CORTICAL EVOKED POTENTIALS AND BEHAVIORAL STATES

MODIFICATION OF CORTICAL EVOKED POTENTIALS DURING INDUCED AND NATURAL BEHAVIORAL STATES IN THE HOODED RAT

By

THOMAS LOREN BABB, B.A.

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AUTHOR: Thomas L. Babb, B.A. (Lake Forest College)

SUPERVISOR: Dr. G.K. Smith

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SCOPE AND CONTENTS: Cortical potentials evoked by flashes or clicks were studied in the CER situation and found to increase by over 200% when these evoking stimuli served as conditional stimuli (classical conditioning), when neutral flashes were paired with click CS's (compound conditioning), and when flashes followed painful shock (backward conditioning) which sometimes suppressed bar pressing. Similar increases were evoked in other animals during high voltage slow electroencephalographic sleep or drowsiness. Only cortical components (not afferent activity) of the evoked response were found to increase with these different behavioral conditions, but the changes are not attributed to conditioning per se or dependent on immobility.

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CONTENTS

HISTORICAL INTRODUCTION

The most important property of nervous systems in general is their remarkable ability to transform sensory information. But in spite of the recent extensive development of the theory of information processing and the resulting increase of interest focused upon the biological examples of it, we still find ourselves unable to specify the neural variables responsible for the changes of the organism's responses in phenomena such as perceptual constancy and attention.

The primitive state of our understanding is illustrated by the difficulties we face in defining the role of the receptor in sensory information processing. It is clear, for example, that there is little the eye can do with the image optically projected upon the retina to preserve size or shape constancy in the face of changes of position or orientation of the outside world. These transforms are ascribed to "central" mechanisms acting upon the neural output of the receptor. Other cases are not so clear. The selection of certain "important" auditory signals for attention at the expense of "redundant" signals has often been ascribed to selective filtering by the receptor.

This latter alternative might seem to be easily controlled and its relative contribution in various perceptual situations easy to assess. But, since we have not yet been able to control central mechanisms, which now has the position of a residual category, the role of the receptor is naturally confounded with the former, such that if the independent variable (receptor change) is applied at the periphery and the dependent

variable (perception) recorded centrally, a third variable (our elusive central mechanism) may be the critical one occurring coincidently but not as a result of the receptor change. This point will become clear later in the light of experimental evidence designed to account for certain nervous system changes in terms of receptor effects alone. The preferred method has been to control the receptor and attribute any sensory differences to post-receptor central states such as attention or arousal.

William James reports a demonstration by Helmholtz in the following way:

"He was trying to combine in a single solid percept pairs of stereoscopic pictures illuminated instantaneously by the electric spark. The pictures were in a dark box which the spark from time to time lighted up; to keep the eyes from wandering between whiles, a pin-hole was pricked thru the middle of each picture, through which the light of the room came, so that each eye had presented to it during the dark intervals a single bright point. With parallel optical axes, these points combined into a single image; and the slightest movements of the eyeballs was betrayed by this image at once becoming double. Helmholtz now found that simple linear figures could, when the eyes were thus kept immovable, be perceived as solids at a single flash of the spark. But when the figures were complicated photographs, many successive flashes were required to grasp their totality. 'Now it is interesting', he says, 'to find that, although we keep steadily fixating the pin-holes and never allow their combined image to break into two, we can nevertheless, before the spark comes, keep our attention voluntarily turned to any particular portion we please of the dark field, so as then, when the spark comes, to receive an impression only from such parts of the picture as lie in this region. In this respect, then, our attention is quite independent of the position and accommodation of the eyes, and of any known alteration in these organs, and free to direct itself by a conscious and voluntary effort upon any selected position of a dark and undiffentiated field of view. This is one of the most important observations for a future theory of attention' (Physiol. Optik p. 741)"

Berger was the first to explain that the arrest of the 10/sec alpha rhythm of the human brain was more closely related to attention to a stimulus situation than to the nature or intensity of the stimulus per se.

Berger, incidentally, thought that this arrest represented a generalized inhibition over large areas of the cortex to allow focusing of attention on one small area subserving the incoming significant stimulus (Jasper, 1958). Durup and Fessard (1935) successfully conditioned arrest of the occipital alpha rhythm to an auditory stimulus by following the sound with a bright illumination.

In 1956, Hernandez-Peon, Sherrer, and Jouvet, (1956) reported an experiment in which click-evoked potentials in the dorsal cochlear nucleus were reduced when the cat "attended-to" the presence of a rat in a nearby glass jar but were returned to normal size the instant the cat "ignored" the rat. Peon concluded that during attention there is a selective awareness of certain sensory messages with the simultaneous suppression of others. In other words, the attended-to stimulus is transmitted unaltered while other stimuli are blocked or reduced (termed "afferent neuronal inhibition") in their trajectory to higher levels of the brain. This is, if you will recall, almost identical to Berger's interpretation of alpha block during attention, in the sense of overall suppression except in the single, attended modality. Peon and his co-workers have, since then, demonstrated similar decreases in post-synaptic potentials at retina (Hernandez-Peon, Sherrer, and Velasco, 1956), optic tract (Palestini, Davidovich, and Hernandez-Peon, 1959), lateral geniculate nucleus (Hernandez-Peon, Guzman-Flores, Alcaraz, and Fernandez-Guardiola, 1957), reticular formation (Hernandez-Peon, Lavin, Alcocer-Cuaron, and Marcelin, 1960), and cortex (Hernandez-Peon, Guzman-Flores, Alcaraz, Fernandez-Guardiola, 1958) due to shifting behavioral states and/or direct

stimulation of the mesencephalic reticular system. These findings organize into a few very reliable phenomena:

1. evoked responses to a stimulus of one modality may be reduced or otherwise modified by attention to distracting stimuli of another or the same modality.

2. monotonous repetition of a stimulus will eventually lead to reduced evoked potentials to that stimulus (habituation).

3. any alteration in the monotony of the habituated stimulus sufficient to "alert" the organism, will return the evoked potentials to their pre-habituated level immediately and for some time thereafter (dishabituation).

4. reticular stimulation usually depresses but can also enhance evoked potentials.

5. consistent association with shock will usually result in the evoked potential remaining at the "attended-to" level, but it may also become enhanced.

6. reticular lesion usually prevents habituation and even may enhance evoked potentials.

Although these phenomena do not appear in all preparations, when they do they show a curious consistency of characteristics from animal to animal:

Attention

1. with mild degree of attention, secondary repetitive waves first to be reduced.

2. there is no correlation between intensity of distracting stimulus and amount of depression.

3. the sizes of evoked potentials vary greatly in animals easily alerted by environmental stimuli, i.e., co-fluctuation of evoked potentials and behavorial shifts.

4. effects of attention not confined to specific sensory pathway; reticular potentials may also be reduced.

Habituation

1. reduction of evoked response increases with number of stimulus presentations.

2. habituation always follows a "waxing and waning" time course.

3. the rate of habituation increases as the interstimulus interval decreases.

4. the rate of habituation increases with regularity or constancy of interstimulus interval; although arhythmic presentations will not prevent eventual habituation.

5. the rate of habituation is inversely related to stimulus intensity.

6. habituation develops specifically to the particular qualities of the stimulus. Altering any of its characteristics (e.g., wavelength or pitch) will produce dishabituation.

7. the reduction in evoked potentials due to habituation develops more slowly than the immediate reduction due to loss of attention, i.e., habituation is not simple inattentiveness.

8. the effects of habituation are of longer duration than the brief suppressor effects due to attention switch, i.e., habituation effects may be recalled on test presentations days apart.

9. habituation is always associated with a relaxed state and a synchronized EEG rather than with alertness and an activated cortex as in attention.

Dishabituation

1. recovery of habituated potentials by rest (cessation of stimulation) requires a variable length of time roughly proportional to the number of presentations required to establish the habituation.

2. sudden presentation of extraneous stimulation of the same or another modality than the habituated stimulus.

3. occurs by association with noxious stimulation.

4. occurs during barbiturate anesthesia, under which habituation cannot be reestablished.

5. may occur with extensive lesion of mesencephalic tegmentum.

6. occurs by altering some aspect of the habituatory stimulus itself, regardless of direction of change. Subsequent habituation is rapid to this slightly altered stimulus.

Reticular Formation

1. stimulation of the mesencephalic or pontine reticular formation abolishes post-synaptic secondary wave of evoked potentials from various thalamic sensory synapses.

2. lesion of mesencephalic tegmentum can result in an increased secondary wave of a sensory nucleus potential.

3. mesencephalic lesion can result in dishabituation.

4. reticular stimulation can lead to either diminished or potentiated afferent secondary potentials.

5. reticular evoked potentials display all the salient characteristics of "afferent neuronal inhibition" (i.e., reduction due to attention and habituation) found in primary pathways.

Though the complementary results of these numerous experiments are certainly unequivocal, Peon's interpretation of them in terms of "afferent neuronal inhibition" seems more questionable. First of all, almost all of the modifications in the evoked potentials occur on the secondary and late components and virtually none on the primary portion. This, of course, suggests that the effect is not truly a blockade of afferent impulses. Moreover, recordings of reticular potentials show reductions similar to those of the primary pathways, again suggesting that the effect of attention or habituation is not directed to the sensory paths in so specific a manner as suggested by Peon's doctrine of afferent neuronal inhibition. More recently, these objections have been answered with the assertion that modification of such non-specific sensory systems as the reticular core account for the rapid diminution of cortical late waves during attention or habituation. There is no evidence that this is true and it still does not answer the problem of whether or not these phenomena represent true centrifugal inhibition. It seems that reticular stimulation, which Peon considers the source of such centrifugal control, may either increase or decrease evoked potentials. In the former, he assumes it causes a release of tonic centifugal control, in the latter an increase.

It is also interesting that habituation of cochlear nucleus potentials occurs mostly for low intensity clicks and in one study (Hernandez-Peon, Jouvet, and Sherrer, 1957) was difficult to obtain except

with near-threshold tones. In view of the fact that in most of Peon's reports no mention is made of attempts to control for receptor changes, it seems possible that they may play some role in phenomena such as evoked potential changes due to reticular stimulation, habituation, and attention. Hugelin, Dumont, and Pallais, (1960) found that disinsertion of tympanic muscles prevented a decrease in the evoked cochlear nucleus potential due to reticular stimulation while the side with intact middle ear muscles demonstrated obliterated cochlear nucleus potentials during the reticular stimulation. Diminution of cochlear microphonic potentials recorded from round window were found to be never greater than 13 db and usually less than 5 db, a relatively unimportant attenuation for most audible levels of sound, but obviously quite critical for near-threshold intensities as were required to obtain cochlear nucleus obliteration. Reticular stimulation, then, according to these authors, exerts its effect on contraction of middle ear muscles to altenuate the amount of sound input; it does not exert an inhibitory control at the first synapse of the auditory pathway.

The possibility of a peripheral explanation for auditory habituation has occurred to many researchers, especially Guzman-Flores who proposed that the reason short interstimulus intervals lead to faster habituation is that a temporal discrimination between the last and next stimulus is more easily learned than for long interstimulus intervals and that middle ear muscle contractions are conditioned to occur at the end of these short intervals. However, Webster, Dunlop, Simons, and Aitkin (1965) show, as did Peon, that irregular stimulus presentations which would prevent the learning of an interstimulus interval will eventually lead to habituation. The possibility that in irregular interval presentations the organism merely exerts tonic

muscle contraction was checked by Webster when he showed that i.p. sodium pentobarbital, which blocks the middle ear muscle reflex, did not abolish habituation. And finally, Moushegian, Rupert, Marsh, and Galambos (1961) demonstrated that absence of middle ear muscles did not preclude changes in evoked cortical potentials during habituation, conditioning, or distraction.

Naquet, Regis, Fischer-Williams, and Fernandez-Guardiola (1960) found a similar "independence" of cortical potentials, though they did not manipulate behavioral states. They found that evoked response amplitudes from optic chiasma and lateral geniculate depend on the conditions of illumination of the retina and stay constant with an atropine-fixed pupil. The specific visual cortex, on the other hand, depends on the state of relative synchronization or desynchronization of the whole cortex rather than on the amplitude of the evoked potential in the chiasma and the lateral geniculate. These results are taken to indicate that there is a control of specific afferents as judged by the evoked potential amplitude, but that it does not occur by an inhibitory influence at the level of the first synapse but probably takes place after the thalamic relay and is indicated by the cortical response.

It may now seem that a sizeable gap exists between the Peon experiments which demonstrated all of these phenomena while recording almost exclusively subcortical and these experiments by Hugelin and Naquet who suggest the unchangeability of subcortical afferents. Perhaps the disparity lies in the different role the receptor plays in each kind of experiment. It seems clear that these latter experiments were designed to demonstrate conditions in which receptor control <u>can</u> be critical. Hugelin,

for example, only demonstrated that for juxtaliminal clicks evoked potential reduction due to reticular stimulation could be accounted for by decreases in cochlear microphonics up to 13 db caused by middle ear muscle contraction. But for greater intensities, which others have habituated (Hernandez-Peon 1955; Marsh, McCarthy, Sheatz, and Galambos, 1961), Hugelin's data shows that muscle contractions due to reticular stimulation will have no appreciable effect in reducing sound input and "appears", in his words, "no more important than other aleatory modifications occurring through active behavior (masking effect, head orientation, and so forth)".

In reporting the stability of subcortical flash potentials when the pupil is fixed in complete mydriasis, Naquet also reports observations on the normal eye. He points out that whenever the cortex is desynchronized the pupil dilates and subcortical potentials are large while cortical potentials are reduced. When the cortex is synchronized the opposite is true; the pupil remains in myosis and subcortical potentials fall while cortical potentials are large. He suggests that the changes in the subcortical potentials (lateral geniculate and optic chiasma) are due to changes in pupil diameter elicited by cortical arousal, and he presumes to have demonstrated that there is no change in evoked subcortical potentials due to synchronization or desynchronization when the pupil size is fixed. However, Naquet actually fixed the pupil in an unnaturally extreme state, complete dilatation, a condition in which the energy input could likely be so high as to mask the effect of a mechanism which has evolved to operate on a more moderate range of intensities. A more convincing demonstration would have been that a pupil fixed at a normal diameter is a condition sufficient to prevent subcortical evoked potential modification by arousal or cortical

activation. If this could not be demonstrated, i.e., if potentials were modified by desynchronization, then pupillary diameter would prove to be an irrelevant and correlated event possibly affected by the same mechanism causing desynchronization but not directly responsible for the observed evoked potential changes. Affani, Mancia, and Marchiafava (1962) have anticipated this objection and equipped their cerveau isole cats with artifical pupils fissurated to simulate a constant normal myosis. These preparations, contrary to preparations with natural pupils who exhibited evoked potential habituation accompanied by extreme myosis, showed no evidence of habituation or enhancement following reticular stimulation. The authors admit, however, that pupillary constriction alone cannot explain all of visual habituation, especially when the stimulus is very strong, nor the observation that cortical responses may sometimes decrease before those recorded from the lateral geniculate. Thus, it seems that while Peon's overworked notion of direct afferent inhibition seems wholly unsatisfactory, attempts to account for his persistent plastic phenomena by purely peripheral explanations have met with only limited success. The obvious approach has been to delineate what conditions are essential to these phenomena; what characteristics of the organism are common to short- or long-term modifications in evoked potentials?

Most of the research directed toward answering these questions in recent years have attempted to maintain better control over the behavioral state to which the brain potentials are related. The belief was always there that perhaps the phenomena resulted from behavioral artifacts. Horn (1960), for example, closely monitored the head and eye movements of a cat

when distracted with either tones or a white mouse. Evoked responses to flash were reduced by attention to mouse or by a series of tones "only if there was some visual searching component in the cat's response to the acoustic stimuli". Click evoked responses were reduced on the first few occasions that the mouse was presented, conceivably because the cat was listening for auditory cues associated with the mouse. Horn argues that during attention the attenuation of cortical evoked responses represents increased sensitivity in the appropriate region rather than signifying a reduction of incoming information. He supports this contention by showing that although the evoked potential was decreased by 20-30 per cent, the background ECoG was reduced by 52 per cent - yielding a net increase in signal to noise ratio.

Jane, Smirnov, and Jasper (1962) recorded simultaneously from thalamic and cortical auditory and visual areas during either auditory or visual distraction. The results showed that both forms of distraction decreased the variability and increased the amplitudes of all potentials in both auditory and visual system. The effects of distraction were never found to be selective, as Peon requires; however in some animals alert prior to distraction, distraction of either kind reduced the amplitudes in both auditory and visual systems. The direction of change of potentials seemed to depend reliably on the state of alertness or quiescence prior to the distraction. In the former alert state distraction probably introduces excessive nonspecific activation and decreases potentials due to occlusive blockage or some internuncial inhibitory process; in the latter, distraction "sharpens" the waveform due to suppression of later components,

presumably by improved synchronization of dispersed responding elements (Jane, Smirnov, and Jasper, 1962). In any case, the inescapable conclusion is that whatever the exact mechanism operating during states of attention and habituation, its recorded effects may be either facilitative or depressive.

Attempts to gain even better control over the animal's disposition, and presumably also some brain mechanism, have lead to the use of conditioning, in which the animal is usually maintained in the same behavioral state during stimulus presentations and thereby not subject to momentary fluctuations of attention which characterizes the distraction technique. Peon, in fact, used shock pairings to dishabituate signals and regarded such Pavlovian conditioning as prolonged attentiveness to the conditioned stimulus, preventing habituation to it by a continuous release of centrifugal inhibition. In short, the reticular formation was conditioned not to inhibit the particular sensory pathways carrying the conditioned stimulus. Galambos has essentially repeated Peon's observations of attention and habituation on cats and monkeys. And, in the past few years, he has presented evidence of evoked potential increases as a result of classical conditioning, but this evidence is tempered by the addition of these generalizations:

1. Low amplitude evoked responses appear throughout the normal brain and are modified with conditioning. In one experiment, average increases in non-auditory areas were greater than in auditory areas following click conditioning.

2. Modifications of evoked responses may occur anywhere along the auditory pathway from cochlear nucleus to the auditory cortex in the form of reduction of increases but not necessarily in a consistent relationship.

3. During Pavlovian conditioning, limbic system structures (hippocampus, septal area, and head of caudate nucleus) exhibit enhanced evoked responses to the conditioned stimulus, while clicks which require a lever press to avoid shock or procure sugar (instrumental conditioning) yield reduced potentials in these limbic structures. Withdrawal of lever, thereby preventing any instrumental response, produced a striking increase in these evoked amplitudes.

