CONDITIONING OF CORTICAL NEURONS

USING ANTIDROMIC ACTIVATION AS THE UNCONDITIONED STIMULUS

by

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(Chairman, Graduate Council)

For Tricia,

Whose belief in me

inspires belief

in myself.

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INTRODUCTION

The idea that learning is due to changes in the efficiency of synaptic transmission is quite old, having been suggested by Tanzi in 1893. While many researchers in the area today would agree with this statement, the details of the processes involved are not yet worked out after three quarters of a century. The hypothetical modifications of neural connections induced by the learning process have been investigated primarily through two methods, which Bures terms static and dynamic (Bures and Buresova, 1970). The static method, first employed by Ricci, Doane and Jasper (1957) and Jasper, Ricci and Doane (1960), involves comparison of neural activity in a naive, untrained animal with that of a trained one. This method suffers from the disadvantage that it cannot differentiate neurons which change their activity during conditioning from those which are unaffected by learning. The neural basis of learning is better investigated by use of the dynamic method, in which the activity of single neurons is examined throughout the conditioning process.

The dynamic method was first applied by Yoshii and Ogura (1960) who studied the activity of reticular neurons in curarized cats during classical conditioning. Acoustic or visual stimuli were used as the conditioned stimuli (CS) and stimulation of the sciatic nerve served as the unconditioned stimulus (US). After 10-40 reinforcements the CS began to elicit increases or decreases in the spike rate of 46% of the neurons studied. The results of Yoshii and Ogura have been replicated many times using a variety of stimuli, both exteroceptive

and interoceptive, as the CS and US. Such studies have examined neuronal activity in the ponto-bulbar reticular formation (Bures & Buresova, 1965), the non-specific thalamus (Kamikawa, McIlwain & Adey, 1964; Hori, Toyohara & Yoshii, 1967) and cortex (Adam, Adey & Porter, 1966; Morrell, 1967; O'Brien & Fox, 1969). These studies all found that a conditioned response could be recorded from individual neurons. However, this by no means indicates that the recorded neuron or its immediate input connections are actively participating in the learning process. For example, the recorded neuron could be an element in a complex neuronal chain and its firing would thus simply reflect changes occurring in areas remote from the recording site.

The authors just cited have made a valuable contribution to our understanding of the neural basis of learning; however, there are several important questions which remain unanswered. Among these are: (1) What is the distribution within the brain of neurons which are directly modified by conditioning procedures? and (2) What is the nature and location of the changes which occur in single neurons during learning? In order to answer these questions we must be able to specify that the observed changes in response of the recorded neurons arise locally and are not projected to the recording site from neurons in other areas of the brain. In effect this means that we must show that our recorded neurons are actively participating in, rather than passively reflecting the elaboration of the conditioned response (CR).

Unfortunately, there are no simple methods of differentiating neurons which actively participate in learning from those which are

passively involved. Olds and his associates have attempted to obtain this type of information by carefully examining the latency of a CR in various areas of the rat brain (Olds, Disterhoff, Segal, Kornblith & Hirsh, 1972) as well as the time in the course of the conditioning sequence when the CR develops (Disterhoff & Olds, 1972; Segal & Olds, 1972; Kornblith & Olds, 1972). The latency criterion assumes that areas in which neurons with the shortest-latency CRs are found can be considered sites of local learning because prior CRs in other brain areas are ruled out. The trials criterion simply states that conditioned responses which appear first in the course of conditioning cannot be secondary to others which arise later in the trial sequence.

When Olds et al. (1972) mapped the rat brain by measuring latencies of conditioned unit responses, very short latency responses (< 20 ms) were found in only 7% of the neurons examined. However, these neurons were widespread throughout the brain, being found in cortex, subiculum, dentate, CA3 field of hippocampus, tegmentum, pontine reticular formation and, most frequently, in posterior thalamus. Areas which did not show short latency responses included the CA1 field of hippocampus, the tectum and the ventral thalamus. These data were not based on a fine enough time analysis to assign precedence to responses occurring closer together than 20 ms. However, if one accepts the assumptions of the latency criterion, they do provide evidence that learning occurs in widely separated areas of the brain.

When the point in the conditioning sequence at which a CR first appeared was determined for various brain areas (Disterhoff & Olds, 1972, Segal & Olds, 1972; Kornblith & Olds, 1972), a slightly

different picture emerged. While responses with short latency were found throughout the brain, the earliest responses in terms of trials were found in the posterior regions of the brain and, in general, more anterior regions began responding progressively later in the sequence. Conditioned responses appeared first in the posterior pons and regions bordering the medial forebrain bundle; second, in the ventral nucleus of the thalamus and the dentate gyrus; third, in the posterior nucleus of the thalamus and basal ganglia. The appearance of a response in all these areas preceded the development of the behavioral conditioned response. Following the appearance of the behavioral response, conditioned unit responses developed in sensory cortex and CA3 of hippocampus, and lastly in motor cortex and CA1 of hippocampus (Kornblith & Olds, 1972). While this research does establish that the conditioned responses recorded in reticular formation and thalamus are not secondary projections of responses in higher centers, it cannot determine whether responses recorded in a given area, such as the cortex, arise independently or are simply a reflection of responses in lower centers. Thus, these experiments leave open the question of whether cortical neurons exhibit local learning changes independently of subcortical input.

One of the most extensive and systematic attempts to demonstrate local neural changes during learning has been carried out by Woody and his associates. In this series of studies both evoked potentials (Woody & Brozek, 1969a; 1969b; Woody, 1970) and unit activity (Woody & Engel, 1972; Woody, Vassilevsky & Engel, 1970; Woody & Yarowsky, 1971; Engel & Woody, 1972) were studied in the facial nucleus and

precruciate cortex of cats. Woody's aim was first to establish the participation of a specified neural circuit in a simple reflex behavior and then to study changes in the circuit as the reflex was conditioned.

In order to accomplish this end, Woody performed a careful study of evoked potential activity in the facial nucleus resulting from a tap to the glabella which produced an unconditioned eye blink (Woody & Brozek, 1969a). Pentobarbital was administered to the subjects in dosages sufficient to abolish the blink response to glabella tap but not to abolish the corneal reflex. The earliest component of the evoked potential recorded from the facial nucleus following glabella tap was found to persist under these conditions. Thus, Woody concluded that: "The persistence of the early component in the absence of an associated blink suggested that the electrophysiological response reflected the input to the facial nucleus rather than the action potential output" (Woody & Brozek, 1969a).

He then demonstrated that conditioning of the eye blink to a click CS resulted in an increased amplitude of the evoked potential elicited by a click in the facial nucleus (Woody & Brozek, 1969b). Extinction was accomplished by presenting both the CS and US but in reverse order (US-CS). Since the response did extinguish under these conditions it is unlikely that it was due to sensitization or pseudoconditioning. Woody (1970) then demonstrated that evoked potentials recorded in coronal-precruciate cortex reflected activity related to production of the conditioned blink. This conclusion was based on the findings that (1) the latency of the cortical response was consistent with conduction through cortex preceding activity in the facial nucleus

and the eye blink muscles, (2) cortical loci at which the largest evoked potentials were recorded were generally the loci at which an eye blink could be elicited by the minimal electrical stimulation and (3) application of KCl to the cortex abolished the conditioned blink but not the unconditioned blink in response to glabella tap.

Having demonstrated that the precruciate cortex participates in the circuit which mediates the conditioned eyeblink, Woody examined the responses of individual neurons in this region to the conditioning procedure. Woody, Vassilevsky and Engel (1970) found that the spike rate of precruciate neurons changed more in response to a click CS in conditioned than in naive cats and that the increased response was found preferentially in neurons which projected to the orbicularis oculi, the muscle which effected the conditioned blink response. This was determined by microstimulation through the recording electrode and recording of EMG activity from the obicularis oculi. In subsequent studies (Engel & Woody, 1972; Woody & Engel, 1972) some of the subjects were trained to blink by pairing a click (CS) with a glabella tap (US) as before and the remaining subjects were trained to twitch their nose by pairing a hiss (CS) with a puff of air to the nose (US). In subjects trained to blink, more neurons were found to respond to the click than the hiss. In subjects trained to twitch their nose, the reverse was true. In addition, the response to click was greater in animals trained with a click CS than in animals trained with the hiss CS. The reverse relationship held for the hiss stimulus.

Woody and Engel (1972) also found, in cats trained to blink, that more neurons projected to the muscles which controlled the eyeblink

than to muscles which produced the nose twitch, but in cats trained to twitch, the highest proportion of neurons projected to nose musculature. Also, and most importantly for a demonstration of local learning, they found changes in the sensitivity of cortical neurons following conditioning that were specifically related to the reflex which had been conditioned. Thus, in cats that were trained to blink, a stimulus of 6.2 ua applied to cells which projected to the orbicularis oculi would elicit an EMG response at the eye, while neurons of nose projection required a stimulus of 9.9 ua to elicit an EMG from nose muscles. In contrast, in cats trained to twitch, the threshold for neurons which projected to nose muscles was 6.2 ua and that for neurons which projected to the orbicularis oculi was 8.6 ua. The differences were significant in both cases. Most convincing of all was the observation that extinction of the CR to one stimulus followed by training with the other produced a reversal in the threshold relationship. This kind of evidence strongly suggests that the cortical neurons recorded by Woody changed during conditioning in ways specifically related to the significance of the applied stimulus. The evidence is very difficult to reconcile with the proposal that changes seen at the cortical level reflect changes in activity projected from elsewhere.

In a subsequent study Woody and Yarowsky (1971) found that electrical stimulation of precruciate cortex could serve as a CS in the blink conditioning paradigm. They found that levels of stimulating current which would not initially elicit an eye blink began to do so after repeated pairing with the glabella tap US. This

lowering of threshold was limited to the area immediately subjacent to the conditioning electrodes and was not found at sites 1.5 mm removed. These data again argue very strongly for the likelihood of local changes in neural responding at the cortical level during conditioning; however, all these studies employ the static method and so are subject to its limitations (Bures & Buresova, 1970).

The amount of stimulating current employed by Woody and Yarowsky (1971) was sufficient to stimulate a rather large population of cells in the vicinity of the stimulating electrode. This increases the difficulty of interpretation since it cannot be known if the effect produced by stimulation was mediated by the recorded neuron. In a series of studies by Bures, Buresova and Gerbrandt this problem was avoided by using such small currents (15-30 nA) that they would not be expected to spread much beyond the site of application (Bures & Buresova, 1965b; 1967a; 1967b; Gerbrandt, Skrebitsky, Buresova & Bures, 1968). In these studies a mild sensory stimulus was used as the CS and electrical stimulation delivered through the recording electrode served as the US. This conditioning procedure produced a modification of the response to the CS in 12% of the cells studied; however, the figure was somewhat higher among cells initially responsive to the CS.

By using such small polarizing currents these investigators localized the area of unconditioned stimulus spread and thus the area of possible convergence between CS and US. For this reason they felt justified in concluding that "the present experiments suggest that conditioning is not necessarily a result of complex co-operation of

large neuronal populations, but that even intracerebral events limited to single cells can induce plastic changes when associated with external stimuli" (Bures & Buresova, 1970).

However, it was unclear in these experiments whether the changes seen were due to conditioning or merely sensitization of the recorded neurons by the polarizing currents. Experiments on the effects of polarization have shown that these results can be produced by non-specific factors not requiring the pairing of the CS and US. These non-specific effects of polarization were first noted by Rusinov (1953) who found that after anodal polarization of the motor cortex, previously ineffective sensory stimuli would induce contralateral limb movements. These results were confirmed and extended by Morrell (1961) using electrophysiological recording techniques. Furthermore, Bindman, Lippold and Redfearn (1962, 1964) found that transcortical anodal polarization induced, in cortical neurons of rats, an increment in the spontaneous firing rate which outlasted the current application for up to several hours. The same effect was found when the polarization was produced intracortically through the recording microelectrode (Bindman, et al., 1962; 1964), the method used by Bures. Evidence of this sort renders the data of Bures and his associates (Bures & Buresova, 1965b; 1967a; 1967b; Gerbrandt et al., 1968) uninterpretable because of their failure to provide controls for the non-specific effects which their US has been shown to be capable of producing. An appropriate control procedure would be to present both the CS and US, but not paired as in the conditioning series.

The localization of the modifications which occur within individual neurons during learning is also an important question in the neurophysiology of learning. This problem, however, has been approached experimentally much less often, though it has aroused considerable interest. The majority of theoreticians have tended to favor the hypothesis that the presynaptic terminal is the site of the changes which occur in neurons during learning. This position is well illustrated by the model presented by Kosower (1972). However, more recently the postsynaptic membrane has been suggested as the site of neural modifications during learning (Huttunen, 1973); a position prompted largely by studies on the nature of the acetylcholine receptor (Feltz & Mallart, 1971). In addition, two studies have recently produced evidence which indicates that the postsynaptic membrane is indeed involved in the neural mediation of learning and memory.

