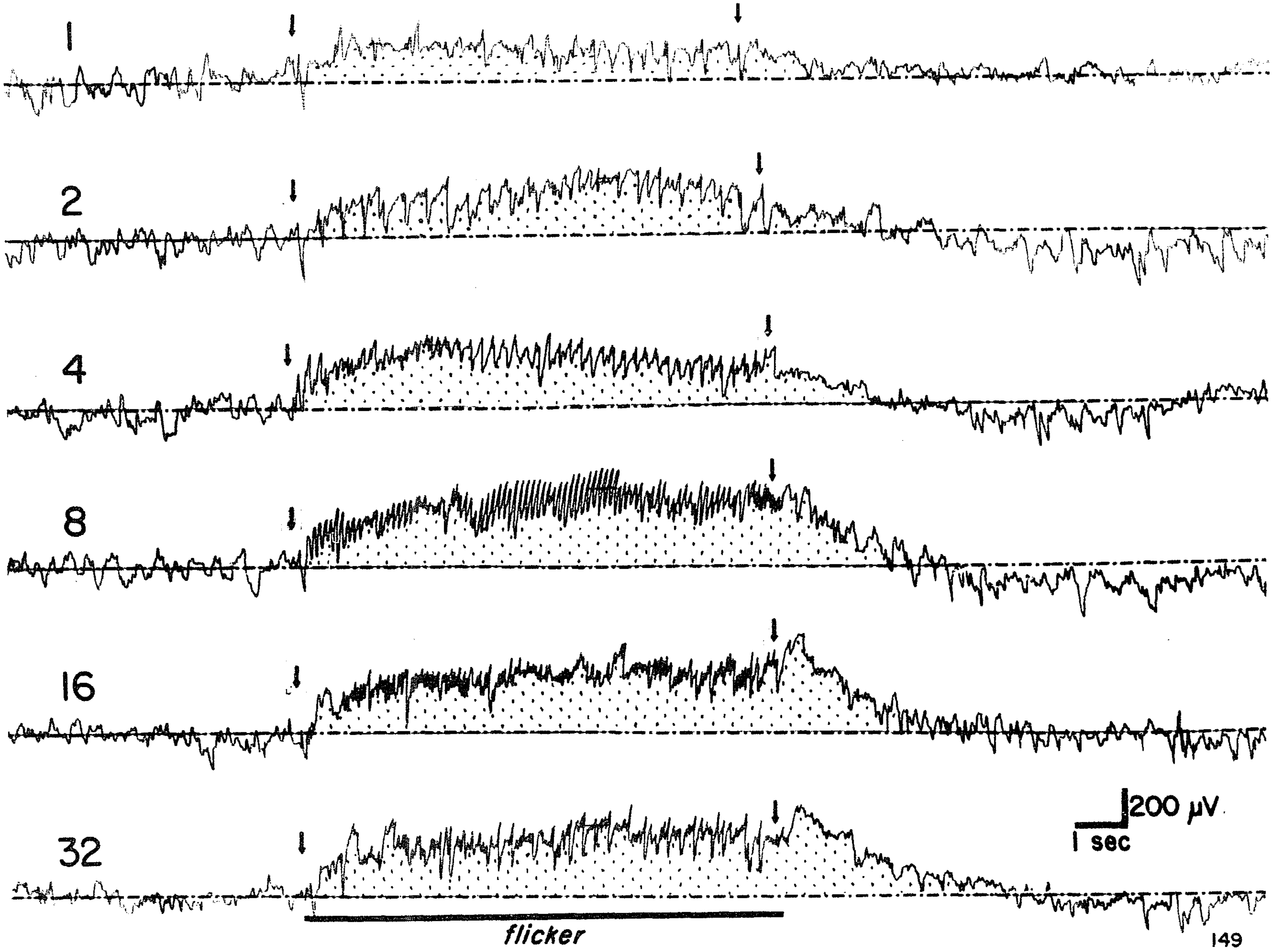
Testing was conducted with the monkey seated in a restraining chair placed in the electrically shielded soundproof conditioning chamber previously described. DC recordings were obtained in the usual way. Photic stimuli were presented by a Grass Photo-Stimulator lamp situated 30 cm in front of the monkeys' eyes. The onset and duration of the stimuli were automatically controlled by a panel outside the chamber and both the frequency and intensity of the stimuli were set manually by the experimenter.

Procedure

Three different experimental procedures were employed. Procedure 1, aimed at evaluating the effects of varying flicker frequency, consisted of presenting 10 second trains of flicker at the rate of one train every two minutes. The frequencies of flicker used were 1, 2, 4, 8, 16, and 32 flashes per second, with the intensity of the flashes being held at a moderate constant level (Stimulator settings: I = 4). The order of presentations of successive stimulus trains followed a latin square design, modified so that two successive trains were always of different frequencies. Each flicker frequency was presented six times in one experimental session. Procedure 2 was similar to Procedure 1 except that the stimuli were one minute trains of flicker presented at the rate of one train every four minutes. This procedure was aimed at evaluating SP responses to stimuli of relatively long durations. Finally Procedure 3, directed at determing the extent to which the SP response is a function of the intensity of the flicker, consisted of presenting one minute trains of 8 per sec flicker at five different intensity levels. The levels, denoted as settings on the Grass Photo-Stimulator, 1, 2, 4, 8, and 16, indicate relative measures of brightness, with approximately doubling of stimulus intensity for each higher setting. Again, the order in which the differing stimuli were presented was balanced. Stimuli were presented at each intensity setting.

Data analysis.--SP shifts recorded from occipital cortex in response to flicker were analyzed in the following manner. First, a 12 second section of the ECG trace preceeding the stimulus presentation was examined to determine the pre-stimulus SP baseline. A straight line was then fitted to this section of the record such that the sum of areas enclosed by the ECG trace above and below the line were equal. The task of establishing the baseline could be accomplished accurately and reliably by means of a highly sensitive compensating polar planimeter. The drift of the entire system was less than 10 μV/mm. Once the pre-stimulus baseline was established it was extended through the recorded response and various response measures were made with reference to it. As can be seen by Figure 1, it was possible to measure: (1) the peak amplitude of the response or the maximum dis-

Figure 12.--Typical SP responses of occipital cortex to 10 second trains of flicker obtained from a monkey with chronically implanted transcortical non-polariable electrodes and DC amplifiers. Negativity is upwards. Numbers to the left indicate the flicker frequency of the stimuli. Arrows indicate onset and offset of the stimuli. Dashed lines through the traces indicate the prestimulus SP baseline. The data indicate the relation of SP response amplitude, duration,, and magnitude (stipled area enclosed by SP shift and baseline) to stimulus frequency. Note the regular ECG (superimposed on the negative SP shift) specific to the frequency of the stimulus.


1
2
4
8
16
32
200 µV
1 sec
flicker
149

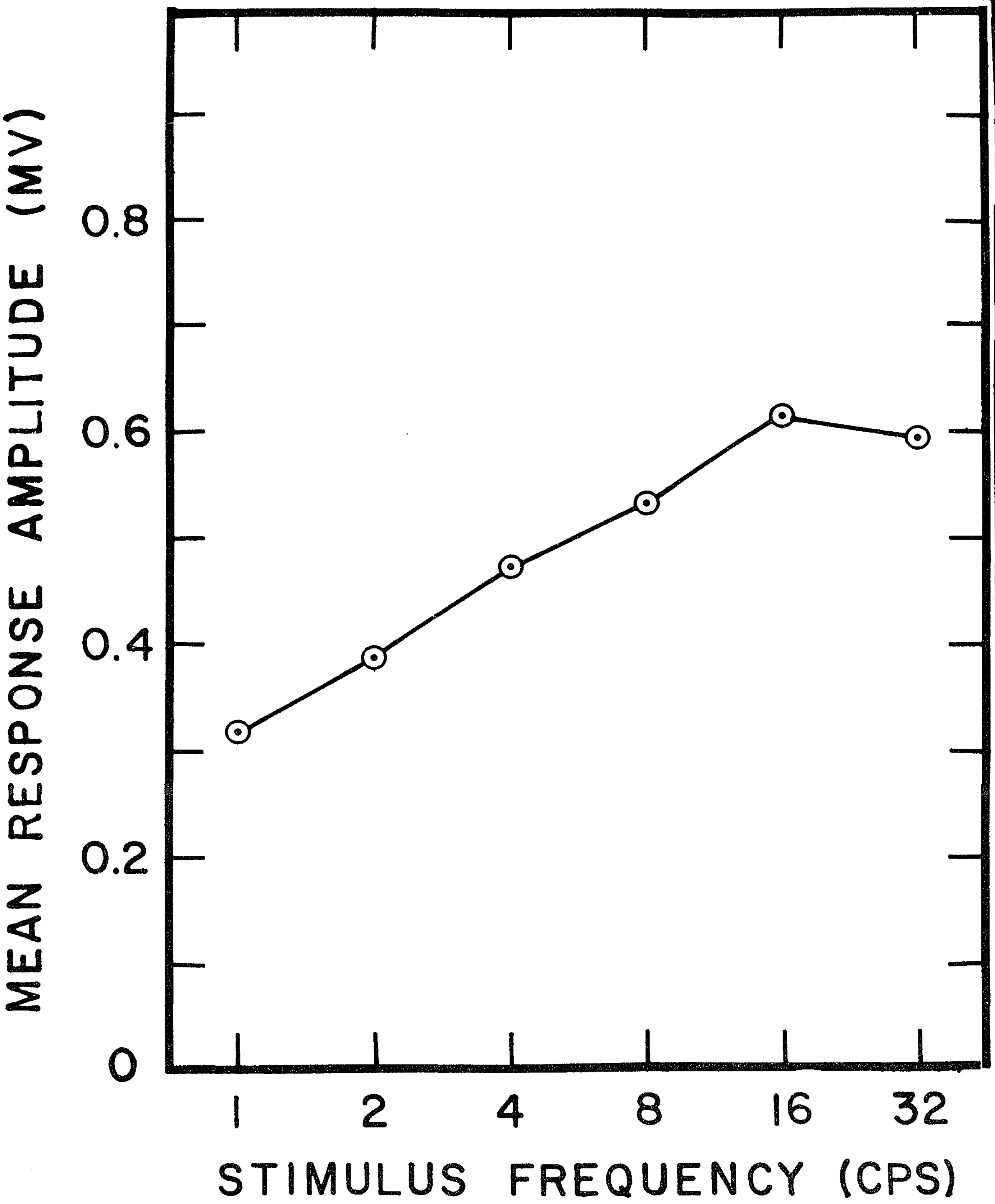
placement of the SP shift from the baseline, (2) the duration of the response or the total time before the SP shift returns to the baseline, and (3) the total area enclosed by the SP shift and the baseline, seen as stipled area. This latter measure of SP response magnitude (expressed in mV. sec) is interpreted as reflecting the total energy of the response and may be regarded as the integrated response measure.

Results

Effects of varying flicker frequencies

The responses to 10 second trains of flicker, as seen in Figure 12 consisted of two components: (1) a shift of the SP in the direction of greater surface negativity, and (2) regular ECG waves superimposed on the SP component, which corresponded to the frequency of the flicker. Figure 12 also shows that after termination of the stimulus the baseline returns slowly to the pre-stimulus level, continues to shift to slight surface positivity, and eventually returns to the baseline. The data indicate that the amplitude, duration, and the total area between the shift and the baseline vary as a function of the flicker frequency. The amplitudes of the SP responses as a function of the frequency of the stimulus are shown by Figure 13 for one monkey. Data for the second monkey are

Figure 13.--SP response amplitude as a function of flicker frequency. Each point represents the mean amplitude of six responses for one monkey at a given stimulus frequency. Note the linearity of the resulting function.

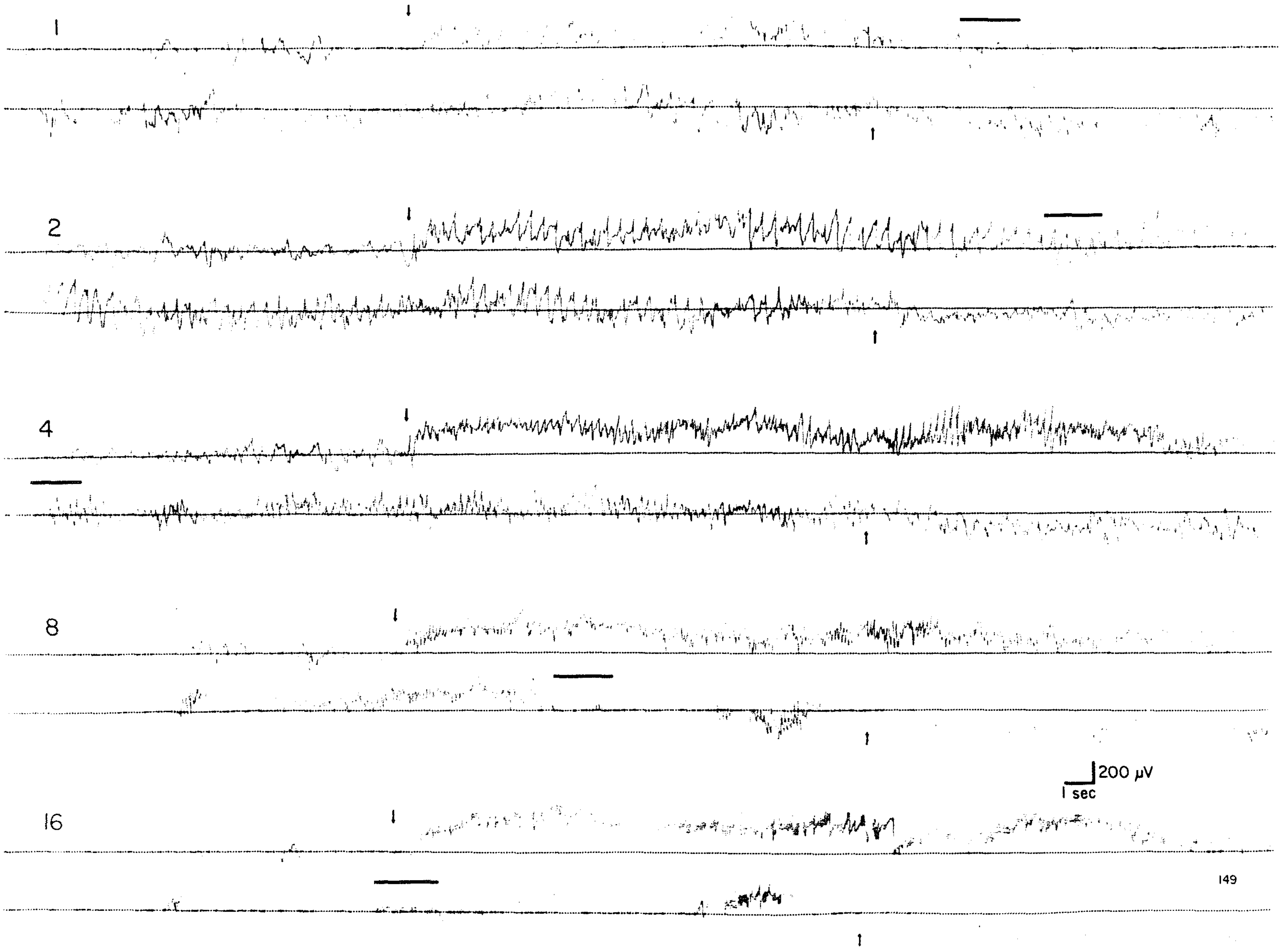

MEAN RESPONSE AMPLITUDE (MV)
0
0.2
0.4
0.6
0.8
1
2
4
8
16
32
STIMULUS FREQUENCY (CPS)

shown in Figure 31 of Appendix B. The mean amplitudes ranged between .3 and .6 mV with a maximum amplitude being obtained with the 16/sec flicker frequency. Of particular note is that the amplitude measures appear to be linearly related to a geometric function of increasing stimulus frequency. Integrated response (area) measures also revealed correlative functions. The mean response amplitudes or area measures were found not to be significantly different.

Effects of duration of stimulus

When flicker of one minute duration was presented negative SP shifts and frequency specific activity were again observed. However, these shifts did not outlast the stimulus, but as indicated by Figure 14 returned to baseline before termination of the stimulus. The duration of the response, i.e., the period before the negative SP shift returned to the pre-stimulus baseline for a period of at least two seconds, appears to be a function of the frequency of the stimulus. Following the return to baseline, the SPs would wax and wane and periodic bursts of frequency specific activity independent of the SP shifts continued as long as the stimulus was maintained. After termination of the stimulus, positive SP shifts were recorded which lasted one minute or more and whose peak

Figure 14.--Continuous traces of SP responses in occipital cortex to one minute trains of flicker. Negativity is upwards. Numbers to the left of each pair of continuous traces indicate the stimulus frequency. Dotted line through the traces indicate the prestimulus SP baseline. Downward pointing arrows indicate the stimulus onset. Upward pointing arrows indicate the stimulus offset. Heavy black line segments indicate two second intervals in which the DC response shift was judged to return to the prestimulus SP baseline level. Note that the amplitude, duration and magnitude of the surface negative SP responses appear to be a function of the stimulus frequency.