What Galambos has hoped to show is that the nervous system will "interpret" a signal differently as it takes on significance or becomes informative to the animal through conditioning. The distinction between Pavlovian and instrumental procedures mentioned in point three above may suggest support for this view. But the fact of such diffuse effects (point two) and that potentials may change in some areas more than others, suggests that the effect of conditioning in <u>not</u> mediated at each site (if we crudely assume equal lability of nervous structures) but is probably communicated throughout the brain from some single central influence. The most likely candidate for such a function is the brain stem reticular formation. All the available evidence on the role of the reticular system indicates that activity in these polysensory areas is correlated with primary sensory area recordings (Hernandez-Peon, Lavin, Alcocer-Cuaron, and Marcelin, 1960) and may facilitate or inhibit activity in sensory areas upon stimulation in different regions (Hagbarth and Fex, 1959). Furthermore, EEG activation associated with various behavioral states ranging from awakening through alterness and even flight has appeared, accompanied by these same activities, at lower thresholds during stimulation of the midbrain reticular formation than when cortical sites were stimulated as a control (Segundo,

Arana, and French, 1955).

Sharpless and Jasper (1956) have hypothesized a bimodal reticular control with a tonic influence originating in the lower brainstem maintaining wakefulness over long periods of time and a phasic activation, mediated by the diffuse thalamic system, subserving transient alerting and attention in response to slight environmental change. Twenty years earlier, Jasper had recognized that "the form of electrical activity of the cortex in response to significant afferent stimuli depended upon the excitatory state of the cortex at the time of arrival of the stimulus" (Jasper, 1958). As, for example, during drowsiness, especially in narcoleptics, arousing stimuli may produce a return of alpha rhythm rather than its block. It seems that we are now on safe enough ground to assert that electrical brain modifications related to attention, habituation, and conditioning is not a predictable matter. Rather, our present knowledge of such proposed mechanisms as "afferent neuronal inhibition" originating from reticular activation only suggests that any influence from reticular structures may at first be anatomically either excitatory or inhibitory or secondly may interact with the afferent volley at the synapse either facilitatively (due to subliminal fringe) or impediently (occlusive block). And, with a look back at the evidence, it is clear that nowhere is the simple notion of arousal, its specific effect on synaptic activity being dependent on other yet undetermined factors as comprise the background activity, insufficient to explain the observed electrical changes. Habituation to a signal is always accompanied by high voltage slow wave drowsiness and never by an activated EEG, which results from, produces, or is related to dishabituation.

Jane, Smirnov, and Jasper (1962) reported evoked potential increases almost identical in both auditory and visual pathways during distraction which aroused the cat's attention. Horn (1960) showed similar non-selectivity in sensory modification during distraction, but the absolute size of his recorded potentials decreased.

In simplest terms, it seems that all studies which employ behavioral shifts to study changes in evoked responses to constant sensory stimuli inadvertently produce overall shifts in the excitability of the brain which may not parallel or in any way represent these dispositional changes. The problem then becomes to decide when an electrical change is due simply to general brain arousal and when it changes because the stimulus means something significant. Perhaps this is an academic distinction; for it seems highly unlikely that we may ever encounter a modified cortical potential without some alteration in the ECoG. At any rate, it is obvious that better techniques are required to sort out the difference between a "significant" stimulus and merely an arousing one. For example, in the classic case of Pierre Dupont, a patient of Fischgold, whose slow wave EEG could not be changed by loud sounds or even pinching but immediately changed to a rapid low voltage record when his name was called (Jasper, 1958).

The notion that what is directly affected by a switch of attention or with conditioning is the state of the reticular activating system or some general form of arousal somewhat vitiates Galambos' contention that the information increase or decrease of the stimulus exerts its effect directly on the lability of the various nervous sites. But, recently Hall (1964) has reported evidence where evoked cortical and geniculate potentials to

irrelevant background clicks in the rat were enhanced in the presence of a conditioned stimulus (change in ambient illumination) which produced a fearful conditioned response. Clearly, here, the information-value of the evoking stimulus in the conditioning procedure is not the critical variable determining the enhanced response to it.

The research which is reported here had its origins in the consideration of such sensory mechanisms relating to behavior as have been reviewed so far. To be precise, this research began as an attempt to provide evidence for or against the existence of a phenomenon of "perceptual block", (similar to Peon's centrifugal inhibition), which was thought to be the mechanism responsible for the prevention of classical conditioning to a neutral stimulus when it was added to an already-conditioned stimulus to form a compound conditioning situation (Kamin, 1965, personal communication). Why does the added stimulus remain not conditioned after an ample number of compound conditioning trials?

This failure-to-condition phenomenon was observed by Palladin (Pavlov, 1926, p. 141) who first trained a dog to salivate to the application of a tactile stimulus, then combined it with a thermal stimulus of 0°C. for many more reinforced, salivation trials, and finally observed that when the thermal stimulus was presented alone conditioned salivation did not occur. Pavlov called this overshadowing; the resultant cortical radiation of the tactile analyzer was so great that the cortical analyzer of the thermal stimulus was inhibited. A similar interpretation was applied to the situation where a more intense neutral stimulus overshadowed a less intense neutral stimulus of the same analyzer during compound conditioning (Pavlov, 1926, p. 142).

Kamin, (1965, personal communication of unpublished data) using the classical conditioning technique of the C.E.R., has found, however, that on the first trial of the compound situation, (i.e., when the neutral stimulus is first added to the already-conditioned stimulus) the on-going conditioned behavior is disrupted. Also, if some information is added to the compound trials, (if the shock reinforcement is increased), then some conditioning will occur to the added stimulus. However, increased shock reinforcement occurring after trial one (i.e., not occurring on trial one but occurring on all other compound trials) did not lead to conditioning of the added stimulus as it did when included in trial one. These results tend to the conclusion that trial one is the only trial in which the added stimulus is perceived or attended to. It is tempting to take the position that the novelty of the added neutral stimulus on trial one distracts the animals attention from the CS, thereby disrupting the conditioned response. If the new stimulus proves to be redundant, (i.e., is not followed by any increase in information), then on subsequent compound conditioning trials that added stimulus is ignored and attention focused on the CS. An evoked potential analysis was undertaken in search of such a sensory-gating mechanism.

It soon became obvious that this behavioral procedure would provide the opportunity to acquire more information about brain activity and concomitant behavior than the mere existence of sensory inhibition. First of all, it allows examination of the modification of cortical potentials evoked by a neutral stimulus (flash or click) unavoidably followed by shock (i.e., classical conditioning). Secondly, this design provides a situation

where a neutral stimulus, in the presence of a conditional stimulus, is unavoidably followed by shock (compound conditioning). Remembering that some researchers report evidence of increased evoked potentials during classical conditioning, it is clear that this procedure allows investigation of such increases during compound stimulation when one stimulus is and the other is not a conditional stimulus. And, finally, as a means of further investigating what might be the critical or at least an effective variable determining evoked potential increases occurring with conditioning, a backward conditioning design was employed to evaluate the necessity of the CS-US contingency and the possibility of effects comparable to those resulting from conditioning occurring after the arousing experience of shock.

METHOD

Subjects and Surgical Procedure

All subjects were adult, male hooded rats ranging in weight from 240 to 360 grams, following at least one week of ad libitum feeding at the time of surgery. The animals were anesthetized with intraperitoneal injections of pentobarbital sodium (Nembutal) at approximately 36 mg./kg. and given a supplementary dose of chloral hydrate (approximately 180 mg./kg.), according to dosages suggested by Vallenstein (1960). Blunt-tip ear plugs were inserted to make contact with the tympanic bulla, avoiding the tympanic membrane, and securing the rats into a Scientific Prototype stereotaxic instrument. A mid-line incision exposed the dorsal skull surface, which was scraped clean, and the temporal muscle was then retracted to expose the temporal bone overlying the auditory cortex. In some of the initial operations it was found that complete removal of the temporal muscle apparently interferred with the rat's ability to chew large pellets of food. Superficial bleeding was controlled by topical application of epinephine hydrochloride (Adrenaline Chloride 1:1000). Four stainless steel screws were inserted into the skull and two specially-constructed electrodes were positioned on the cortex through burr-holes 4 mm. in diameter. Ground electrodes consisting of uninsulated wire were either soldered to the mounted jeweller's screws (using 10% phosphoric acid as flux), implanted in the frontal cortex, or protruded into the saggital sinus. The electrodes, with attached connector, were imbedded in dental cement and the incision closed around the mound of cement. Approx-imately two hours from induction, the operation was completed; animals

were given 30,000 units potassium penicillin G and returned to their home cages for a recovery period of at least two weeks. During this period body weight was maintained on an ad libitum feeding schedule, and terramycin was added to the drinking water.

Acute Preparations and Cortical Mapping

In an attempt to locate cortical areas responding maximally to visual and auditory stimuli respectively, five rats were lightly anesthetized, (only pentobarbital sodium), craniotomies were performed, and evoked cortical responses to contralateral flash, click, or both were recorded monopolarly and measured on an oscilloscope or, in some cases, averaged with a Computer of Average Transients (see Recording Apparatus). In four rats, a large portion of the right hemisphere corresponding to Areas 17, 18, and 18a of Krieg's atlas (Krieg, 1946) was exposed and maximal visually-evoked potentials of 100-250 microvolts were recorded in a region 7.5 mm. posterior from bregma and 3 to 4 mm. lateral from the saggital suture, positioned slightly in front of the lambdoidal suture. In one of these four rats and one other rat, removal of pieces of the temporal bone exposed a large region of the auditory cortex overlapping the temporal portion of Area 18a and most of Area 41. Monopolar recordings of evoked responses to clicks were measured in each of four sub-quadrants and maximal responses were located where Areas 18a and 41 meet, roughly 7 mm. posterior from bregma and 3 mm. down the temporal bone from the parieto-temporal suture. Subsequent bipolar recordings from unanesthetized preparations indicated that these areas are not given over to either visual or auditory responsiveness exclusively. In some cases auditory implants have given larger

responses to flashes than to clicks. However, in no case has a visual implant shown larger auditory than visual responses. Nevertheless, attention to this point makes it clear that the rat cortex is not organized into simple sensory partitions, (e.g., LeMessurier and Woolsey, 1948), and for this reason presentation of both visual and auditory stimuli must be alternating, with the interval between each visual and auditory stimulus lasting at least the duration of the evoked response to the prior stimulus.

Electrodes and Connectors

The electrodes were constructed of two enamelled nichrome wires ,010 inch diameter (Driver-Harrison Company, Harrison, N.J.) cemented through the holes of miniature buttons so that the tips were separated by 2 to 3 mm. These wire tips were cut to protrude no more than 2 mm. from the bottom surface of the button, which during implantation rested on the skull. In some experiments only the cut ends of the wire tips were exposed for recording. In others, spherical tips were formed by attaching one side of a 30 volt A.C. source to a bared portion of the wire and the other side to a tungsten electrode situated in a mercury bath covered with a layer of mineral oil. Bringing the nichrome tip close to or touching the bath created a spark and heat sufficient to curl the tip into a ball only slightly larger in diameter than the wire itself.

The recording and ground electrodes were attached to crimp-type contacts situated in the socket portion of Amphenol "Mighty Mite" (222-12NO7-SO1) connectors. Biological potentials were led from Amphenol plugs (222-11NO7-PO1) through shielded phono pickup arm cables (Belden 8419) to the recording apparatus.

Recording Apparatus

In the acute preparations, potentials were amplified by a Grass P5 C-R AC amplifier (amplification -28K; filters: low -7 cps, high -.1kc) and displayed on a Tektronix 502 oscilloscope. Average potentials for 50 stimuli into the contralateral receptor (atropinized eye or ear) were measured on a Mnemotron C.A.T. 400B computer, printed out on a Technical Measurement Corporation Printer Model 500, and plotted with a Moseley Autograf Model 2D-2.

For the chronic preparations, which were employed in the conditioning program, the potentials were amplified through a Beckman Type R Dynograph and then capacity-coupled with the Mnemotron C.A.T. The average waveform computed from 180 ($\pm$3) stimuli was printed on the same T.M.C. Printer and plotted with the same Moseley Autograf. In one experiment, potentials were recorded on a four-channel Ampex SP-300 tape recorder and later analyzed off-line.

Conditioning Apparatus

All experimentation took place in two nearly identical experimental boxes with slight modifications to accommodate rats with cumbersome recording leads attached to their heads. The front wall of the boxes consisted of an aluminum panel with openings through which protruded a metal bar which could be operated as a first class lever to close a microswitch and provide a pulse to operate a Gerbrand's feeder which delivered a 45 mg. Noyes food pellet into a brass cup protruding through the other of these two openings. The bar and cup were side by side, separated by only 5/8 inch, four inches above the grid floor. This arrangement reduced gross movements between

bar and cup, which can produce recording artifacts. Two speakers were mounted on the back of the rear wall and played through perforations in the aluminum wall. One speaker carried a continuous 60 db. level of white noise to attenuate possible extra-experimental noises. The other speaker was driven intermittently by an 18 volt battery supply to produce a discrete click, used as the auditory CS (conditional stimulus). Both side walls were covered from grid floor to ceiling with mirrors to increase the constancy of the overall illumination from the visual stimulus. Situated on the ceiling of transparent plexiglas was the flashlamp and parabolic reflector of a Grass PS-2 photo-stimulator which delivered a 10 microsecond flash of moderate stroboscopic intensity (setting number 2) to produce the visual CS. It was found by sampling a number of evoked potentials at all PS-2 intensities that for settings above number 2, all evoked responses were supra-maximal, (i.e., did not increase with an increase in flash intensity). A sub-maximal response was desired for these experiments; therefore setting number 2 was chosen.

All of this apparatus was contained in large wooden chests, approximately 3'x3'x3', insulated with either sand or felt and equipped with ventilation fans, both serving to further attenuate extraneous laboratory noises. Conditioning trials and the variable interval reinforcement schedule as well as the remote control of the recording apparatus were programmed on standard Grason-Stadler relay equipment located in another room. The unconditional stimulus was a one milliampere unavoidable shock of .5 sec. duration delivered to the rat's feet by a Grason-Stadler E1064 GS Shock Generator with grid scrambler.

Stimulus Presentation

The stimuli were controlled by the onset of a one-second sweep of the C.A.T. through a delay circuit from which the leading edge triggered the Grass PS-2 photo-stimulator and the trailing edge (490 msec. later)

supplied an 18 volt pulse to a 45 ohm. speaker. Since most recording sites showed responses to both modalities, the interleaving of stimuli in this way was necessary to eliminate the interference that would be caused by simultaneous afferent volleys. Thus, in the computed waveforms, flash potentials appear at the beginning of the sweep, click potentials are always recorded in the second half of the one-second response epoch. In all cases, averaged responses are actually the summed responses to 180 (± 3) stimuli.

The Electrical System and Grounding Techniques

The block diagram (Diagram 1) illustrates the relation between the components of the recording and control system. Brain potentials are small and in these experiments were led from relatively high resistance electrodes (50 to 100 kilohms) through several feet of wire to the recording amplifiers. With these conditions in the vicinity of various power equipment, problems with electrical interference were severe and some techniques had to be employed to eliminate them.

The interference encountered was of three types:

1. Radiated interference from nearby 60 cps. fields and from relay equipment inducing transient surge currents.

2. Conducted interference due to eddy currents or leakage currents between the animal and associated apparatus.

3. Artifact potentials due to movement of the animal. These originate either from bodily potentials such as heart beat or neck muscle potentials which may be picked up by the electrode tips, or from potentials due to flexion of the recording leads and connector contacts.

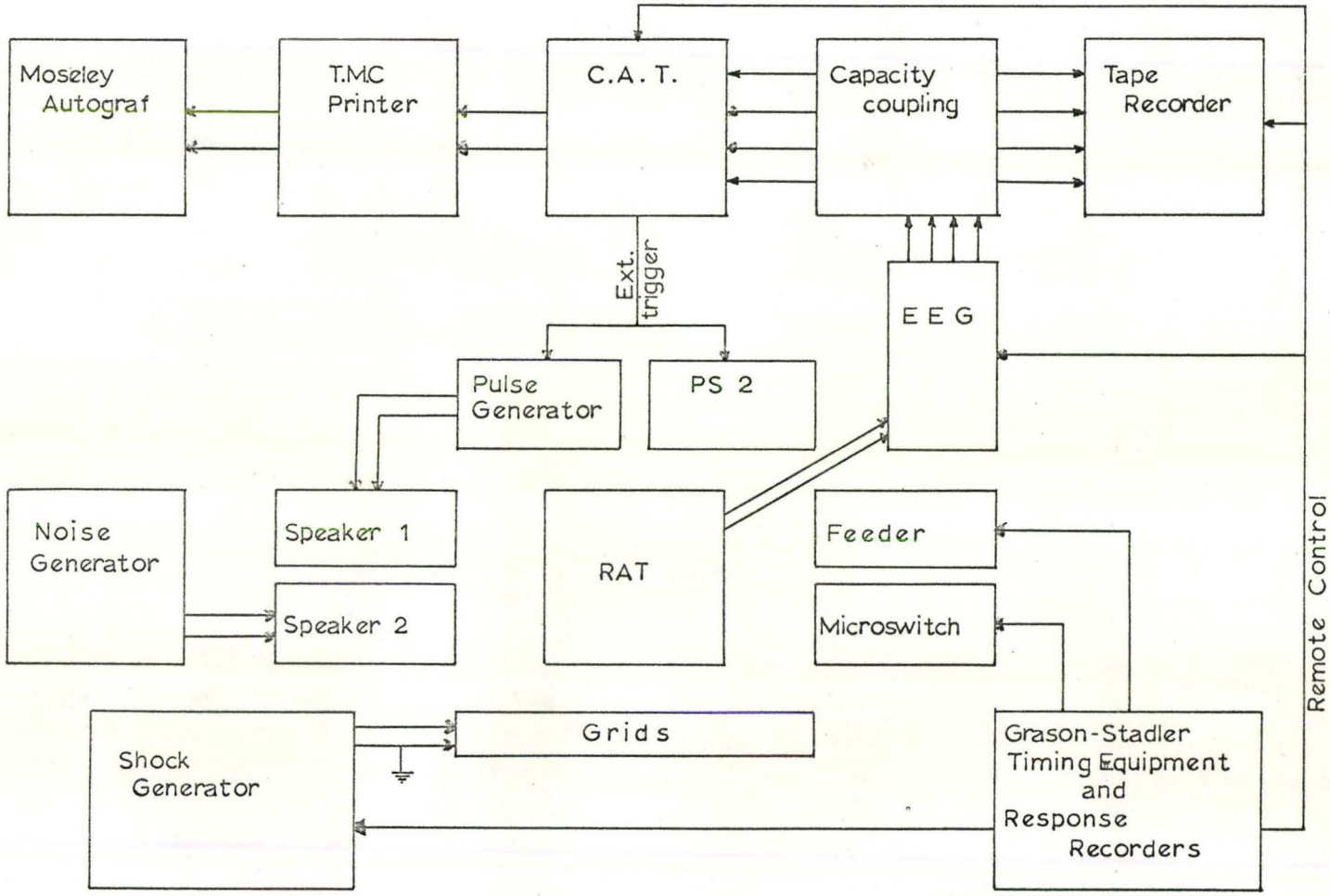

Moseley Autograf
T.M.C Printer
C.A.T.
Capacity coupling
Tape Recorder
Ext. trigger
E E G
Pulse Generator
PS 2
Noise Generator
Speaker 1
Speaker 2
RAT
Feeder
Microswitch
Shock Generator
Grids
Grason-Stadler Timing Equipment and Response Recorders
Remote Control

Diagram 1..