In a series of pharmacological studies on the cholinergic synapse and memory in rats (Deutsch, 1973) it was found that 7-day-old maze habits were blocked by a dose of carbamylcholine which left 3-day-old habits unaffected. Carbamylcholine is a cholinomimetic; it acts on the postsynaptic membrane much like acetylcholine but is not susceptible to destruction by the enzyme acetylcholinesterase. For this reason carbamylcholine can be used to test the sensitivity of the postsynaptic membrane. The results reported by Deutsch (1973) show that the postsynaptic membrane increases in sensitivity to this transmitter analogue from the third to the seventh day following acquisition of a maze habit. The results obtained using carbamylcholine were in essential agreement with those from earlier experiments employing treatment with anti-

cholinesterase (Deutsch, Hamburg & Dahl, 1966; Deutsch & Leibowitz, 1966) although the earlier studies could not distinguish between increased sensitivity of the postsynaptic membrane and increased output of acetylcholine. The use of carbamylcholine, however, did allow this distinction and led Deutsch (1973) to conclude that memories are mediated by changes in the sensitivity of the postsynaptic membrane to acetylcholine rather than by increases in the amount of transmitter emitted at the presynaptic ending. Deutsch's work is impressive and provocative, but the changes in membrane sensitivity which he found occurred from 3-7 days after training. Thus, these changes cannot account for the initial learning or early retention of the maze habit.

The problem was also approached through the use of electrophysiological techniques by Woody and Black-Cleworth (1973). Using cats which had been conditioned to blink in response to a click CS, they classified 221 cells in the coronal-precruciate cortex according to muscle projection on the basis of elicited EMG activity in the facial musculature. After this procedure the cells were tested to determine the amount of intracellularly injected current which was required to initiate a spike discharge in the cell. It was found that the amount of current required to initiate a spike potential was lower in cortical neurons which projected to the target muscle of the conditioned blink response than in adjacent cortical neurons which projected elsewhere. These results are best explained by changes in the sensitivity of the postsynaptic membrane caused by conditioning. In a previous study (Woody, Vassilevsky & Engel, 1970) it was shown that less current (extracellular) was required

to produce activity in cell populations after conditioning than in the naive state or after extinction of the CR. This controls for the possibility that neurons which project to the orbicularis oculi are inherently more excitable than adjacent neurons which project to other muscle systems.

Thus, two recent investigations on the locus of plasticity in neurons, using widely different methodologies, suggested the same conclusion--that the locus of plasticity is the postsynaptic cell. The validity of this conclusion could be tested by attempting to condition neurons by pairing a sensory afferent CS with a US which affected only the postsynaptic cell. The use of such a paradigm would limit convergence of the CS and US to the postsynaptic cell; presynaptic terminals impinging on the cell would not receive input from the US. Since classical conditioning requires pairing of the CS and US, conditioning could occur under these conditions only if it was mediated by the postsynaptic cell.

The experiment described in this report attempted to carry out this test in the following manner. Pyramidal cells in the postcruciate cortex of cats were subjected to a conditioning procedure in which paw shock was used as the CS and stimulation of the pyramidal tract, which produced antidromic activation of the neurons, as the US. Antidromic activation is not transmitted backwards across the synapses of the cells being stimulated. Thus, pairing of the CS and US was limited to the postsynaptic cells. This led to the prediction that a conditioned change in the responses of these cells to the CS would occur only if the conditioning could be mediated within the postsynaptic cells.

There are assumptions implicit in this plan which should be made clear at the outset. It is necessary to assume that: (1) stimulation of the pyramidal tract does not affect cortical pyramidal cells via orthodromic collateral pathways in addition to its direct antidromic path; and (2) depolarization of a neuron does not, in itself, affect the presynaptic terminals impinging on the neuron. Evidence regarding the validity of these assumptions is brought out and evaluated in the discussion.

In addition, the procedure used for US delivery in this experiment avoided passing polarizing currents through the cortical tissue. In this way it was hoped to avoid the problems of non-specific sensitization encountered by Bures (Bures & Buresova, 1973) and so to extend the findings of Woody and Olds concerning the localization of learning changes in the cortex. In order to accomplish this end it was necessary to control for the possibility that changes in the responses of the cortical neurons to the CS simply reflected changes produced by conditioning in subcortical areas. In order to control for this possibility, in the present experiment, evoked potential activity from the centromedian nucleus or the nucleus ventralis posterolateralis of the thalamus was recorded during the conditioning procedure.

AIM OF THIS STUDY

This thesis was designed to provide answers to two questions. First, can the method of Bures and Buresova (1965b; 1967a; 1967b), modified to control for sensitization effects, be used to demonstrate localized conditioned changes in the response of cortical neurons. And, second, can a conclusion be reached regarding the locus of the modifications induced by conditioning within single neurons.

METHODS

Animal preparation

Experiments were performed on 19 acutely prepared cats with operative procedures performed under ether anesthesia. An endotracheal tube was inserted, the saphenous vein was cannulated, and the subject was mounted in a Kopf stereotaxic apparatus. After a complete midline incision in the scalp and retraction of the temporal muscles with a periostial elevator, all cut surfaces and stereotaxic pressure points were infiltrated with procaine. Using stereotaxic coordinates, three burr holes were made in the skull and the recording and stimulating electrodes were lowered into position.

Subsequent to electrode placement, ether anesthesia was terminated and gallamine triethiodide (Flaxedil) administered for immobilization. Artificial ventilation was maintained at 26 strokes per minute with stroke volume adjusted to maintain tracheal CO_2 levels at 3.6-4.0% as monitored on a Godart capnograph. In preparation for the delivery of subcutaneous electric shock, 21 gauge hypodermic needles were introduced into the paw pad region of the right and left hind limbs. Procaine was administered to head muscles and stereotaxic pressure points every 4 hours to maintain local anesthesia. Flaxedil was administered by injection through the cannulated saphenous vein at the rate of 20 mg per hour (one injection per hour).

Recording and stimulating electrodes

The pericruciate cortex was exposed at stereotaxic coordinates of AP +21 and Lateral 2.0. The microelectrodes were either (1) etched stainless steel insect pins, tip 2-5 u or (2) insulated wire electrodes

with a 25 u diameter glass coated platinum alloy wire tip. The microelectrode was guided manually until the tip penetrated the cortex, the point of entry being inside the boundaries of 2 mm lateral from the midline and 2 mm posterior to the cruciate sulcus. Additional adjustment of the microelectrode was accomplished using a hydraulic drive calibrated in microns. The microelectrode was lowered until a single cell spike of appropriate signal-to-noise ratio could be isolated, allowing unequivocal counting of the activity of the cell. A hemostat attached to the temporal muscle was used as a reference for recording.

A concentric bipolar macroelectrode (.01 inch-diameter stainless steel wire in hypodermic stock) was introduced either into the centromedian nucleus of the thalamus or into the nucleus ventralis posterolateralis. The stereotaxic coordinates for the centromedian electrode were: AP: 7.5, L: 2.5, V: 0.5. For the ventralis posterolateralis electrode the coordinates were: AP: 9.5, L: 7.0, V: less than 6.0. The final vertical placement of the electrodes was determined by monitoring the evoked potential elicited by shock to the hind paws.

A concentric bipolar stimulating electrode, slanted 30° from vertical, was introduced into the pyramidal tract just rostral to the decussation (P: -9.0, L: 0.5, V: 0.0). The final vertical placement was set by monitoring antidromic responses in the postcruciate cortex. All macroelectrodes were placed ipsilateral to the cortical microelectrode site.

Recording procedure

The signal from the microelectrode was led through a Bak cathode follower and then amplified and filtered (500 Hz-3Khz) by a Tektronix 122 amplifier. The signal was displayed on an oscilloscope, converted into digital form by a Schmitt trigger and stored on-line by a PDP-12 computer. The signal was constantly monitored on the oscilloscope in order to assure that the trigger level reliably excluded noise.

Evoked potentials from the electrodes in centromedian or ventralis posterolateralis were amplified and filtered (0.2-250 Hz) by Tektronix 122 amplifiers and displayed on an oscilloscope. A signal of 1.0 volt peak-to-peak was recorded on a Sangamo FM magnetic tape recorder and maintained as the data record.

Stimulation apparatus

Peripheral somatic stimuli were delivered to the right or left hind limb by means of 21 gauge hypodermic needles placed subcutaneously in the paw pad region. The shock was delivered by a Devices MK IV isolated stimulator as 3 pulses of 0.2 msec duration at a frequency of 250 Hz. The temporal occurrence of the peripheral stimuli was regulated by a Digitimer crystal clock. The intensity of the shock was adjusted to the minimum value which produced a perceptible muscle twitch in the stimulated paw, generally 5-10 volts. The pyramidal tract stimulation was a 100 msec train of 0.1 msec pulses delivered at a frequency of 200 Hz. The intensity was set to a voltage just above the threshold for a clear antidromic response in the recorded cortical neuron.

A differential trace conditioning paradigm was used to assess changes in cell behavior. The differential paradigm was used because it controls not only for sensitization and pseudoconditioning, but also for momentary changes in the state of the animal such as blood pressure, CO_2 level, electrode movement, etc. In each subject stimulation of one hind paw, selected at random, was paired with the US at a CS-US interval of 550 msec; this stimulus was designated the CS+. Stimulation of the other hind paw was never paired with the US; this stimulus was designated the CS-. Electrical stimulation of the pyramidal tract served as the US. For each cell the CS+ was presented alone for 75 trials (habituation), paired with the US for 225 trials (conditioning), and presented alone again for 75 trials (extinction). The CS- was presented unpaired on 375 trials, interspersed among the CS+ trials in a sequence randomized within a block of 50 trials, so that each cell received a total of 750 trials. The intertrial interval was randomized with a mean of 11 seconds and a range of 8-15 seconds.

Both the sequence of CS+'s and CS-'s and the intertrial intervals were controlled by a mechanical film programmer. Closure of a microswitch on the film programmer triggered a Devices Digitimer which generated a time zero pulse signalling the beginning of a trial to the PDP-12 computer. A second microswitch on the film programmer determined whether the stimulus to be presented was a CS+ or a CS-. A CS was presented 1.0 second after the time zero pulse and, on CS+ trials only, was followed after 550 msec by the US. The PDP-12 computer controlled the course of the experiment, insuring that the proper number of trials was given during habituation, conditioning, and extinction and that the US was presented only during conditioning.

Cortical units which were successfully isolated were tested to determine their response to the CS+, CS-, and US prior to the beginning of conditioning. Cells chosen for conditioning were required to give a response to the CS+ and CS-, although no explicit magnitude or type of response was required. They were also required to show an antidromic discharge in response to pyramidal stimulation with a latency of 2.0 msec or less and to follow stimulation at a minimum frequency of 250-300 Hz.

Data analysis: Evoked potential

Data from the thalamic electrodes were stored in analogue form on magnetic tape by a Sangamo FM tape recorder. Responses to the CS+ and CS- were analyzed independently as follows. The thalamic signals were led into the A/D converters of the PDP-12 computer and the time zero signal was led into the Schmitt trigger of the KW12 clock on the computer. The time zero marker initiated computer sampling. The evoked potential signal was sampled every 2.0 msec following the occurrence of the CS+ or CS- stimulus. Two hundred and fifty-six samples provided the measurements necessary to characterize an evoked potential of 512 msec duration. Average evoked potentials were formed for each block of 25 trials and stored on LINCtape in digital form.

The average evoked potential for each block of 25 trials was examined by integrating the evoked potential waveform, and quantifying the area described by the evoked potential. This permitted an assessment of the overall neural activity which comprised the waveform. The evoked potential in its original form was transformed, without changing the shape, to provide equal positive and negative area around

a zero baseline. The evoked potential waveform was then rectified, giving all positive values, and subsequently integrated. The integrated waveform represents all the area described by the evoked potential waveform which deviates from the mean in either the positive or the negative direction (see Figure 1). Responses to the CS+ and CS- were compared for each block of 25 trials for each cell using this procedure.

