CORTICAL AND THALAMIC CORRELATES OF DIFFERENTIAL CLASSICAL CONDITIONING: A CRYOGENIC STUDY

by

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DEDICATION

Plasticity, then, in the wide sense of the word, means the possession of a structure weak enough to yield to an influence, but strong enough not to yield all at once. Each relatively stable phase of equilibrium in such a structure is marked by what we may call a new set of habits. Organic matter, especially nervous tissue, seems endowed with a very extraordinary degree of plasticity of this sort; so that we may without hesitation lay down as our first proposition the following: that the phenomena of habit in living beings are due to the plasticity of the organic materials of which their bodies are composed.

William James, 1892

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INTRODUCTION

Localization of learning

One of the ambitious goals for the realm of physiological psychology is to describe the changes taking place in the central nervous system underlying the learning process. This basic problem has been of long-standing interest, couched in terms of the site of the memory to the clinical neurologists of the nineteenth century. In line with the basic concept of the localization of function Prochaska and Gall among others applied the localization of function concept to specific memories (57, 62). Flechsig considered that the association areas of the cortex were critical sites for memory storage. Having distinguished sensory and motor areas as separate from association areas, he then proposed that memory traces are stored in the association areas adjacent to the corresponding primary sensory area (6). Henschen extended the concept of localization of function to speculate that single ideas or memories resided within single cells (32).

The other main thrust in describing the changes underlying learning was with regard to the reflex arc concept (49). Psychologists of the behaviorist school, based upon the work of Bechterew and Pavlov, extended the conditioned reflex to include all behavior (68). The neurological analogy to the S-R formulation was to view the reflex arc as extending across the central nervous system. As such the learning pathway involved the excitation of the sense organ, transmission to the cerebral sensory area, which was in turn transmitted to an association area, then to the motor cortex, and subsequent

pyramidal pathway activity, eventually manifested by overt activity of the organism.

One of the most influential investigations in physiological psychology was carried out by Karl S. Lashley (6, 32, 33). In an empirical manner he examined the question of the localization of learning. Lashley's work was prepared for by Shepherd Ivory Franz, who combined the study of animal learning with that of surgical extirpation of brain tissue. In fact in 1915 he attacked the "fact" of localization of function by questioning the precise cerebral localization of motor function as described by Fritsch and Hitzig (19, 20).

Lashley initially worked with Franz investigating the effect of differential extirpation of rat cerebral cortex upon learning. All of Lashley's experiments were based upon the method of surgically destroying part of the brain of animals trained in various tasks. The surgical destruction either preceded or followed training, thus testing either learning or retention, respectively. After the postoperative testing, the animal was sacrificed and the extent of brain damage assessed.

Lashley rejected the reflex arc conception of learning in the central nervous system. At the outset he minimized the importance of the motor cortex for performance of a particular active avoidance task. Rats were required to jump toward a visual cue with a striped background. He found that the animals were able to perform the differential response with almost the entire motor cortex removed. Similarly monkeys, after undergoing surgical ablation of much of the motor cortex, could still open various latch boxes. Thus, Lashley concluded

. . . "that the motor cortex does not participate in the transmission of the conditioned reflex pattern" (33).

The reflex arc theorists posited transcortical conduction as an important element in the underlying process of the conditioned reflex. To test this hypothesis Lashley severed the underlying fibers to separate functional cortical areas. Finding that the animals were able to perform complicated habits postoperatively, he concluded that . . . "the transmission of impulses over well-defined isolated paths from one part of the cortex to another is inessential for performance of complicated habits" (33).

The findings most associated with Lashley's work are that of equipotentiality and mass action. The concept of equipotentiality applied to the primary sensory area for the given task. Within the sensory area there was an apparent lack of topographic organization of learning, in that learning remained intact as long as there was not total loss of the primary sensitivity. Successful performance was demonstrated in rats with a fraction of the sensory area intact and it did not matter which particular portion of the sensory area remained (33).

The concept of mass action referred to performance of complex tasks such as maze running. A simple relationship was found in which the amount of loss in performance was directly proportional to the amount of the cortex destroyed (32).

The interpretation of Lashley's experiments has been the rejection of the theory of well defined conditioned reflex pathways from the sense organ via association areas to the motor cortex. Furthermore, Lashley has rejected the importance of the motor cortex for the

retention of sensori-motor habits. These findings are consistent with a Gestalt view of motor learning in which the overt response is not a set patterning of muscle contractions. Neurologically this would be equivalent to a lack of a fixed efferent pathway. Thus, Lashley has maintained that it is not possible to demonstrate the isolated localization of a memory trace anywhere in the nervous system.

The effect of Lashley's findings was to discourage the examination of the localization of learning. Lashley's negative finding with regard to the localization of the engram encouraged behavioral psychology to disregard central nervous system functioning. However, a more recent re-examination of the issue of the localization of learning has offered additional information which encourages re-evaluation of the problem.

The literature concerning the effects of lesions on learning is extremely extensive. It is clear that numerous variables effect the empirical findings of this research. For example, it has been shown that the nature of the lesion, the lesion technique, the postoperative recovery period, and the nature of the learning task are critical variables affecting the relationship between lesion experiments and learning (15, 27, 50). However, it is evident that discrete lesions have been successfully utilized to show impairment of acquisition and/or retention. It is of particular interest that Delacour, for example, was able to show a deficiency in learning and retention resulting from medial thalamic lesions in the rat (15). Testing rats in a conditioned avoidance response situation, he showed a significant difference between a control group and those with medial thalamic lesions. Thus, the studies utilizing lesion techniques have given an equivocal answer concerning the localization of learning.