1
2
4
8
16
200 μV
1 sec
149

amplitudes often exceeded .5 mV. Measurements of the responses revealed that the amplitude, duration, and area of the SP response varied monotonically as a geometric function of the stimulus frequency. In addition, the amplitude and duration of the positive after-potentials, which were quite reliable events, also varied as a function of the stimulus. The effects of varying the frequencies of one minute stimuli on duration and magnitude of the SP response in one monkey are shown in Figure 15 and 16, respectively. Stimulus frequencies of 8 and 16 cps consistently gave the largest responses in terms of both response duration and area. The differences in the mean response magnitudes illustrated in Figure 16 were statistically significant ($p < .02$) as can be seen in the analysis of variance summarized in Table 5. Similar functions were obtained for response amplitude measures.

Effects of intensity of stimulus

The magnitude of SP responses to one minute trains of 8/sec flicker clearly reflect the intensity of the flicker. As seen by Figure 17 and 18 both the duration of the negative SP shift and its magnitude increased linearly as functions of stimulus intensity. An analysis of variance of the mean data presented in Figure 18

TABLE 5

ANALYSIS OF VARIANCE SUMMARY TABLE. DIFFERENCES IN SP RESPONSE MAGNITUDE OBTAINED WITH FLICKER OF VARIED FREQUENCY

Source of Variation	Sums of Squares	d.f.	Mean Squares	F
Between groups	1044.57	5	208.91	25.54*
Within groups	245.25	30	8.18	
Total	1289.82	35		

TABLE 6

ANALYSIS OF VARIANCE SUMMARY TABLE. DIFFERENCES IN SP RESPONSE MAGNITUDE OBTAINED WITH FLICKER OF VARIED INTENSITY

Source of Variation	Sums of Squares	d.f.	Mean Squares	F
Between groups	-2906.23	4	-726.56	5.27**
Within groups	3449.36	25	137.97	
Total	543.13	29		

*$p < 0.01$

**$p > 0.05$

Figure 15.--Mean SP response duration as a function of flicker frequency. Each point represents the mean duration of six responses for one monkey at a given stimulus frequency.

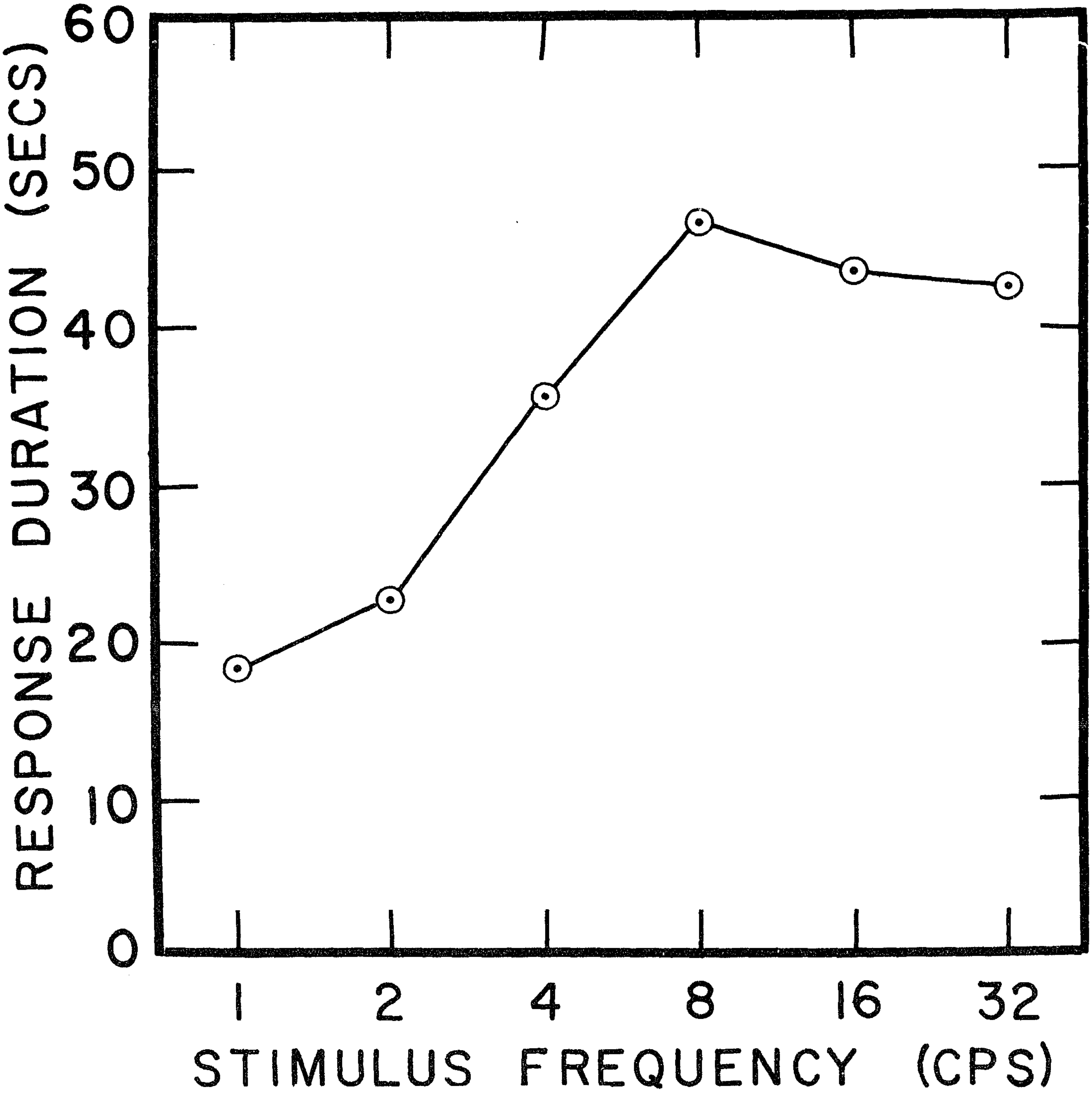

RESPONSE DURATION (SECS)
60
50
40
30
20
10
0
1
2
4
8
16
32
STIMULUS FREQUENCY (CPS)

Figure 16.--Mean SP response magnitude (expressed in Millivolt-seconds) as a function of stimulus frequency. The SP response magnitude was determined by measuring the area enclosed by the negative SP shift and the pre-stimulus SP baseline.

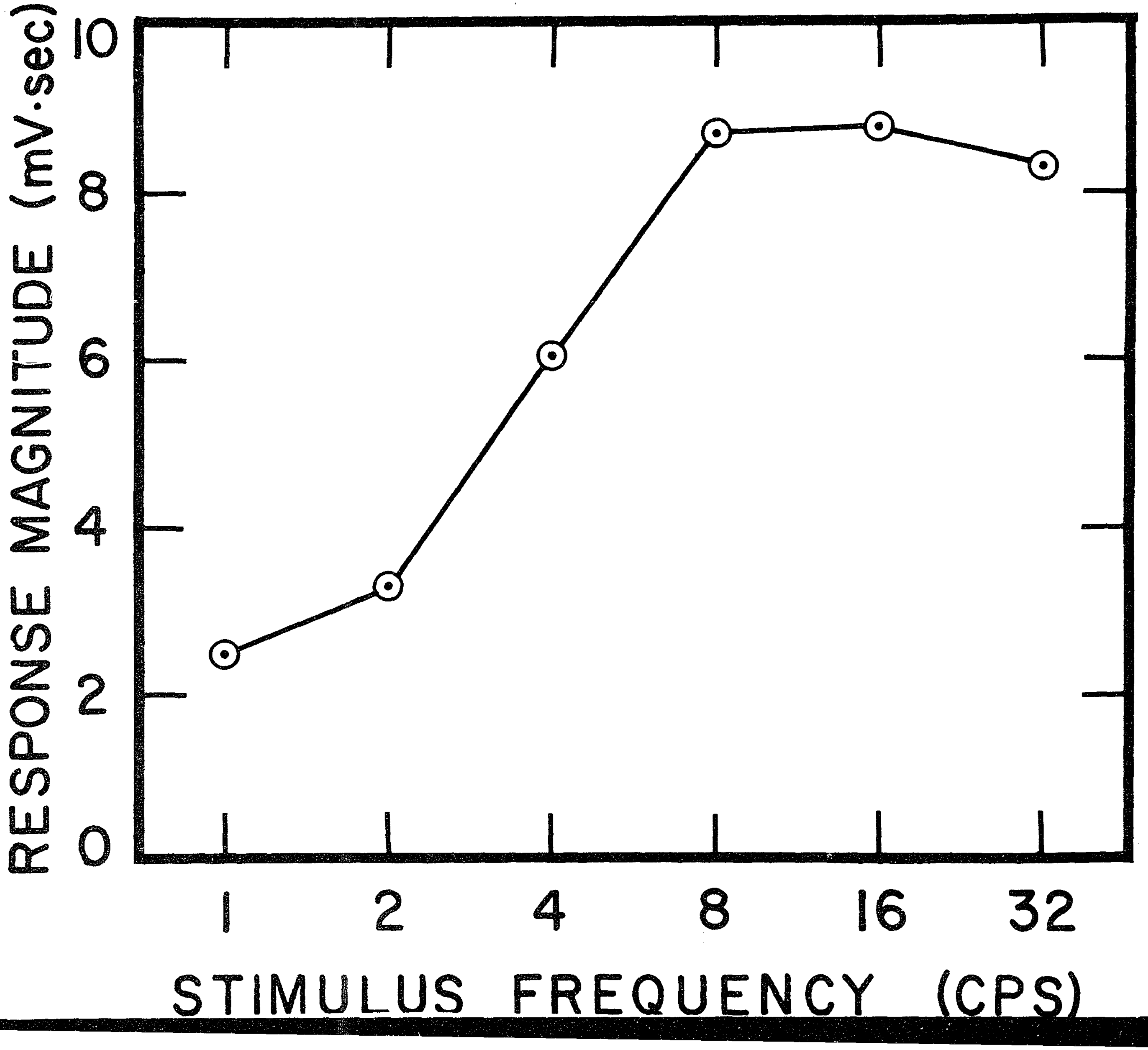

RESPONSE MAGNITUDE (mV·sec)
0
2
4
6
8
10
1
2
4
8
16
32
STIMULUS FREQUENCY (CPS)

Figure 17.--SP response duration as a function of relative stimulus intensity. The stimuli were one minute trains of 8/sec flicker. Intensity values, corresponding to settings of Grass Photo-Stimulator, are scaled values of brightness such that brightness at intensity 4 is twice that at intensity two, or four times that at inensity one. Each point represents the mean of six responses at a given intensity level.,

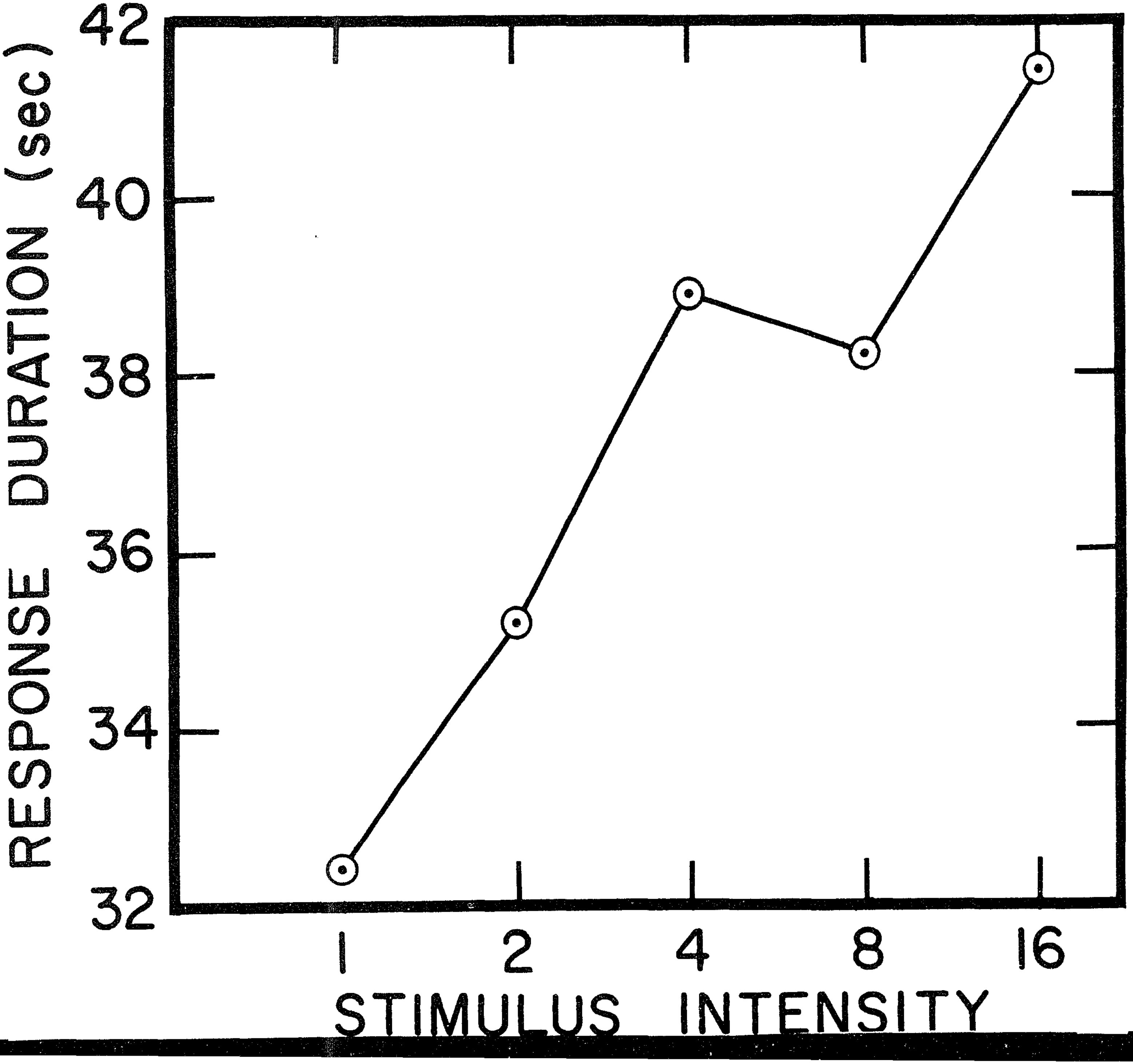
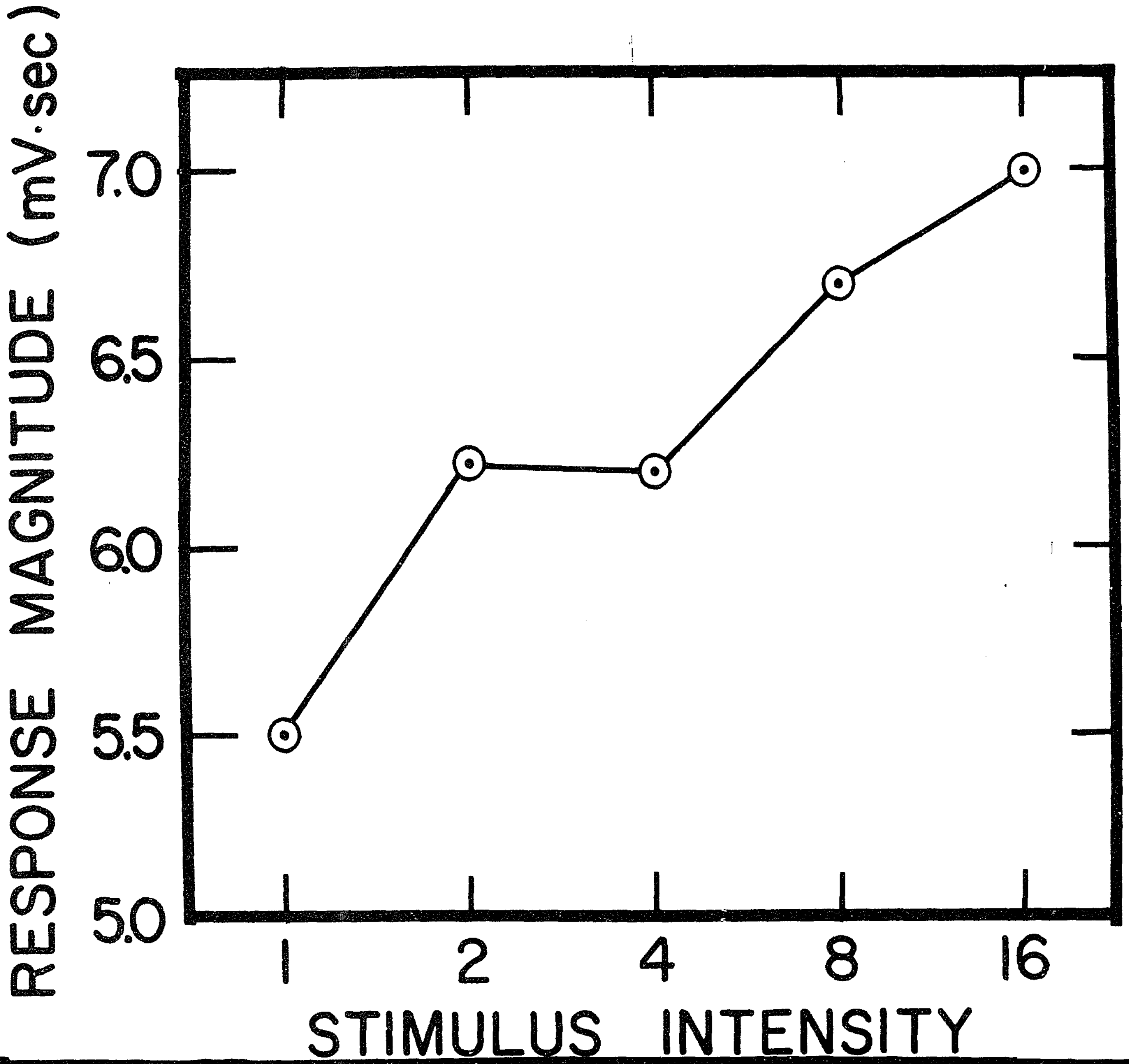

RESPONSE DURATION (sec)
32
34
36
38
40
42
1
2
4
8
16
STIMULUS INTENSITY

Figure 18.--SP response magnitude (expressed in mV-sec) as a function of stimulus intensity. Stimuli were one minute trains of 8/sec flicker, varied intensity. Intensity values are relative values corresponding to settings on the Grass Photo-Stimulator. Each point represents the mean for six responses at a given intensity level.