Data analysis: Single cell data

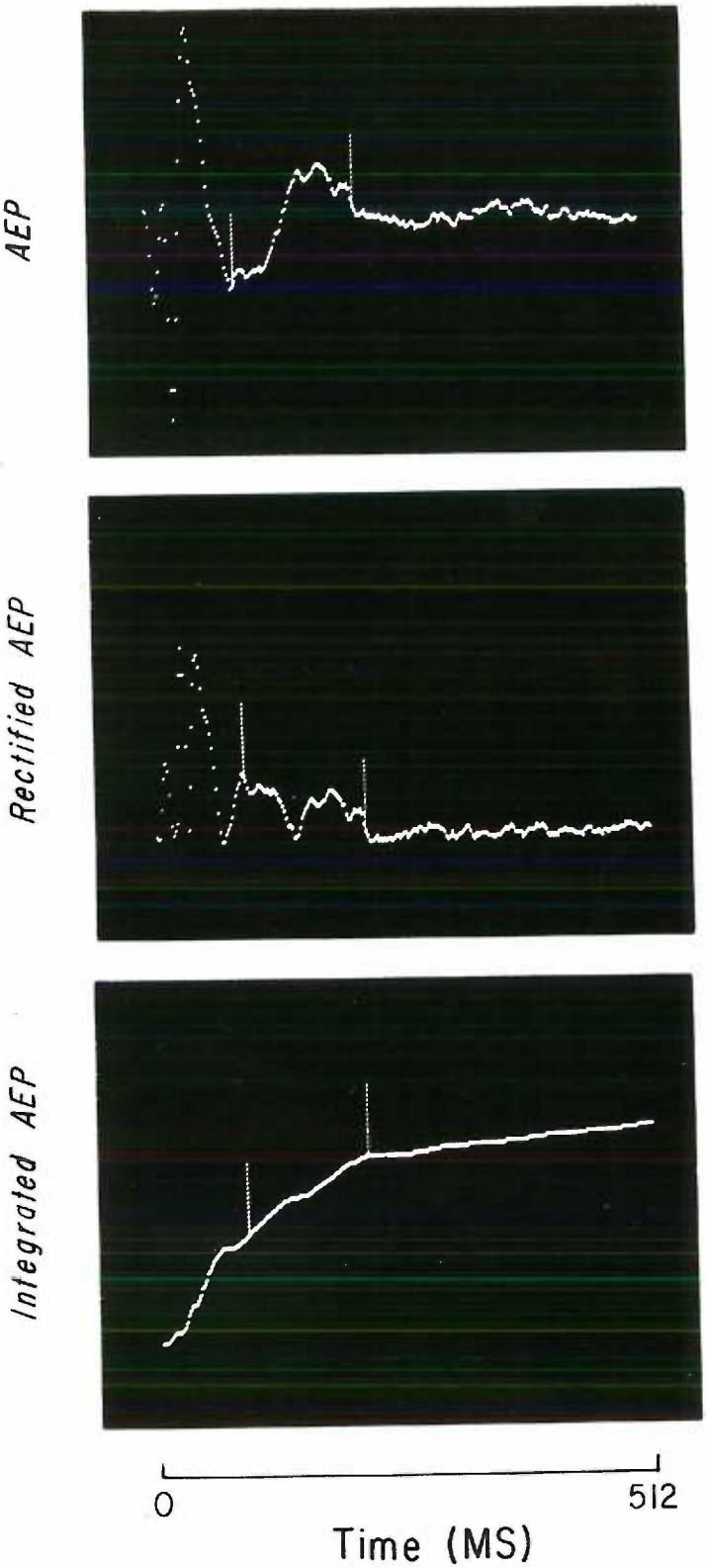
Spike discharges from the cortical microelectrode, converted to Schmitt trigger pulses, were collected trial-by-trial in digital form on magnetic LINCtape by the PDP-12 computer. The data were collected for 3.5 seconds following the time zero pulse on each trial. The first second of this sampling period was spontaneous background or inter-trial activity, the period from 1.0-1.55 seconds was the response to the CS and the period from 1.66-3.5 seconds was the response to the US.

Unit data were also accumulated in blocks of 25 trials by the PDP-12 computer in the form of peri-stimulus histograms, including the 1.0 second period just preceding the presentation of the CS. It was then possible to consider the mean spike rate during any block of trials and during any portion of the response interval as an instantaneous mean firing rate. Thus, the post-stimulus histogram (PSH) is a statement of the probability of the firing of a neuron at a particular latency following the CS presentation. On the basis of these histograms portions of the CS-US interval which appeared to show the greatest change during conditioning were selected independently for both the CS+ and the CS- trials. It was necessary to consider certain periods of the response individually because most of the cells

Figure 1. Area analysis of evoked potentials. This figure illustrates the steps in the area analysis of averaged evoked potentials. The paw stimulus was presented 10 msec prior to the beginning of the displayed trace. The scale of the vertical deflections is arbitrary. The vertical dotted lines are cursors which could be used to measure the duration of particular response components. The averaged evoked potential was summed over 100 presentations of ipsilateral paw stimulus (top). The averaged evoked potential was rectified by using absolute values of difference from baseline (middle). The cumulative values of the rectified averaged evoked potential were summed to give the area under the curve (bottom).

AREA ANALYSIS

Cat 39A, Ipsilateral Paw Stimulus



showed complex post-stimulus histograms, exhibiting both excitatory and inhibitory components. Excitatory components were simply defined as those which exceeded the pre-CS rate and inhibitory components were those which were below the pre-CS rate. Consequently, rather than using an *a priori* interval for analysis of the response, it seemed preferable to select an interval individualized for each cell. This approach is more sensitive to subtle changes since the use of an arbitrary interval could misrepresent the data by equating the presence of an excitatory and an inhibitory response in the same interval with the absence of a response. Response components were visually selected with the criterion of selecting as long an interval as possible without combining excitatory and inhibitory components within the same interval. The selected component could have any location in the CS-US interval and a duration ranging from 50-300 msec.

The mean firing rate of the cell during the first 980 msec of each trial (pre-CS interval) was subtracted from the mean rate during the portion of the CS-US interval selected for analysis. The values thus obtained were divided by the variance of the difference scores to yield a modified student's t-score. These t-scores were computed for each block of 25 trials throughout the experimental sequence and served as the basic unit of quanitification for subsequent statistical analyses.

RESULTS

Unit data: Graphical analysis

Thirty-two neurons were recorded throughout a complete habituation-conditioning-extinction sequence. A graph was prepared for each cell showing its response to the CS+ and CS- (t-score) during each block of 25 trials throughout the trial sequence. Examples of these individual cell graphs may be seen in the upper portion of Figure 2 and Figure 3. These graphs were used to determine whether the response of a cell followed the pattern seen in traditional classical conditioning studies. Based on an evaluation of these graphs the neurons were divided into a group of 24 cells which were judged to show good conditioning and a group of 8 cells which were judged to exhibit poor conditioning. Cells were classified as showing poor conditioning because of (1) failure to show a developmental response increment during conditioning, or (2) failure to exhibit extinction, or (3) lack of differentiation between the response to the CS+ and CS-. All statistical tests and grouped data curves, however, include the data from the entire group of 32 cells.

Unit data: Habituation

An analysis of variance performed on the unit responses to the conditioned stimuli during habituation indicated that the response magnitude did not change across the 75 habituation trials (F = .15, df = 2/155). A further examination of the data revealed that this result was due to the fact that 15 cells decreased their response to the CS during habituation while the responses of 14 cells increased in magnitude. Three cells showed no orderly change in response.

Figure 2. Changes in response to the CS over trials. Top: Response of single neuron recorded from the postcruciate cortex. The t-score response measure is a mean rate difference score (post-CS minus pre-CS, divided by the variance). The CS-US analysis period in milliseconds is shown in parentheses (CS occurs at 1000). Bottom: Area of the averaged evoked response recorded from the centromedian nucleus of the thalamus. Note that the cortical neuron shows differential conditioning to the CS+ and CS- whereas the centromedian response does not.

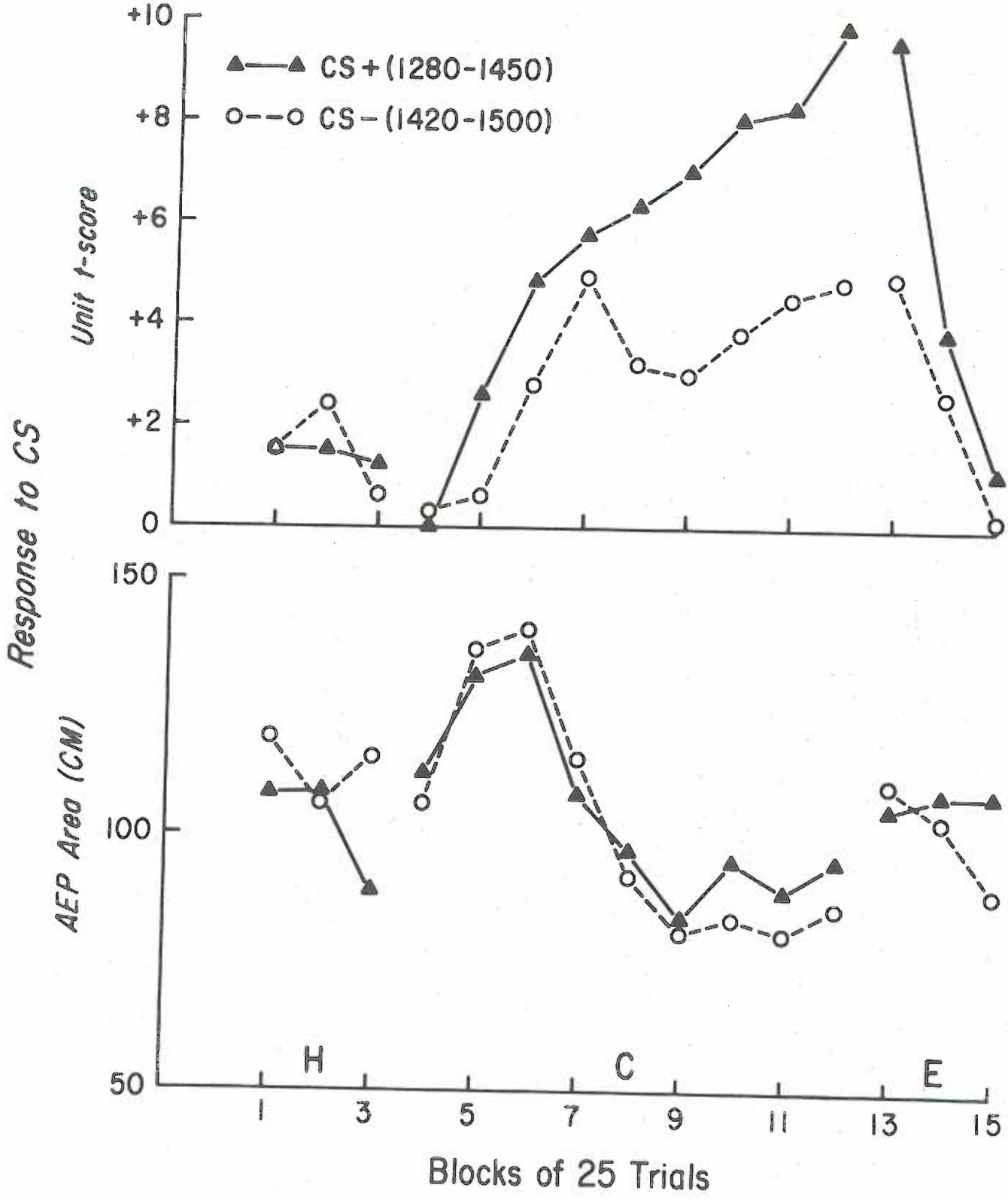
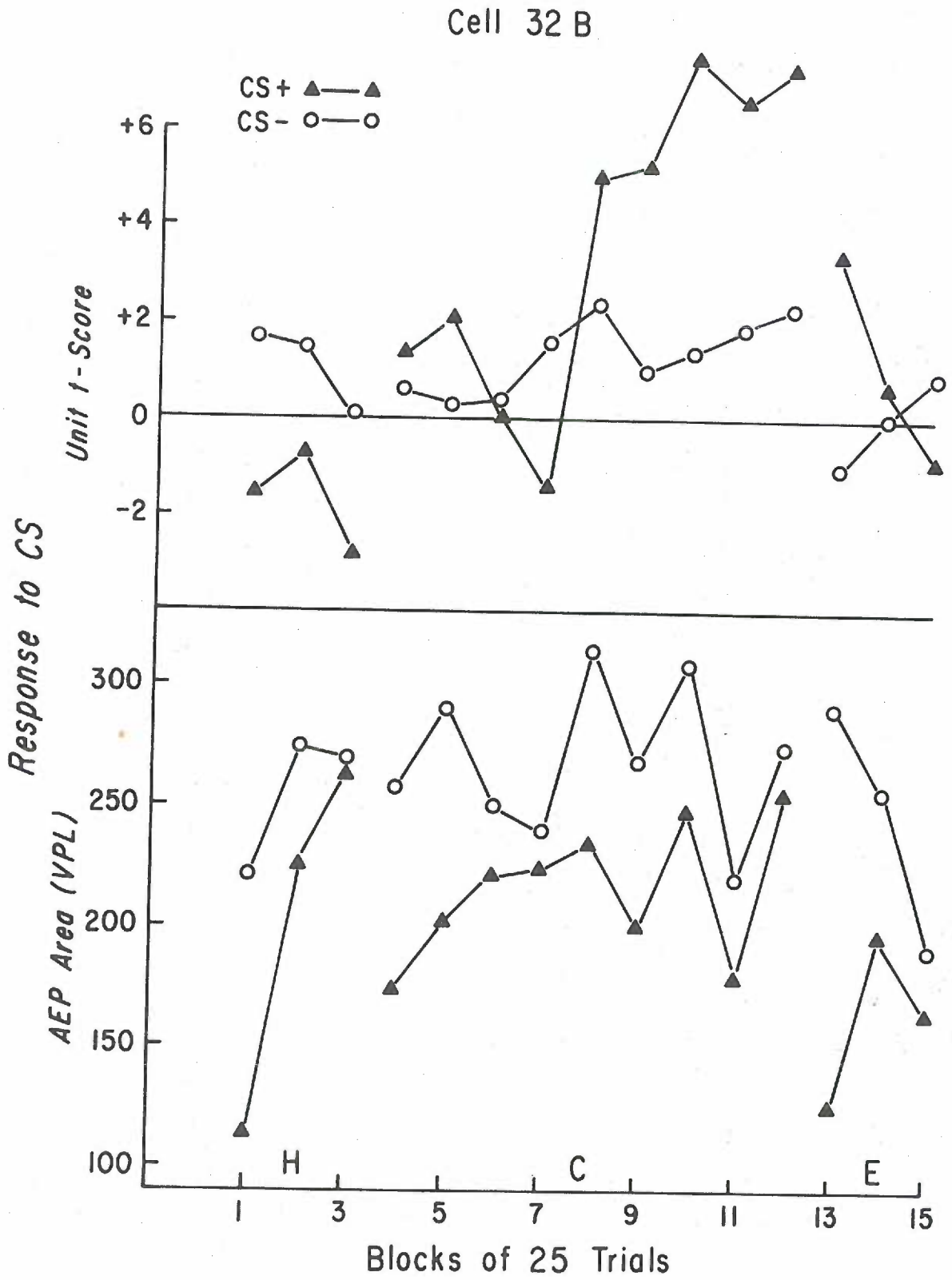

Cell 20A
Response to CS
Unit t-score
+10
+8
+6
+4
+2
0
CS+(1280-1450)
CS-(1420-1500)
AEP Area (CM)
150
100
50
H
C
E
1
3
5
7
9
11
13
15
Blocks of 25 Trials

Figure 3. Changes in response to the CS over trials. Top: Response of single neuron recorded from the postcruciate cortex. Format is the same as Figure 2. Bottom: Area of the averaged evoked response recorded from the nucleus ventralis posterolateralis of the thalamus. Format is the same as Figure 2. Note that the response recorded from the nucleus ventralis posterolateralis is not differentiated whereas the cortical neuron showed differential conditioning to the CS+ and CS-.