Radiated interference was eliminated mainly by moving equipment generating A.C. fields as far as possible from the experimental box, screening all power leads, screening the recording leads and the experimental chamber, and shunting nearby relay contacts and microswitch with spark-suppressing capacitors. Conducted interference derived mainly from the shock grids which carried a small A.C. voltage when the shock generator was inoperative. The shock generator output was grounded through a relay except during shock application. Customarily, the animal ground was made through two stainless steel screws placed in the frontal cranium and the average potential between them provided a better respresentation of the animal's bodily potential. Occasionally, one of these electrodes became high resistance relative to the other and the resulting eddy currents required that the high resistance lead be switched to open circuit. The intracranial electrodes placed in the frontal area and used as the animal ground had to be switched off ground to open circuit during shock application to avoid passing current through the brain electrode.

Artifact potentials generated from skeletal muscle are of such high frequency that the 22 cps. low pass filters used eliminated them from the recording. The low frequency electrocardiogram was not a problem since there is virtually no phase or amplitude difference picked up between closely-spaced bipolar brain electrodes. Noise from recording wire friction was eliminated by encasing the shielded recording cables in a lightweight jacket of adhesive tape which prevented lead flexion without seriously hampering the animals mobility. Contacts joining the mounted electrodes to the recording wires were encased in a tight-fitting connector designed to eliminate friction potentials.

Procedures

Prerecording Following at least two weeks post-operative recovery, animals were placed in the experimental box-recording chamber, and a variable number of samples were taken of evoked responses to flash alone, click alone, and alternating flash-click compound. These recordings were used to evaluate sizes of evoked potentials for each subject (to adjust amplification), to record and compute the within-day and between-day variability of each response, and also as a control for the possibility that enhanced potentials during conditioned suppression may be due simply to the absence of movement, which is a common characteristic of the Conditioned Emotional Response. For, during Prerecording sessions, it was generally the case that after a short period of exploration, rats would situate themselves in a corner of the box completely motionless and in some cases would become drowsy or fall asleep, as judged by the appearance of high voltage slow wave ECoG (even during sensory stimulation). This latter situation, slow wave ECoG, showed the limitations of this condition as a control, as will be clearly shown in the results comparing ECoG and form of evoked response.

Bar-Press Training Following the Prerecording sessions, animals were reduced to 75% ad lib body weight and placed on a 24-hour feeding rhythm, so that they were 22 hours hungry at the beginning of each two-hour bar-pressing session. On the first day of training, animals were placed in the experimental box with the house lights on and administered 40 pellets automatically on a one-minute V.I. (variable interval) schedule, during and after which time any bar press was also reinforced with a food pellet. After 120 pellets had been acquired, animals were returned to their home cages. On subsequent days animals were placed and maintained on either a

one-minute or a two and one-half minute V.I. schedule, depending on individual response rates. Eventually, all animals were brought to a reasonably high and consistent rate of bar pressing in complete darkness, though in some cases the heavy recording leads mounted on their heads produced considerable variability.

Pretesting Each of the three conditioning stimuli (flash, click, and flash-click compound), were presented for four three-minute periods unevenly spaced over the two-hour bar-pressing session, with an average intertrial interval of 28 minutes. On some days, order of presentation of stimuli was counterbalanced, on others it was not. Pretesting was carried on for two or three days, in order to evaluate the unconditioned effects of the stimuli on the rate of bar pressing as well as to record the preconditioned level of evoked responses to the stimuli while the animals were bar pressing.

Conditioning and Extinction Trials presented in time sequence exactly as described in the Pretest section were followed by shock in conditioning programs extending as long as thirty days including extinction periods. Although the exact number of trials to acquisition and extinction varied from one experiment to the next, all animals may be categorized into two basic designs:

1. The "block" group, schematized below, consisted of animals who were first trained to suppress fully to clicks, i.e., given from 16 to 32 trials, then given 8 reinforced trials of the flash-click compound (during which they remained under the control of the clicks), but when subsequently tested in four unreinforced trials the following day to flash alone showed no suppression, i.e., no acquisition to the flash took place during the

flash-click compound conditioning.

I	II	III	IV	V
16-32 N+ Shock	8LN+ Shock	4L- No Shock	16L+	Extinction: 12-32 N- 12-40 L-

2. The "backward conditioning" animals received either flash or click alone, no compound stimulus conditioning. Sixteen trials with the shock preceding the stimulus for both the flash and the click was followed by 16 trials of traditional conditioning with the shock following each stimulus period.

I	II	III	IV	V
16 +L	16 +N	16N +	16L +	Extinction

In both of these designs conditioning and extinction was continued until a suitable number of both flash and click evoked responses were recorded in the suppressed and unsuppressed behavioral state. The relevance of these designs by themselves and to each other as possible control comparisons will be evident from the results. At the beginning of each session, a few drops of 1-2% atropine sulphate was placed into each rat's eyes to dilate pupils and abolish the pupillary reflex for at least the 2-hour session and control possible pupillary dilation or constriction occurring with conditioning.

Measures Four types of data are reported. The degree of conditioning as judged by interference with or suppression of bar pressing is expressed as a ratio of the number of presses in the three-minute CS period to the total number of responses occurring in the six minutes of Pre-CS and CS:

$$\text{Suppression Ratio} = \frac{CS}{\text{Pre-CS} + CS}$$

Since the amount of time devoted to the denominator is twice that of the numerator (CS period), trials with no suppression occurring (i.e., CS = Pre-CS) will yield a ratio of .50; complete suppression (no CS responses) is .00.

All evoked potential data are bipolar recordings presented in two ways:

1. as post-stimulus histograms of the averaged potentials plotted on-line by a Moseley X-Y writer and reproduced by hand copy for visual inspection of the waveforms.[1]

2. as measured values of the peak-to-peak amplitude, always expressed in microvolts, for each averaged evoked potential. Since an evoked potential consists of a multiple response of a number of positive and negative-going excursions, more than one peak-to-peak measure may be chosen. In all cases, the figure quoted represents the largest unidirectional excursion in the multiple response.

The electrocorticogram was recorded on chart paper throughout the experiments. Selected samples are presented to indicate changes in the background cortical activity.

The presence of cortical spindles throughout all of the experiments were monitored on the chart paper. Measurements of the number and duration of spindles were computed for two animals, only one of which is presented.

[1] It should be noted and remembered that these waveforms are not always plotted at comparable amplification and ordinates may differ. For this reason, peak-to-peak amplitudes are always given to indicate the absolute size of each displayed potential.

RESULTS

I. Evidence for evoked potential enhancement with conditioning.

Figures 1 through 4 demonstrate before (L,N) and after conditioning comparisons of evoked responses to both flashes (L+) and click (N+). Following extinction (L-, N-), potentials return to pretest (L,N) levels. Graphs 1 through 3 plot the peak-to-peak amplitude changes over pretest, conditioning, and extinction trials in all of the animals (e.g., C9, C3) which exhibited the reversible enhancement of potentials in either the auditory (AC) or visual cortex (VC). Tables 1 through 7 present the raw data with variability measures and significance tests between the three treatments or behavioral states: preconditioned, conditioned, and extinguished. Since the variability of the peak-to-peak measures is related to the mean, the average standard deviation ($S/\bar{x}$) is presented as the best estimate of the within-treatment variability. Despite the fact that this, in turn, negates the assumption of equal variance between treatments, t-tests have been computed to indicate significance levels in the absence of a more satisfactory non-parametric statistic.

Recordings were taken from 35 different animals in the course of four experiments; but due to epidemics, which accounted for 13 deaths, and grounding failures, which caused the elimination of 10 others, only 12 animals were under experimental control throughout the three treatments with the opportunity to exhibit evoked potential modification. Of these 12, 5 rats did show evoked potential changes coincident with behavioral shifts. The remaining 7 gave no evidence of evoked potential modification. In these five animals which did show changes, it was often the case that one of the