RESPONSE MAGNITUDE (mV·sec)
7.0
6.5
6.0
5.5
5.0
1
2
4
8
16
STIMULUS INTENSITY

produced an F ratio of 5.27 which was not significant at the .05 level. The results of the analysis are summarized in Table 6. Within the limited range of intensities explored in this experiment the significant characteristics of these functions is their apparent linearity. Corroborative data for a second monkey are presented in Figure 32 of Appendix B.

Discussion

The results of the present study clearly indicate that intermittent photic stimuli (flicker) evoke surface negative SP shifts in areas of visual cortex of the monkey. The data are consistent with results of both acute experiments (Gumnit, 1960; Kohler, et al., 1955; Libet and Kahn, 1946; Lickey and Fox) and chronic studies (Gumnit and Grossman, 1961; Rowland and Goldstone, 1963) of sensory SP responses in the cat. Collectively, these data suggest that surface negative SP shifts are post synaptic dendritic potentials arising in the superficial layers of the cortex. Recordings obtained in the present experiment with transcortical (surface to depth) non-polarizable electrodes lend particular support to this view.

It has been further suggested that SP shifts reflect processes of cortical excitation (O'Leary and Goldring, 1964). The finding that the magnitude of the

SP response (output) is a function of the parameters of the sensory stimulus (input) provides supportive data for this hypothesis heretofore lacking.

In the present experiment amplitude, duration, and integrated response (area) measures of negative SP shift were found to be functions of frequency and intensity parameters of the stimulus. Rise times too, appeared to be related to the stimulus although measurements failed to uncover quantifiable relationships. Gumnit and Grossman (1961) reported that rise times of SP shifts recorded from auditory cortex of the cat in response to repetitive acoustic stimuli (clicks) were a function of the repetition rate of the clicks. Differences in electrodes and their placements as well as species and modality differences may account for discrepancies in the results. However, the suggestion of Lickey and Fox (1966) that sensory SP responses consist of two components, one local and presumably related to specific sensory inputs and the other diffuse and related to diffusely projecting systems, each distinguishable from the other in terms of its time course, makes it likely that the relative contribution of each may account for the variability in results encountered in the present experiment. Further studies are required to clarify this point. Studies which attempt to correlate the incidence of frequency specific activity to negative SP shifts may be particularly useful in this respect.

With respect to the signifance of SP shifts in sensory cortex, the findings that plots of response magnitude as log functions of stimulus frequency or intensity result in monotonic functions, within limits of the parameters explored, suggest that cortical SP shifts are relevant concomitants of sensory processes which should be included in any discussion of the neural substrates of sensation and perception.

Summary

Transcortical steady potentials (SPs) were recorded from occipital cortex of monkeys with chronically implanted nonpolarizable electrodes in response to trains of intermittent photic stimuli (flicker). Amplitude, duration, and integrated (area) response measures of surface negative SP shifts were found to be positive functions of both frequency and intensity parameters. Measures of response magnitude plotted for log values of flicker frequency and intensity resulted in essentially linear functions within limited ranges explored. The findings were interpreted as supporting the hypothesis that surface negative SP shifts reflect neuronal processes associated with cortical excitation which may relate to neural mechanisms underlying sensation and perception.

SUPPLEMENTARY EXPERIMENT 2

STEADY POTENTIAL RESPONSE MAGNITUDE AS A FUNCTION OF CHANGES IN CORTICAL EXCITABILITY

Introduction

Reports of cortical steady potential (SP) shifts recorded directly from the cerebral cortex of unrestrained rats and cats with chronically implanted nonpolarizable electrodes, during sleep and wakefulness and during behavior (Caspers, 1960; Rowland, 1960) provide important data in support of a hypothesis that SP shifts reflect relatively long term changes in cortical excitability. This hypothesis is suggested by two findings reported in prior experiments with monkeys; (1) that "conditioned" SP responses once established in visual cortex of the monkey show considerable variability, particularly when elicited by procedures in which the interval between conditioning trials was of variable duration but averaged one minute (Experiments 1 and 2); and (2) that intermittent photic stimuli (flicker), in addition to evoking surface negative SP shifts in visual cortex associated with the onset and duration of the stimulus, resulted in positive shifts associated with termination of the stimulus (Supplementary Experiment 1). The duration of the positive shifts often exceeded one minute. These data point to a relation of positive steady afterpotentials to SP responses evoked by subsequent stimulation. Therefore, the present experiment was aimed at evaluating the extent to which the magnitude of the SP response to flicker is a function of the conditions of prior photic stimulation, particularly the time interval between prior (conditioning) and subsequent (test) stimuli, when positive steady afterpotentials are in various states of growth and decay.

Method

The subjects were two normal stumptail monkeys used in the previous supplementary exeriment. The electrodes were implanted approximately 5 to 6 months prior to testing. Recordings of SP shifts in response to sensory stimuli were obtained with DC amplifiers in the usual manner (see Experiment 1). Again a Grass Photo Stimulator and lamp was the source of intermittent photic stimuli, and the sequence and duration of stimuli were controlled by an automatic testing panel.

Procedure

Two experimental testing procedures were employed to determine the extent to which SP response magnitude is a function of conditions of prior photic stimulation.

Procedure 1.--was concerned with evaluating the effects of varying the interval between successive presentations of a stimulus. The stimulus in this case was a six second train of 8/sec flicker of moderate intensity.

Following a ten minute period of baseline recording, the stimulus was presented to the subject and then repeated at interstimulus intervals (ISIs) of 5, 10, 20, 40, 80, and 160 seconds until ten responses were obtained for each ISI. The order of ISIs was balanced so that each interval occurred only once in a random block of six stimulus presentations. This procedure was selected because of its similarity to prior conditioning studies to which it may relate, however, it should be noted that conclusions drawn from the results of this procedure are limited to the extent that a response to a given stimulus may not only be a function of the stimulus immediately proceeding it, but to several others, since the duration of positive afterpotentials frequently exceeds one minute. In order to compensate for effects which may have confounded the results, Procedure 2 was subsequently conducted.

Procedure 2.--This procedure was concerned with the effects of varying the interval between a conditioning stimulus and a subsequent test stimulus. The conditioning stimulus was a one minute train of 8/sec flicker presented at the rate of one every four minutes. The test stimulus was a six second train of 8/sec flicker which followed termination of the test stimulus at ISI of 2, 4, 8, 16, 32, 64, and 128 seconds. The intensity of all stimuli were the same as for Procedure 1. Again, the order of ISI was balanced and six SP responses were obtained for stimuli with each ISI.

Data analysis

The magnitude of SP responses were measured with reference to prestimulus SP baseline levels. The method of determining baselines is described elsewhere (see Experiment 1), however, it should be noted that baseline determinations were made with 5 second sections of record immediately preceeding stimulus presentations, except in Proceudre 2 with ISIs of 2 and 4 seconds. In these cases determinations were based on 2 and 4 seconds, respectively.

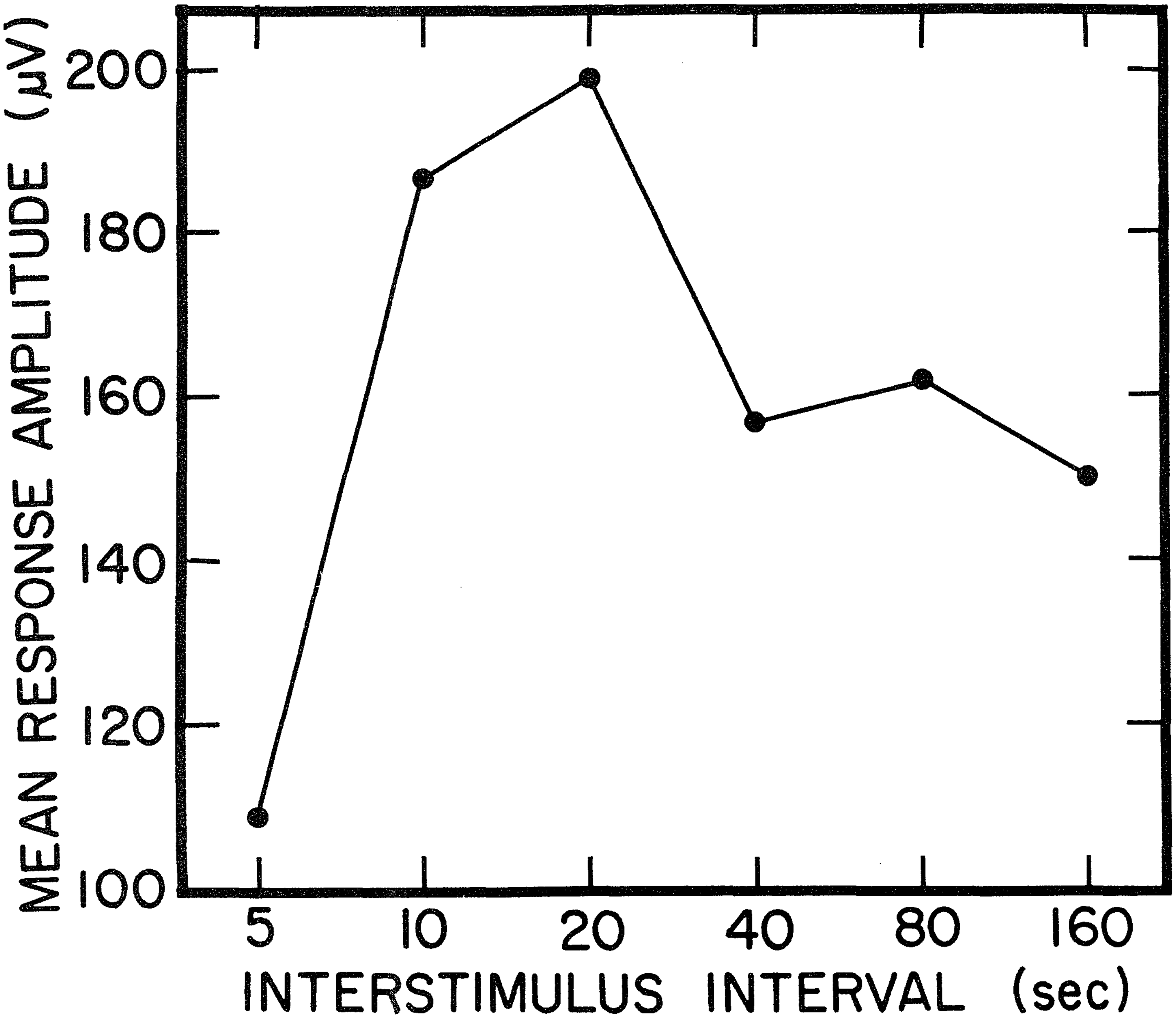
Results

Procedure 1

The SP response to six second trains of 8/sec flicker consisted of ; (1) a surface negative SP shift assoicated with the onset and duration of the stimulus, and (2) a gradual return of the shift to baseline with subsequent positivity, associated with the offset of the stimulus. The duration of the positive shift (after-potential) ranged between 10 and 40 seconds.

The magnitude of the SP response was variable and dependent upon the interval between successive stimuli. Figure 19 shows the mean response amplitude of SP responses for one monkey associated with each ISI. The

Figure 19.--Mean amplitude of SP response to 8/sec flicker as a function of the interval between successive stimuli. Each point represents the mean of ten responses obtained for one monkey.


MEAN RESPONSE AMPLITUDE (μV)
200
180
160
140
120
100
5
10
20
40
80
160
INTERSTIMULUS INTERVAL (sec)

largest means of 186 and 198 μV were found with ISIs of 10 and 20 seconds, respectively whereas, the means for the longer ISIs of 40, 80, and 160 seconds were approximately 160 μV. With an ISI of 5 seconds the mean response was slightly greater than 100 μV. This low value more accurately reflects a rapid positive shift in the prestimulus record than it does the amplitude (i.e., peak to peak) of the response. The positive shift in the prestimulus record resulted in an average prestimulus baseline that was considerably more negative (approximately 50 μV) than the SP level just prior to response. Therefore, the measure of the negative response amplitude with reference to the baseline was lower than it might otherwise have been.

Inspection of the curve illustrated in Figure 19 indicates that the mean amplitude response was greatest for interstimulus intervals of 10 and 20 seconds when positive SP shifts were most evident. These data suggest a relation of positive afterpotentials to SP response magnitude. Similar results were obtained with amplitude measures for the other monkeys (see Figure 33 of Appendix B). The differences between means were not found to be statically significant in either monkey.

Procedure 2

The presentation of one minute trains of 8/sec flicker as conditioning stimuli resulted in surface negative SP shifts which returned to baseline prior to stimulus termination. Highly reliable positive SP shifts, often exceeding 200 μV, resulted from offset of the stimulus. Maximum displacements of the positive SP from the baseline were usually seen within 4 to 10 seconds of stimulus offset and the duration of the positive shift often exceeded one minute.

The presentation of six second trains of 8/sec flicker as test stimuli resulted in negative SP responses whose amplitudes were related to the ISIs between conditioning and test stimuli. Maximum amplitude responses seen as means of 146 and 136 μV in Figure 20 were obtained with ISIs of 4 and 16 seconds, respectively. Longer ISIs of 32, 64, and 128 seconds resulted in mean responses less than 120 μV. The mean response for the 2 second ISI was approximately 100 μV. Again, this comparatively low value could be explained by a rapidly shifting prestimulus record, and a resulting relatively negative baseline. An Analysis of Variance for the mean response amplitudes presented in Figure 20 is summarized in Table 7. The findings of this procedure clearly indicate that maximal SP responses were associated with positive displacements of the SP resulting from prior stimulation.