Cell 32 B
CS+
CS-
+6
+4
+2
0
-2
Unit t-Score
Response to CS
300
250
200
150
100
AEP Area (VPL)
H
C
E
1
3
5
7
9
11
13
15
Blocks of 25 Trials

This analysis also showed that the response (t-score magnitude) of all cells to the CS+ was significantly different from the response of the cells to the CS- during habituation ($F = 8.53$, $df = 1/155$, $p < .005$). This difference is evident in the habituation trials shown in Figure 4 (top). This level difference was unexpected since the stimuli (CS+ and CS-) were assumed to be equivalent and any systematic difference in the intrinsic responsiveness of the neurons to one of the stimuli could make interpretation of the conditioning results difficult.

In this experiment the habituation-conditioning-extinction sequence was presented to more than one neuron from each animal. Thus, the second and third cells recorded in each animal had been previously exposed to the conditioning procedure and could be expected to differ from first cells in their responses to the CS. In fact, an inspection of the response curves of the individual cells revealed that the level difference seen during habituation in Figure 4 (top) was produced in the overall curve by the contribution of records taken from second and third cells in each animal. First cells did not show this difference in habituation response magnitude. This observation was supported by a separate analysis of variance performed on the responses of first cells to the conditioned stimuli during habituation ($F = .10$, $df = 1/70$). However, the level difference was highly significant for second and third cells ($F = 14.79$, $df = 1/80$, $p < .001$). A comparison of the response of first and subsequent cells during habituation is shown in Figure 5.

This level difference during habituation, which appears only after the animal has undergone the conditioning procedure once, is almost

Figure 4. Grouped response data. Top: Mean response of cortical neurons to the CS+ and CS- over trials. The sign of all response values was inverted for neurons which had an excitatory response during habituation which became inhibitory during conditioning. The difference in response level to the CS+ and CS- during habituation is discussed in the Results. Middle: Mean of evoked response areas recorded from the centromedian nucleus of the thalamus. The difference in the level of response evoked by the CS+ and CS- is discussed in the Results. Note that the form of the response curves is nearly identical. Bottom: Mean of the evoked response areas recorded from the nucleus ventralis posterolateralis of the thalamus.

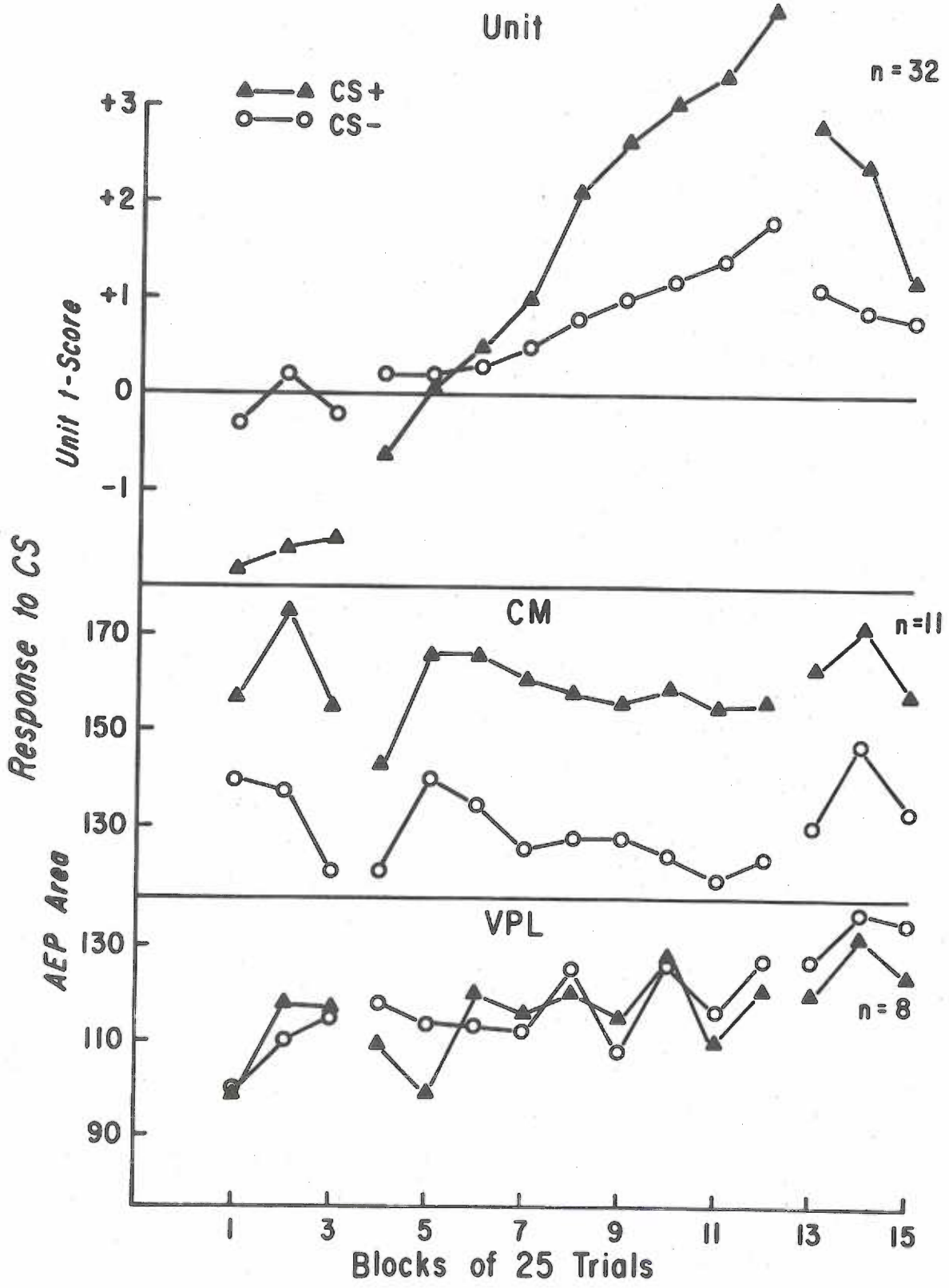
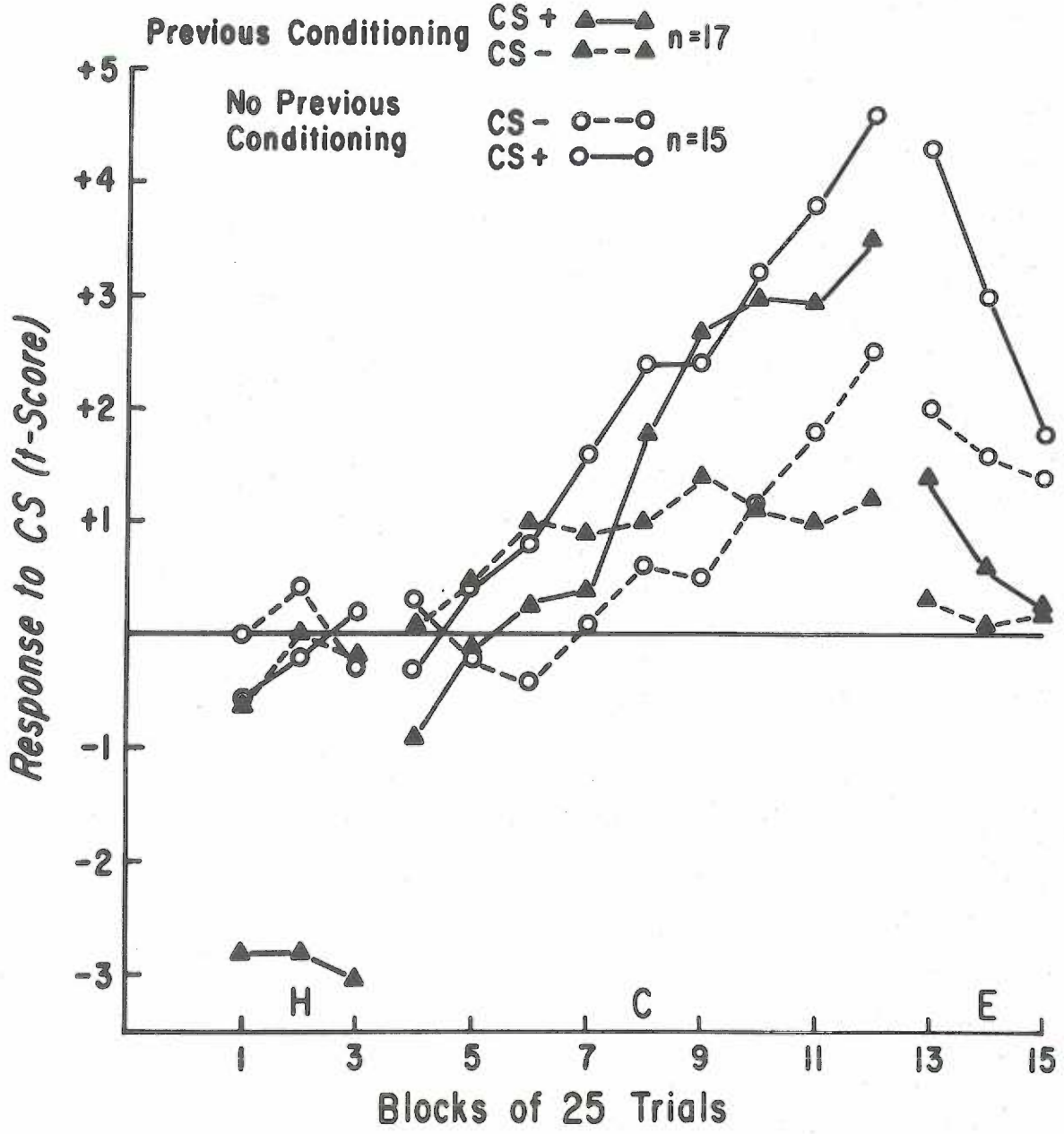

Unit
n = 32
CS+
CS-
+3
+2
+1
0
-1
Unit t-Score
Response to CS
CM
n=11
170
150
130
AEP Area
VPL
130
110
90
n = 8
1
3
5
7
9
11
13
15
Blocks of 25 Trials

Figure 5. Effect of previous conditioning on the acquisition and extinction of the neural conditioned response. It is important to note that cells recorded during the first conditioning series in each subject (No Previous Conditioning) (1) show a smaller change in response from habituation levels during the first block of conditioning trials, (2) show less discrimination between the CS+ and CS-, and (3) extinguish more slowly and less completely than cells recorded during subsequent conditioning sequences (Previous Conditioning).


Previous Conditioning
CS +
CS -
n=17
No Previous Conditioning
CS -
CS +
n=15
+5
+4
+3
+2
+1
-1
-2
-3
Response to CS (1-Score)
H
C
E
1
3
5
7
9
11
13
15
Blocks of 25 Trials

certainly a result of the conditioning but it is difficult to interpret. Evidently the conditioning process produces a change in the cortical neurons such that larger responses occur selectively to the CS+. This change is quite long-term since a few hours often elapsed between the experimental runs of first and second cells.