FIGURE 1
Rat 35 Auditory Cortex
Flash Evoked Responses

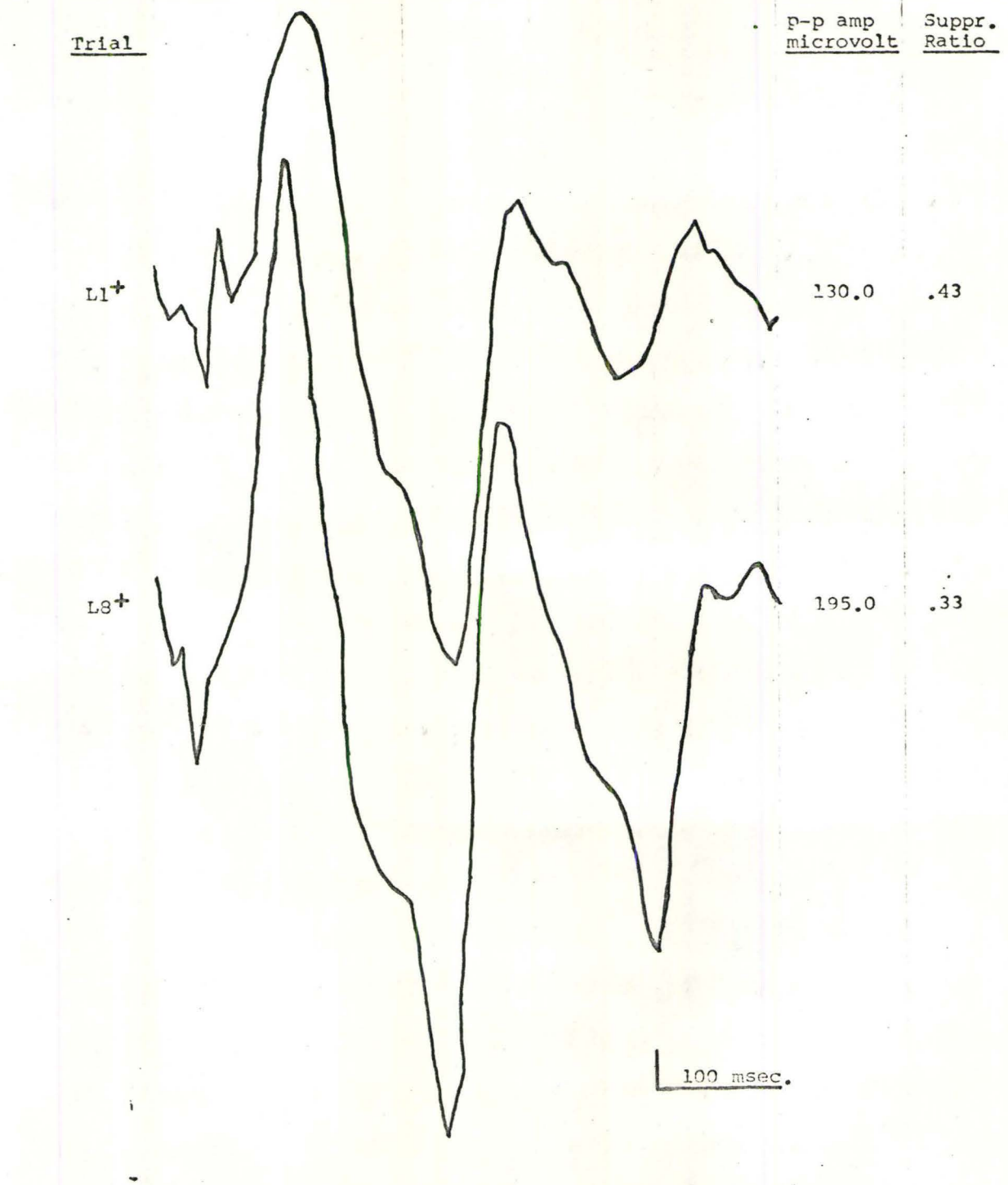


FIGURE 2
Rat C3 Auditory Cortex
Flash Evoked Responses

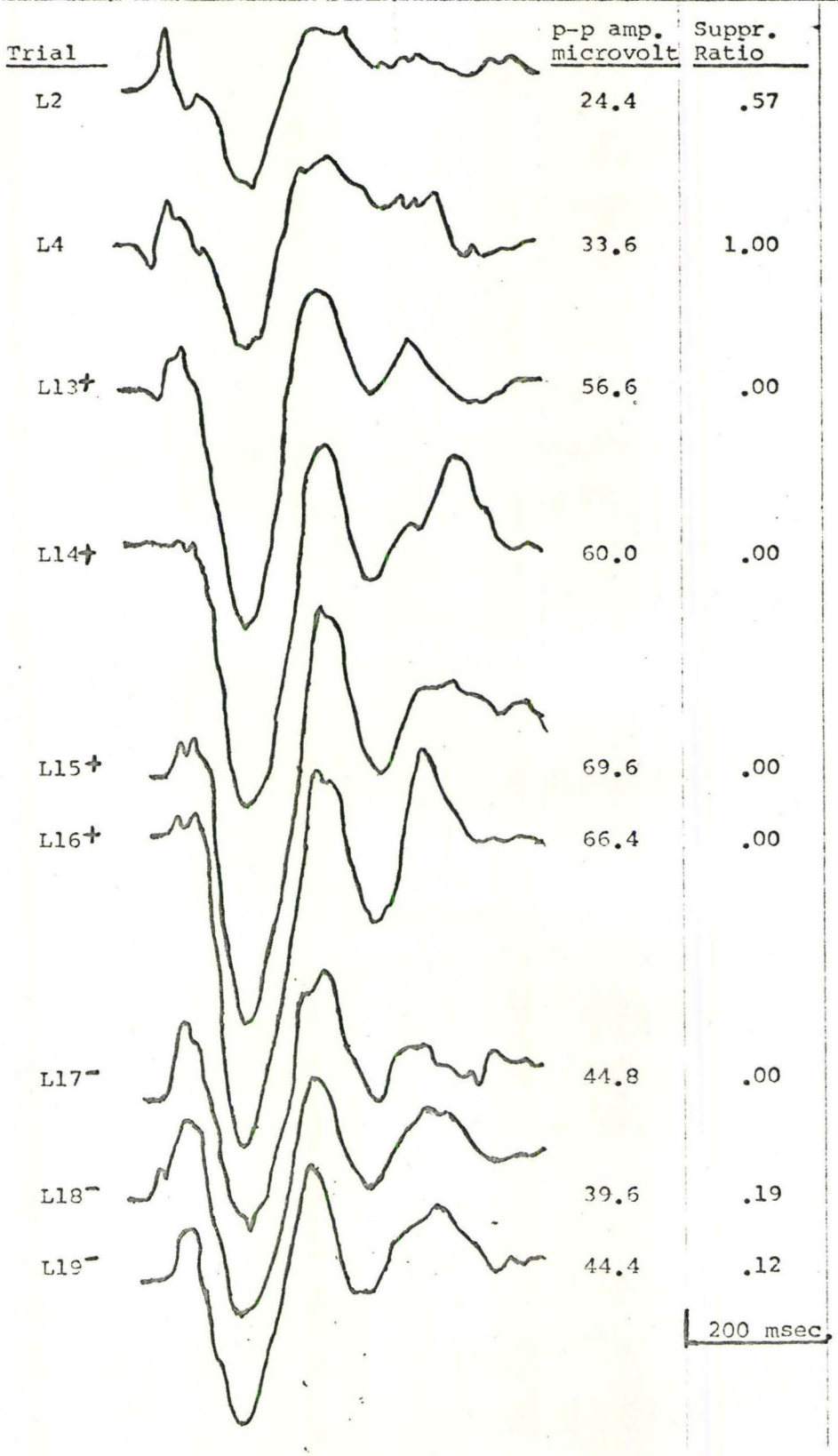


FIGURE 3
Rat C8 Auditory Cortex
Click Evoked Responses

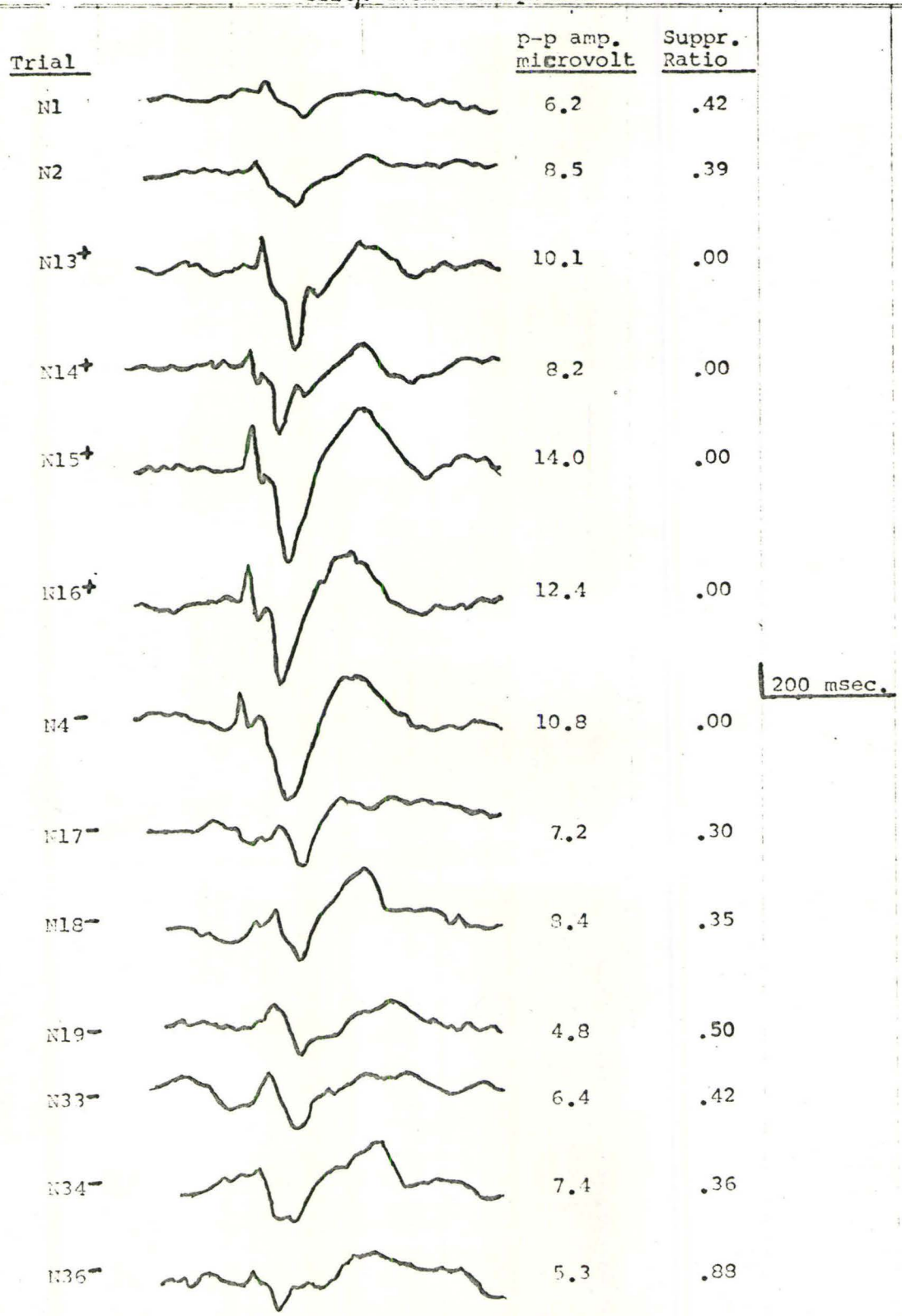
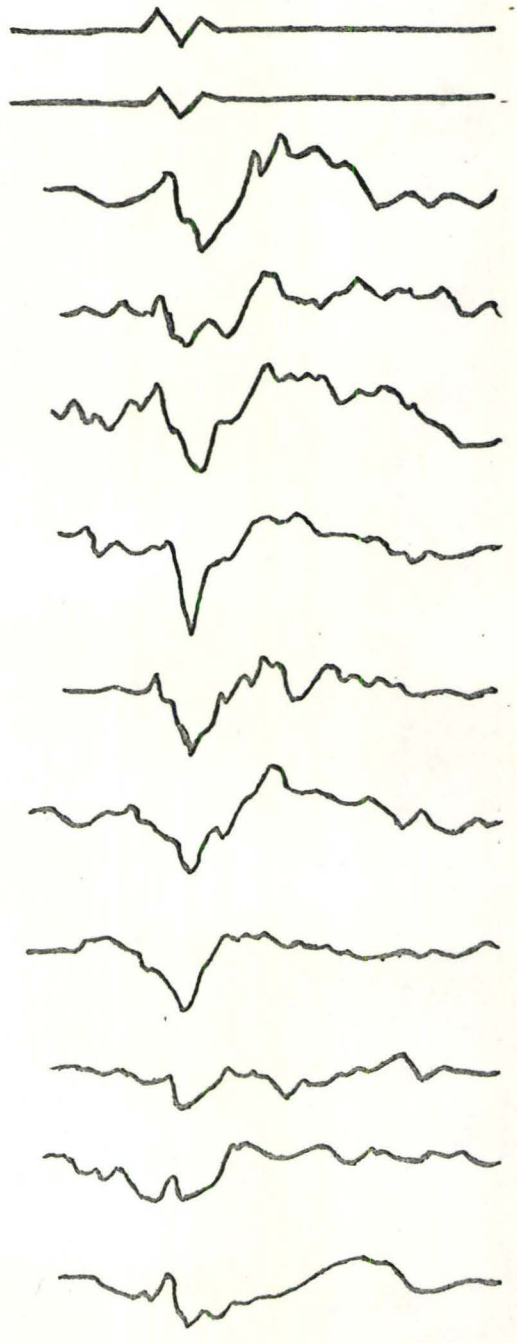
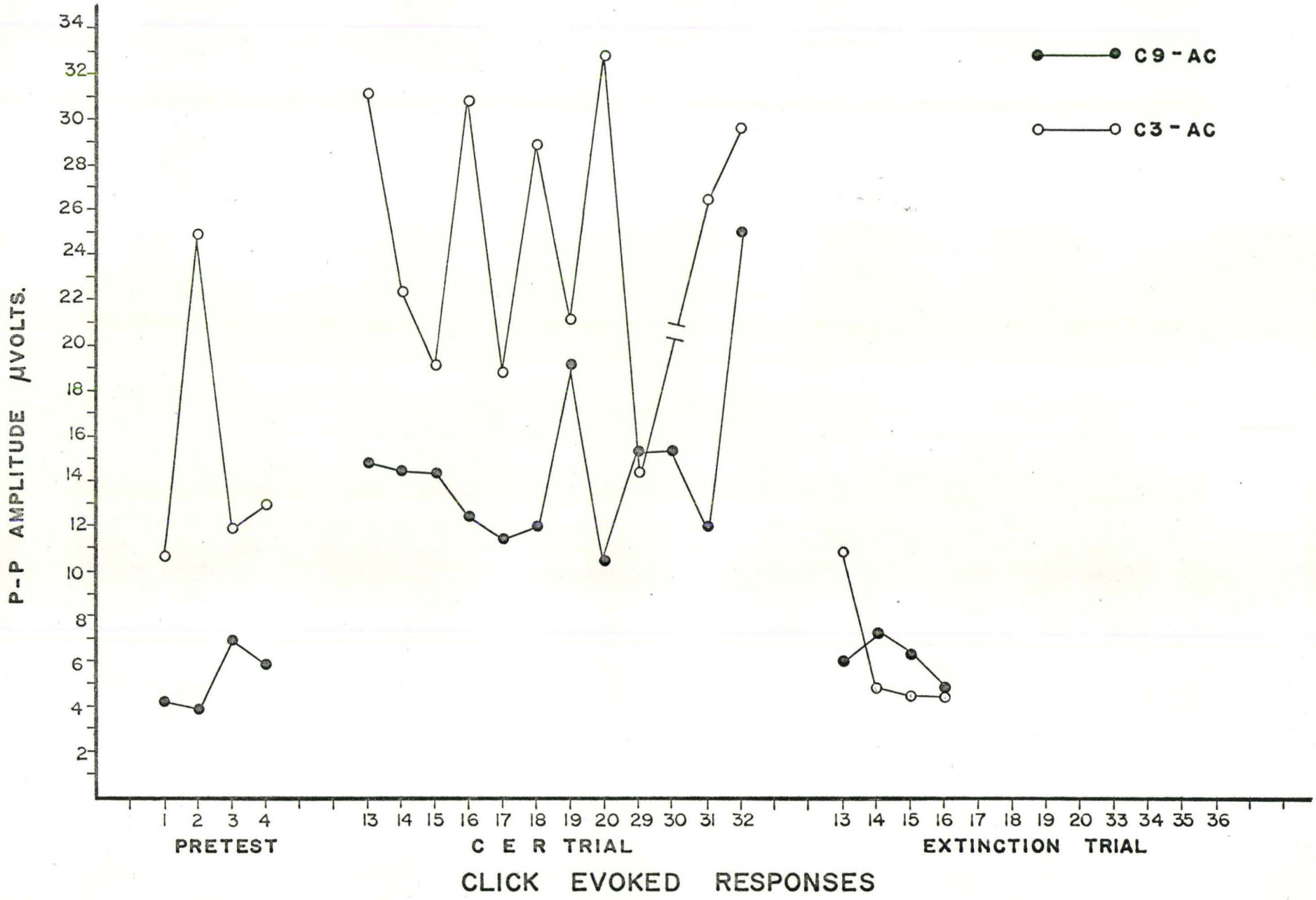


FIGURE 4
Rat C3 Auditory Cortex
Click Evoked Responses



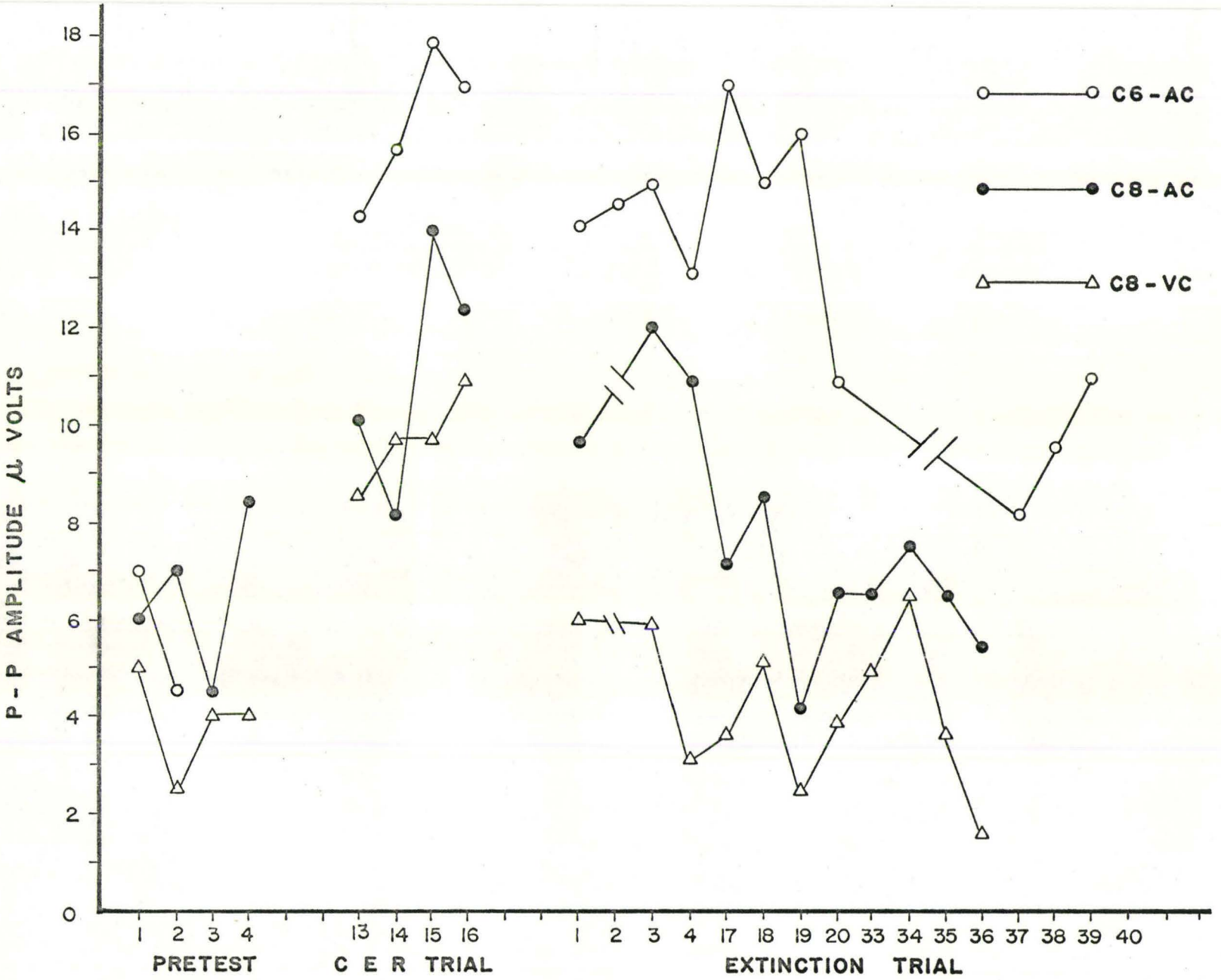
Trial	p-p amp. microvolt	Suppr. Ratio
N1	13.0	.41
N2	12.0	.42
N13+	31.2	.07
N15+	19.2	.37
N16+	30.8	.60
N20+	32.8	.00
N31+	26.4	.14
N32+	29.6	.57
N13-	10.8	.50
N14-	4.8	.56
N15-	4.4	.57
N16-	4.4	.42

200 msec.



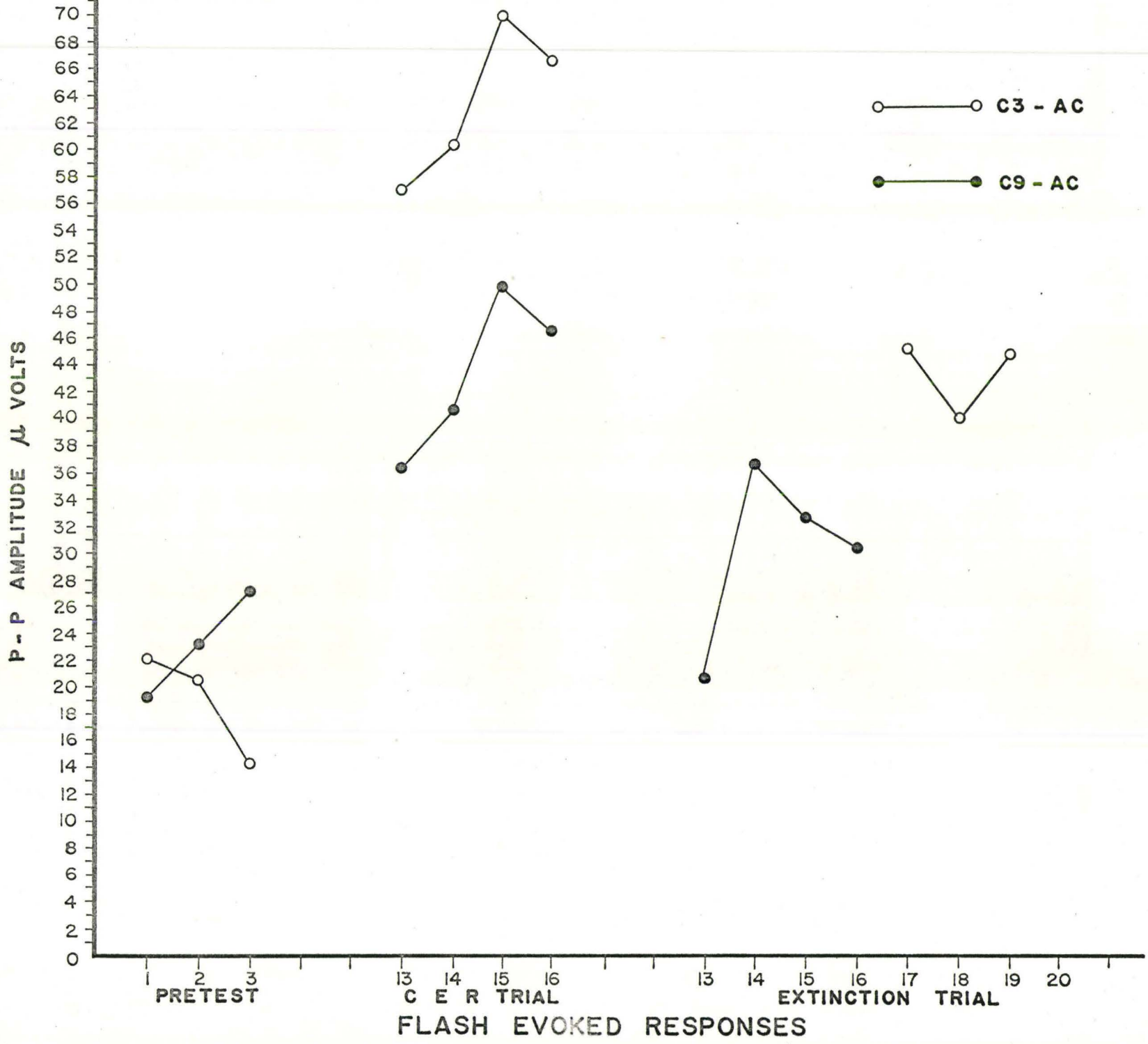
CLICK EVOKED RESPONSES

Graph 1



CLICK EVOKED RESPONSES

Graph 2



FLASH EVOKED RESPONSES

Graph 2

TABLE 1

Rat C3 Auditory Cortex
Click Evoked Responses (microvolts)

Preconditioned	Conditioned	Extinguished
10.7	31.2	10.8
25.0	22.4	4.8
12.0	19.2	4.4
13.0	30.8	4.4
	18.8	
	28.8	
	21.2	
	32.8	
	14.4	
	26.4	
	29.6	
$\bar{x}$ 15.2	25.1	6.1
$\bar{D}$ 4.9	5.3	2.4
s^2 43.8	28.8	9.9
s 6.6	5.3	3.2
$\bar{D}/\bar{x}$ .3224	.2112	.3934
$s/\bar{x}$ .4342	.2112	.5245
	$t = 3.193$	$t = 7.307$
	$p < .01$	$p < .01$

TABLE 2

Rat C9 Auditory Cortex
Click Evoked Responses (microvolts)

Preconditioned	Conditioned	Extinguished
7.0	14.8	6.0
4.2	14.4	7.4
4.4	14.4	6.4
4.0	12.4	4.8
7.0	11.5	
6.0	12.0	
	19.2	
	10.5	
	15.4	
	15.3	
	12.0	
	25.0	
$\bar{x}$ 5.4	14.7	6.2
$\bar{D}$ 1.2	2.7	0.8
s^2 1.7	14.6	1.6
s 1.3	3.8	1.3
$\bar{D}/\bar{x}$ .2222	.1837	.1290
$s/\bar{x}$ .2407	.2585	.2096

$t = 5.705$ $t = 4.292$

$p < .01$ $p < .01$

TABLE 3

Rat C6 Auditory Cortex
Click Evoked Responses (microvolts)

	Preconditioned	Conditioned	Extinguished
	7.0	14.4	8.2
	4.5	15.6	9.4
		17.8	10.8
		16.8	
$\bar{x}$	5.8	16.2	9.5
$\bar{D}$	1.3	1.2	0.9
s^2	1.6	1.6	1.7
s	1.3	1.3	1.3
$\bar{D}/\bar{x}$	.2241	.0741	.0947
$s/\bar{x}$	.2241	.0802	.1368

$t = 7.819$ $t = 5.826$

$p < .001$ $p < .01$

TABLE 4

Rat C8 Auditory Cortex
Click Evoked Responses (microvolts)