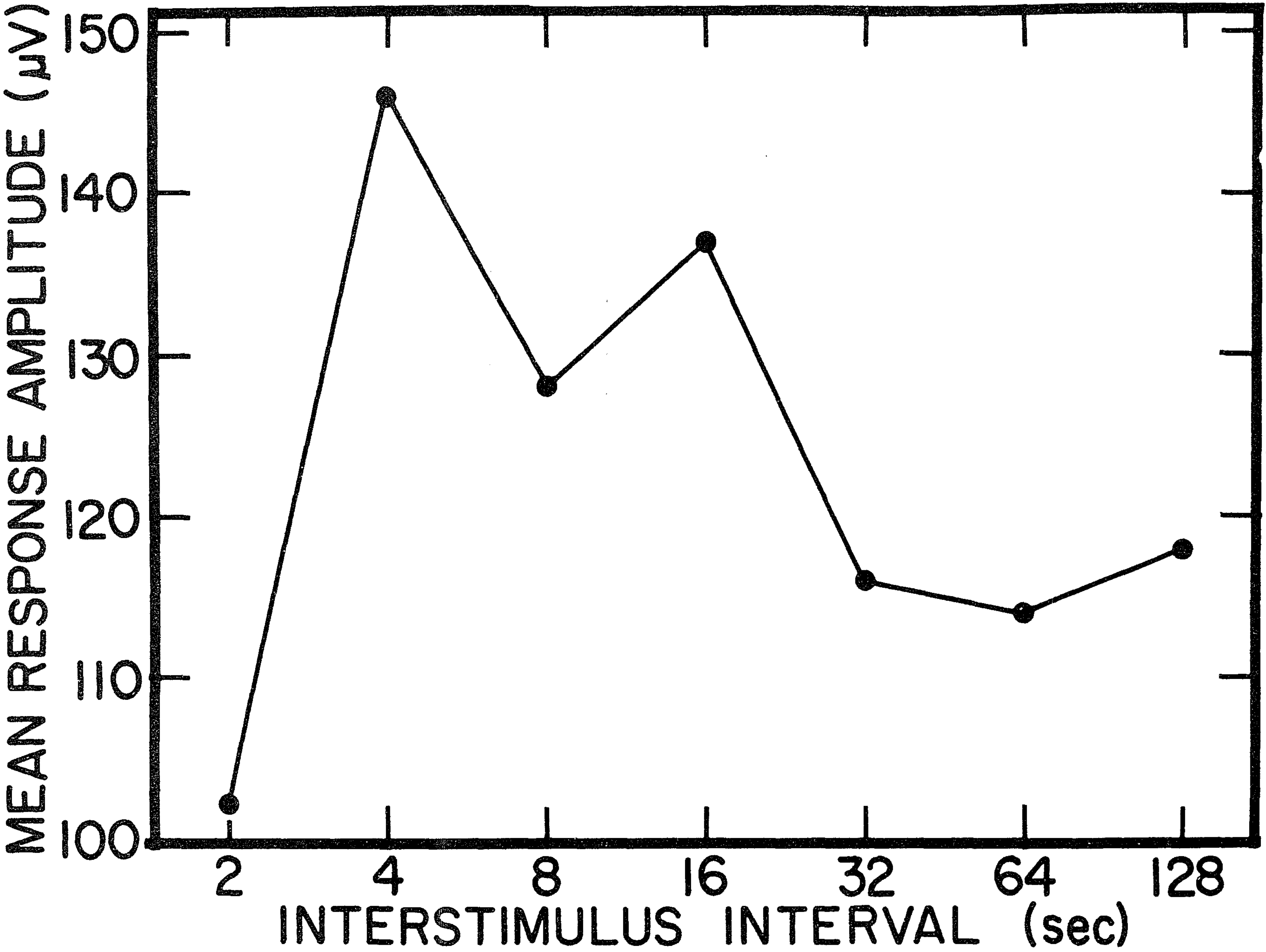
TABLE 7

ANALYSIS OF VARIANCE SUMMARY TABLE. DIFFERENCES IN SP RESPONSE AMPLITUDE OBTAINED WITH VARIED INTERVALS BETWEEN CONDITIONING AND TEST STIMULI

Source of Variation	Sums of Squares	d.f.	Mean Squares	F
Between groups	81.55	6	13.59	64.71*
Within groups	7.39	35	0.21	
Total	88.94	41		

$*p < 0.01$

Figure 20.-- Mean amplitude of SP response to test stimuli as a function of the interval between conditioning and test stimuli. The conditioning stimuli was a one minute train of 8/sec flicker and the test stimulus was a six second train of 8/sec flicker. Each point represents the mean response of six test stimuli for one monkey.


MEAN RESPONSE AMPLITUDE (μV)
150
140
130
120
110
100
2
4
8
16
32
64
128
INTERSTIMULUS INTERVAL (sec)

Discussion

The results of the present experiment clearly indicate that the magnitude of the SP response of occipital cortex to flicker is a function of the conditions of prior photic stimulation particularly,,the interval between successive stimuli. Further, it was found that the largest SP responses were associated with intervals corresponding to the time when positive steady afterpotentials attained their maximum voltage displacements from prestimulus baseline levels. These data, therefore, suggest that SP shifts reflect relatively long term changes in cortical excitability with positive shifts indicative of raised, and negative shifts lowered excitability levels. It should be noted however, that cortical excitability was measured in the present experiment in terms of the magnitude of SP shifts to trains of moderately intense photic stimuli. The findings do not preclude the possibility that threshold measures of cortical excitability may yield dissimilar results which would be consistent with the hypotheses stated in previous sections.

One interpretation of these findings is that positive SPs reflect the capacity of the cortex to shift towards surface negativity.. If we accept the suggestion of O'Leary and Goldring (1964) that the cortex behaves as a polarized layer of cells with the surface electropositive with respect to the depth, and if we further assume that there is a normal physiological limit of the extent to which the surface can shift towards negativity, then it is clear that a relatively surface positive SP level represents a greater capacity for a shift toward the limit of negativity than does a negative SP level. It should be noted, however, that this interpretation holds only insofar as the test stimuli in the present experiment were capable of evoking shifts of maximum negativity. Studies involving manipulation of the parameters of the test stimuli are required for clarification of this point.

The results of the study also revealed that whether 6 seconds or one minute trains of flicker were used as conditioning stimuli, the periods of poststimulus hyperexcitability were approximately the same, except perhaps with very short ISIs. In Procedure 1 minimal responses were observed with an ISI of 5 seconds whereas maximal responses were observed in Procedure 2 with a 4 second ISI. This latter result may be explained in terms of the fast rise time of positive SP shifts following termination of the one minute conditioning stimulus in Procedure 2. In any event these results point to the need for other investigations of the extent to which parameters of the conditioning stimulus, such as frequency, intensity, and duration, affect resulting excitability functions. It should also be pointed out that in the present experiment evaluations of cortical excitiability were based on only SP resonse amplitude measures rather than on more traditional measures of thresholds for responding. The whole issue of cortical excitability can only be understood when different avenues of investigation of the subject are fully explored.

Summary

Surface negative steady potential (SP) shifts in response to six second trains of 8/sec flicker were observed in two monkeys with transcortical nonpolarizable electrodes chronically implanted in occipital cortex. Recordings made with DC amplifiers showed the magnitude of the SP shifts to be related to the time interval between successive presentations of the stimulus. In one procedure in which flicker was presented with randomly ordered interstimulus intervals (ISIs) of 5, 10, 20, 40, 80, and 160 seconds, maximal SP responses with mean amplitude of 186 and 198 μV were associated with ISIs of 10 and 20 seconds, respectively, whereas mean responses of about 160 μV were associated with longer ISIs. Similar results were obtained with a second procedure in which flicker followed a conditioning stimulus, consisting of a one minute train of 8/sec flicker, at randomly ordered ISIs of 2, 4, 8, 16, 32, 64, and 182 seconds. Maximum apmlitude responses seen as means of 146 and 136 µV were associated witn ISIs of 4 and 16 seconds respectively whereas, mean responses of less than 120 µV were observed with longer ISIs. In both procedures, responses of maximal amplitude were associated with ISIs during which positive steady afterpotentials, resulting from prior stimulation, were most evident. These data suggest that relatively long term SP shifts reflect changes in cortical excitability with relatively positive SP baselines associated with maximal negative SP responding.

SUPPLEMENTARY EXPERIMENT 3

TEMPORAL CONDITIONING OF EPILEPTIC DISCHARGES

Introduction

During the course of intersensory electrocortical conditioning of steady potential (SP) shifts in epileptic monkeys an increase in the incidence of focal epileptic discharges (spikes) was observed in the interval between successive presentations of paired stimuli, particularly in portions of the interval immediately prior to stimulation (Experiment 2). Although the interstimulus was of variable duration, the data nonetheless suggest that the time interval between stimuli served as a cue for epileptic spiking. In order to determine whether epileptic spiking can indeed be affected by temporal conditioning procedures the following experiment was conducted.

Since evidence for temporal conditioning was seen in both occipital lobe and temporal lobe epileptic subjects, and because both photic and acoustic stimuli were presented, it was not clear whether the effect was related to modality specific mechanisms or to more general non-specific mechanisms associated with anticipatory or orienting phenomena. In order to provide relevant data for clarification of this point only a photic stimulus (US) was presented at regular intervals to both an occipital lobe and a temporal lobe epileptic.

Method

Subjects

Two experimental epileptic monkeys, one with an alumina cream implant on lateral occipital cortex (no. 156) and the other with a similar epileptogenic implant on superior temporal cortex (no. 154) served as subjects (Ss) for the experiment (see Experiment 2 for details). Each S had transcortical Ag-AgCl nonpolarizable electrodes chronically implanted adjacent to its epileptic focus some 3 to 4 months prior to testing. Both Ss were familiar with the conditioning chamber and stimuli since they served in a prior intersensory electrocortical conditioning study.

Apparatus

The conditioning chamber, modes of stimulus presentations, and recording techniques were all similar to those described previously. (See Experiment 1 for details.)

Procedure

The temporal conditioning procedure consisted of periodic presentations of a photic stimulus (US) consisting of a six second train of 8/sec. flicker of moderate intensity. Following fifteen minutes of baseline recording (adaptation), the US was presented repeatedly at the rate of one every two minutes. From time to time, however, the US was omitted from the sequence of presentations and was presented again two minutes later. These omissions provided test periods for observations of the progress of conditioning. During the conditioning phase of the experiment, which consisted of 48 successive two minute periods, there were 8 random test periods occurring once in a block of six stimulus presentations. An extinction phase, consisting of fifteen minutes during which no stimuli were presented, followed the conditioning phase. The US was then reintroduced for a single presentation and fifteen additional minutes of recordings were obtained.

Data analysis

Recordings of focal epileptic activity made with DC amplifiers and nonpolarizable electrodes revealed high amplitude spikes of suprisingly long durations, i.e., up to .5 sec. Gumnit and Takahashi (1965) reported similar findings using DC recording techniques for the analysis of epileptic activity in acute preparations with cats. Accordingly, epileptic spikes were defined as 200 μV or greater waves whose duration (base width) did not exceed 400 msec.

Results

Baseline recordings of epileptic activity made with DC amplifiers and nonpolarizable electrodes showed relatively low levels of criterion epileptic spiking. The baseline rate for each S was found to be less than 5 spikes/min. However, greatly increased rates of spiking resulted from presentations of the US. The rates of spiking during stimulus presentations exceeded 20 spikes/min. for each monkey.

In order to chart the progress of temporal conditioning of spikes, measurements were made of spikes in the eight test periods in which the US was omitted. The test periods were divided into six successive 40 second intervals such that the beginning of the fourth interval corresponded to the time when the US was usually presented. Computation of the numbers of criterion spikes in each of these intervals resulted in a distribution of spikes for the entire test period. In both occipital lobe and temporal lobe epileptics the number of spikes in the fourth 40 second interval increased as a function of the conditioning procedure. As can be seen in Figures 21 and 22, for the occipital lobe and the temporal lobe epileptic respectively, the distributions of spikes seen for test periods 1 to 4 and 5 to 8 (each curve represents the sum of four distributions) show marked differences, particularly in the number of

Figure 21.--Summed incidence of occipital lobe spikes as a function of successive 40 second intervals of test periods in which the US was omitted. The dashed line represents the sum of spikes in respective intervals of test periods 1 to 4. The solid line represents the sum of spikes of test periods 5 through 8. Note that the incidence of spikes differs for interval 4. This interval corresponds to the point in time when the US was usually presented.

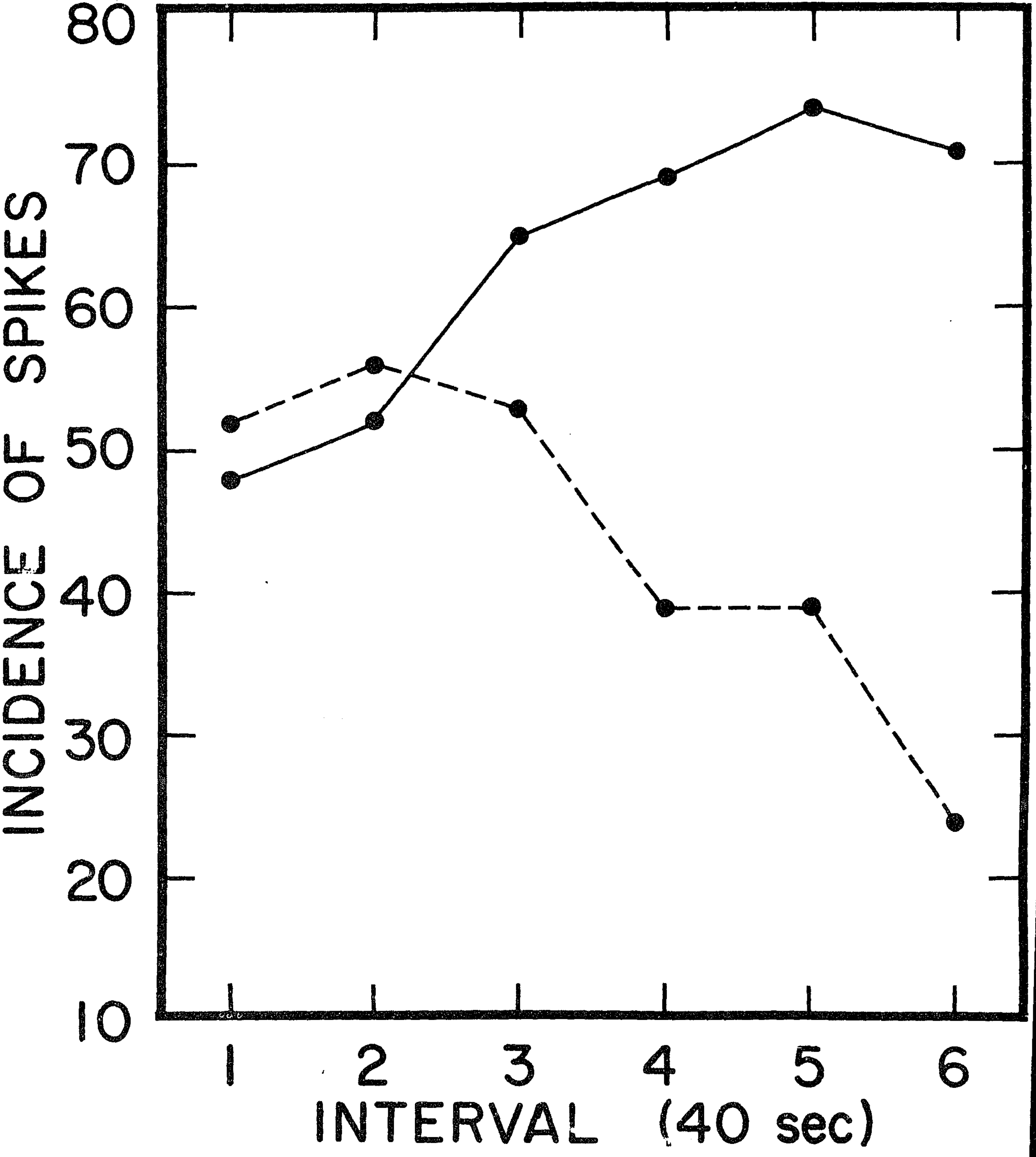
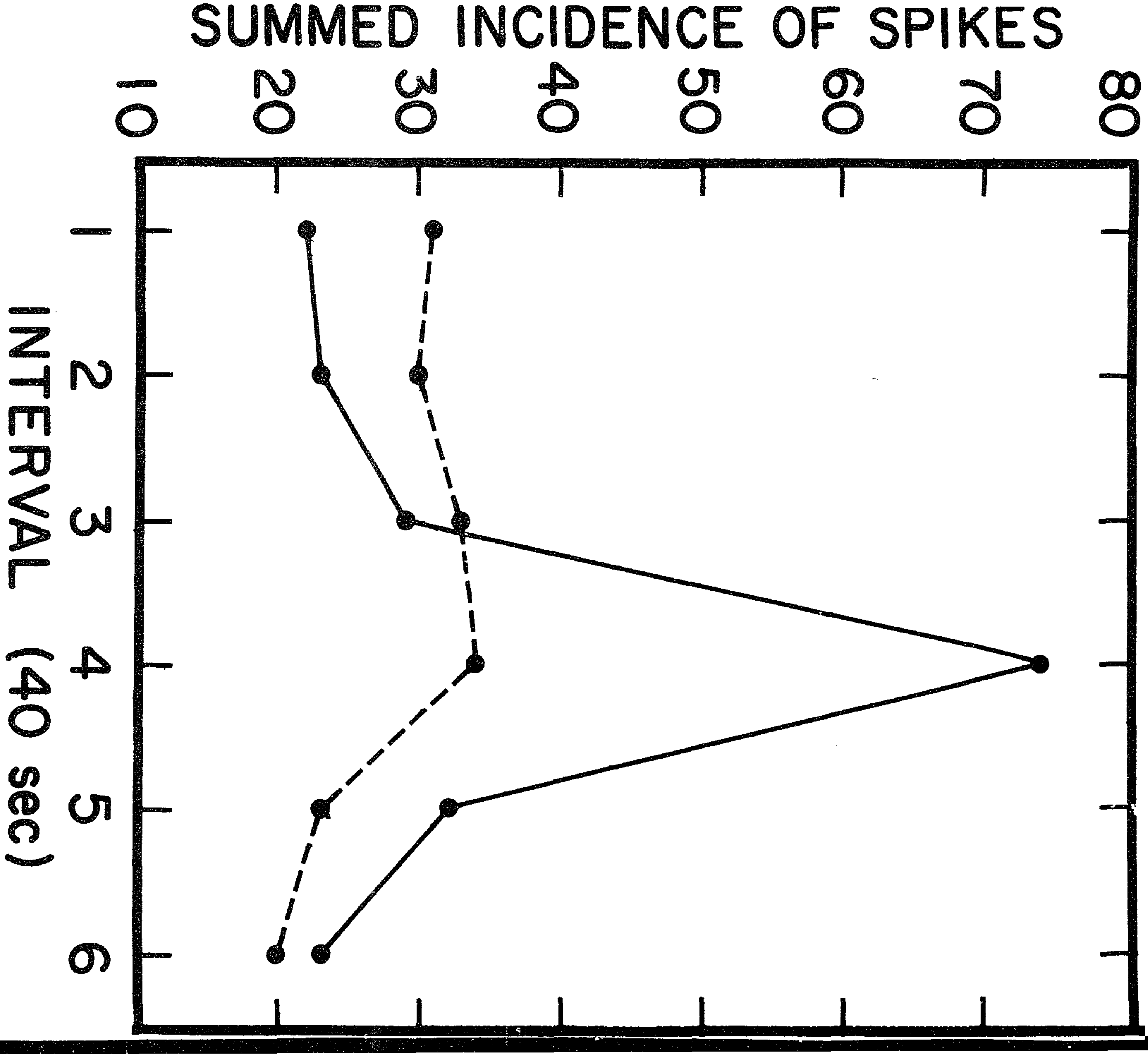

INCIDENCE OF SPIKES
80
70
60
50
40
30
20
10
1
2
3
4
5
6
INTERVAL (40 sec)

Figure 22.--Summed incidence of temporal lobe spikes as a function of successive 40 second intervals of test periods in which the US was omitted. The dashed line represents the sum of spikes in respective intervals of test periods 1 to 4. The solid line represents the sum of spikes of test periods 5 through 8. Note that the incidence of spikes differs for interval 4. This interval corresponds to the point in time when the US was usually presented.