Unit data: Conditioning

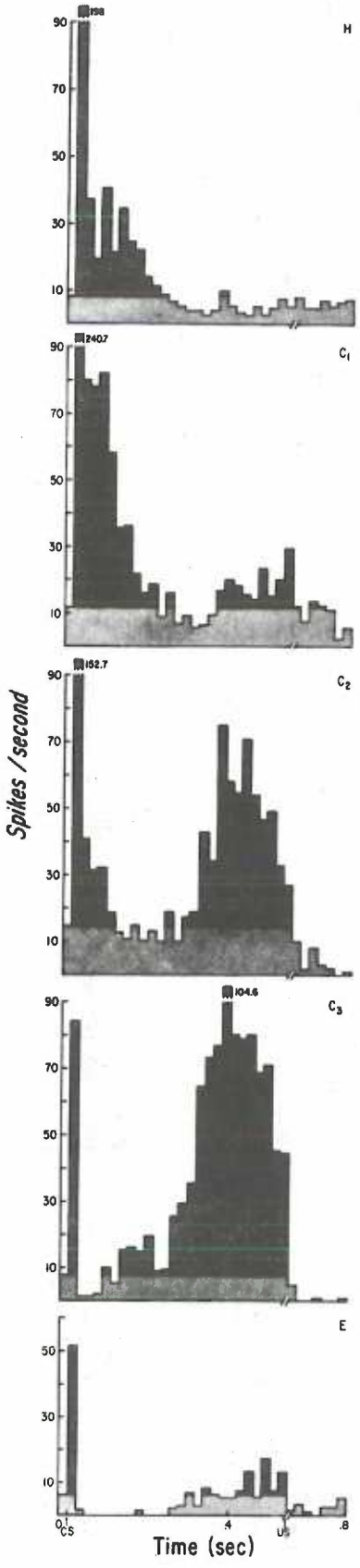
The development of a response to the CS+ in one neuron is shown as a series of post-stimulus histograms in Figure 6. During habituation (H) the neuron responded to the CS with a period of increased firing which returned to pre-CS rates by 200 ms post-CS and fell below the background rate for much of the remaining sample period.

During the first 75 trials of conditioning (C_1) the firing rate during the early excitatory component approximately doubled, but the duration of the response decreased to approximately 140 ms. Also during this trial segment a less pronounced excitatory component appeared during the 200 ms period just preceding the US.

During the second block of 75 conditioning trials (C_2) the excitatory component immediately following the CS returned to the level it exhibited during habituation. The later excitatory response, however, continued to develop during this period. In the third conditioning interval (C_3) the early excitatory response to the CS disappeared completely and the early interval began to show inhibition. The excitatory response just preceding the US showed even more development, however, reaching its greatest duration and peak firing frequency during this last block of conditioning trials.

Figure 6. Post-stimulus histograms showing the development of a neural conditioned response. Each histogram represents the mean of 75 trials. Mean pre-CS rate is represented as stippled area. The excitatory response (darkened) is that which exceeded the pre-stimulus rate. Occurrence of CS and US is marked by arrows beneath the bottom histogram. The break in the time line represents the duration of the US; this interval is not shown because of the large artifact. The bin width is 20 msec.

Cell 32 C-Post Stimulus Histograms



During the 75 extinction trials (E) the response of the cell during this interval returned almost to habituation levels. Extinction was, in fact, more complete than is indicated since the histogram represents an average of all 75 extinction trials while the response of this cell did not completely extinguish until the last 25 trials of extinction.

An analysis of the post-stimulus histograms was done for each cell and served as the basis of interval selection for subsequent statistical analyses. For the cell shown in Figure 6, the interval from 300-500 ms post-CS was chosen because it exhibited progressive development during conditioning followed by a return to habituation levels during extinction.

Figure 4 (top) shows the grouped unit data for the 32 neurons which were recorded throughout the trial sequence. The response of the neurons to the CS+ and CS- was compared across the conditioning trials using an analysis of variance. The analysis showed that the response to the conditioned stimuli changed significantly during conditioning ($F = 9.47$, $df = 8.527$, $p < .001$). An inspection of Figure 4 reveals that the response to the CS- also increased in magnitude during the early portion of the conditioning sequence. The increase in response to the CS-, however, was small while the response to the CS+ increased throughout the conditioning interval at a greater rate. This parallel development of the response to the conditioned stimuli during the early conditioning trials might be ascribed to sensitization, but such an effect would be expected to be prominent during the first 25 conditioning trials. A more likely

explanation is that the effect is due to generalization. It should be noted in this regard that there is neural convergence for the right and left hindpaw afferents used as the CS+ and CS- on the recorded cortical neurons.

The analysis of variance also revealed that the response of the cells to the CS+ during conditioning was greater than their response to the CS- (F = 16.17, df = 1/527, $p < .001$). An analysis of the interaction effect (trials X condition) was also significant (F = 2.23, df = 8/527, $p < .05$) which indicates that the rate of change in response to the CS+ was greater than the rate of change in response to the CS-. As a result of these differing rates of change the response to the CS+ significantly exceeds that to the CS- after the fourth block of conditioning trials (for conditioning trial block five: t = 4.3, df = 60, $p < .001$). These differences are shown clearly in the conditioning segment of the unit group response curve, Figure 4 (top).

Unit data: Extinction

An analysis of variance was also performed on the response of the cells to the conditioned stimuli during extinction. Even though the responses to the CS+ and CS- are different at the beginning of extinction (see Figure 4), the analysis indicated that the response to the CS+ did not differ from the response to the CS- over extinction (F = 3.22, df = 1/155). However, a significant trials effect (F = 3.10, df = 2/155, $p < .05$) indicated that the magnitude of response to the conditioned stimuli decreased significantly during extinction. No interaction between CS and trials was present (F = .40, df = 2/155)

indicating that the change in response over trials to the CS+ and CS- was nearly the same.

Unit data: Factors possibly contributing to the conditioning effect

The unit data were further examined to determine the possible contribution of cortical recurrent inhibitory collaterals (see Figure 7) to the conditioning effect. It was assumed that if these collateral inputs were mediating the conditioning effect then cells which were inhibited following the US would show a greater difference in response to the CS+ and CS- during conditioning than those which did not. On the basis of an inspection of the resonse of the cells during the 350 ms immediately following the offset of the US, the cells were separated into a group of 9 cells which were inhibited during this period and a group of 20 cells which were not. Three cells were not classified with either group because their resonse during the post-US interval was not consistent.

To obtain a measure of the overall change in response to the CS during conditioning, a T-score was computed. The mean firing rate during the selected portion of the CS-US interval during habituation and extinction was determined for each cell. This mean rate was subtracted from the cell's mean rate across the same interval during conditioning. The resulting difference score was divided by its variance to yield an overall T-score for each cell. This overall T-score was used as an indication of the amount of conditioning shown by the cells. The T-scores of cells which showed a developmental response increment during conditioning, extinction following the termination of reinforcement and differential responding to the CS+

and CS- were compared to those of cells which failed to show these response properties using a student's t-test. The results of this comparison showed that the groups differed significantly on this measure (t = 3.5, df = 30, $p < .01$), indicating that the T-score constitutes a valid measure of conditioning.

The overall T-scores for the cells showing post-US inhibition were then compared with the T-scores of the cells which did not, using a student's t-test. It was found that the difference between these groups was not significant (t = 2.01, df = 27). Thus, it seems unlikely that the conditioning effect is mediated via recurrent inhibitory collaterals.

The possible relationship between the nature of the response to the CS exhibited by the cells during habituation and their subsequent conditioned response was also examined. Fifteen cells decreased their firing rate in response to the CS during habituation and 14 cells increased their rate. The overall T-scores of these two groups of cells were compared and it was found that they did not differ significantly (t = .85, df = 27). The relationship between the absolute magnitude of the response of the cells during habituation and their overall T-score was also examined, using a Pearson product-moment correlation. The correlation was found to be -0.18 which was not significant. Thus, it appears that the parameters of the response to the CS shown by the cells during habituation do not serve to predict the outcome of subsequent conditioning.

Average evoked potential data: Centromedian nucleus

Figure 4 (middle) shows the group curve for the evoked potentials recorded in the centromedian nucleus. There is very little change in

centromedian during conditioning, and the pattern is almost identical for the CS+ and CS-. This observation is supported by an analysis of variance which demonstrated that the responses to the conditioned stimuli do not change in magnitude across the conditioning trials (F = 1.48, df = 8/170). An analysis of the interaction of trials with condition was also not significant (F = .14, df = 8/170), indicating that the rate of change in response to the CS+ and CS- did not differ. It was also found that the responses to the conditioned stimuli recorded in CM did not change significantly in magnitude during extinction (F = 2.11, df = 2/55). There is, however, a very clear difference in the level of response throughout the entire habituation-conditioning-extinction series. This level difference was found to be highly significant (for habituation: F = 11.45, df = 1/55, $p < .005$; for conditioning: F = 61.77, df = 1/170, $p < .001$; for extinction: F = 18.05, df = 1/55, $p < .001$). The level difference is almost constant across trials, however. A student's t-test reveals that the mean difference between the response to the CS+ and CS- during conditioning does not differ from the mean difference during habituation and extinction (t = .67, df = 163). This result indicates that the factors responsible for the level difference are not affected by the conditioning procedure used.

When the data from the centromedian nucleus are separated into that from first conditioning series and that from subsequent series in the same animal, the level difference is present in both groups. This indicates that the difference is not the result of previous conditioning, as was the case for the habituation level difference

seen in the unit data. A single signal line from centromedian carried both the CS+ and CS- responses (coded by separate marker channels on the tape recorder), so any amplifier or signal reproduction changes would influence both responses equally. Stimulation of the hindpaw ipsilateral to the recording site served as the CS+ equally as often as stimulation of the contralateral hindpaw so that paw selection should not be a biasing factor. It is also unlikely that the stimulator for the CS+ was set to a higher level since the difference is not present in the response of the cortical neurons or in evoked potentials recorded from the nucleus ventralis posterolateralis. The most important point to note with regard to the data from the centromedian nucleus is that the trend of the responses recorded in CM cannot account for the change observed during conditioning in the response of the cortical neurons.

Average evoked potential data: Nucleus ventralis posterolateralis

Figure 4 (bottom) shows the group curve for the evoked potentials recorded in the nucleus ventralis posterolateralis. There is almost no change in response to the conditioned stimuli in ventralis posterolateralis during conditioning. An analysis of variance revealed that the change in response across the conditioning trials was not significant (F = 1.01, df = 8/119). The responses to the CS+ and CS- in ventralis posterolateralis are also very nearly the same in magnitude (F = .45, df = 1/119), and in rate of change over trials (F = .29, df = 8/119). In the curves shown in Figure 4 (bottom) the magnitude of response to both the CS+ and CS- appears to increase throughout the trial sequence from habituation through extinction indicating a possible non-specific effect of the stimuli. However, when a t-test was used to compare

response magnitude during the first block of 25 trials with the magnitude of response during the last block of trials, the results were not significant (for CS+: t = .76, df = 14; for CS-: t = .93, df = 14). Thus, the changes seen in the response of the cortical units may not be accounted for by changes in response at the level of the nucleus ventralis posterolateralis.

DISCUSSION

The results reported here indicate that the pairing of antidromic activation with a somatic afferent signal produces changes in the neuronal response to this afferent which do not occur for an unpaired somatic afferent. This difference in response to the paired and unpaired stimuli convincingly demonstrates that the changes seen are due to learning rather than to nonspecific effects of stimulation. The development of the neural response to the reinforced stimulus is incremental and continues throughout the reinforced trials (Figure 4, top). The form of the acquisition curve is, in fact, quite similar to those obtained in human eyelid conditioning experiments (cf. Kimble, 1961, Fig. 3.4; Pennypacker, 1967, Fig. 8.2). The extinction curve shown in Figure 4 (top) is also quite orderly. The magnitude of the CR decreases within each block of 25 extinction trials, but the CR does not return completely to preconditioning levels. However, this is probably the result of presenting an insufficient number of CS alone trials to obtain complete extinction.

A comparison of the responses of cells recorded during the first conditioning sequence of each subject with cells recorded during subsequent conditioning series also reveals several characteristics typical of classical conditioning. First, as is shown in Figure 5, the discrimination between the CS+ and CS- appears to be better for second and third cells than for first cells. This effect is due to a decreased tendency of second and third cells to respond to the

CS-. In addition, the response to the CS+ of second and third cells changes greatly in magnitude from habituation levels during the first 25 trials of conditioning. The response of first cells, however, changes much less rapidly, exceeding habituation levels only after 75 conditioning trials (see Figure 5). Extinction of the CR is also much more rapid for second and third cells than for first cells, as well as being more complete after the 75 CS alone trials. All of these results would be predicted for a classical conditioning experiment involving repeated conditioning and extinction of a response. Thus, they support the conclusion that the phenomenon which we have studied is a neural analogue of true classical conditioning.

The results of the discrimination procedure provide further convincing evidence that effective conditioning was obtained under the conditions of the present experiment. Procedurally, the only systematic difference between the CS+ and CS- was that the presentation of the US regularly followed the CS+. Yet, as can be seen in Figure 4 (top), the response to the CS+ changed significantly more during conditioning than did the response to the CS-. This condition was considered by Kimble to be sufficient to demonstrate the occurrence of learning. In his opinion: "Particularly if the two stimuli excite the same sensory modality, it appears justifiable to refer to an increase in the tendency to respond to the reinforced stimulus as learned if no comparable increase occurs to the nonreinforced stimulus" (Kimble, 1967). Thus, the conclusion that the pairing of sensory afferent input to a cortical pyramidal cell with antidromic activation of the cell is capable of producing effective classical conditioning is strongly supported by the results of this experiment.