	Preconditioned	Conditioned	Extinguished
	8.0	10.1	6.4
	6.0	8.2	7.4
	7.0	14.0	6.4
	4.5	12.4	5.3
	8.5		
$\bar{x}$	6.8	11.2	6.4
$\bar{D}$	1.2	2.0	0.5
s^2	2.1	4.9	0.7
s	1.4	2.2	0.8
$\bar{D}/\bar{x}$	.1765	.1786	.0781
$s/\bar{x}$	.2059	.1964	.1250

$t = 3.165$ $\quad$ $t = 3.529$

$.01 > p > .005$ $\quad$ $p < .01$

TABLE 5

Rat C8 Visual Cortex
Click Evoked Responses (microvolts)

	Preconditioned	Conditioned	Extinguished
	5.0	8.6	4.8
	2.5	9.6	6.4
	4.0	9.6	3.6
	4.0	10.8	1.6
$\bar{x}$	3.9	9.7	4.1
$\bar{D}$	0.7	0.6	2.5
s^2	0.8	0.6	4.1
s	0.9	0.8	2.0
$\bar{s}/\bar{x}$	.2308	.0825	.4878
$D/\bar{x}$	.1795	.0619	.6097

$t = 8.285$ $t = 4.480$

$p < .001$ $p < .01$

TABLE 6

Rat C9 Auditory Cortex
Flash Evoked Responses (microvolts)

Preconditioned	Conditioned	Extinguished
19.0	36.0	20.4
23.0	40.4	36.4
27.0	49.6	32.6
	46.4	30.2
$\bar{x}$ 23.0	43.1	29.9
$\bar{D}$ 2.7	4.9	4.8
s^2 10.7	27.7	35.0
s 3.3	5.3	5.9
$\bar{D}/\bar{x}$ .1174	.1137	.1605
$s/\bar{x}$ .1435	.1230	.1973

$t = 4.938$ t 1.444

$.001 < p < .01$ $.10 > p > .05$

TABLE 7

Rat C3 Auditory Cortex
Flash Evoked Responses (microvolts)

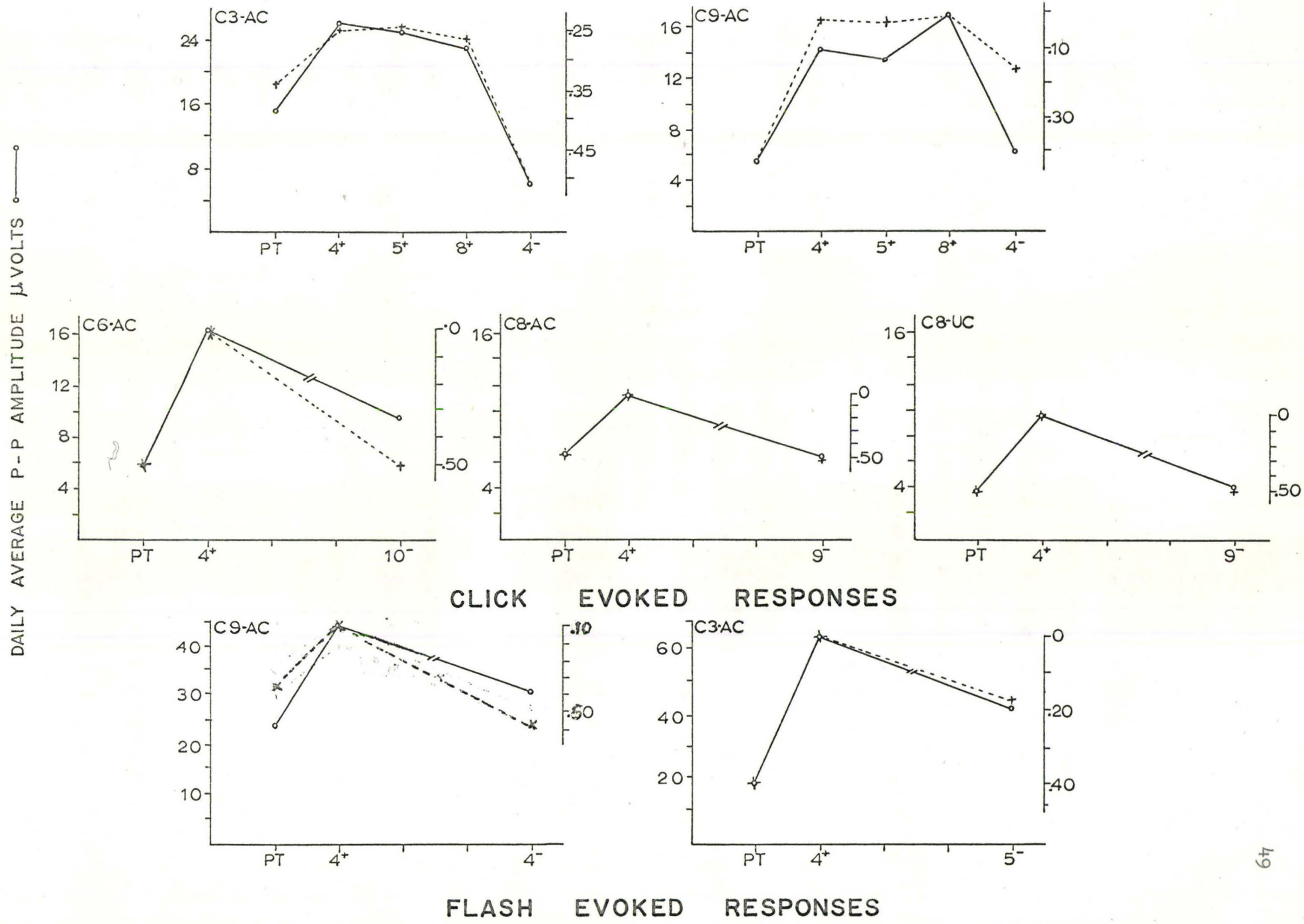
Preconditioned	Conditioned	Extinguished
20.0	56.6	44.8
14.0	60.0	39.6
21.7	69.6	44.4
	66.4	
$\bar{X}$ 18.6	63.2	42.9
$\bar{D}$ 3.0	4.9	2.2
s^2 10.9	26.3	5.6
s 3.3	5.1	2.4
$\bar{D}/\bar{x}$ .1613	.0775	.0513
$s/\bar{x}$ .1774	.0807	.0559

$t = 11.177$ $p < .001$

$t = 5.398$ $p < .01$

two electrode sites revealed evoked potential increases while the other remained unchanged. In addition, evoked response changes to either sensory stimulus was not restricted to its primary receiving area. In fact, all instances of response increases to flash occurred in auditory areas. However, it should be made clear again that in the rat the visual and auditory zones overlap to some degree (e.g., Le Messurier & Woolsey, 1948), and it is likely that some or all of the auditory implants were situated on the fringe of the visual area.

Although these increases are clearly significant, it is not clear to which variable they are most closely related. Figures 2 and 4, for example, contain instances where an enlarged potential is accompanied by no suppression (Figure 4: N16+) or a moderate potential by partial suppression after at least sixteen extinction trials (Figure 2: L17-, L18-, L19-). Other cases, as convincing as these lead to the conclusion that on a trial-by-trial basis the amplitude of the evoked response need not be related to the suppression ratio. It is true, however, that the average suppression ratio over many trials is related to the average evoked potential amplitude (i.e., the average of averaged evoked potentials) for those trials. Graph 4 traces this relationship for all the animals, comparing the average daily suppression ratio to the average evoked response size for that day across the three treatments. In each individual graph, the suppression ratio ordinate has been constructed as an equal-interval scale with the physical distance between high and low data points equal to the physical distance between the high and low data points on the evoked response ordinate.

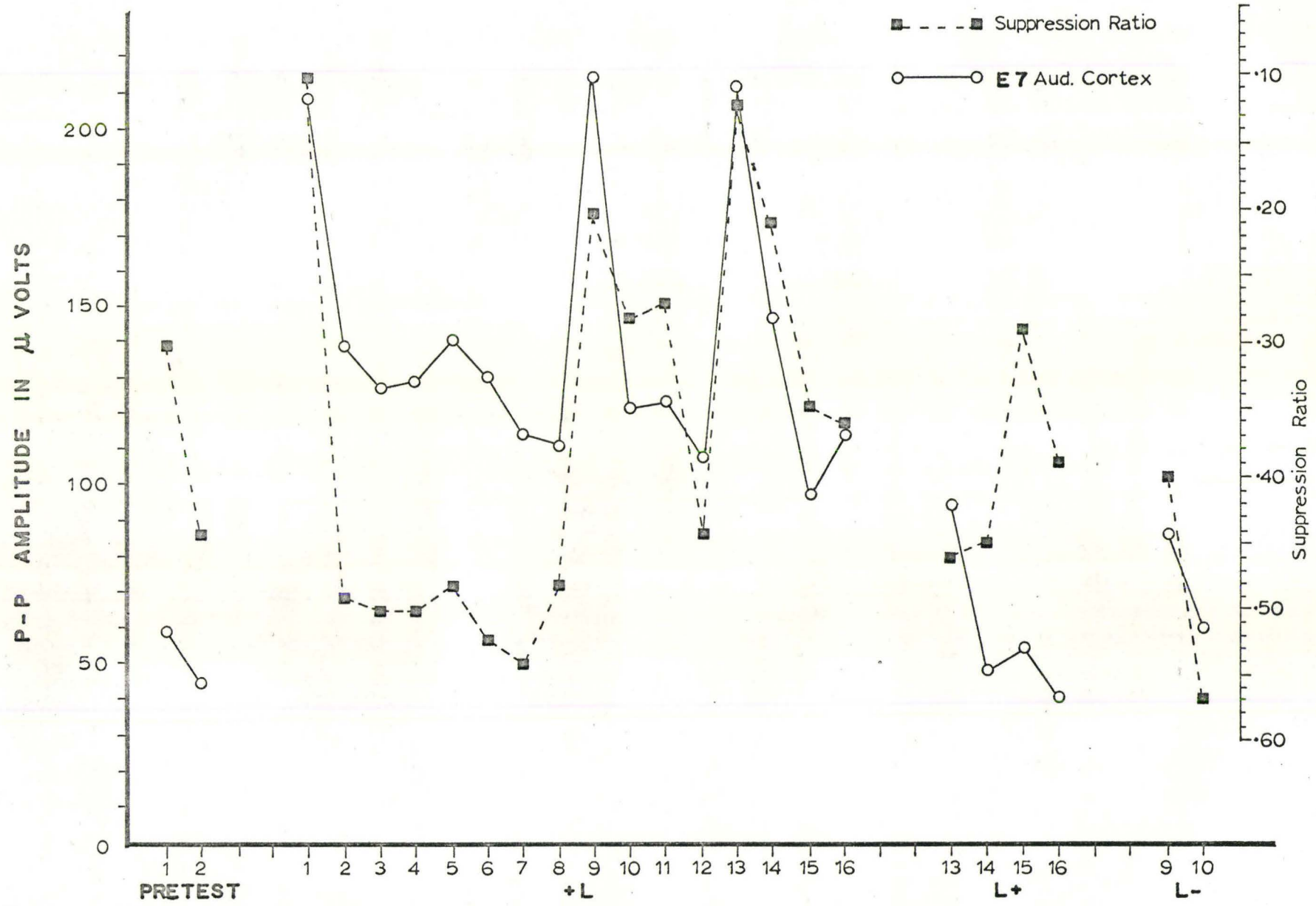

DAILY AVERAGE P-P AMPLITUDE μVOLTS
C3-AC
C9-AC
C6-AC
C8-AC
C8-UC
PT
4+
5+
8+
4-
10-
9-
CLICK EVOKED RESPONSES
C9-AC
C3-AC
FLASH EVOKED RESPONSES

Since the suppression ratio, as calculated, depends on the baseline rate of bar pressing prior to the CS, perhaps the actual number of presses during the CS is a better indication of the fear-arousing properties of the stimulus and, in turn, might be expected to be related to the evoked response size trial-by-trial. However, in this experiment this is not true; rather the number of CS responses is closely related to the suppression ratio and is therefore only related to the evoked potential size in the average sense, as the suppression ratio is shown to be in Graph 4.

In terms of strict classical conditioning, it is also not true that the evoked potential increases with the number of reinforced trials (e.g., Graph 1: N29+, N30+). It is unfortunate that the data available from these experiments are insufficient to trace the course of acquisition or extinction completely. It is not known whether these cortical changes are sudden or incremental. It must be concluded that increases in averaged cortical potentials evoked by a stimulus serving as a CS are related, on the whole, to the presence or absence of conditioned suppression. However, none of the measures of conditioning examined is an ideal predictor of the coincident size of evoked response. This indicates that the process responsible for the potentiation of the evoked response is not necessarily common but may be related to some processes controlled by classical aversive conditioning.

II. Evidence for evoked potential enhancement with backward conditioning.

Graph 5 presents a trial-by-trial measure of the evoked potential magnitude when the evoking stimulus comes after (+L; backward conditioning) and before (L+; forward conditioning) shock. This graph includes the trial-



FLASH EVOKED RESPONSES

Graph 5

by-trial suppression ratios inverted on the ordinate to demonstrate the inverse relation between the potential size and the suppression ratio. Note that on the first trial of days one (+L1), three (+L9), and four (+L13) low post-shock suppression ratios are accompanied by larger evoked responses. But, note also that all post-shock evoked responses are larger than those in pretest or forward conditioning (L+), regardless of suppression ratio.

Of the two animals and four conditional stimuli employed in the backward to forward conditioning paradigm, all four instances revealed retardation in the acquisition of the CER during forward conditioning, with conditioned suppression occurring to clicks on the sixteenth trial for one animal but no suppression in the three other cases after as many as twenty trials of CER training. This indicates that during the backward conditioning the evoking stimulus may have become a discriminative stimulus signaling safety, and that perhaps the evoked potential increases are due to the discriminative properties of the evoking stimulus alone.

However, the close relation between the evoked potential magnitude and the low suppression ratio argues oppositely to a discrimination interpretation, since larger potentials should indicate more safety and higher suppression ratios. Rather, it seems more plausible that the post-shock state of arousal elicits a process or processes in common with the state of conditioned fear present in the CER and probably in communication with the hypothetical potentiation process. At any rate, this single result, not obtained in another animal tested under identical circumstances, clearly demonstrates that evoked potential enhancement can be obtained in the

absence of an evoking stimulus - shock contingency.

III. Evidence from the compound conditioning procedure.

Figures 5 and 6 demonstrate increased responses to flash when that evoking stimulus is added to clicks which serve as the CS producing suppression. Note that on the first compound trial (LN1+) of both figures the averaged cortical potential is not as greatly enhanced as on subsequent trials (LN2+, LN4+). In Figure 5, the trial one (LN1+) potential is only slightly increased, and is accompanied by attenuated suppression. The trial one (LN1+) potential in Figure 6, however, shows a greater increase but still less than the increase seen on the following trials (LN2+, LN4+). Suppression was not attenuated on this first compound trial. When the clicks are removed on test trials to flash alone, the evoked response sizes (Figure 5: L1, L4; Figure 6: L1+) return to normal, pretest levels (e.g., Figure 5: pretest L2) and no suppression occurs, indicating that the flash did not serve as a CS during the compound conditioning trials. However, following trials with the flashes alone employed as the CS the flash responses (Figure 5: L15+; Figure 6: L8+) again increase over the pretest sizes and return to normal sizes following extinction (Figure 5: L16-; Figure 6: L8-).

These results, obtained in the only two animals successfully tested in the "block" design described in the Procedures section, lead to the conclusion that the increases in cortical evoked potentials observed when an evoking stimulus is a CS producing conditioned suppression may also be seen before that stimulus becomes a CS, if the evoking stimulus is presented in the presence of conditioned suppression controlled by any other CS. In short, as regards, these cortical enhancements, the role of the evoking