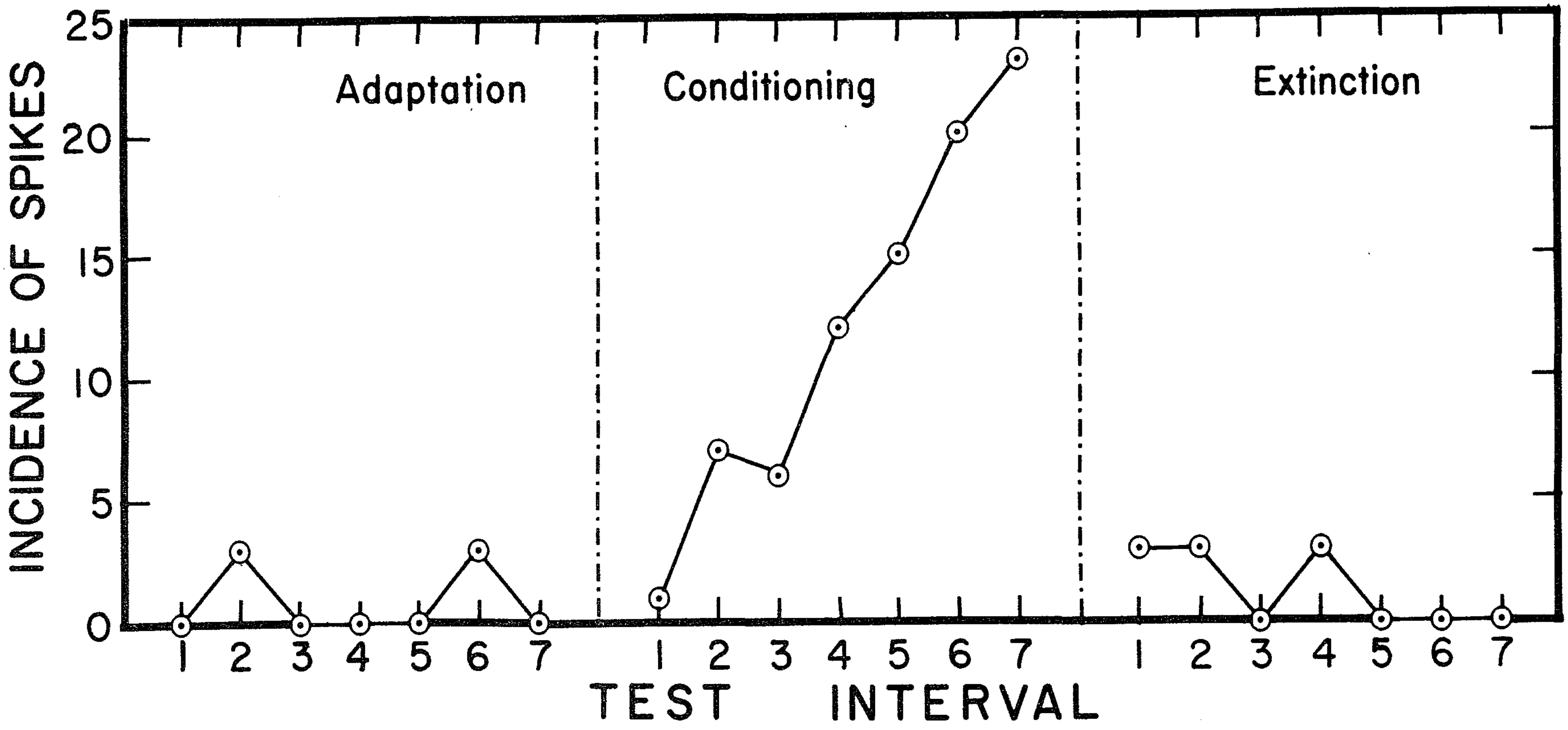

SUMMED INCIDENCE OF SPIKES
10
20
30
40
50
60
70
80
INTERVAL (40 sec)
1
2
3
4
5
6

spikes in the fourth interval. The sum of spikes in the fourth interval of periods 5 to 8 is nearly twice that of periods 1 to 4. However, statistical tests for the differences between the means of spikes for test periods 1 to 4 and 5 to 8 failed to uncover significant differences.

The acquisition of the conditioned response, namely the relative increase in the number of spikes associated with the time interval when the US was presented, can best be seen in Figure 23 for the temporal lobe epileptic S. Figure 23 shows that in a 20 second interval of the test periods, corresponding to 10 seconds before and 10 seconds after the US is usually presented, the incidence of spikes increases as a function of successive test periods, or more accurately, as a function of the intervening conditioning between the test periods. The figure also shows that the incidence of spikes for adaptation and extinction control periods remain at constant low levels. Corroborative, though less clearcut data were obtained with the occipital lobe epileptic.

Following the US presentations, the numbers of spikes observed during the extinction phase decreased rapidly in both epileptic subjects and a baseline rate of less than 5 spikes/min. was again attained. However, when the US was presented once at the end of the extinction period, the number of spikes in successive

Figure 23.--Incidence of spikes in 20 second intervals corresponding to US omissions as a function of successive test intervals.during Conditioning. The incidence of spikes in similar random 20 second intervals of Adaptation and Extinction phases are also presented.


INCIDENCE OF SPIKES
0
5
10
15
20
25
Adaptation
Conditioning
Extinction
1 2 3 4 5 6 7
1 2 3 4 5 6 7
1 2 3 4 5 6 7
TEST INTERVAL

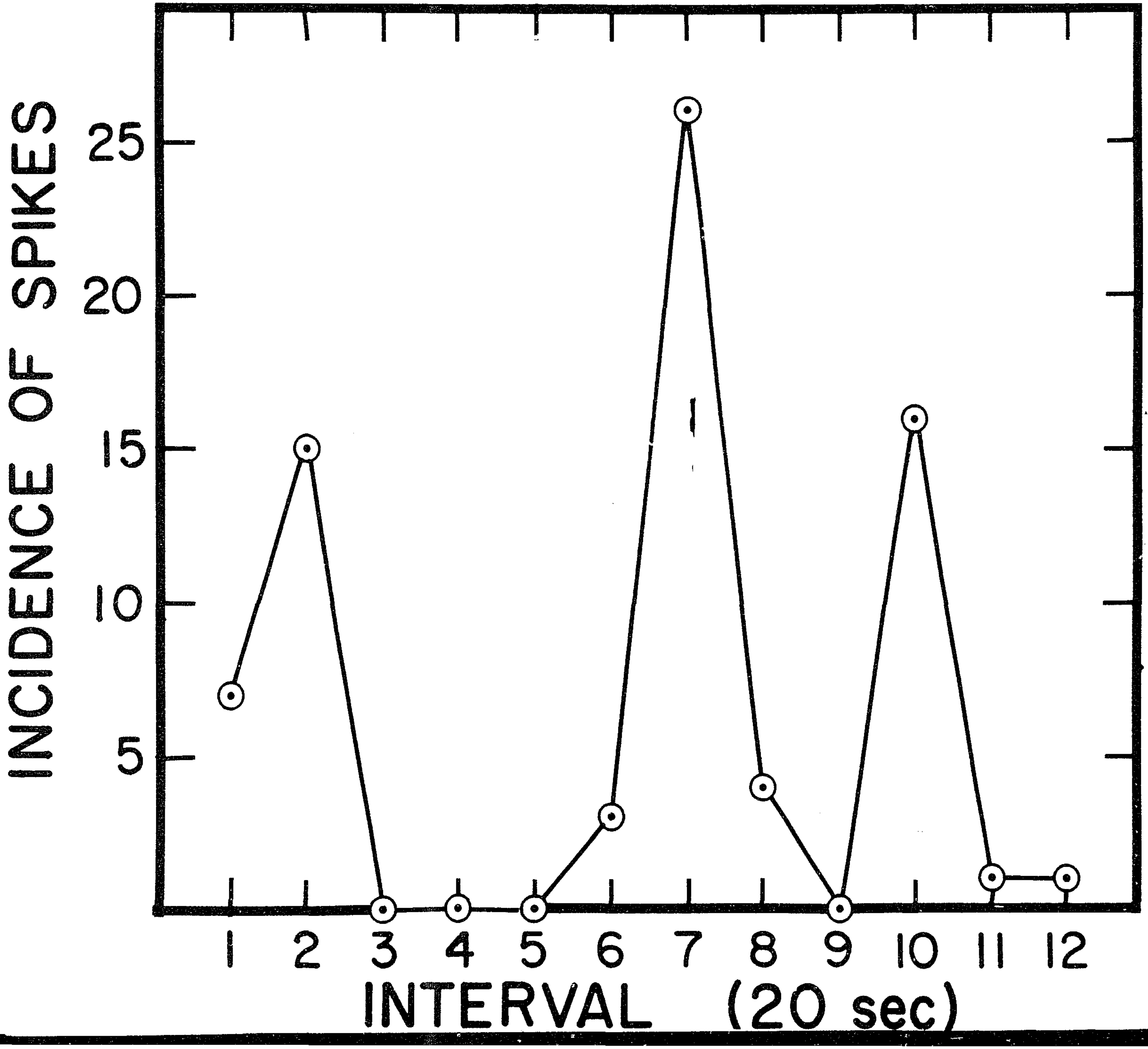
twenty second intervals increased sharply with the greatest number of spikes occurring approximately two minutes after the US presentation. Figure 24 shows the data for the temporal lobe epileptic. Similar results were obtained for the occipital lobe epileptic. These findings are analagous to spontaneous recovery phenomena seen in behavioral conditioning.

Discussion

The results of the present experiment indicate that temporal conditioning of focal epileptic discharges (spiking) is a possible, if not highly probable phenomenon. Periodic presentations of a photic US resulted in the acquisition of conditioned spiking responses associated with specific time intervals (CSs) when the US was presented. Extinction or a dimmnition of spiking was observed following cessation of US presentations and spontaneous recovery or the reappearance of the conditioned spiking response occurred following a subsequent US presentation. These electrocortical data appear analogous to phenomena observed in behavioral conditioning.

The finding that a photic US results in conditioned spiking in a temporal lobe as well as in an occipital lobe epileptic suggests that the neural mechanisms underlying

Figure 24.--Incidence of temporal lobe spikes in successive 20 second intervals following the presentation of a single US during Extinction. The distribution shows a sharp increase in the incidence of spikes in the 7th interval corresponding to the point in time when a subsequent US was usually presented.


INCIDENCE OF SPIKES
25
20
15
10
5
1
2
3
4
5
6
7
8
9
10
11
12
INTERVAL (20 sec)

the conditioning process are not modality specific, but rather, they reflect diffuse or nonspecific excitatory mechanisms associated with expectancy or orienting phenomena. However, modality specific mechanisms may not entirely be ruled out since projection fibers in the visual system transverse the temporal lobe and anatomical connections between occipital and interotemporal cortex are well known. Precisely how these anatomical relations affect the activity of the epileptogenic lesion in auditory cortex is yet unknown. However, it should be pointed out that the extent of the lesion is not under strict experimental control and secondary epileptic foci are known to occur in temporal and occipital lobes, particularly in monkeys with long standing lesions, Secondary lesions may account for the apparent conditioning in the temporal lobe epileptic subject. Additional experiments involving other temporal and occipital lobe epileptics as well as acoustic and photic USs are required for further clarification of the mechanisms involved in temporal conditioning.

Summary

Nonpolarizable electrodes were chronically implanted adjacent to epileptogenic lesions, induced in one monkey by an alumina cream implant in occipital cortex, and

in a second monkey by an implant in temporal cortex. Subsequent recordings of focal epileptic discharges (spikes) showed accelerated rates of spiking in response to presentations of a six second train of 8/sec. flicker.. In order to determine if spiking was a response of epileptogenic cortex capable of being affected by relatively simple temporal conditioning procedures the flicker, used as an unconditioned stimulus (US), was repeatedly presented every two minutes. Once in a block of six presentations, the US was ommitted so that it was possible to observe acquisition of the conditioned spiking response (CR). In both monkeys the incidence of spikes during periods of stimulus ommissions, particularly during intervals when the US was usually presented, increased as a function of prior US presentations. The data were interpreted as demonstrating that temporal conditioning of spikes was a possible if not highly probable phenomena. In addition, the finding that CRs were seen in both occipital lobe and temporal lobe epileptics when a photic US was used suggests that nonspecific or diffuse rather than modality specific mechanisms underly the conditioning process.

SUMMARY AND CONCLUSIONS

The findings in the preceding experiments support two related hypotheses concerning the significance and relevance of cortical SP shifts to problems of brain function; (1) that SP shifts reflect processes of cortical excitation with surface negative shifts indicative of increased, and positive shifts decreased, excitation levels, and (2) that SP shifts reflect other processes which serve to "gate" or "modulate" or in other ways modify subsequent cortical activity. The first of these hypotheses is supported by the findings of experiments concerned with intersensory electrocortical conditioning of SP shifts in normal monkeys. Intermittent sensory stimuli (i.e., trains of clicks and flicker) were found to evoke reliable surface negative SP shifts in areas of sensory cortex corresponding to the modality of the stimulus (auditory and visual cortex, respectively). These shifts were accompanied by lower voltage oscillatory potentials (frequency specific activity) with frequency characteristics corresponding to those of the stimulus, which have been traditionally regarded as indicative of cortical excitation. Further, it was found that when clicks (CS) were paired with flicker (US), both components of cortical responses to flicker could be conditioned to clicks alone. Conditioned responses (CRs) seen in the CS-US interval consisted of 25 to 50 μV surface negative SP shifts and conditioned frequency specific activity, superimposed on the peaks of conditioned negative SP waves. These latter findings suggest that SP shifts also reflect processes of conditioned cortical excitation. The findings are consistent with earlier reports of conditioned electrocortical activity in chronic preparations (Chow et al., 1957; Morrell and Jasper, 1956; and Yoshii and Hockaday, 1958). The findings of Morrell and Jasper (1956) are of particular interest in that they reported conditioned frequency specific activity late in the CS-US interval during the early phase of conditioning, as seen in the present experiment. Conditioned activation patterns (i.e., transitions from high voltage slow to low voltage fast EEG activity) were the early signs of conditioning. In the present experiments, activation patterns were seen as concomitants of conditioned negative shifts during early conditioning but the observations were restricted by the limited frequency response of the DC amplifiers used for recording.

DC amplification did however, uncover an additional phenomenon associated with intersensory electrocortical conditioning not previously reported in investigations employing more conventional capacitor-coupled (AC) amplifiers. In the present studies conditioned positive SP shifts were found in auditory cortex during the CS-US interval. During early conditioning the presentation of flicker (US) resulted in a diminution of the primary negative auditory response to clicks. As conditioning proceeded with negative SP shifts being elicited in occipital cortex, a diminution of the negative auditory shift (relative positive shift) was observed prior to US presentations in the CS-US interval. This finding was interpreted as evidence for electrocortical conditioning of positive shifts in sensory cortex corresponding to the modality of the CS and supports the contention that positive SP shifts are indicative of decreased cortical excitation presumably associated with anticipation of a different sensory stimulus. Additional research is required to clarify the significance of positive SP shifts in conditioning.