It is also interesting to compare the results of the present study with those of previous experiments in which classically conditioned changes in neural responses were obtained. In the present experiment the CR failed, in most cases, to completely extinguish during the 75 CS alone trials. This is in contrast to the data presented by Braun and Geiselhart (1959) for human eyelid conditioning, in which the CR typically extinguished within 20 nonreinforced trials. However, Woody and Brozek (1969b) found a result similar to the present study in an experiment in which evoked potentials were recorded from the facial nucleus of cats during conditioning procedures. In their study the response to the CS was larger after extinction than it had been prior to conditioning even when as many as 600 extinction trials were used. Furthermore, an inspection of Figure 6 shows that the pattern of the neural response during extinction was not the same as during habituation. The short latency excitatory response to the CS seen during habituation disappeared during conditioning and did not recover in extinction. Woody, Vassilevsky and Engel (1970) also noted that the response to the CS elicited from units in pericruciate cortex prior to conditioning was no longer present following extinction. This led them to conclude that: "Electrophysiologically, the state of extinction is quite different from the naive state" (Woody, Vassilevsky and Engel, 1970). The data obtained in the present experiment support this conclusion.

The majority of the cells recorded in this study responded almost equivalently to the CS+ and CS- during the early conditioning trials, as is shown in Figure 2 (top) for an individual cell and in Figure 4 (top) for the group as a whole. Most of these cells began to respond

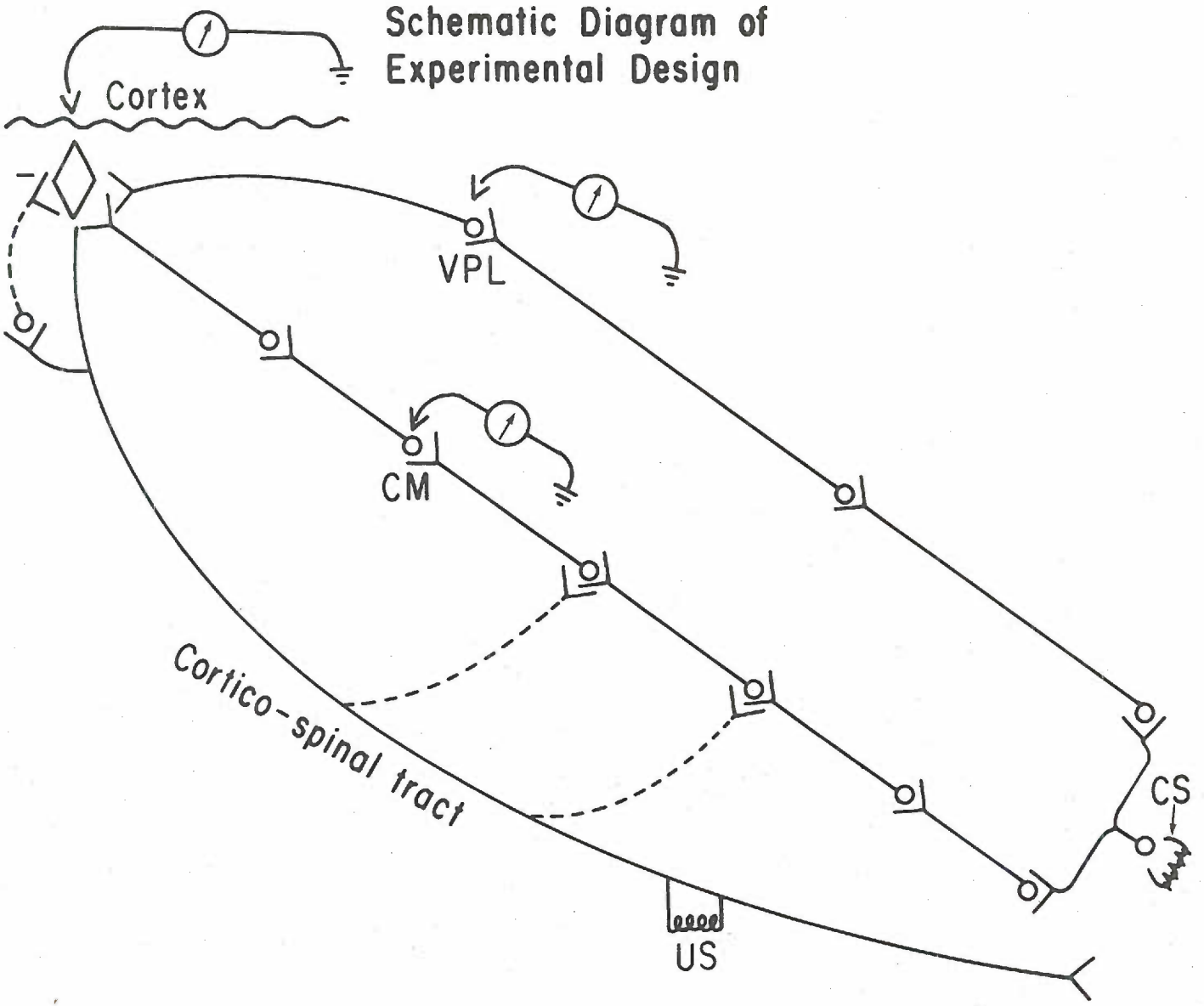
differentially to the CS+ and CS- after about 75 conditioning trials, a time course which fits well with the data reported for cortical cells by Disterhoff and Olds (1972). In their study the development of the CR in cortical cells occurred after 60-70 conditioning trials.

Of the 32 cells which were recorded throughout the entire habituation-conditioning-extinction sequence in the present experiment, eight were classified as showing poor conditioning (see page 22). One reason for such classification was failure of the cells to develop differential responding to the CS+ and CS-. This equivalence of response to the CSs in these cells can be attributed to generalization rather than sensitization for two reasons. First, the development of the responses of the cells to the CS+ and CS- was not sudden following the introduction of the US. Instead, the responses increased gradually across the conditioning trials. Second, extinction was gradual for both stimuli following termination of reinforcement. Disteroff and Olds (1972) also found cells which responded equivalently to the discriminative stimuli which they employed. In their study, these cells were found to be widely distributed within the brain, being located in the thalamus in the dorsomedial nucleus, ventral nucleus, lateral nucleus and posterior nucleus as well as in posterior regions of the cortex. It is possible that such cells mediate a different function within the brain than those which respond differentially to discriminative stimuli. If so, it is interesting to note that in the present experiment both types of responding were found in a relatively homogenous cell population--cortical pyramidal cells.

One of the aims of this experiment was to determine if pyramidal stimulation could be utilized as a US to localize the learning change to cortical neurons which had axons in the pyramidal tract. The data strongly indicate that the paradigm used resulted in conditioned changes in the response of the recorded cortical neurons. However, before we can conclude that the observed learning occurred locally within the cortex, we must examine other possible sites of interaction between the CS and US. Figure 7 shows a schematic representation of the stimulation and recording sites used in this experiment as well as possible sites of convergence between the CS and US. A brief consideration of the anatomy of the pyramidal system will help make some of these possibilities more clear.

The pyramidal bundle accumulates in the white matter underneath the precentral gyrus and proceeds to the internal capsule. As the fibers leave the internal capsule to enter the midbrain they join the middle portion of the cerebral peduncle. At the level of the midbrain a portion of the corticobulbar fibers separate from the corticospinal tract and distribute to the cranial nerve nuclei. Other corticobulbar fibers separate from the pyramidal tract well above their levels of termination to enter the medial lemniscus, forming the aberrant pyramidal tracts. At the lower border of the medulla 70-90% of the corticospinal fibers cross in the motor decussation to form the lateral corticospinal tracts of the spinal cord. However, in addition to these well known pathways, it is also claimed that certain of the corticospinal fibers terminate in sensory nuclei--the nucleus gracilis and nucleus cuneatus (Crosby, Humphrey & Lauer, 1962). Thus, the

Figure 7. Schematic diagram of experimental design. The sites of stimulus presentation and recording of neural responses are shown, as are the most probable pathways by which the stimuli converge on the cortical pyramidal cell. Locations other than the cortical neuron which could be influenced by the US are indicated by dashed lines. The possible projection of the corticospinal tract to brainstem sensory relay nuclei (gracilis and cuneatus) is not shown.


Schematic Diagram of
Experimental Design
Cortex
VPL
CM
Cortico-spinal tract
CS
US

pyramidal system may well affect sensory as well as motor systems as has been suggested by Chambers and Liu (1957) and Hagbarth and Kerr (1954). Activation of the motor cortex or pyramid has been shown to inhibit the discharge of sensory neurons in the dorsal columns, midbrain, cerebellum and sensory cortex (Hagbarth & Kerr, 1954). The pathways mediating these inhibitory functions are considered to be relayed through the reticular formation (Chambers & Liu, 1957).

Thus, anatomical locations other than the cortical neuron where a CS-US interaction could take place are (1) spinal cord, (2) thalamic and brainstem locations which receive input from the pyramidal tract, and (3) cortical loci influenced by recurrent inhibitory collaterals of cortical neurons. In order to assess the possible contribution of these sites to the observed conditioning, recordings of neural activity were made in the centromedian nucleus and nucleus ventralis posterolateralis of the thalamus.

It is well known that the major projection of the reticular formation is through the ipsilateral centromedian; thus, any effects mediated via the pathway mentioned by Chambers and Liu (1957) should be detectable in centromedian. Additionally, a recent experiment (O'Brien & Rosenblum, 1974) has shown that about 50% of the activity elicited in the postcruciate cortex by a hindpaw stimulus is mediated by centromedian. Ventralis posterolateralis is the primary sensory relay nucleus for somatic afferent input to cortex. Thus, any changes occurring as a result of interaction between the CS and the pyramidal stimulus at the level of the brainstem relay nuclei (gracilis and cuneatus) should be transmitted through ventralis posterolateralis. However, no changes in response at the level of centromedian or ventralis posterolateralis were observed

in the present experiment which could account for the changes seen in the cortical neurons. Therefore, it seems unlikely that peripheral (thalamus, brainstem, spinal cord) changes are responsible for the conditioned response observed in the cortex.

The possible contribution of intracortical recurrent collaterals to the development of the conditioned response is more difficult to assess. It has been frequently observed that activation of the pyramidal tract results in a brief inhibition of cortical pyramidal cells (Stefanis & Jasper, 1964; Kameda, Nagel & Brooks, 1969). This inhibition is thought to be mediated through recurrent inhibitory collaterals which affect both their cell of origin and surrounding cortical cells. Thus, it is possible that the conditioning which was found in this experiment was not due simply to the pairing of the CS with antidromic activation of the recorded cell; it could have been dependent upon activity in these recurrent circuits. Our control for this possibility was a functional one. An interval of 340 msec immediately following the presentation of the pyramidal stimulus was analyzed for each of the recorded neurons as described in the Results. The duration of the recurrent inhibition found by Stefanis and Jasper (1964) was no more than 120 msec and its onset following stimulation occurred with a maximum latency of 50 msec. Thus, the post-US interval analyzed in the present experiment would certainly have included any recurrent inhibitory effects which were present. Some of the neurons which were conditioned showed a dramatic inhibition during this post-US interval while others were not inhibited at all. When the overall degree of conditioning obtained by these two groups was compared, the

difference between them was found to be non-significant. Thus, it appears that activation of the cortical recurrent inhibitory collaterals does not contribute significantly to the development of the CR recorded from cortical pyramidal cells. Thus, all the evidence we have obtained in this experiment supports the conclusion that the site of learning is localized within the cortex.

To determine whether the site of the neural change during learning is presynaptic or postsynaptic with respect to the recorded neuron is even more difficult than demonstrating that the learning occurs locally within the cortex. In principle, since an antidromic spike cannot cross a synapse, antidromic activation of pyramidal cells by stimulation of their axons should affect only the cell bodies from which the axons project and not the presynaptic terminals making contact with these cell bodies. However, while it is true that antidromic activity is not transmitted across synapses, there is evidence that activity in post-synaptic neurons can influence presynaptic terminals. Decima and his associtates have investigated this type of interaction in a series of experiments on the spinal cord of cats (Decima, 1969; Decima & Goldberg, 1970, 1973). Decima (1969) demonstrated that centrifugal discharge in cat sensory fibers could be induced by antidromic motor neuron activation if this activation was preceded by a conditioning stimulus. Adequate conditioning stimuli were found to include shocks to the dorsal roots or the dorsal columns one or two segments above the test location or natural stimuli applied to the hindlimbs. The conditioning stimuli were presumed to act by increasing presynaptic excitability (Decima & Goldberg, 1970). A study of conduction velocities (Decima & Goldberg,

1973) indicated that only alpha motoneurons and group I muscle afferents participated in this interaction. In all these studies the latency of the centrifugal dorsal root response to ventral root stimulation was found to be shorter than that of the monosynaptic reflex mediated by the same fibers. This finding suggested an electrical coupling between motoneurons and presynaptic terminals. It led the authors to put forth the hypothesis that "via an antidromic electrical interaction, the motoneuron discharge can depolarize the same presynaptic terminals which, through chemical synaptic mechanisms, induce the orthodromic firing of the same neuron" (Decima & Goldberg, 1973). The type of interaction described by Decima and Goldberg has not been studied in cortical cells. However, there is no reason to assume that this type of relation could not exist between pyramidal cells and their presynaptic terminals.