FIGURE 5
Rat C9 Auditory Cortex
Flash Evoked Responses

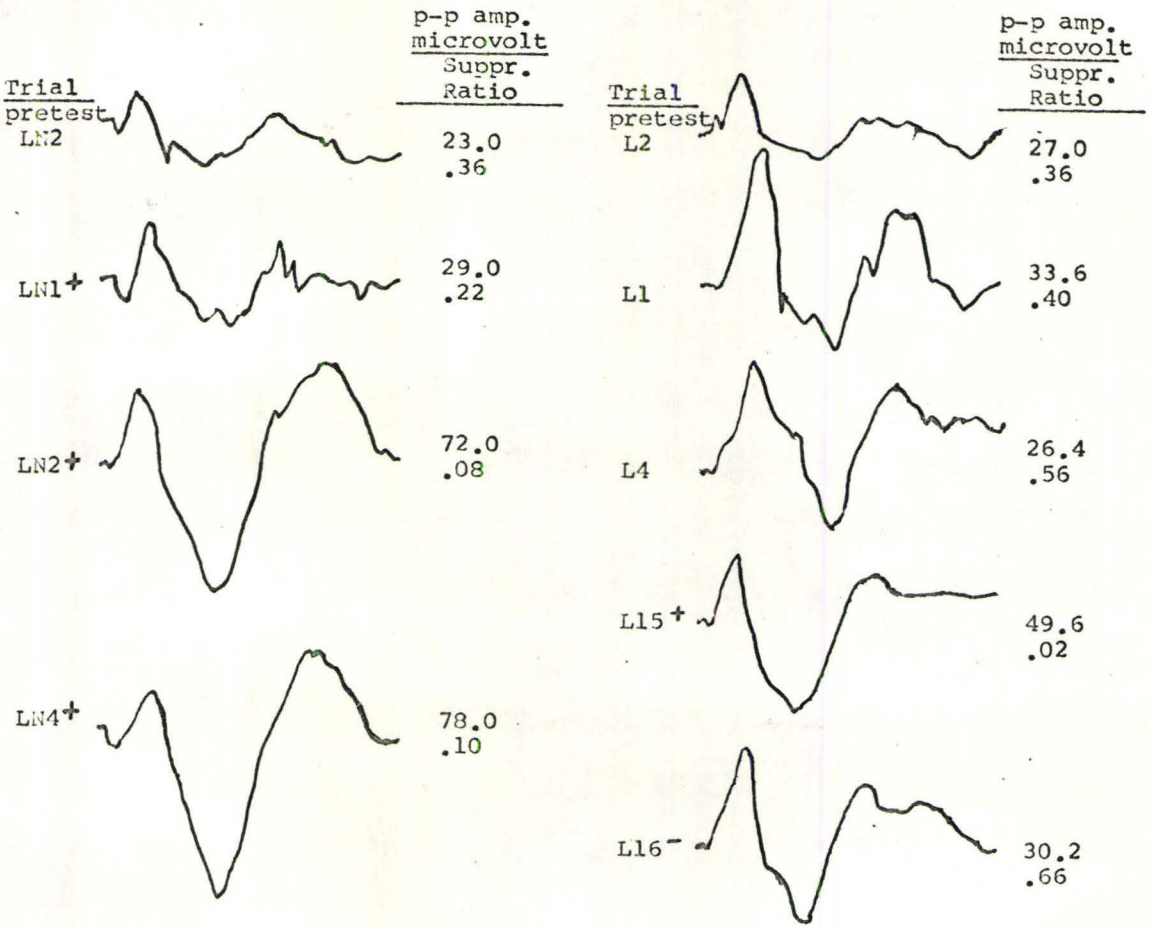
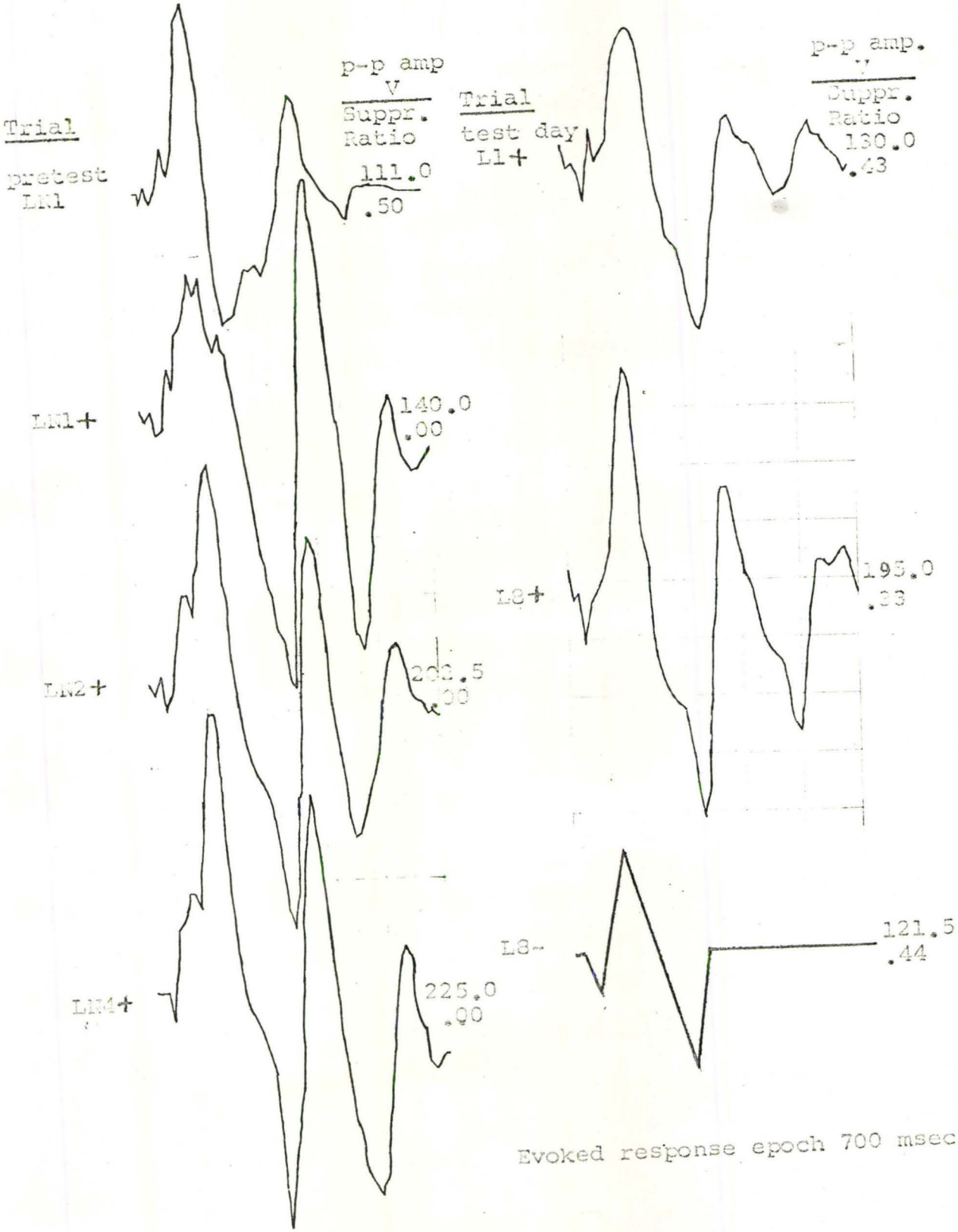


Figure 5. The four waveforms in the left column demonstrate enlargement of averaged evoked potential to flashes which do not serve as the CS during conditioned suppression controlled by clicks, each presented 490 msec. after each flash. Note that on the first trial of the compound conditioning (LN1+) the flash response is not enlarged, and, accordingly, suppression is attenuated. Subsequent test trials with flash alone (right column: L1,L4) reveals that no conditioning occurred to the flash during these compound conditioning trials. Accordingly, the flash evoked responses (L1,L4) remain a magnitude similar to the pretest level (L2) until subsequent conditioning to the flash leads to an increase in the flash evoked potential (L15+). Following classical extinction, the flash response returns to a normal, preconditioned level (L16-). Evoked response epoch 400 msec.

FIGURE 6
Rat B5 Auditory Cortex
Flash Evoked Responses



stimulus as a conditional stimulus is a coincidental one. This finding more fully supports the point made earlier that cortical potentiation may result from a process or processes which have something in common with processes elicited in classical fear conditioning or with painful arousal, as with post-shock suppression.

It is amply clear from these evoked potential results during compound conditioning that the existence of a sensory-receptive block occurring because the clicks would so engage the attention that the interspersed flashes would be ignored is not true. At least neurologically the opposite is true; flash responses increase. Thr flash evoked potential size on trial one is also opposite to the "attention" prediction, which suggested that trial one responsiveness would be increased due to the introduction of a novel stimulus, (i.e., a conditionably neutral stimulus).

IV. Evidence concerning the form and locus of the evoked potential modification.

Figures 7 and 8 exemplify a single before and after conditioning comparison. The identifiable components of the multiple response of the flash evoked potential are labeled a through e. Table 8 lists the peak-to-peak amplitudes of these components. Tables 9 and 10 give similar comparisons of before and after conditioning amplitudes for each identifiable component in two other animals.

Component a, generally considered to represent the afferent input alone, remains strikingly constant. Components b through e, taken to be of cortical origin (Bishop & Clare, 1952), are clearly susceptible to increase with conditioning. This generality alone, that the effect of

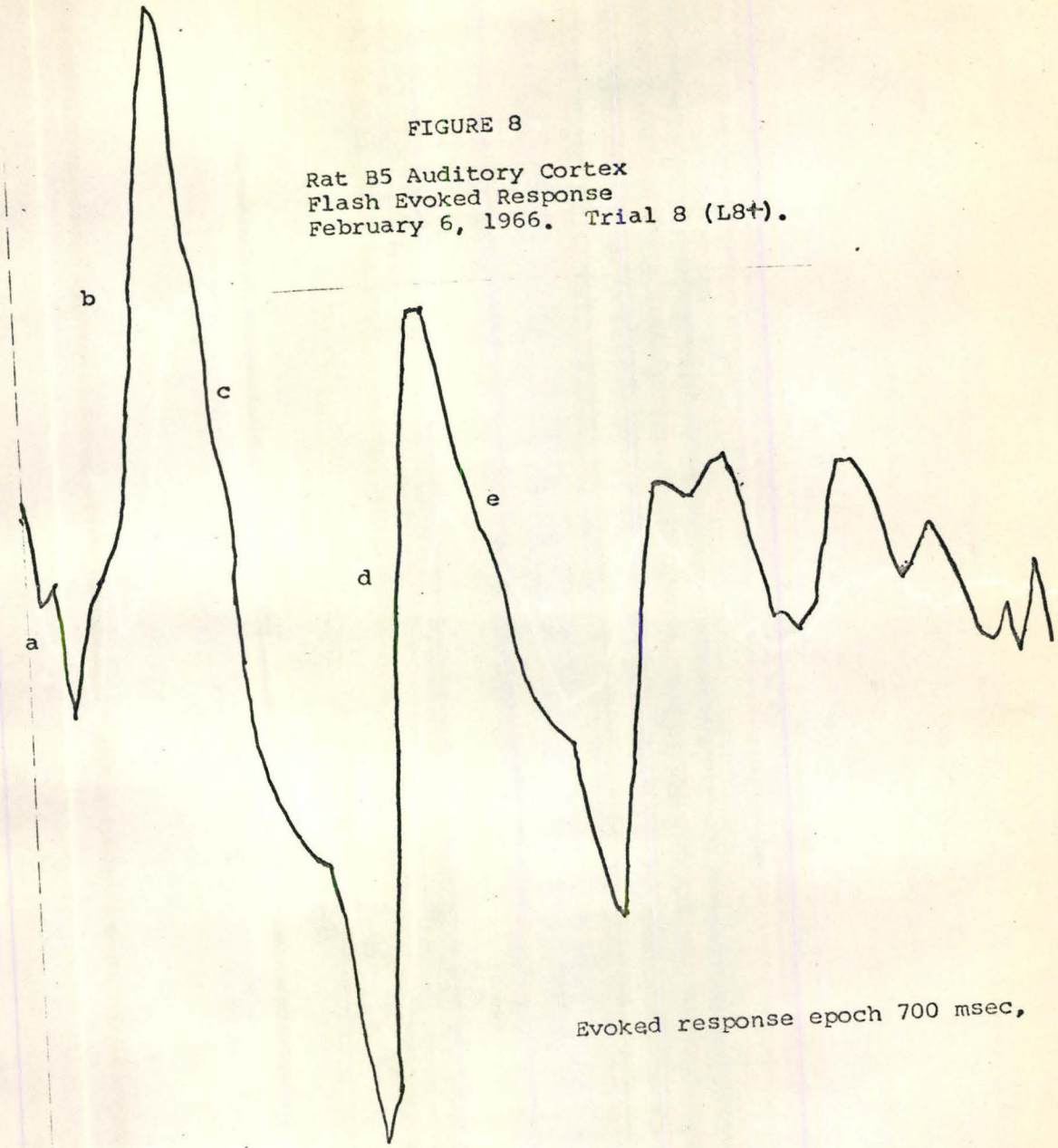
FIGURE 7

Rat B5 Auditory Cortex
Flash Evoked Response
February 5, 1966. Trial 1(L1).

Evoked response epoch 700 msec..

FIGURE 8

Rat B5 Auditory Cortex
Flash Evoked Response
February 6, 1966. Trial 8 (L8+).



Evoked response epoch 700 msec.

TABLE 8

Rat B5 Auditory Cortex
Flash Evoked Responses (microvolts)

Evoked Potential Component	February 5, 1966 L^+ Trial 1 .43	February 6, 1966 L^+ Trial 8 .33
a	18.5	20.0
b	125.0	120.0
c	130.0	195.0
d	92.5	142.5
e	35.0	105.0

TABLE 9

Rat C3 Auditory Cortex
Flash Evoked Responses (microvolts)

Evoked Potential Component	April 23,1966 L^- Trial 2 .57	April 27, 1966 L^+ Trial 15 .00
a	9.2	6.9
b	24.4	48.0
c	24.4	69.6
d	7.2	27.4

TABLE 10

Rat C9 Auditory Cortex
Flash Evoked Responses (microvolts)

Evoked Potential Component	April 23, 1966 L⁻ Trial 4 .56	April 27, 1966 L⁺ Trial 15 .02
a	14.4	13.4
b	26.4	49.6
c	22.6	41.8
d	5.8	5.8

conditioning is on the late intracortical potentials, indicates that the potentiating influence observed during conditioned and post-shock suppression has its effect on the receptive zone of the cortex itself rather than on some stage of the subcortical transmission.

An attempt to find further evidence implicating a drastic alteration in cortical excitability during suppression led to the evaluation of the amount of spindling, a high amplitude synchronous waveform often evoked by photic stimulation and lasting from a fraction of a second to fifteen seconds or longer. However, data from two animals whose rate of spindling was considered high enough for reliable computations revealed no increased or decreased propensity for spindling as a result of conditioning. Table 11 presents these calculations for two conditioning and one extinction day in one of these two animals. The total and mean amount of spindling remains unchanged over the treatments. Although the median duration appears slightly less in extinction than during conditioning, the fact that the other measures of central tendency remain unchanged suggests that the sample size is too small to make this difference between medians a significant one. It is also clear that there is considerable within-treatment variability across trials for the median as well as for other measures. The comparison between conditioning and extinction, rather than a preconditioning comparison, is given because it has been found that the propensity for photically-evoked cortical spindles in the rat increases with the number of stimulus presentations alone (Kimura, 1962). If an increase in spindling was found after conditioning, when compared to preconditioning, it would not be known whether the difference was due to the

TABLE 11

Rat D1 Visual Cortex
Photically Evoked Spindles

June 18, 1966 L+	Trial 17	Trial 18	Trial 19	Trial 20
Suppression Ratio	.07	.52	.14	.23
Number of Spindles	21	17	16	9
Total Spindle Time *	54.5	35.5	57.5	28.5
Mean Duration	2.6	2.1	3.6	3.2
Median Duration	2.0	2.0	2.8	2.5
Modal Duration	2.0	2.0	1.5 2.5	2.0

June 19, 1966 L+	Trial 21	Trial 22	Trial 23	Trial 24
Suppression Ratio	.07	.29	.02	.26
Number of Spindles	27	15	15	12
Total Spindle Time	48.5	31.5	76.5	31.0
Mean Duration	1.8	2.1	5.1	2.6
Median Duration	2.0	2.0	3.0	2.0
Modal Duration	2.0 1.5	1.5	1.5	2.0

July 1, 1966 L-	Trial 21	Trial 22	Trial 23	Trial 24
Suppression Ratio	.30	.35	.37	.50
Number of Spindles	15	17	22	15
Total Spindle Time	28.5	46.0	55.0	46.5
Mean Duration	1.9	2.7	2.5	3.1
Median Duration	1.5	1.5	1.5	2.3
Modal Duration	1.5	1.5	1.5	1.5

* All four measures of duration expressed in seconds.

effect of the treatment or to the mere increase in number of photic stimuli presented.

V. The averaged evoked potential and the electrocorticogram.

Figures 9 through 14 present evoked responses recorded from one animal (Rat E1) during a Prerecord session in which several shifts occurred from wakefulness to drowsiness or sleep, as indicated by the ECoG. In general, during the high voltage slow (HVS) wave activity which characterizes sleep, evoked responses are of greater magnitude and later components of the multiple response more enhanced (e.g., Figures 9, 11, and 12) than during wakeful cortical activation where the low voltage fast (LVF) activity may obliterate (e.g., Figures 9 and 11) or alter the basic waveform of the response (e.g., Figures 12 and 14). Figures 9, 11, 12, and 14 indicate a monotonic relation when potentials are averaged over a partly-awake, partly-asleep period of stimulation.

Although these comparisons are a dramatic illustration of the effect of altered background cortical activity on responsiveness to sensory stimulation, that parameter alone would not be sufficient to explain the increases observed under conditioned or post-shock suppression. First of all, once an animal has been trained to bar press and is maintained at 75 per cent of its normal body weight, the ECoG remains activated (LVF) during the remaining sessions and slow wave activity (HVS) is no longer encountered in the recording chamber. Secondly, it is clear from the before and after conditioning comparisons that the waveform is never altered to any great extent as it is in Figures 12 and 14. And, finally, the increases occurring during slow wave (HVS) sleep are in the wrong direction to allow an

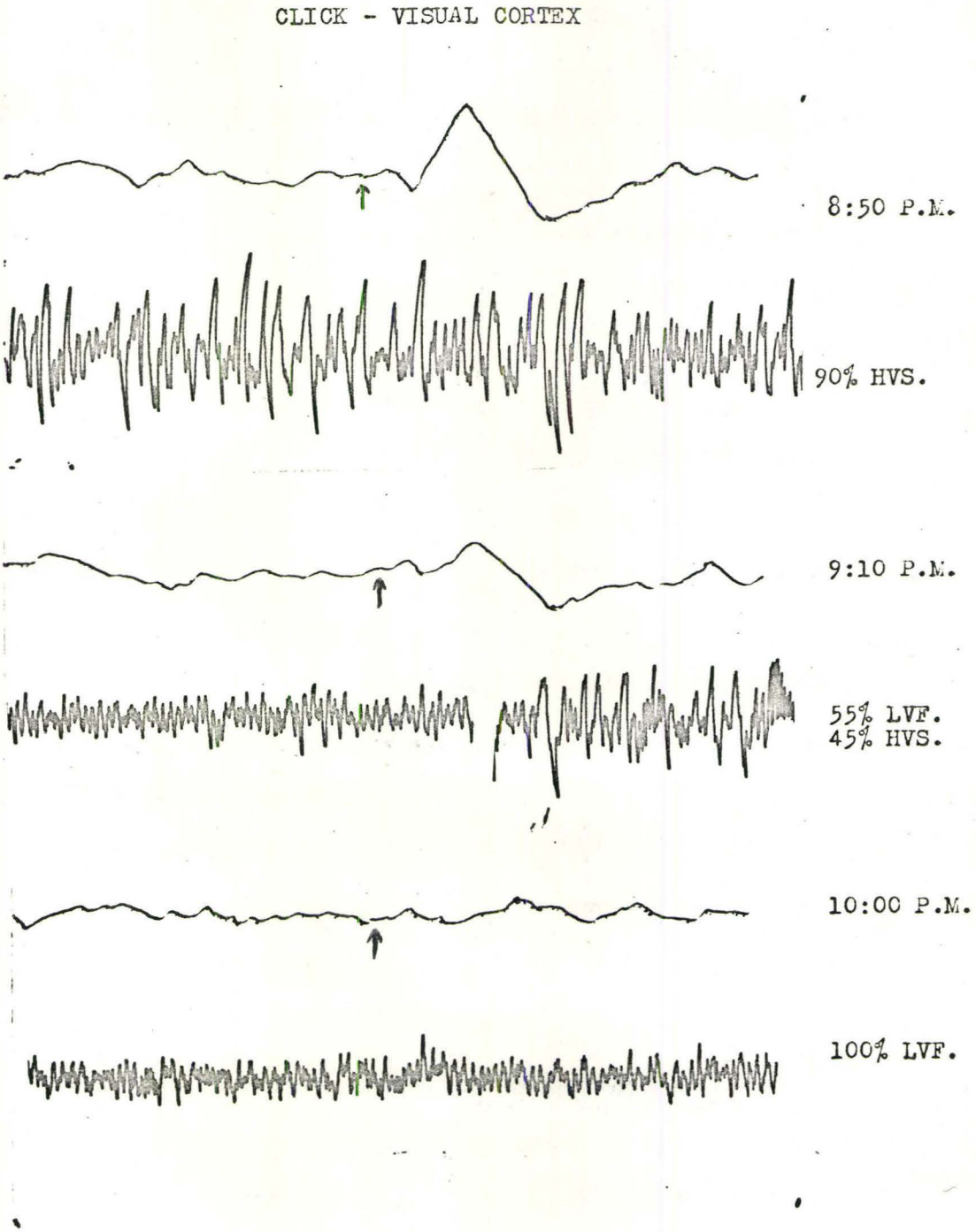


Figure 9 The relation between averaged evoked response and the electrocorticogram. Evoked response epoch one second; stimulus presented at 490 msec. ECoG samples approximately 13 seconds taken from 180 sec. trial.