The first hypothesis gained additional support from the first supplementary experiment in which the magnitude of the negative SP shifts in occipital cortex were found to be a positive function of both flicker frequency and intensity. If SP shifts do indeed reflect cortical excitation then one would expect to find shifts of greater magnitude related to stimulus conditions, presumably effecting greater cortical excitation. Such was the finding in this experiment. It should be noted that measures of the amplitude of concomitant frequency specific activity were not obtained in the experiment. Such measures should provide important additional data concerning the relation of SP shifts to frequency specific activity in view of Rowland's (1963) suggestion of a dissociation of SP shifts from ECG frequency components. Rowland has suggested that SP shifts increase in amplitude with increased cortical excitation whereas ECG rhythms desynchronize with a corresponding decrease in amplitude as seen the the activiation pattern. Further investigations are required for clarification of this potentially signifcant distinction.

The second hypothesis that SP shifts reflect processes related to the modification of subsequent cortical activity or to the more general problem of cortical excitability is supported by the findings of intersensory electrocortical conditioning of SP shifts in epileptic monkeys. Conditioning in monkeys with epileptogenic lesions in either visual or audtiory cortex resulted in increased epileptic discharges (spikes) associated with conditioned negative SP shifts in occipital cortex and decreased spikes with positive shifts elicited in temporal cortex by the same conditioning procedure. Moreover, spikes seen in occipital cortex during the CS-US interval were superimposed upon the peaks of conditioned surface negative shifts. The findings are reminiscent of reports by Goldring and O'Leary (1963) of SP shifts "preceding" seizure discharges in normal and epileptogenic cortex and the report of Gumnit and Takahashi (1965) that the transition from interictal spiking to full blown seizures occurs during surface negative afterpotentials associated with each spike at pencillin-induced epileptic foci. Further, the findings in the present experiment of suppression of spontaneous temporal lobe seizures during the CS-US interval of conditioning trials brings to mind reports of surface negative (depth positive) polarizing currents terminating seizures while in progress (Goldring and O'Leary, 1963). Collectively, the data point to SP shifts as being related to mechanisms involved in the initiation of seizures. However, the extent to which SP shifts may be regarded as reflecting more general processes of cortical excitability, based on the present findings, is restricted to the extent that epileptic spiking is an index of normal neuronal excitation of the cortex. Evidence that single units at an epileptic focus discharge at high rates during epileptic spiking or seizure discharges has been presented by Ward (1961) and Morrell (1961). But, no study thus far, has related conditioned shifts specifically to changing rates of single unit activity in normal cortical tissue. Additional supportive evidence, however, is to be found in studies of the effects of applied cortical polarization on single unit discharges. Creutzfeld _et al_. (1962) and Bindman _et al_. (1962) have found that surface positive (depth negative) polarizing currents accelerate the rate of single unit discharges and that surface negative depth positive currents have opposite effects. Further, studies by Von Euler and Green (1960) have shown that local cat-and an-electrotonus can effect all membrane excitability. According to Strumwasser and Rosenthal (1960) such effects can be mediated by currents in the range of nanoamperes. These reports suggest that current densities in the extracellular environment occurring normally during negative SP shifts are sufficient to effect subsequent neuronal activity. Contrasting views have be put forth by Creutzfeld _et al_. (1962) and Lickey and Fox (1966). Further investigations are required to verify either view.

The hypothesis that SP shifts reflect processes of cortical excitability was also supported by the findings of the second supplementary experiment. The magnitude of the SP response to flicker was found to be related to the interval between successive stimulus presentations when positive steady afterpotentials were in various steady of growth and decay. The larger shifts were associated with maximal positive afterpotentials. This finding was interpreted as indicating that positive shifts were associated with greater cortical excitability, and negative shifts decreased excitability in contrast to the hypothesis previously stated. Further investigations are required to evaluate the significance of positive steady afterpotentials with reference to other measures of cortical excitability such as thresholds for responding.

With regard to the relevance of the present findings to other problems of brain function, it appears that the recording of SP shifts provides a readily quantifiable measure of cortical excitation which exhibits a certain degree of localization. DC recording techniques may be useful in providing information concerning the excitation, and hence involvement of cortical structures in the performance of behavioral tasks. Subsequent experiments conducted by the author have indeed shown that negative SP shifts can be related to specific components of behavioral tasks. Spatially and temporally distinct SP shifts can be recorded simultaneously from different cortical areas which correspond to distinct components of the behavioral task (Stamm and Rosen, 1969).

A second area to which the results of the present study apply is the control of epilepsy by conditioning processes. The finding that conditioned positive shifts may be associated with the suppression of epileptic activity suggests a possible therapeutic means of controlling and preventing seizures. Additional studies with epileptic patients as well as with animals are required for further elucidation of the neural mechanisms involved.

APPENDIX A

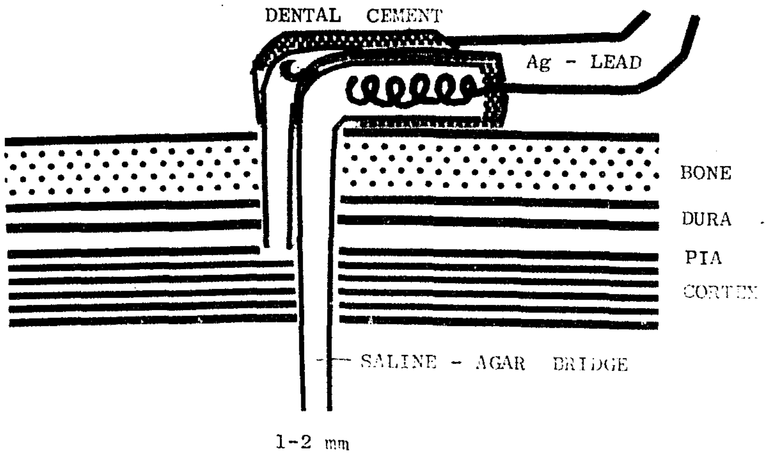


Figure 25.--Schematic representation of transcortical bipolar Ag-AgCl nonpolarizable electrodes. Note that one electrode tip rests on the pia and the other is inserted into adjacent subcortical white matter. Tip diameter is 1 to 2 mm.

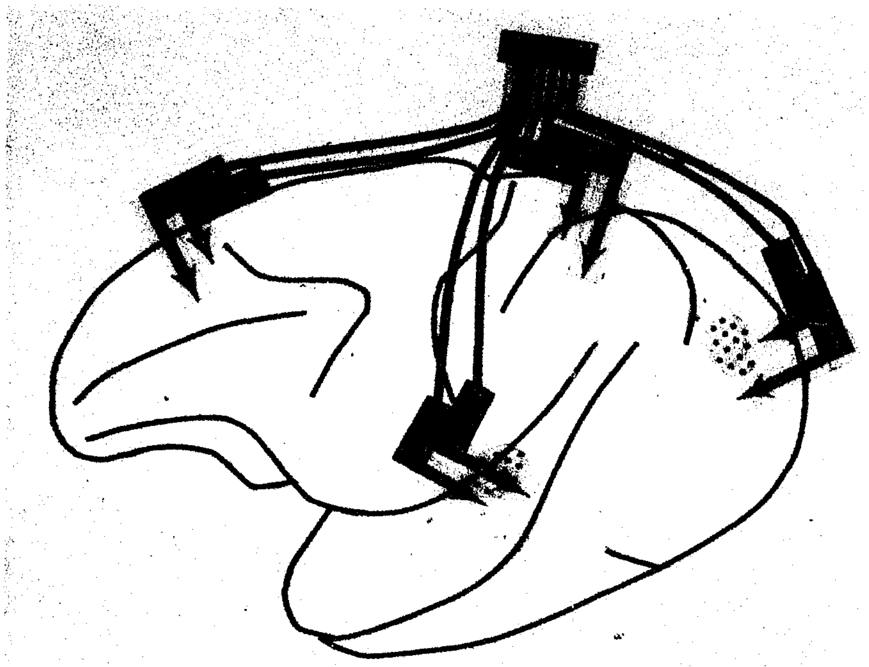


Figure 26.--Schematic representation of electrode and alumina cream placements. Alumina cream implants (stipled areas) were placed on either lateral occipital cortex or superior temporal cortex (near the supratemporal plane of superior temporal gyrus). Bipolar electrodes were placed in frontal, temporal, occipital, and parietal cortex. Surface electrodes in epileptogenic sensory cortex were usually placed within 8 to 10 mm of the perimeter of the alumina cream implants. Electrode leads were soldered to plug seen at top of figure.

INTERSENSORY ELECTROCORTICAL CONDITIONING PARADIGMS

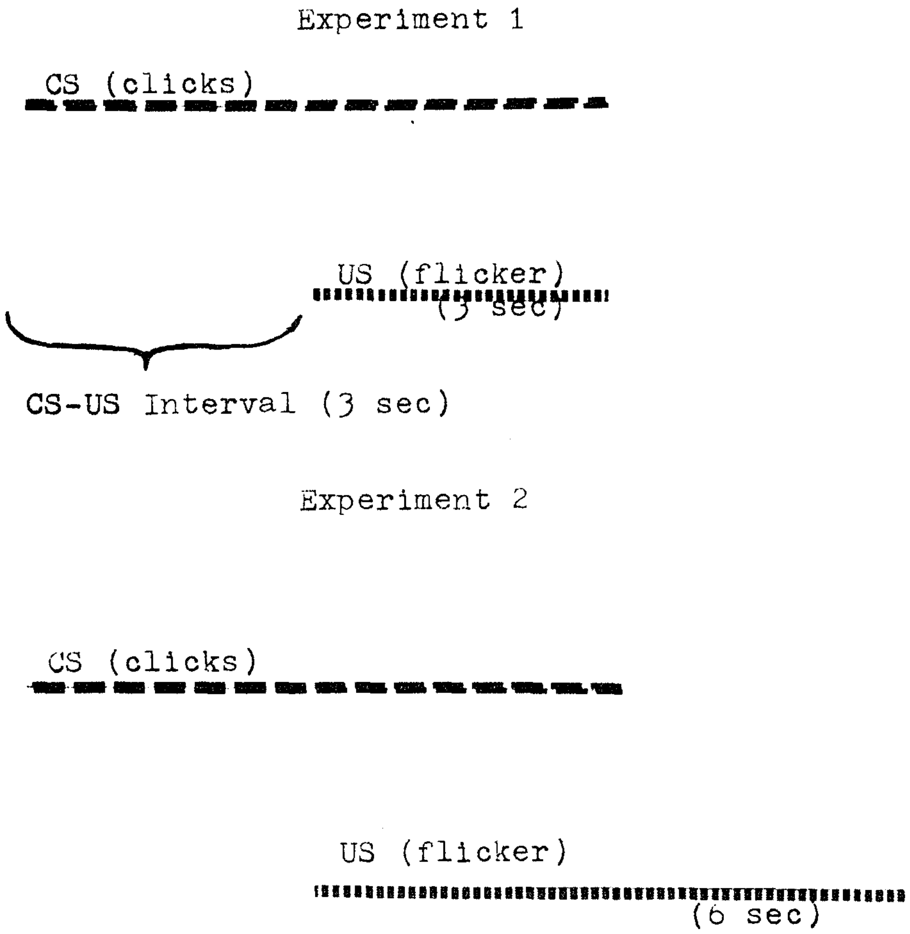
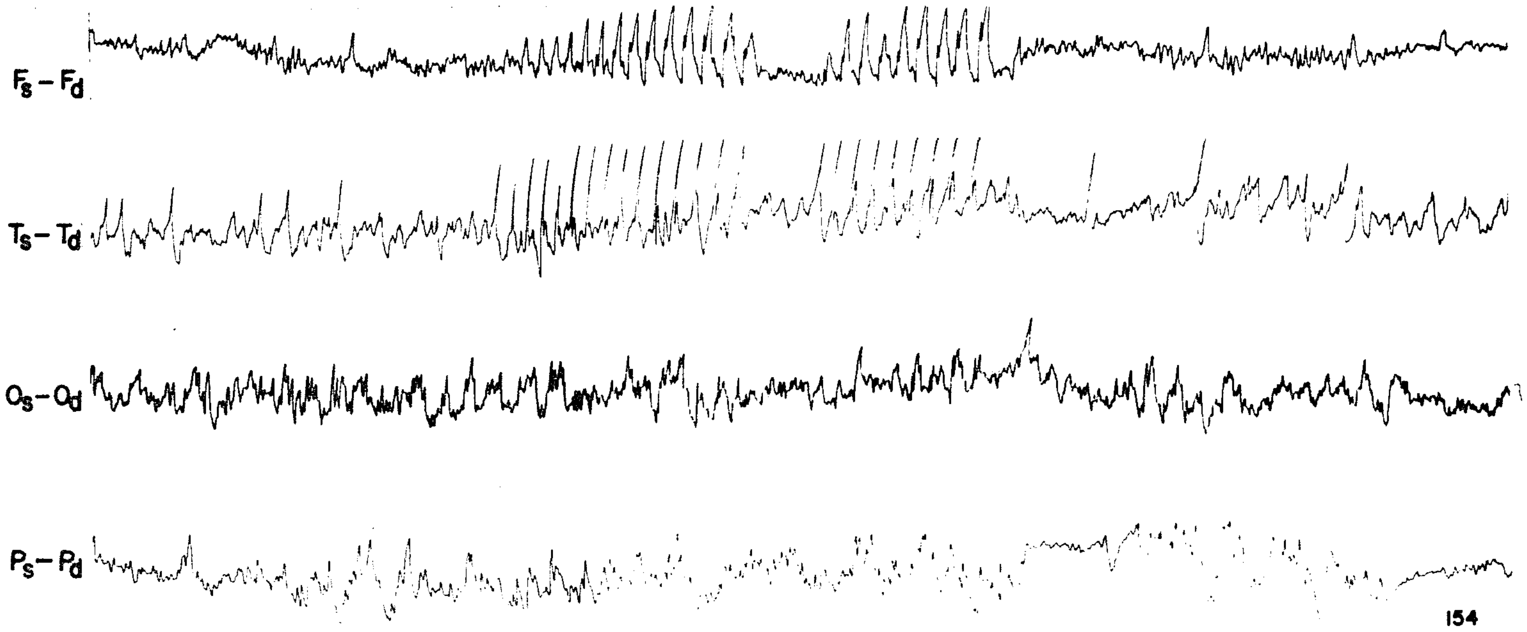


Figure 27.--Schematic representations of intersensory electrocortical conditioning paradigms used in Experiments 1 and 2. In both experiments the CS was a train of 6/sec clicks and the US was 8/sec flicker. The intertrial interval was varied but averaged one minute in duration.

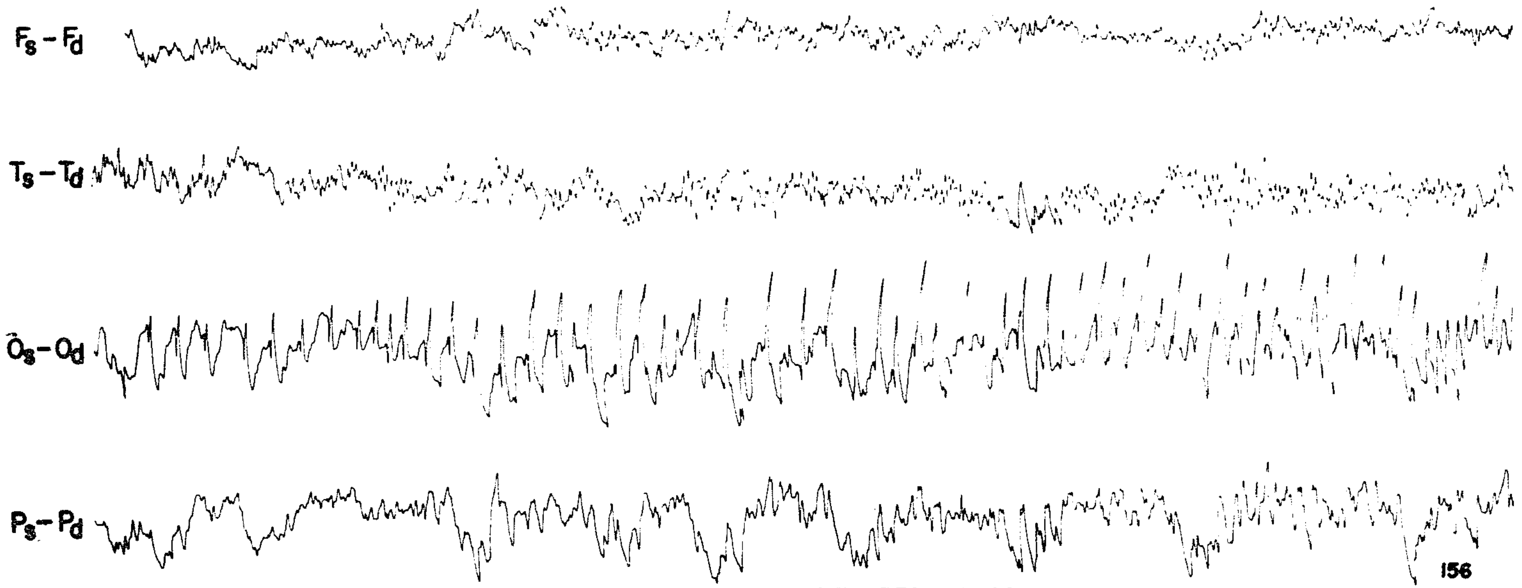
APPENDIX B

Figure 28.--Electrocorticograms obtained with DC amplifiers and chronically implanted nonpolarizable electrodes showing typical epileptic discharges from a temporal lobe and an occipital lobe epileptic monkey..