An additional difficulty arises in that it is possible that stimulation of the pyramidal tract reaches the cortical pyramidal cells by longer orthodromic pathways as well as directly, via the stimulated axons. Pathways by which this could occur were mentioned above. Our control procedures showed that the conditioned response probably was not learned in a subcortical locus and projected to the cortex. However, orthodromic transmission of the pyramidal stimulus through these areas is still possible. In fact, an examination of the period following US presentation in the average evoked potentials recorded from centromedian and ventralis posterolateralis revealed the presence of a response to the US in these nuclei. The response of the cortical units during the post-US period was also analyzed by the methods previously described for the CS-US interval

analysis. The units were found to respond significantly more during the post-US interval on reinforced trials than on nonreinforced trials. This indicates that the responses seen were elicited by the US, however, their latencies were much too long to be antidromic. The magnitude of these responses also tended to be correlated with the degree of conditioning attained by the cells ($p < .1$). Thus, the possibility that the conditioning found in this experiment was due to the pairing of orthodromic synaptic inputs to the recorded cell cannot be rejected.

Because of these possibilities we are unable to conclude on the basis of this experiment that the conditioned response recorded from cortical pyramidal cells is mediated postsynaptically. However, while the data do not demonstrate that the locus of the conditioned change is the postsynaptic cell, they do support the conclusion that the site of the learning change is in the cortex.

SUMMARY AND CONCLUSIONS

The spike activity of single cortical pyramidal cells in the postcruciate cortex of acutely prepared cats was examined during an habituation-conditioning-extinction trial sequence. A differential conditioning paradigm was used to control for sensitization and pseudoconditioning effects. Left and right hindpaw stimulation was used for the conditioned (CS+) and differential (CS-) stimuli, and stimulation of the pyramidal tract, which produced antidromic activation of the cortical neuron, was used as the unconditioned stimulus.

This procedure produced large changes in neural responsiveness to the CS+ analogous to a behavioral conditioned response. The response to the unpaired CS- did not show a comparable change. Activity in the centromedian nucleus of the thalamus, the major multisynaptic relay to the cortex, and the nucleus ventralis posterolateralis, the primary somatic afferent relay to the cortex, did not show any changes which could account for the changes in the response of the cortical neurons. The contribution of inhibitory cortical recurrent collaterals to the conditioned response was also judged to be insignificant.

It was concluded that the learning produced by this paradigm was localized in the cortex. The question of whether the conditioned response was mediated presynaptically or postsynaptically within the recorded neuron was also addressed, but the issue could not be resolved on the basis of the available evidence.

REFERENCES

Adam, G., Adey, W. R., & Porter, R. W. Interoceptive conditional response in cortical neurones. Nature, Lond., 1966, 209, 920-921.

Bindman, L. J., Lippold, O. C. J., & Redfearn, J. W. T. Long lasting changes in the level of the electrical activity of the cerebral cortex produced by polarizing currents. Nature, Lond., 1962, 196, 584-585.

Bindman, L. J., Lippold, O. C. J., & Redfearn, J. W. T. The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. J. Physiol. Lond., 1964, 172, 369-382.

Braun, H. W., & Geiselhart, R. Age differences in the acquisition and extinction of the conditioned eyelid response. J. exp. Psychol., 1959, 57, 386-388.

Bures, J., & Buresova, O. Discussion: Plasticity at the single neuron level. In Proc. Int. Union Physiol. Sci., 4, XXIII Int. Congr., Tokyo, 1965. pp. 359-364.

Bures, J., & Buresova, O. Plastic changes of unit activity based on reinforcing properties of extracellular stimulation of single neurons. J. Neurophysiol., 1967a, 30, 98-113.

Bures, J., & Buresova, O. Spreading depression and corticosubcortical interrelations in the mechanism of conditioned reflexes. Progr. Brain Res., 1967b, 22, 378-387.

Bures, J., & Buresova, O. Plasticity in single neurons and neural populations. In G. Horn & R. A. Hinde (Eds.), Short term changes in neural activity and behavior. Cambridge: Cambridge University Press, 1970. pp. 363-404.

Chambers, W. W., & Liu, C. N. Corticospinal tract of the cat. An attempt to correlate the pattern of degeneration with deficits in reflex activity following neocortical lesions. J. comp. Neurol., 1957, 108, 23-55.

Crosbey, E. C., Humphrey, T., & Lauer, E. W. Correlative anatomy of the nervous system. New York: Macmillan, 1962.

Decima, E. E. An effect of postsynaptic neurons upon presynaptic terminals. Proc. Nat. Acad. Sci. (USA), 1969, 63, 58-64.

Decima, E. E., & Goldberg, L. J. Centrifugal dorsal root discharges induced by motoneurone activation. J. Physiol., 1970, 207, 103-118.

Decima, E. E., & Goldberg, L. J. Antidromic electrical interaction between alpha motoneurones and presynaptic terminals. Brain Res., 1973, 57, 1-14.

Deutsch, J. A. The cholinergic synapse and the site of memory. In J. A. Deutsch (Ed.), The physiological basis of memory. New York: Academic Press, 1973. pp. 59-75.

Deutsch, J. A., & Leibowitz, S. F. Amnesia or reversal of forgetting by anticholinesterase, depending simply on time of injection. Science, 1966, 153, 1017-1018.

Deutsch, J. A., Hamburg, M. D., & Dahl, H. Anticholinesterase induced amnesia and its temporal aspects. Science, 1966, 151, 221-223.

Disterhoff, J. F., & Olds, J. Differential development of conditioned unit changes in thalamus and cortex of rat. J. Neurophysiol., 1972, 35, 665-679.

Engel, J., Jr., & Woody, C. D. Effects of character and significance of stimulus on unit activity at coronal-precruciate cortex of cat during performance of conditioned motor response. J. Neurophysiol., 1972, 35, 220-229.

Feltz, A., & Mallart, A. An analysis of acetylcholine responses of junctional and extrajunctional receptors of frog muscle fibers. J. Physiol., 1971, 218, 85-100.

Gerbrandt, L. K., Skrebitsky, V. G., Buresova, O., & Bures, J. Plastic changes of unit activity induced by tactile stimuli followed by electrical stimulation of single hippocampal and reticular neurons. Neuropsychologia, 1968, 6, 3-10.

Hagbarth, K. E., & Kerr, D. I. B. Central influences on spinal afferent conduction. J. Neurophysiol., 1954, 17, 295-307.

Hori, Y., Toyohara, I., & Yoshii, N. Conditioning of unitary activity by intracerebral stimulation in cats. Physiol. Behav., 1967, 2, 255-259.

Huttunen, M. O. General model for the molecular events in synapses during learning. Persp. Biol. Med., 1973, Autumn, 103-108.

Jasper, H. H., Ricci, G., & Doane, B. Microelectrode analysis of cortical cell discharge during avoidance conditioning in the monkey. EEG clin. Neurophysiol., 1960, 13, 137-155.

Kameda, K., Nagel, R., & Brooks, V. B. Some quantitative aspects of pyramidal collateral inhibition. J. Neurophysiol., 1969, 32(4) 540-553.

Stefanis, C., & Jasper, H. Recurrent collateral inhibition in pyramidal tract neurons. J. Neurophysiol., 1964, 27, 855-877.

Woody, C. D. Conditioned eye blink: Gross potential activity at coronal-precruciate cortex of the cat. J. Neurophysiol., 1970, 33, 838-850.

Woody, C. D., & Brozek, G. Gross potential from facial nucleus of cat as an index of neural activity in response to glabella tap. J. Neurophysiol., 1969a, 32, 704-716.

Woody, C. D., & Brozek, G. Changes in evoked responses from facial nucleus of cat with conditioning and extinction of an eye blink. J. Neurophysiol., 1969b, 32, 717-726.

Woody, C. D., Vassilevsky, N. N., & Engel, J., Jr. Conditioned eye blink: Unit activity at coronal-precruciate cortex of the cat. J. Neurophysiol., 1970, 33, 851-864.

Woody, C. D., & Yarowsky, P. Conditioned eye blink using electrical stimulation of coronal-precruciate cortex as conditioned stimulus. J. Neurophysiol., 1971, 34, 242-252.

Woody, C. D., & Engel, J., Jr. Changes in unit activity and thresholds to electrical microstimulation at coronal-precruciate cortex of cat with classical conditioning of different facial movements. J. Neurophysiol., 1972, 35, 230-241.

Woody, C. D., & Black-Cleworth, P. Differences in excitability of cortical neurons as a function of motor projection in conditioned cats. J. Neurophysiol., 1973, 36, 1104-1116.

Yoshii, N., & Ogura, H. Studies on the unit discharge of brainstem reticular formation in the cat. I. Changes of reticular unit discharge following conditioning procedure. Med. J. Osaka Univ., 1960, 11, 1-17.

Table 1. Basic Data: Unit Responses (t-scores) to CS+ and CS-

Cell		Blocks of 25 Trials														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
9A	+	-8.0	-6.8	-7.1	-2.4	-0.6	0.04	-0.9	1.7	0.9	0.3	2.8	0.6	1.7	-5.4	-7.2
	-	-3.2	-4.2	-6.2	0.3	0.2	-1.0	-2.3	-1.0	-0.1	-1.2	-2.2	-0.1	-5.3	-2.4	-0.5
9B	+	-3.3	-5.1	-3.2	-2.4	-2.3	-2.0	-3.6	-2.5	-0.7	-0.9	-1.1	-0.1	-0.4	-1.1	-0.6
	-	2.5	3.7	5.0	-0.1	0.0	-0.2	0.9	-0.1	0.7	-1.0	-0.2	0.7	-0.8	1.5	0.4
9C	+	-9.2	-9.2	-7.8	-2.7	-6.4	-2.7	-3.0	-2.3	-2.3	-2.7	-1.1	-2.1	-1.1	-3.4	-4.1
	-	-1.4	-0.4	-1.1	-0.0	4.2	1.9	5.3	2.9	-.04	2.3	2.9	-0.6	4.5	-0.5	4.3
12A	+	-1.7	-1.2	-0.4	-1.5	3.9	3.6	4.2	7.0	4.7	7.8	10.2	8.9	6.8	5.8	5.5
	-	-1.8	-1.9	-1.2	-4.3	-1.9	-1.8	0.8	1.0	3.8	4.7	6.8	8.9	8.7	4.8	5.8
12C	+	2.0	1.7	0.8	2.6	5.3	3.6	5.4	7.5	10.9	12.1	10.3	10.4	10.4	7.1	5.5
	-	-1.4	-2.5	-2.7	-1.3	1.9	3.5	3.8	3.2	5.5	5.5	6.5	4.2	3.5	1.4	0.5
13A	+	1.3	0.4	-1.0	0.1	0.8	-0.6	4.3	-1.0	-0.2	-0.3	2.9	3.1	0.2	1.4	-0.8
	-	0.8	-0.6	0.6	-0.9	-1.6	-0.6	0.4	-0.8	-1.1	-0.5	0.5	0.5	0.7	0.7	-1.1
13B	+	-6.3	-4.0	-8.8	-12.1	-5.9	-3.9	-1.2	-2.9	-1.9	-1.8	-1.6	0.5	-1.3	-1.7	-1.4
	-	-5.3	-1.4	-0.8	-1.1	-1.8	-3.2	-0.7	1.2	-0.3	-0.2	0.3	0.0	1.7	0.9	0.9

Table 1. Continued

Cell		Blocks of 25 Trials													
14B	+	0.0	0.7	-1.7	0.6	2.9	2.1	2.8	5.4	4.1	2.6	3.6	5.1	1.2	-0.8	-1.0
	-	0.5	0.5	-0.3	0.4	0.6	0.7	0.6	-1.8	1.1	-0.2	-0.8	0.8	-0.3	1.6	0.2
15B	+	4.1	2.3	0.5	2.1	1.7	3.2	5.8	6.1	5.9	6.8	4.5	4.0	0.6	0.1	0.9
	-	3.2	2.8	1.2	0.4	0.5	-1.2	0.3	-1.1	-0.9	-0.2	0.2	0.9	-4.1	-0.5	-3.6
17C	+	-2.1	3.4	0.7	6.0	7.2	9.1	8.7	6.8	8.3	7.6	9.9	11.1	0.9	3.7	2.5
	-	-6.7	-1.5	-1.8	-3.7	-0.8	1.7	-0.4	0.8	-1.4	0.4	-2.5	-1.2	0.1	0.5	0.7
18B	+	-2.1	4.0	2.9	-1.5	-2.3	-2.8	-2.7	-1.0	-1.5	0.1	0.0	0.8	2.6	2.8	10.5
	-	-3.8	-0.5	0.5	4.2	1.3	3.9	2.8	2.1	3.7	3.2	2.9	2.2	3.6	1.1	1.7
18C	+	-1.5	-0.8	-2.7	1.5	2.2	0.1	-1.4	5.0	5.2	7.5	6.5	7.3	3.4	0.7	-0.9
	-	0.2	0.3	1.8	-0.1	0.7	1.2	1.2	2.6	1.3	2.4	2.2	2.7	0.7	0.3	1.0
20A	+	1.6	1.5	1.2	-0.0	2.6	4.8	5.7	6.4	7.1	8.1	8.2	9.9	9.4	3.8	1.2
	-	1.6	2.4	0.6	0.3	0.6	2.9	4.9	3.2	3.0	3.8	4.5	4.8	4.9	2.7	0.2
21A	+	5.6	8.5	9.5	3.7	2.6	2.8	5.8	10.1	8.8	7.1	6.6	2.6	9.6	17.2	12.7
	-	-0.8	-0.9	-1.1	1.1	-0.2	0.9	-0.5	-0.0	-0.9	2.1	0.8	0.5	1.5	1.2	0.3