FLASH - VISUAL CORTEX

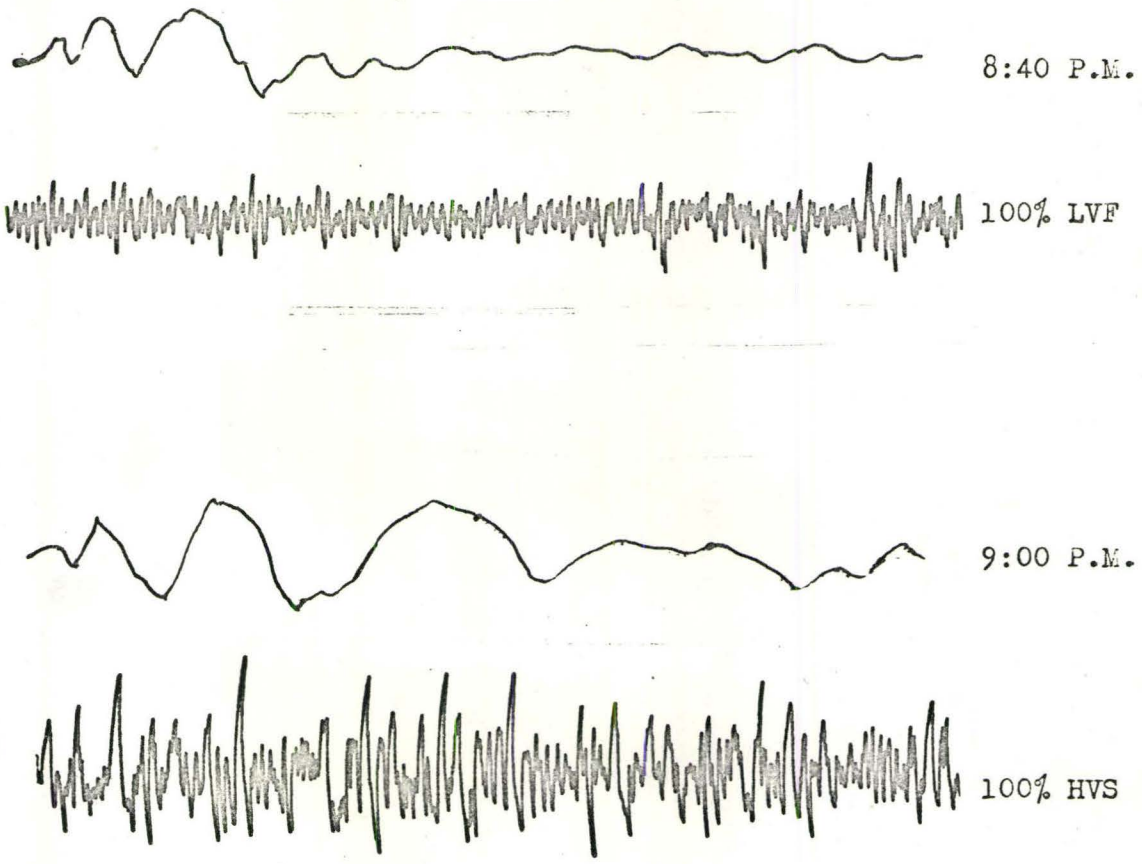


Figure 10 The relation between averaged evoked response and the electrocorticogram. Evoked response epoch one second; stimulus presented at 0 sec. ECoG samples approximately 13 seconds taken from 180 sec. trial.

FLASH/CLICK - VISUAL CORTEX

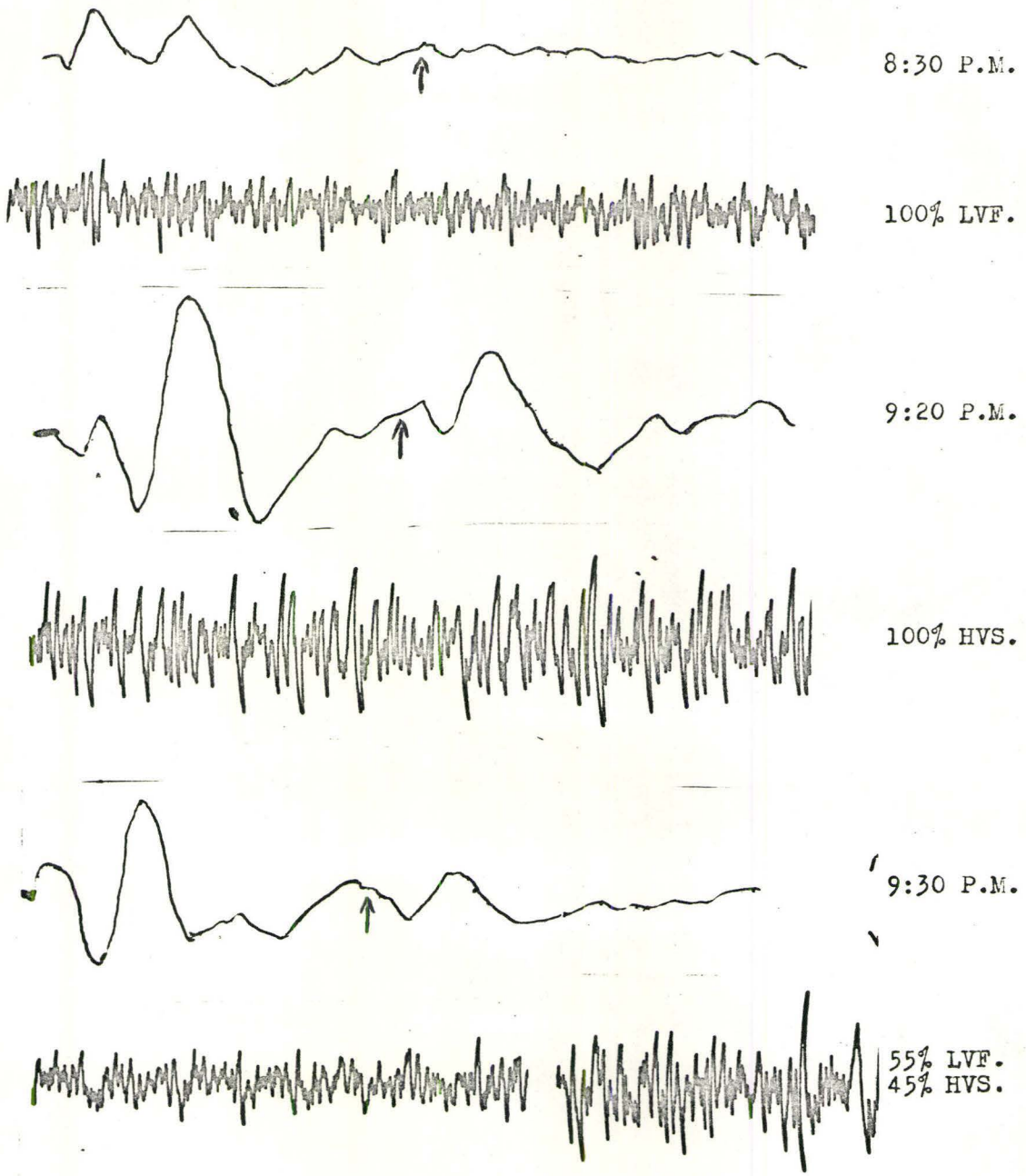


Figure 11 The relation between averaged evoked response and the electrocorticogram. Evoked response epoch one second; flash presented at 0 sec., click at 490 msec. ECoG samples approximately 13 seconds taken from 180 sec. trial.

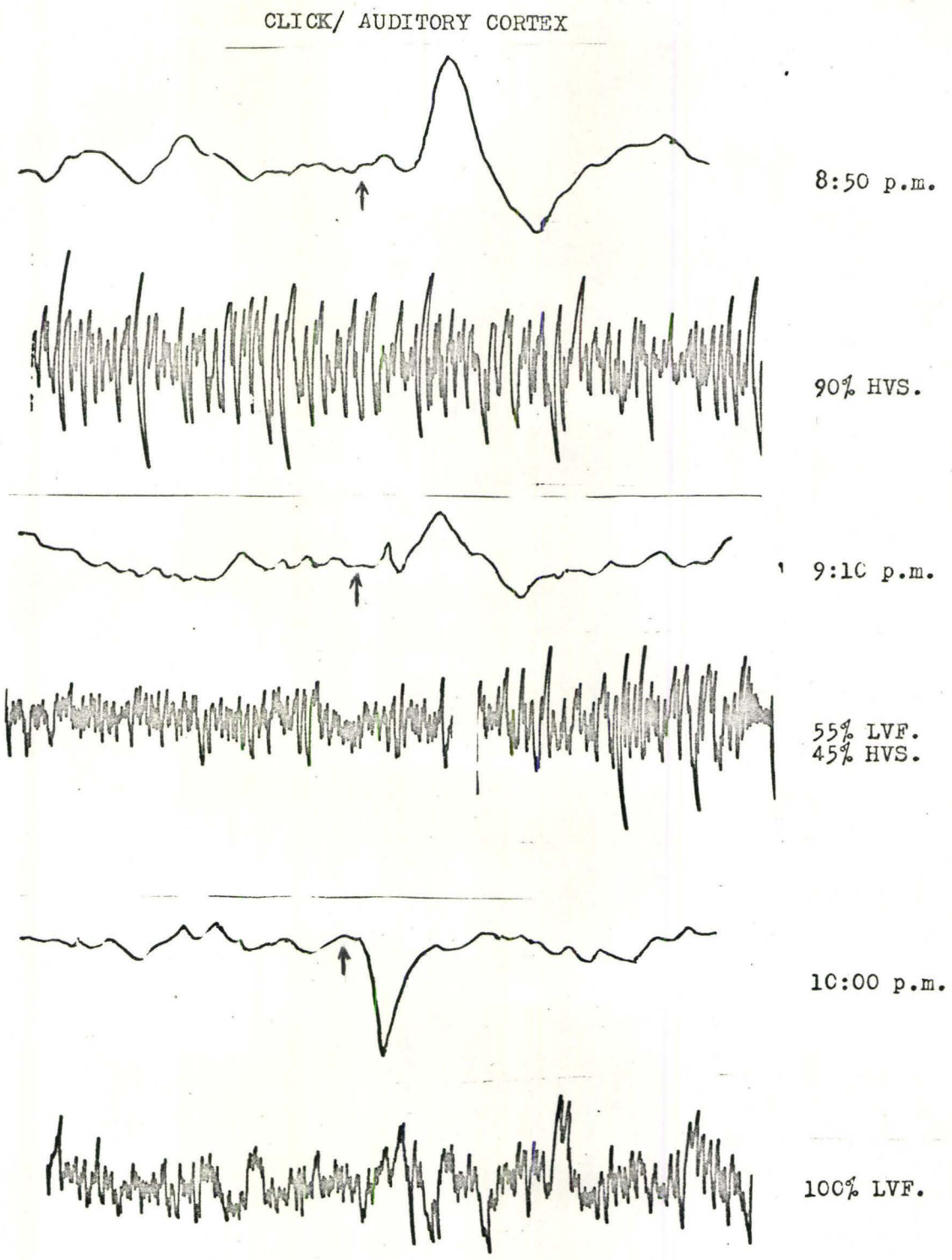


Figure 12 The relation between averaged evoked response and the electrocorticogram. Evoked response epoch one second; stimulus presented at 490 msec. ECoG samples approximately 13 seconds taken from 180 sec. trial.

FLASH / AUDITORY CORTEX

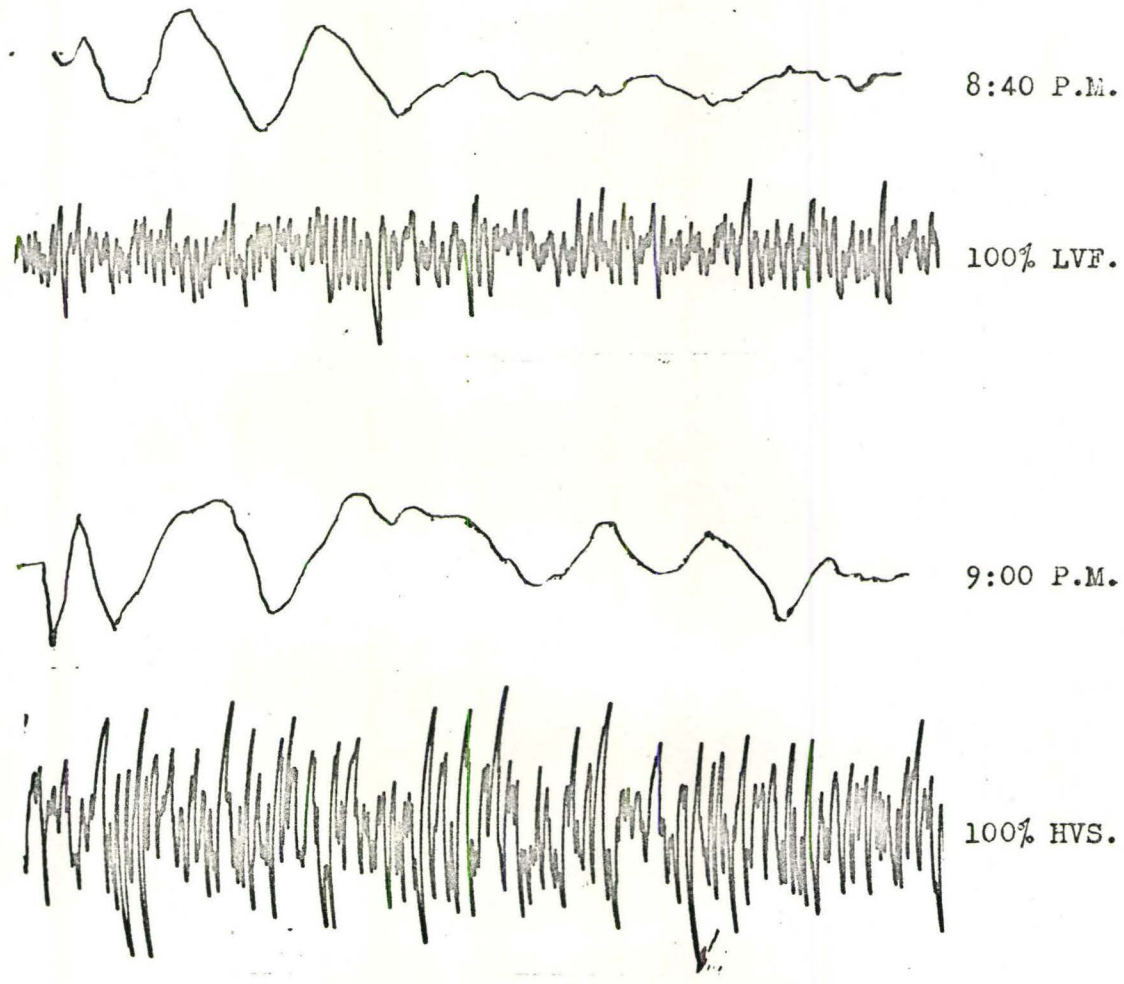


Figure 13 The relation between averaged evoked response and the electrocorticogram. Evoked response epoch one second; stimulus presented at 0 sec. ECoG samples approximately 13 seconds taken from 180 sec. trial.