TEMPORAL LOBE EPILEPTIC

100 µV
1 sec



OCCIPITAL LOBE EPILEPTIC

Figure 29.--Diminution of surface negative SP shift in temporal cortex during successive Adaptation, Conditioning, and Extinction sessions. Presented are mean SP shifts per trial (μV-sec) in the CS-US interval of Conditioning trials and the first three seconds of CS presentations during Adaptation and Extinction trials. Data based on findings in one monkey.

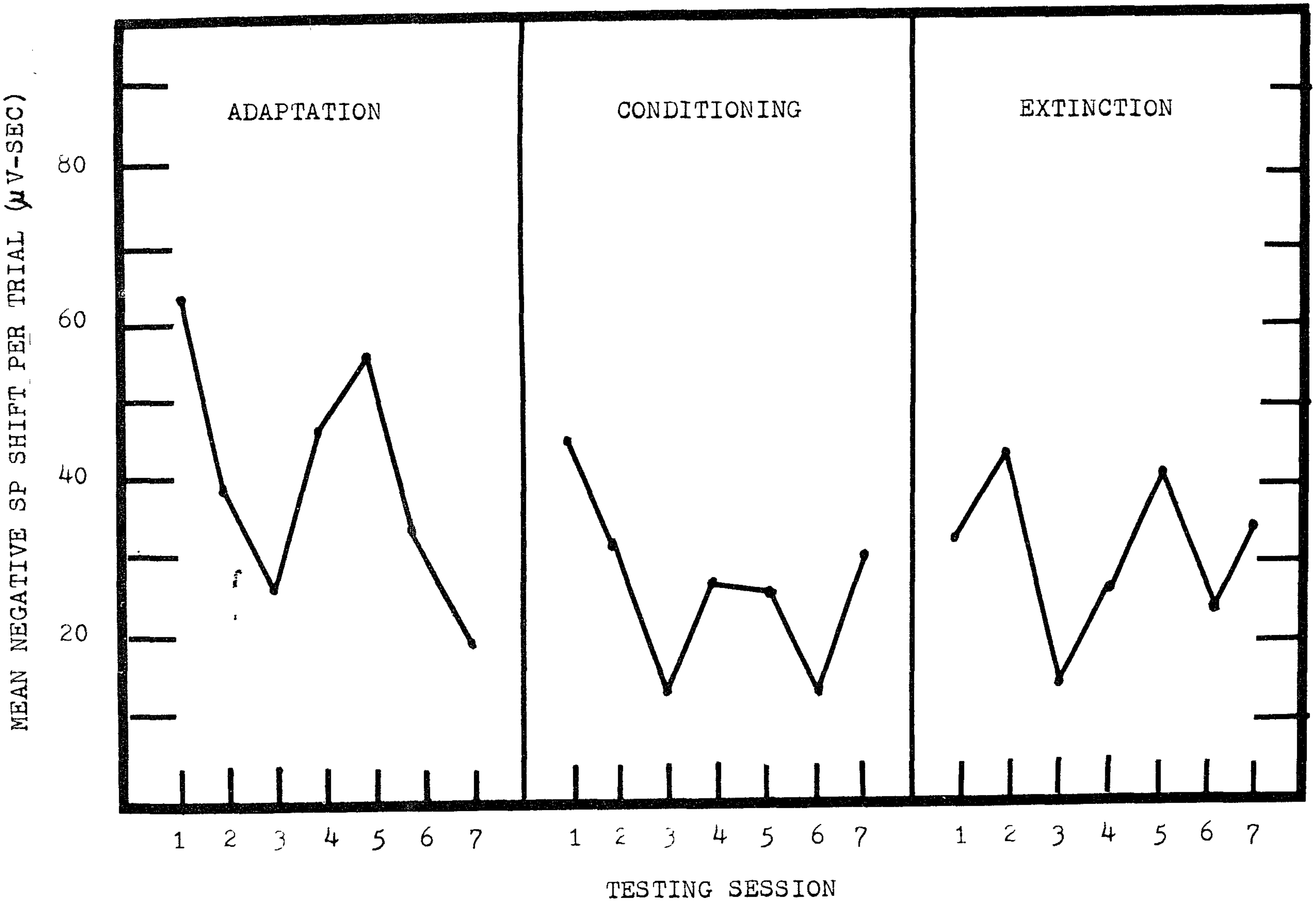
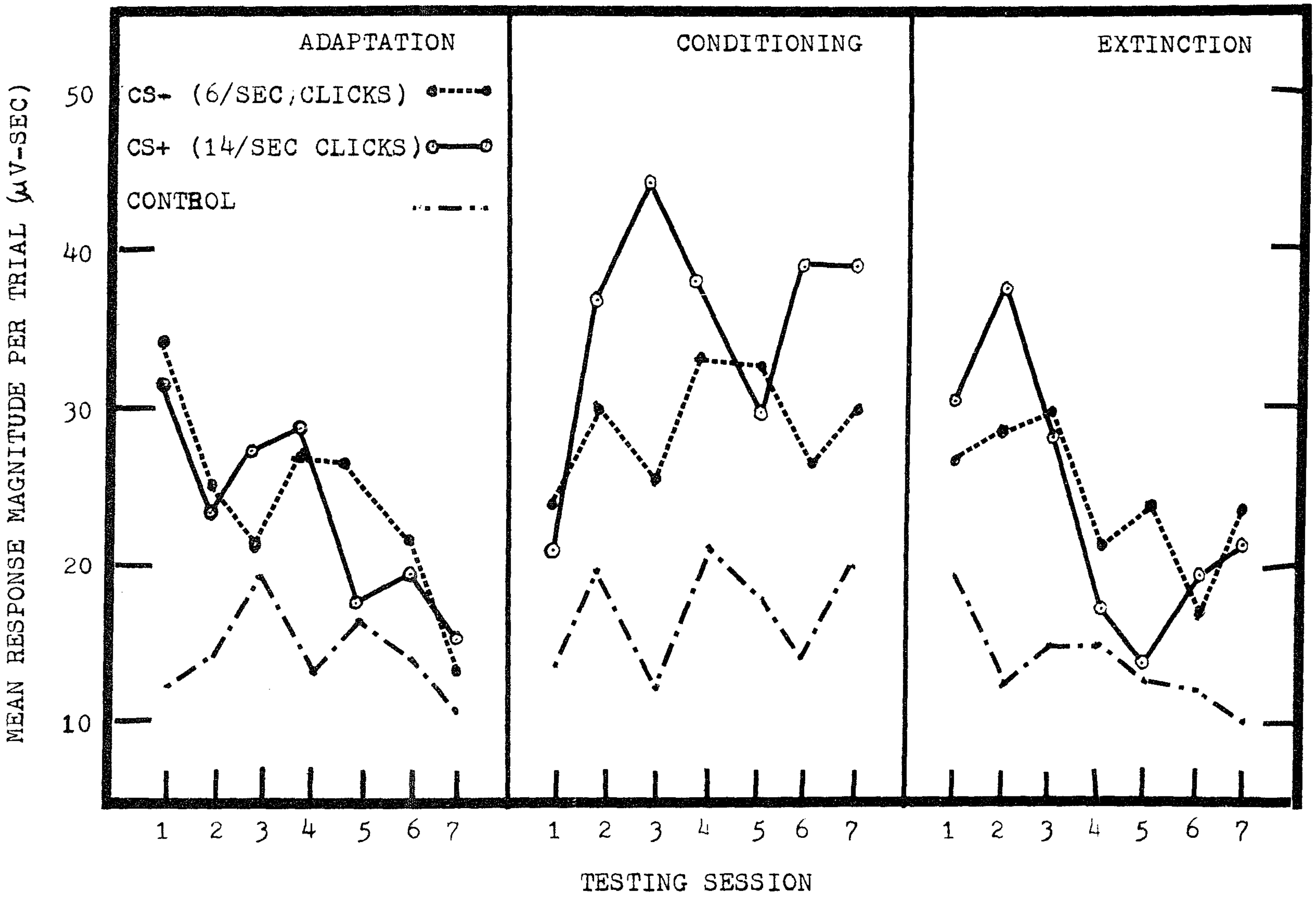

MEAN NEGATIVE SP SHIFT PER TRIAL (µV-SEC)
80
60
40
20
ADAPTATION
CONDITIONING
EXTINCTION
1 2 3 4 5 6 7
1 2 3 4 5 6 7
1 2 3 4 5 6 7
TESTING SESSION

Figure 30.--CR magnitudes (SP shifts) in occipital cortex for a second subject during differential conditioning to CS+ (paired with US) and CS- (no US). Each point represents the mean response over the first 3 seconds of CS presentations for the 20 trials of each session. The control data was obtained for 3 second periods when no stimuli were presented.


ADAPTATION
CONDITIONING
EXTINCTION
CS- (6/SEC CLICKS)
CS+ (14/SEC CLICKS)
CONTROL
MEAN RESPONSE MAGNITUDE PER TRIAL (μV-SEC)
50
40
30
20
10
1 2 3 4 5 6 7
1 2 3 4 5 6 7
1 2 3 4 5 6 7
TESTING SESSION

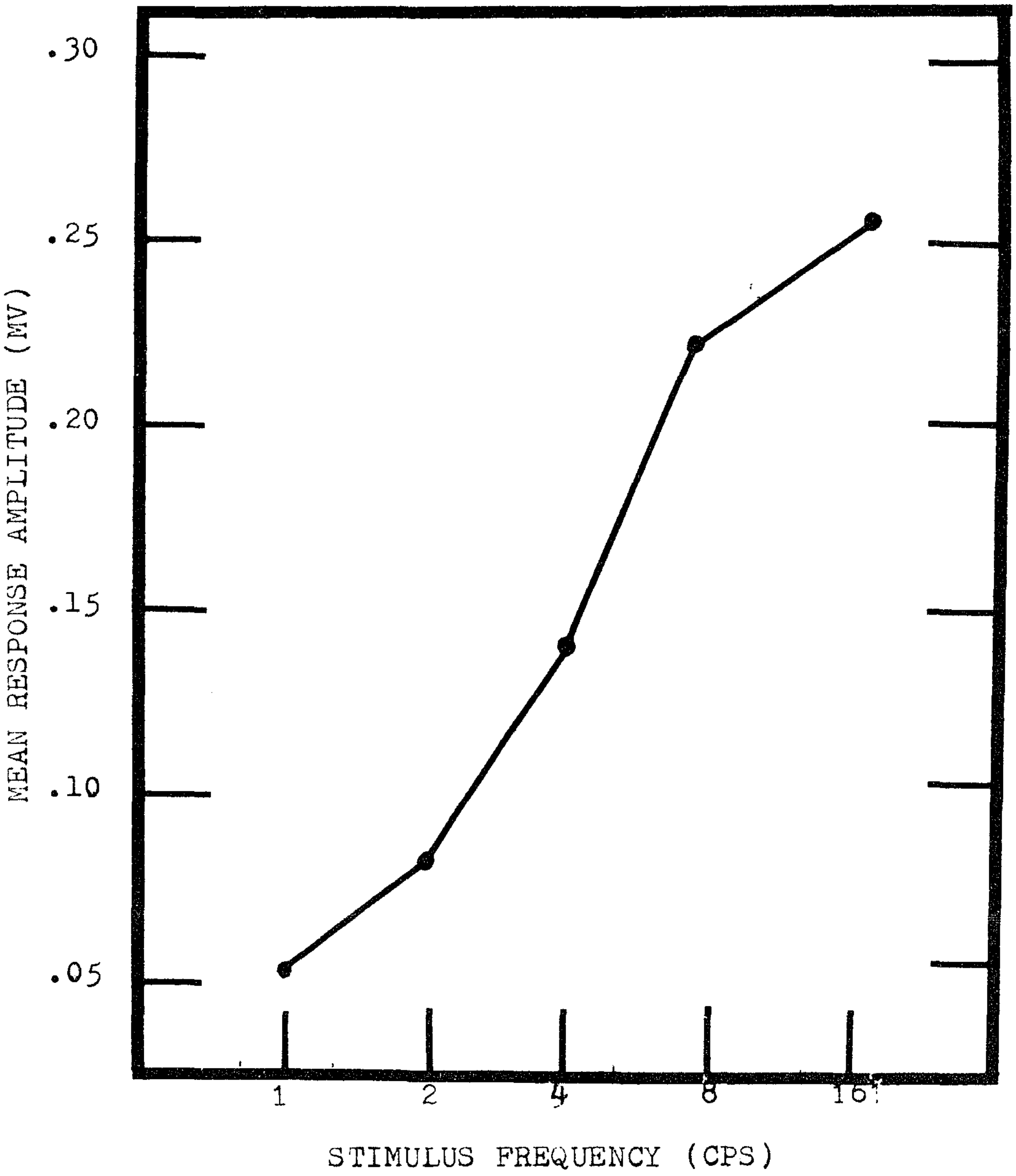


Figure 31.--Mean SP response amplitude (mV) as a function of flicker frequency. Means are based on six responses in one moneky to six second trains of flicker at a given frequency. Intensity was held constant.

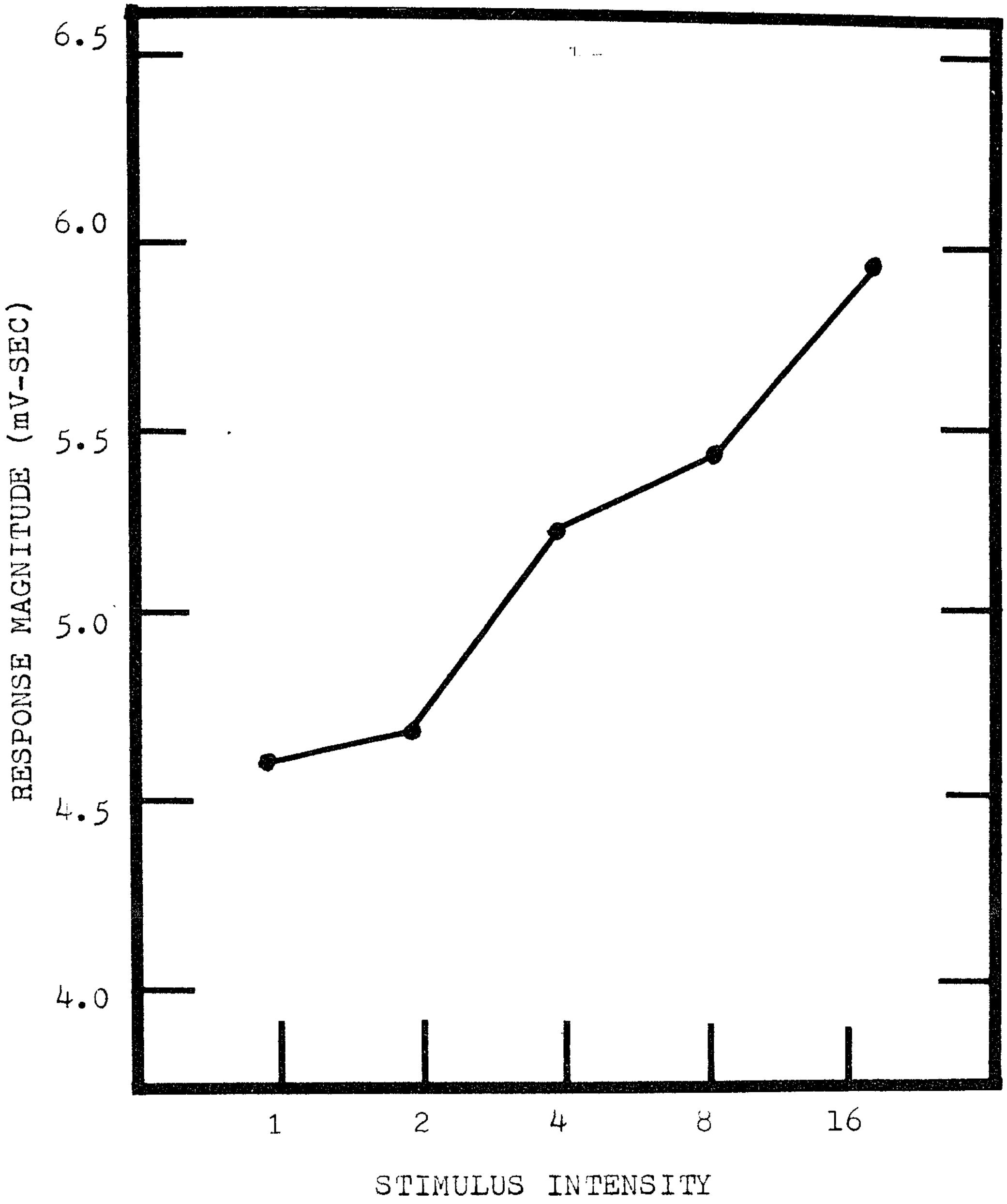


Figure 32.--SP response magnitude (mV-sec) as a function of stimulus intensity. Stimuli were one minute trains of 8/sec flicker of varied intensity. Intensity values are relative values corresponding to settings on the Grass Photo-Stimulator. Each point represents the mean for six responses at a given intensity level.