Table 1. Continued

Cell		Blocks of 25 Trials													
23A	+	-6.6	-6.3	-3.1	-5.9	-7.9	-4.5	-2.5	-2.4	0.2	-0.2	3.4	1.0	0.5	-1.6	-4.4
	-	-2.7	-5.1	-2.8	-3.1	-2.8	-6.5	-3.1	-3.9	-2.3	0.6	-0.3	1.0	-3.6	-4.4	-1.0
23B	+	-7.2	-4.9	-6.4	-2.7	-2.9	-1.8	-3.0	-0.7	1.2	-0.3	0.1	0.9	-2.9	0.2	-1.0
	-	-1.2	-2.2	-2.3	0.5	0.2	0.3	-0.2	-0.3	2.9	-0.1	0.9	0.3	-1.9	-1.5	-1.6
24A	+	0.7	2.7	2.4	1.1	1.1	0.9	-0.4	-0.9	0.8	-0.6	0.8	0.3	0.8	0.3	3.2
	-	0.9	1.8	1.3	2.6	-0.2	-1.3	-0.6	1.0	0.7	-0.1	1.0	1.4	0.4	0.1	0.2
24B	+	0.0	-1.3	-1.7	0.4	1.5	3.3	2.0	1.1	4.6	1.8	1.2	1.8	4.0	4.8	2.4
	-	-0.2	-0.3	-2.1	-2.1	-2.7	-0.1	-1.7	-0.6	-1.4	0.3	0.7	-0.1	-2.6	-2.8	-0.5
25A	+	2.4	2.4	1.7	-0.1	0.6	2.6	1.9	1.6	-0.8	0.0	1.7	9.9	9.0	8.4	5.8
	-	1.1	0.2	-1.0	0.5	-1.2	-0.2	0.7	0.8	-1.0	-0.4	1.2	3.8	8.3	12.5	6.9
25B	+	-1.3	-0.3	-0.3	4.3	2.6	2.8	2.1	3.9	3.0	5.5	3.8	4.8	1.1	2.1	-2.8
	-	-0.5	-0.9	0.6	0.7	5.1	2.5	0.9	3.3	3.8	1.9	0.0	3.5	-0.3	0.9	-1.4
26A	+	-0.7	0.0	1.1	-1.1	-0.1	-0.9	-0.1	0.5	-2.1	0.4	0.3	1.4	-0.4	-0.1	-0.2
	-	-13.3	--.3	-0.8	0.5	-0.1	-1.7	-1.0	-0.0	-0.9	-2.0	-1.9	-2.7	0.0	0.4	-0.1

Table 1. Continued

Cell		Blocks of 25 Trials														
27A	+	3.5	5.5	3.8	3.6	2.9	3.7	4.9	3.0	3.4	5.2	4.9	7.5	8.3	7.4	5.3
	-	-3.8	-2.0	-1.9	-2.1	-2.5	-2.6	-2.2	-1.3	-1.5	-2.4	-0.6	-1.3	0.6	0.3	1.4
28A	+	-7.9	-7.3	-2.3	-3.2	-4.9	-2.0	-1.5	-1.1	-1.0	-0.9	-0.3	0.0	-4.3	-2.9	-1.9
	-	18.3	9.8	3.3	5.5	4.9	6.1	3.3	4.4	4.5	3.6	5.7	9.8	4.0	2.4	1.5
29A	+	-2.0	-3.4	-1.9	-2.1	-1.0	-6.6	-2.5	-1.2	-1.6	-0.2	-2.8	2.4	0.9	-0.6	1.5
	-	-2.7	1.2	-1.4	0.7	-1.9	-2.3	1.2	0.6	0.2	0.2	0.0	0.5	1.4	-0.9	-0.4
29B	+	-5.7	-6.8	-5.5	-3.7	-4.7	-5.8	-4.1	-4.5	-3.7	-6.1	-3.8	-0.6	-3.4	-3.2	-2.3
	-	3.4	1.3	1.7	0.3	3.4	2.4	2.6	2.7	3.2	0.8	1.8	1.6	1.4	0.8	1.0
30A	+	-0.8	-3.6	-3.4	-0.3	-0.2	1.6	2.2	3.2	3.6	4.1	1.3	3.6	1.4	-0.9	-0.8
	-	-0.8	0.6	0.5	-2.1	-2.2	-3.5	-3.3	1.1	-1.3	-0.0	-0.2	1.3	-0.8	0.6	-0.6
30B	+	-16.4	-28.8	-16.8	-14.5	-12.1	-9.1	-11.3	-10.6	-8.3	-6.4	-6.3	-9.3	-9.2	-7.1	-9.5
	-	-2.6	-2.9	-2.3	1.0	-0.9	2.2	0.9	1.6	1.9	3.6	0.7	1.6	0.9	0.0	2.7
30C	+	0.3	0.5	1.2	3.7	3.6	4.9	5.8	6.5	4.8	4.8	5.6	7.7	4.4	-0.9	2.4
	-	0.2	1.7	-1.2	2.9	1.6	3.0	3.3	2.1	3.6	2.1	2.5	1.6	2.1	-2.7	-2.5

Table 1. Continued

Cell		Blocks of 25 Trials														
31A	+	0.9	1.4	1.4	0.4	3.3	4.7	5.9	5.5	6.2	7.4	8.9	8.6	10.5	4.0	1.2
	-	5.9	3.9	3.9	3.9	3.9	2.9	3.5	2.2	1.7	1.8	3.7	2.6	1.8	0.6	0.2
31B	+	2.0	1.4	0.6	2.4	5.2	3.5	5.3	7.7	10.9	12.1	10.4	10.7	10.3	7.0	5.6
	-	1.3	0.5	0.9	-0.4	-4.6	-2.5	-5.6	-3.2	0.0	-2.7	-2.9	0.5	-1.6	0.9	-1.1
32A	+	2.6	3.2	1.3	2.1	3.3	1.8	-2.6	3.3	6.3	6.9	7.7	9.4	10.5	8.4	6.2
	-	0.9	1.8	1.5	1.7	1.7	2.4	-0.1	2.1	3.6	7.4	7.9	6.7	6.8	5.9	8.0
32B	+	-1.5	-0.8	-2.7	1.5	2.2	0.1	-1.4	5.0	5.2	7.5	6.5	7.3	3.4	0.7	-0.9
	-	1.7	1.5	0.0	0.6	0.3	0.4	1.6	2.4	1.1	1.3	1.9	2.3	-1.1	0.0	0.9

Table 2. Basic Data: Area of Average Evoked Responses Recorded From the Centromedian Nucleus

Cell		Blocks of 25 Trials														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
20A	+	108	108	89	112	131	135	108	97	84	95	89	95	105	108	108
	-	119	106	115	106	136	140	115	92	81	84	81	86	110	103	89
21A	+	267	259	252	251	297	283	300	280	301	298	299	287	272	265	265
	-	166	159	164	160	181	185	192	186	189	186	190	178	169	166	170
23A	+	116	78	77	70	73	71	70	73	64	62	55	60	54	55	63
	-	103	52	55	64	76	73	81	73	76	82	68	58	63	68	61
23B	+	98	100	81	53	48	56	61	64	67	81	88	91	90	87	59
	-	83	82	65	51	48	51	53	54	61	63	86	84	102	104	70
24A	+	233	318	234	193	221	189	204	193	195	187	190	195	252	292	251
	-	217	306	214	169	175	167	172	156	149	149	130	145	212	252	217
24B	+	183	191	207	173	173	172	194	195	167	205	181	203	214	260	228
	-	166	167	176	142	154	143	138	119	159	134	139	145	215	195	202
25A	+	193	169	171	105	157	184	152	134	133	128	165	156	161	198	203
	-	197	173	127	149	167	167	114	165	134	142	121	93	146	174	137

Table 2. Continued

Cell		Blocks of 25 Trials														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
25B	+	135	158	117	104	109	128	108	124	138	138	121	127	105	117	108
	-	82	101	93	86	106	110	94	79	119	104	92	113	124	109	108
26A	+	123	129	98	104	103	83	84	72	111	119	87	84	94	134	85
	-	36	28	33	29	35	36	38	40	26	48	65	119	94	97	95
31A	+	160	108	103	90	92	91	91	86	83	76	70	79	77	73	87
	-	81	58	52	46	50	49	46	45	43	42	38	43	38	43	45
31B	+	116	308	280	317	427	435	400	418	377	356	355	335	368	302	279
	-	294	281	242	331	408	366	337	384	365	329	295	287	267	267	311

Table 3. Basic Data: Area of Average Evoked Responses Recorded From the Nucleus Ventralis Posterolateralis

Cell		Blocks of 25 Trials														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
28A	+	49	40	38	33	36	41	46	45	46	25	37	36	29	23	27
	-	34	44	59	34	40	39	39	58	57	51	51	55	46	59	56
29A	+	110	103	116	126	102	98	94	88	90	93	92	88	97	94	79
	-	71	62	71	74	73	62	68	89	69	77	93	79	85	78	73
29B	+	54	58	89	95	66	96	96	114	87	118	104	101	109	118	94
	-	60	54	76	76	65	84	82	92	61	102	69	87	85	81	84
30A	+	56	75	69	58	76	126	91	136	133	160	149	149	221	254	245
	-	69	71	60	67	77	92	91	120	111	131	151	98	141	226	210
30B	+	208	263	158	167	139	101	119	118	109	114	114	86	81	74	75
	-	166	186	146	129	110	87	103	83	79	90	83	73	51	65	58
30C	+	52	49	54	60	69	65	44	59	50	68	46	63	53	71	113
	-	46	67	35	62	53	53	46	48	43	50	50	63	60	86	107

Table 3. Continued

Cell		Blocks of 25 Trials														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
32A	+	149	125	151	157	100	220	184	163	202	193	159	182	249	226	191
	-	122	119	211	245	200	234	230	196	175	198	206	288	258	245	304
32B	+	113	226	263	174	203	212	214	235	200	248	182	255	125	196	165
	-	222	273	271	257	291	249	240	312	268	309	221	274	291	256	190

Table 4. Summary of Analysis of Variance Results For Unit Responses.

HABITUATION

Source	SS	df	MS	F	p
Within Subjects	2108.38	160			
CS+ vs. CS-	109.73	1	109.73	8.53	.005
Trials	3.99	2	1.99	.15	N.S.
Interaction	2.35	2	1.17	.09	N.S.
Error	1992.30	155	12.85		

CONDITIONING

Source	SS	df	MS	F	p
Within Subjects	4987.47	544			
CS+ vs. CS-	126.69	1	126.69	16.17	.001
Trials	593.45	8	74.18	9.47	.001
Interaction	140.08	8	17.51	2.23	.05
Error	4127.25	527	7.83		

EXTINCTION

Source	SS	df	MS	F	p
Within Subjects	1514.60	160			
CS+ vs. CS-	29.54	1	29.54	3.22	N.S.
Trials	56.92	2	28.46	3.10	.05
Interaction	7.44	2	3.72	.40	N.S.
Error	1420.69	155	9.17		

Table 5. Summary of Analysis of Variance Results For CM AEP Data.

HABITUATION

Source	SS	df	MS	F	p
Within Subjects	81807.83	55			
CS+ vs. CS-	14344.38	1	14344.38	11.45	.005
Trials	3546.27	2	1773.14	1.41	N.S.
Interaction	1301.85	2	650.92	.51	N.S.
Error	62615.33	50	1252.31		

CONDITIONING

Source	SS	df	MS	F	p
Within Subjects	165420.94	187			
CS+ vs. CS-	41745.58	1	41745.58	61.77	.001
Trials	8005.09	8	1000.64	1.48	N.S.
Interaction	781.37	8	97.67	.14	N.S.
Error	114888.90	170	675.82		

EXTINCTION

Source	SS	df	MS	F	p
Within Subjects	38635.67	55			
CS+ vs. CS-	9648.55	1	9648.55	18.05	.001
Trials	2255.48	2	1127.74	2.11	N.S.
Interaction	13.73	2	6.86	.01	N.S.
Error	26717.91	50	534.36		

Table 6. Summary of Analysis of Variance Results For VPL AEP Data.

CONDITIONING

Source	SS	df	MS	F	p
Within Subjects	93886.17	136			
CS+ vs. CS-	327.01	1	327.01	.45	N.S.
Trials	5872.88	8	734.11	1.01	N.S.
Interaction	1703.68	8	212.96	.29	N.S.
Error	85982.60	119			