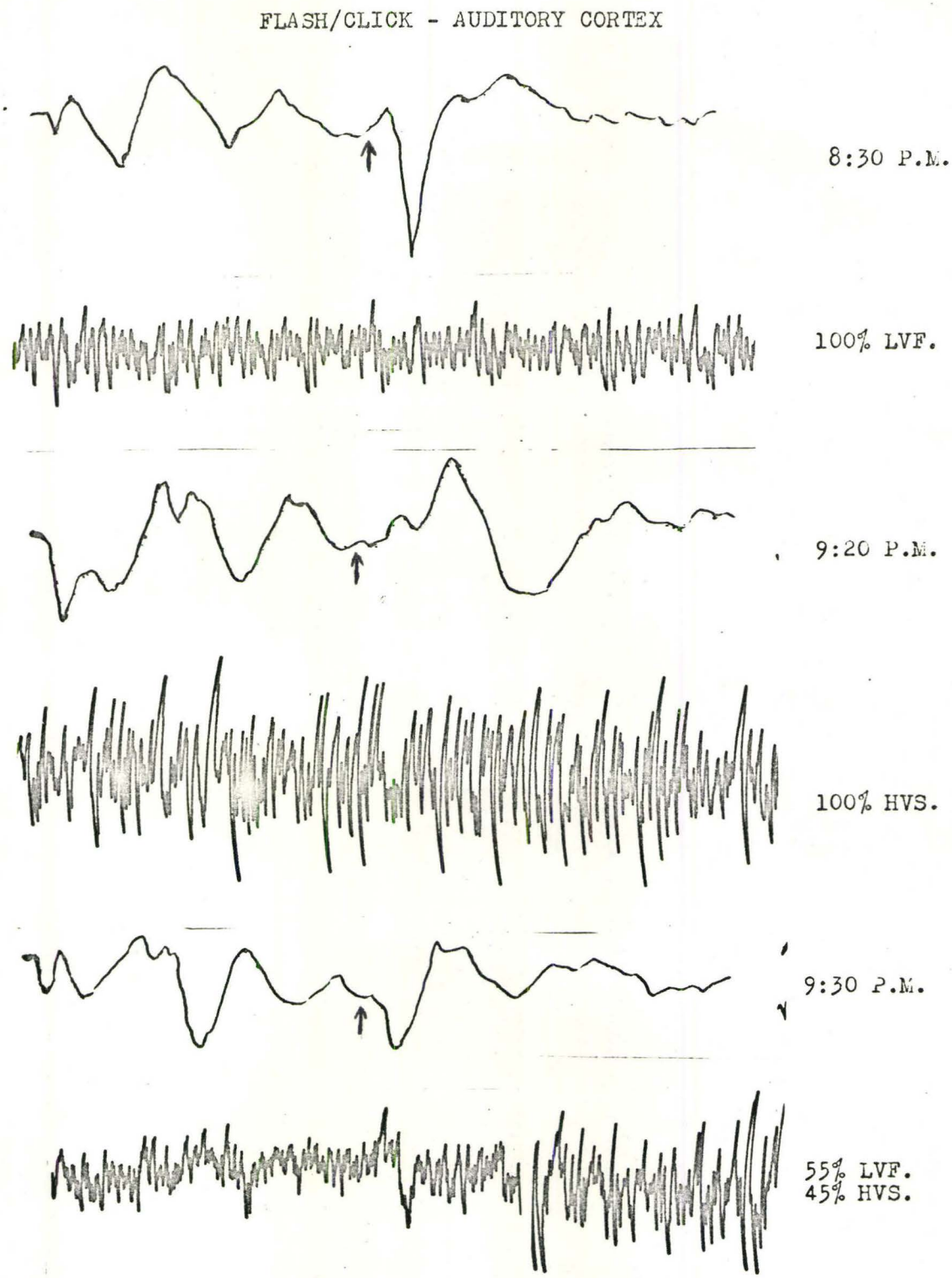


Figure 14 The relation between averaged evoked responses and the electrocorticogram. Evoked response epoch one second; flash presented at 0 sec., click at 490 msec. ECoG samples approximately 13 seconds taken from 180 sec. trial.

explanation of enhanced potentials during suppression in terms of cortical activation alone (LVF).

However, the close relation between background cortical activity and size or form of evoked potential is convincing evidence to suggest that the modifications seen in these various procedures and presumably different behavioral states have their source in cortical behavior alone. But, it is sufficient, at this point, to conclude from the last six figures that enhancement during slow wave ECoG is a condition in which the cortical behavior becomes conducive to super-responsiveness. What relation it bears to the super-responsiveness seen during conditioned or post-shock suppression is not clear. It is clear, however, that evoked response increases due either to a HVS ECoG or to behavioral suppression have two common characteristics:

1. enhanced late potentials, (e.g., Figures 9, 11, and 12; Figure 8), which implicate a cortical locus of effect.

2. diffuse responsiveness, (e.g., Figures 9 and 11; Tables 1, 5, 6, and 7), in which evoked responses to a sensory stimulus become prominent at cortical points distant to the primary projection area.

DISCUSSION

The experiments here reported have demonstrated instances where cortical macropotentials evoked by a neutral intermittent stimulus have become enlarged when that evoking stimulus is:

1. followed by shock for a number of trials, (classical conditioning).

2. preceded by shock (backward conditioning).

3. presented during the performance of an aversively-conditioned response (compound conditioning).

4. accompanied by a high voltage slow wave ECoG.

As was pointed out in the introduction, the goal of studies which relate neural activity and behavior has been to uncover nervous modifications which may reflect information storage. When the physical properties of a conditional stimulus do not change but the behavior and nervous response to it do change covariantly, it is tempting to conclude that this nervous change is a part of the conditioned response, i.e., it is the physical engram or a necessary link in the elicitation of the C.R. This, of course, would be an exaggerated interpretation, since waveforms could scarcely be thought to hold or transmit information; waveshapes merely reflect cellular activity underlying the recording electrodes. A more sober attitude is that these waveforms arise from and represent a distinct patterning of cortical cell activity organized throughout the elaboration of a conditioned response and essential to the persistence of that response. The present observations of evoked potentials increasing after conditioning

(classical conditioning) and returning to normal sizes following extinction support such an interpretation. It is not hard to reason that the evoked cortical response to a stimulus increases as the stimulus plays an increasingly important role in controlling some behavior; the evoked response increases being the reflection of an increasingly non-random patterning developed in the cortical units.

But what is the possibility of these waveforms being changed other than in the course of acquisition or extinction? The evidence obtained from backward and compound conditioning procedures show that similar evoked response increases occur even though the stimulus is clearly irrelevant to the concomitant behavior. In these cases, no informational content can be attributed to the enlarged cortical responses; because the evoking stimulus did not have the status of a signal. Moreover the large potentials seen during high voltage slow waves accompanying sleep or drowsiness is another example of increased responses similar to those seen following the attachment of "meaning" to an evoking stimulus by a conditioning operation. We are left to conclude that if waveform changes really do reflect information processing, then they are not the best neural correlate of that processing, since virtually identical waveform changes may be observed under many and drastically different physiological variations, (e.g., compare conditioned suppression with slow wave sleep). If, however, we concede that the results from backward and compound conditioning suggest an evoking stimulus - shock contingency to be irrelevant and that the cortical potentiation thought to represent conditioning may, instead, <u>result from</u> the effect of conditioning, (i.e., the evoked response enhancement may be a by product of conditioning), then we are forced to consider just what may be a functional denominator

common to these various procedures and behavioral states which gives rise to increased cortical responsiveness.

What seems to be the common denominator in all three of the experimental procedures and during high voltage slow sleep as well is the fact that the appearance of cortical enhancements is invariably accompanied by a distinct change in behavior. With the exception of a few trials in the classically conditioned animals, suppression of bar pressing has been a reasonably good index of the magnitude of the evoked response. That is, while the intermediate values of the suppression ratio can be ambiguous predictors of evoked response size, the extremes of this behavioral index usually relate to the extremes of amplitude variation. This suppression of bar-pressing, moreover, probably represents a drastic decrease in the overall amount of movement by the animal; although it does not, to my knowledge, conform to any "freezing" syndrome in which the animal adopts a rigid posture throughout the presentation of the fearful stimulus. Rather, most rats seem to have a short latency response for getting off their haunches and placing all four feet on the grids. In well-developed conditioned suppression, there is some walking or pacing near the foodcup, animals may groom or lick briefly, and eventually most animals learn to position more body surface across the grids presumably to distribute the current and decrease, to some degree, the effective shock density. In an early experiment, when the shock grids were grounded through high resistances and therefore carried a small A.C. voltage which was picked up by the recording electrodes, it was possible to measure this "density-decreasing" behavior as a trial-by-trial increasing amplitude of A.C. pick-up. The point to be made here is that while it is clear that there is a considerable

gradient of activity between the suppressed and the unsuppressed state, it would be naive to equate suppression of bar pressing with the total absence of movement. Nevertheless, an attempt to uncover causes of evoked potential enhancement in these various procedures will have to consider movement-related variables.

Hall (1964) has completed numerous studies of click potential changes in the rat during the CER and arrived at the conclusion that the observed increases are not due to conditioning per se. In his report he notes some findings of Starr (1964) which show that ".... in the cat, movements of almost any kind may be accompanied by middle ear muscle activity which serves to attenuate the input." However, Hall's recording of eighth nerve potentials fail to show any reliable alterations with conditioning, and this, along with the other control experiments, leads him to conclude that the increases in evoked cortical and sub-cortical potentials are not due to immobility or other systematic movement-related variables, (Hall, 1966: personal comm.). Thompson and Shaw (1965) demonstrated in cats that increased bodily activity is associated with a marked decrease in evoked cortical association area responses. In the same series of experiments, they reported an inverse relation between association cortex responses and degree of behavioral orienting to the evoking stimulus. After habituation of the orienting reflex, associational cortex responses were large and relatively constant. The juxtaposition of these last two findings raises a simple question. If movement, which presumably involves paying <u>less</u> attention to the evoking stimulus, reduces responses, why would orienting toward or paying <u>more</u> attention to a stimulus likewise decrease the response to it? Other

experiments by Thompson and Shaw showed clearly that introduction of any novel stimulus which causes a marked attention-switch always results in decreased evoked responses. The suggestion here is that any transient change of behavioral state results in decreased evoked responsiveness. If this is the case, and it appears to be, then we are again encountering a so-called nervous mechanism which, like the earlier suggestion of information storage in waveforms, is not a very discriminating one and a poor neural correlate for attentiveness or the active state. What these findings do suggest, and it is a point mentioned earlier in the introduction, is that changes in behavioral state or attentiveness may be totally confounded with an alteration in general brain arousal or excitability, without regard for the discriminative properties of the stimulus or the disposition behind the behavior.

Returning to this point of excitability or general arousal which was discussed briefly in the introduction, we can more quickly see that this mechanism, like the other two possibilities discussed so far, offers no improvement in the power of explanation or prediction though it is neurologically closer to reality. We saw from the compound conditioning data that if we regarded enhanced potentials as indicative of "meaning", we were not able to predict the increases to the flash stimulus which was without meaning. The possibility of movement depressing potentials has been discarded by Hall and in these present experiments the number of presses during CS periods has not been closely related to evoked response size. We also note that an attempt to employ an attentional mechanism will meet with differential predictions, since it has been shown that both distraction away from or orienting toward an evoking stimulus will lead to

decreased cortical responses. In a similar manner, regarding evoked response changes as due to alterations in brain excitability allows differential prediction because it does not identify or delimit the physical components responsible for the excitability changes. It is insufficient to say that larger responses represent greater excitability and smaller responses less. But the notion that what we are observing in these instances of cortical response changes is due to only a phasic physiological process coincidentally accompanying the various behavioral states rather than an enduring reorganization of certain nervous structures is a useful one, taking us one step closer to the possible identification of the critical process.

The traditional electrophysiological measure of brain excitability has been the spontaneous rhythm of the E.E.G. Low voltage fast activity has been associated with increased cortical excitation, with a high proportion of cells in the recording area firing rapidly. The high voltage slow ECoG has been thought to be due either to less frequent or more synchronized cell activity. Accordingly, it is well-documented that cortical responses evoked during wakeful cortical activation (LVF) are smaller than when evoked during a synchronized (HVS) ECoG, (e.g., Bremer, 1961; Palestini et al., 1964). The possibility that this occurs because of occlusive phenomena resulting from increased background activity in the LVF ECoG is rendered unlikely by the fact that during wakefulness both the spontaneous and evoked discharges of visual cortex units are smaller than or equal to those recorded during light and activated sleep, (Evarts, 1962). The other possbility is that the occurrence of active inhibition in cortical neurones during wakefulness is depressed during sleep, thereby accounting for the

large amplitude of evoked responses (especially the late cortical components) seen during sleep. However, it has been shown that the depression of responses evoked by exciting peripheral receptors during cortical arousal is replaced by a marked potentiation of the cortical response during E.E.G. activation if evoked centrally by an electrical stimulus applied to the optic nerve or to a specific thalamic nucleus, (Gauthier et al., 1956; Bremer, 1961). In the present experiments, the ECoG has been a paradoxical predictor of evoked response size. Enhancements similar to those seen during slow sleep are seen during continuous cortical activation. In addition, comparison of the ECoG throughout the experiment revealed no changes with different treatments.

Arduini (1958), by recording from the cortical surface with direct coupled amplifiers, observed d.c. shifts negative to an indifferent electrode placed beneath the skin of the temporal side of the head whenever the E.E.G. became activated, either by electrical sensory stimulation or reticular stimulation. Caspers (1961) has found surface-negative d.c. shifts with reference to the nasal bone in the free albino rat during spontaneous movements and sensory stimulation. In the transition from wakefulness to sleep, he observed surface-positive deviations. In 1959 (Caspers, 1961), he noted a correlation between the dendritic potential of the direct cortical response and the d.c. component at the cortical surface. He suggests that the ECoG is apparently produced by the same generator as the epicortical d.c. component and thus may be regarded as an amplitude modulation of the local steady potential, with the degree of polarization in the mass of apical dendrites and/or other elements involved in the production of the direct cortical response playing as essential part

in generating the d.c. component (Caspers, 1961). Accordingly, Caspers has recorded step-wise negative d.c. shifts during locomotion and sensory stimulation ordinally related to the modality, rate of repetitive stimuli, and actual activity phase of the animal. He concludes that "the d.c. component reflects the level of cortical excitations more accurately than the ECoG, in wakeful animals" (Caspers, 1961).

We can now turn to the possibility of the epicortical d.c. potential being a less ambiguous measure of excitability and perhaps fulfilling the role of the functional process common to the various instances we have observed of super-responsiveness. A number of studies have shown that slow potential shifts are related to the occurrence of conditioned responses. Rusinov (1960), for example, reported the appearance of a slow negative wave on the occasion of the first conditioned response to a light which previously did not elicit a d.c. shift. With an increasing number of trials (CS-US pairings), the negative wave irradiated broadly first throughout the occipital and then in the motor area until, after enough trials, the negativity concentrated to more localized portions of the occipital and motor areas. Morrell (1960) was able to condition a negative d.c. shift to a tone, which by itself produced no d.c. alteration, after pairing it for 30 trials with low frequency stimulation of the nucleus center median in the rabbit, which produced a negative d.c. shift. This conditioned d.c. shift was restricted to the hemisphere ipsilateral to the thalamic stimulation and often, though not always, it was associated with desynchronization of the E.E.G. We have noted earlier instances of marked surface-negativity during locomotion and sensory stimulation, and Caspers' finding of surface-positivity during sleep.

Recently, Smith (1966, pers. comm. of unpublished data) has found a positive correlation between the size of cortical response evoked electrically in the cerveau isole cat and the surface positivity of the transcortical d.c. potential. This finding closely relates to the instance of increased evoked response size during slow wave sleep when, according to Caspers' data, the cortex is surface-positive also. Bishop and O'Leary (1950) have shown that applied surface-positive polarization accentuates the negative potential of the evoked response, while surface-negative transcortical polarization accentuates the positive ones. Creutzfeld et al., (1962), using currents of the order of 200-1000 microamps, have shown that surface-positive polarization accelerates both spontaneous and evoked unit firing while surface-negative polarization has the opposite effect. Smith (1966, pers. comm. of unpublished data) has also found that the relative cell spike likelihood decreases with transcortical d.c. negativity and increases with surface-positivity.

While these results relating surface d.c. positivity to increased cortical responses and unit activity seem promising, the fact that conditioned responses and various forms of arousal are reportedly associated with negative d.c. shifts returns us to our original quandry, if we are able to accept the polarity of these various preparations as comparable. There is good reason to question that the selection of an extracranial indifferent point (e.g., the nasal bones) is equivalent to an indifferent electrode situated in the white matter, especially since the latter usually involves a significant injury potential. So while the possbility of a d.c. measure being a suitable index of excitability might result from a re-evaluation of the validity of d.c. polarity, we are at present left with the original

problem, that is to locate a nervous mechanism or electrophysiological measure which is common to such radically different states as slow sleep and conditioned suppression. All of the measures of general brain excitability record opposite electrical events during these two different conditions, but the evoked response measures are quite similar.

If we allow a measure of introspection, we must concede that the induced behavioral states of conditioned (classical and compound conditioning) and unconditioned (backward conditioning) suppression must represent an extreme position on the continuum of general arousal. What the animal is experiencing in these conditions is likely an exaggerated state of nervous activity probably incorporating drastic autonomic changes, hardly comparable to the spontaneous arousal which we identify with the activated E.E.G. It would be difficult indeed to argue that varying degrees of alerting, arousal, or anxiety may be ordinally related to the frequency or voltage characteristics of the ECoG. The spontaneous cortical rhythm of an awake but relaxed animal probably consists of an averaging of thousands of cortical cells firing asynchronously. Increasing the number of active cells would likely result in little if any change in the averaged frequency and amplitude, since a population increase would be ignored by an averaging process. D.C. potential changes probably exhibit a similar limitation in range. It seems a fair appraisal to conclude that with the present level of electrophysiological measures, we are unable to evaluate the behavior of cortical cell populations with much precision. One of the most striking examples of this is the fact that an observed increase in an evoked cortical response may be due to a greater number of cells firings or to improved synchronization of the same number of cells.

Either case (more available cells or better timing) will give rise to larger cortical responses, which are non-random cortical activities. It can be seen, then, that the failure of the search for a common denominator probably lies in the fact that we are ignorant of the existence of some augmenting influence which must supervene during radical states such as have been induced in these experiments. As a final word, it is perhaps wiser if future research were designed to determine the basic neural factors responsible for temporal and intensity characteristics of the evoked response, which, when brought under artificial experimental control, would result in a more useful understanding of the evoked response as a dependent measure as well as the effect of experimental treatments or natural conditions which modify it.

SUMMARY

1. Averaged cortical evoked potential amplitudes may be increased by as much as 250%, with normal variability not exceeding 30%, during the conditioned emotional response in hooded rats, when the evoking stimulus serves as a conditional stimulus (CS) and is unavoidably followed by the unconditional stimulus (US), a shock. Following classical extinction, in which the shock is omitted, the averaged evoked response amplitudes return to normal, pre-conditioned magnitudes. On a trial-by-trial basis, these evoked response changes were found not to be reliably related to the suppression ratio, number of CS presses, or number of reinforced trials. Daily average evoked response sizes were found to be related to daily average suppression ratios.

2. Results from a backward conditioning design, in which the shock precedes the evoking stimulus, indicate that these changes may occur during some unconditioned response to or aftermath of the shock, with the amplitude of evoked response increases being inversely related to the post-shock suppression ratio.

3. The presentation of a neutral, evoking stimulus during conditioned suppression controlled by another stimulus (the CS) results in averaged evoked responses of increased amplitudes comparable to those obtained when that same evoking stimulus is subsequently employed as a CS to produce behavioral suppression.

4. Measurement of individual peak-to-peak excursions in the averaged evoked potential prior to and following conditioning show that the only significant increases that occur are on the late fluctuations, with the primary potentials (considered to represent afferent input) remaining unmodified.

5. Evoked potential modifications during suppression show characteristics similar to evoked responses recorded from a synchronized cortex (i.e., enhanced late potentials, diffuse responsiveness); however, during suppression the ECoG is highly activated and never synchronized.

6. Photically-evoked cortical spindles were found to occur as frequently and last as long during conditioning as during extinction.

7. It is concluded that the observed evoked response changes are not due to the conditioning operation per se but are probably a by product of some physiological alteration coincident and in common with the various behaviors observed.

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