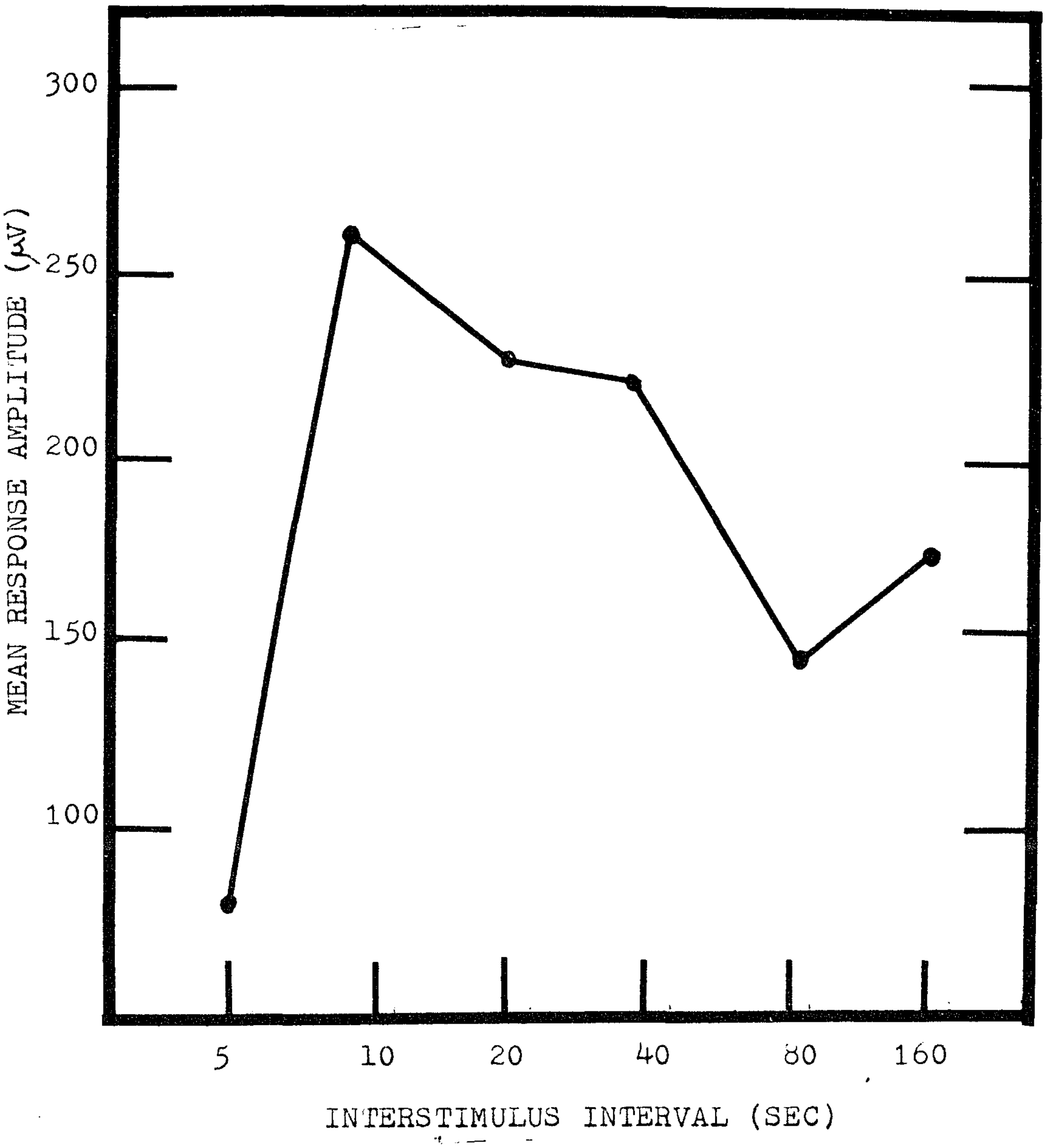


Figure 33.--Mean amplitude of SP responses to 8/sec flicker as a function of the interval between successive stimuli. Each point represents the mean of six responses obtained for one monkey.

References

Aladjalova, N. A. Slow Electrical Processes in the Brain. Moscow: Moscow Publ. House, USSR Acad. Sci., 1962.

Arduini, A. Enduring potential changes evoked in the cerebral cortex by stimulation in brain stem reticular formation and thalamus. In H. H. Jasper, L. D. Proctor, R. S. Knighton, W. C. Noshay, and R. T. Costello (Ed.), Reticular Formation of the Brain. Boston: Little, Brown, and Co., 1958. Pp. 333-349.

Arduini, A., Mancia, M., and Mechelse, K. Slow potential changes elicited in the cerebral cortex. Arch. Ital. Biol., 1957, 95, 127-138.

Bates, J. The unidirectional potential changes in petit mal epilepsy. In M. Brazier (Ed.), Studies in Brain Function, Vol. 1. Los Angeles: Univ. of Calif. Press, 1963. Pp. 237-379.

Bergers, H. Ueber das elektrenkophalogramm des mensche I. Arch. of Psychiat., 1929.

Bindman, L., Lippold, O., and Redfearn, J. The prolonged after-action of polarizing currents on the sensory cerebral cortex. J. Physiol., 1962, 162, 45-46.

Bishop, G., and O'Leary, J. The effect of polarizing currents on cell potentials and their significance in the interpretation of central nervous activity. EEG Clin. Neurophysiol., 1950, 2, 401-416.

Brazier, M. Historical Introduction. The discoverers of the steady potential of the brain. In M. Brazier (Ed.), Studies in Brain Function, Vol. 1. Los Angeles: Univ. of Calif. Press, 1963. Pp. 1-13.

Brookhart, J., Arduini, A., Mancia, M., and Moruzzi, B. Thalamocortical relations as revealed by induced slow potential changes. J. Neurophysiol., 21, 499-525.

Bures, J. Ontogenetic development of steady potential differences in cerebral cortex in animals. EEG Clin. Neurophysiol., 1957, 9, 121-130.

Caspers, H. Changes in cortical D.C. potentials in the sleep-wakefulness cycle. In G. E. W. Wolstenholme and M. O'Conner (Ed.), Ciba Foundation Symposium on the Nature of Sleep. Boston: Little, Brown, and Co., 1960. Pp. 237-259.

Caspers, H. The cortical D.C. potential and its relation with the EEG. EEG Clin. Neurophysiol., 1961, 13, 651.

Caspers, H. Relations of steady potential shifts in the cortex to the wakefulness-sleep spectrum. In M. Brazier (Ed.), Studies in Brain Function, Vol. 1. Los Angeles: Univ. of Calif. Press, 1963. Pp. 177-213.

Caspers, H., and Simmich, W. Cortical D.C. shifts associated with seizure activity. In A. Bischoff and F. Luthy (Ed.), Comparative and Cellular Pathophysiology of Epilepsy. Netherlands: Royal Van Goreim Assoc., 1966. Pp. 151-163.

Creutzfeld, O., Fromm, G., and Kapp, H. Influence of transcortical D.C. currents on cortical neuronal activity. Exp. Neurol., 1962, 5, 436-452.

Doty, R. Conditioned reflexes elicited by electrical stimulation of the brain in macaques. J. Neurophysiol., 1965, 28, 622-640.

Durkovic, R., and Cohen, D. D.C. potential activity in a nervous system lacking neocortex: the pigeon telencephalon. Anat. Rec., 1966, 154, 341.

Durkovic, R., and Cohen, D. Spontaneous, evoked, and defensively conditioned steady potential changes in the pigeon telencephalon. EEG Clin. Neurophysiol., 1964, 24, 474-481.

Durup, G., and Fessard, A. L'electroencephalogrammme de l'homme. L'Annee Psychol., 1935, 36, 1-32.

Gerard, R. Factors controlling brain potentials. Cold Springs Harbor Symp. Quant. Biol., 1936, 4, 292-304.

Gerard, R., and Libet, B. The control of normal and "convulsive" brain potentials. Am. J. Psychiat., 1940, 96, 1125-1153.

Gerard, R., and Libet, B. Steady potential field and neuronal activity. J. Neurophysiol., 1941, 4, 435.

Gloor, P., Sperti, L., and Vera, C. A consideration of feedback mechanisms in the genesis and maintenance of hippocampal seizure activity. Epilepsia, 1964, 5, 213.

Gloor, P., Vera, C., Sperti, L., and Ray, S. Investigations on the mechanism of epileptic discharge in the hippocampus. Epilepsia, 1961, 2, 42-62.

Goldring, S., and O'Leary, J. Experimentally derived correlates between ECG and steady cortical potential. J. Neurophysiol., 1951, 14, 275-288.

Goldring. S., and O'Leary, J. Cortical D.CC changes incident to midline thalamic stimulation. EEG Clin. Neurophysiol., 1957, 9, 577-584.

Goldring, S., and O'Leary, J. Negative steady potential shifts which lead to seizure discharge. In M. Brazier (Ed.), Studies in Brain Function, Vol. 1. Los Angeles: Univ. of Calif. Press, 1963. Pp. 215-236.

Gumnit, R. D.C. potential changes from auditory cortex of cat. J. Neurophysiol., 1960, 23, 667-675.

Gumnit, R. The distribution of direct current responses evoked by sounds in the auditory cortex of the cat. EEG Clin. Neurophysiol., 1961, 13, 889-895.

Gumnit, R., and Grossman, R. Potentials evoked by sound in the auditory cortex of the cat. Am. J. Physiol., 1961, 200, 1219-1225.

Gumnit, R., and Takahashi, T. Changes in direct current activity during experimental focal seizures. *EEG Clin. Neurophysiol.*, 1965, *19*, 63-74.

Jasper, H., and Cruikshank, R. Electroencephalography. II. Visual stimulation and the after image as affecting the occipital alpha rhythm. *J. Gen. Psychol.*, 1937, *17*, 29048.

John, E., and Killam, K. Electrophysiological correlates of avoidance conditioning in the cat. *J. Pharmacol. exp. Ther.*, 1959, *125*, 151-274,

Knott, J., and Henry, C. The conditioning of the blocking of the alpha rhythm of the human electroencephalogram. *J. exp. Psychol.*, 1941, *28*, 134-144.

Kohler, W. Relational determination in perception. In L. S..Jeffries (Ed.), *Cerebral Mechanisms in Behavior*. New York, 1951. Pp. 200-243.

Kohler, W., and Held, R., The cortical correlate of patterm vision. *Science*, 1949. *110*, 414-419.

Kohler, W., O'Connell, D. N. Currents of the visual cortex. in the cat. *J. Cellular Comp. Physiol.*, 49 suppl., 1957, *2*,1-43.

Kohler, W., Neff, W., and Wegener, J. Currents of the auditory cortex in the cat. *J. Cellular Comp. Physiol.*, 45 suppl., 1955, *1*, 1-24.

Kohler, W., and Wegener, J. Currents of the human auditory cortex. *J. Cellular Comp. Physiol.*, 45 suppl., 1955, *1*, 25-54.

Kuffler, S., Nicholls, J., and Orkand, R. Physiological properties of glial cells in the central nervous system of amphibia. *J. Neurophysiol.*, 1966, *29*, 768-787.

Kupfermann, L. Effects of cortical polarization on visual discriminations. *Exp. Neurol.*, 1965, *12*, 179-189.

Libet, B., and Gerard, R. An analysis of some correlates of steady potentials in mammalian cerebral cortex. EEG Clin. Neurophysiol., 1962, 14, 445-452.

Libet, B., and Kahn, J. Steady potentials and neuron activity in mammals. Fed. Proc., 1946, 6, 152.

Lickey, M., and Fox, S. Localization and habituation of sensory evoked D.C. responses in cat cortex. Exp. Neurol., 1966, 15, 437-454.

Mahnke, J., and Ward, A. A.. Standing potential characteristics of the epileptogenic focus. Epilepsia, 1961, 2, 161-169.

Morrell, F. Some electrical events involved in the formation of temporary connections. Reticular Formation of the Brain. Boston: Little, Brown, and Co., 1958.

Morrell, F. Microelectrode and steady potential studies suggesting a dendritic locus of closure. EEG Clin. Neurophysiol., 1960, 13, 1553-1593.

Morrell, F. Effect of anodal polarization of the firing pattern of single cortical cells. Ann. N. Y. Acad. Sci., 1961, 92, 860-876.

Morrell, F. Electrophysiological contributions to the neural basis of learning. Physiol. Rev., 1961, 41, 443-494.

Morrell, F. Microelectrode studies in chronic epileptic foci. Epilepsia, 1961, 2, 81-88.

Morrell, F., and Jasper, H. Electrographic studies in the formation of temporary connections in the brain. EEG Clin. Neurophysiol., 1956, 8, 201.

Morrell, F., and Naitoh, P. Effect of cortical polarization on a conditioned avoidance response. Exp. Neurol., 1962, 6, 507-523.

Morrell, F., Roberts, R., and Jasper, H. H. Effect of focal epileptogenic lesions and their ablation upon conditioned electrical responses of the brain in the monkey. *EEG Clin. Neurophysiol.*, 1956, *8*, 217-236.

O'Leary, J., and Goldring, S. D.C. potentials of the brain. *Physiol. Rev.*, 1964, *44*, 91-125.

Orkand, R., Nicholls, J., and Kuffler, S. The effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *J. Neurophysiol.*, 1966, *29*, 788-806.

Ralson, B. L. The mechanism of transition of interictal spiking foci into ictal seizure discharges. *EEG Clin. Neurophysiol.*, 1958, *10*, 217.

Rosen, S. C., and Stamm, J. s. Inter-sensory electro-cortical conditioning of slow potentials. *Fed. Proc.*, 1966, *25*, 305.

Rosen, S. C., and Stamm, J. S. Conditioning of cortical slow potentials in epileptic monkeys. *Fed. Proc.*, 1967, *26*, 28.

Rowland, V. Electrographic responses in sleeping conditioned animals. In G. E.,W. Wolstenholme and M. O'Connor.(Ed.), *The Nature of Sleep*. Boston: Little, Brown, and Co., 1960. Pp. 284-303.

Rowland, V. Steady-potential phenomena of cortex. In G. C. Quarton, T. Melnechukm, and Fl Schmitt (Ed,), *The Neurosciences*. New York: The Rockefeller Univ. Press, 1967. Pp. 482-495.

Rowland, V. Cortical steady potential (direct current potential) in reinforcement and learning. In E. Stellar, and J. M..Sprague (Ed.), *Progress in Physiological Psychology*, Vol. 2. New York: Academic Press, 1968. Pp. 1-77.

Rowland, V., and Goldstone, M. Appetivtively conditioned and drive related bioelectric baseline shift in cat cortex. EEG Clin. Neurophysiol., 1963, 15, 474-485.

Rowland, V., and Goldstone, M. Steady potential shifts in cortex and learning. In M. Brazier (Ed.), Studies in Brain Function, Vol. 1. Los Angeles: Univ. of Calif. Press, 1963. Pp. 136-174.

Rusinov, V. An electrophysiological analysis of the connecting function in the cerebral cortex in the presence of a dominant region area. Communications XIX Internat. Physiol. Congr., 1953, 719-720.

Rusinov, V. General and localized alterations in the EEG during the formation of conditioned reflexes in man. EEG Clin. Neurophysiol., 1960, 13, 133-320.

Stamm, J. S., and Rosen, S. C. Electrical stimulation and steady potential shifts in prefrontal cortex during delayed response performance by monkeys. Acta Biol. Exp., in press.

Strumwasser, F., and Rosenthal, S. Prolonged and patterned direct extracellular stimulation of single neurons. Am. J. Physiol., 1960, 198, 405-413.

Svaetichin, G., Negishi, K., Fatehchand, R., Drujan, B., and Selvin de Testa, A. Nervous function based on interactions between neuronal and non-neuronal elements. In Progress in Brain Research, Vol, 15. New York, 1965. Pp. 243-266.

Terzuolo, C., and Bullock, T. Measurement of imposed voltage gradient adequate to modulate neuronal firing. Proc. Natl. Acad. Sci. U.S., 1956, 42, 687-694.

Von Euler, C., and Green, J. Activity in single hippocampal pyramids. Acta Physiol. Scand., 1960, 48, 95-109.

Ward, A. The epileptic neurons. Epilepsia, 1961, 2, 70-80.

Watanabe, A., and Bullock, T. H. Modulation of activity of one neuron by subthreshold slow potentials in another in lobster cardiac ganglion. *J. Gen. Physiol.*, 1960, *43*, 1031-1045.

Wurtz, R. Steady potential shifts during arousal and sleep in the cat. *EEG Clin. Neurophysiol.*, 1965, *18*, 649-662.

Yoshii, N., and Hockaday, W. J. Conditioning of frequency-characteristic repetitive electroencephalographic response with intermittent photic stimulation. *EEG Clin. Neurophysiol.*, 1958, *10*, 487-502.