More recent research has been directed at an attempt to localize the active sites of learning using refined techniques. For example, Bures and Buresova have simplified the reflex pathways by using microstimulation as the unconditioned stimulus (US) (10, 11, 12, 13). The small anodal or cathodal current was applied via the same extracellular microelectrode used for recording. They used small currents (15-30 nA) to limit the spread from the site of application. According to the authors they view the situation as "injecting information" into the central nervous system, with the localized polarization as being analogous to a peripheral US (21). However, it is questionable as to whether the highly synchronized and aphysiological nature of the microstimulation could be viewed as influencing the neural processes of conditioning in the comparable manner as a peripheral US. As such, it is especially critical when using microstimulation as a US to also use appropriate sensitization or pseudoconditioning controls. Both sensitization and pseudoconditioning are factors which may contribute to changes in behavior yet neither are due to the experimentally arranged contingency between the CS and US. In the case of sensitization, the occurrence of the US potentiates the response to CS without an established CS-US contingency. Pseudoconditioning refers to the possibility that the unconditioned response to the US may come to be elicited by other stimuli in spite of the absence of any association between them. Unfortunately, appropriate controls were absent from the initial Bures and Buresova studies, and it appears as if the conditioning changes reported can be best attributed to sensitization (22).

Thus, although this technique allows a localization of function, it does not appear relevant for specifying bona fide conditioning processes.

Olds et al. have utilized a sophisticated technique of electrophysiological mapping to determine the sites of learning (17, 31, 47, 54, 55). The initial study of the rat brain suggests two overall conclusions; (1) that the active sites of learning are widely distributed across brain regions, including the pons, midbrain, diencephalon, paleocortex, and cortex; and (2) that although the active sites are widely distributed across brain levels, they are nevertheless localized at a finer scale of organization. For example, conditioning changes were evident in the posterior nucleus of the thalamus, yet conspicuously absent from the tectum and dorsal reticular formation (47).

Olds et al. stated that the active site of learning needs to be differentiated from the secondary consequences of learning changes occurring at a distant point. They reasoned that a projected change would occur later in the CS-US sequence, and that by using a latency criterion, it would be possible to specify the active site of learning. The active locus would then be defined as changes in neural activity correlative with learning occurring the earliest in the CS-US interval. In fact, learning centers were labelled with post-CS latencies of 20 milliseconds, which after allowing air travel time for the auditory stimulus, was equivalent to conditioning changes 16 milliseconds after the CS (47).

The definition of active learning sites with respect to the shortest post-CS latency neglects the critical importance of the acquisition curve. The parameter of the number of pairings should not be disregarded. A site that changes early in the pairing process could exert tonic influences on distant neurons, which would be seen as conditioned changes

at some point prior to behavior but anywhere in the CS-US interval. A later finding by Disterhoft and Olds substantiates this position. They found that while there appeared to be very short post-CS latency conditioned changes in cortical units, the thalamic units changed earlier in the trial-to-trial sequence. In fact, the mean number of trials to criterion showed that the cortical changes were subsequent to the behavioral changes, with the latter perhaps being more related to the elaboration of an already conditioned response, the mean trials to criterion being 47 and 69 for the behavior and cortical units, respectively. In addition, Disterhoft and Olds uncovered changes in the pre-CS unit activity which developed in sequence with the pairing of the CS and US. In this case the background activity changes in cortical units preceded that of thalamic changes (17).

Woody has attempted to characterize a reflex pathway and to analyze the neuronal changes involved (9, 71-78). The conditioning paradigm is that of classical conditioning in which an auditory stimulus is the conditioned stimulus (CS) and a glabella tap is unconditioned stimulus (US). Woody has recorded the overt response, the electromyogram from the orbicularis oculi muscles, evoked potentials from the facial nucleus, and single cell and evoked potential activity from the coronal-precruciate cortex. The electrophysiological data showed changes in a naive or extinguished state. The findings suggest that the conditioned eye blink is mediated cortically. For example, the cortical application of KCl is followed by the loss of the conditioned blink response (78). In cats with extensive bilateral lesions of the cortical motor areas, eye blink conditioning was severely impaired; however, the lesioning

did not simply alter the performance of the eye blink response, since a loud auditory stimulus could elicit an unconditioned blink response for the lesioned animals (78).

The cortical involvement in the acquisition of the conditioned eye blink response is further suggested by the electrophysiological changes in the coronal-precruciate cortex. Using electrical stimulation the thresholds of cells were lower in conditioned animals than in those in the naive or extinguished states. There was specificity in terms of the cells which did exhibit threshold changes. The threshold to electrical stimulation was specifically lowered for those cells that projected to the orbicularis oculi motoneurons (75).

Subcortical and cortical conditioning

It appears as if conditioning can be described in terms of both cortical and subcortical components. The fact that conditioning can be achieved in decorticate animals suggests the importance of subcortical components (49). Other investigators have shown a variety of interfering effects from various cortical and subcortical lesions. It has been my intent to extend these investigations into an examination of the nonspecific thalamus and sensori-motor cortex during conditioning. Using an appetitive classical conditioning procedure, Disterhoft and Olds were able to show the existence of thalamo-cortical and cortico-thalamic interactions during conditioning (17). However, they were unable to resolve the nature of the relationship, with the cortex appearing to be the active locus according to the criterion of prestimulus CS activity while the posterior thalamic nucleus was the active site of conditioning with respect to post CS unit activity. To better enable a resolution of this problem, I used a reversible

cryogenic blockade to interfere with the pathways from the nonspecific thalamus to the cortex, while recording the neural changes concomitant with conditioning at the cortical level and at a site posterior to the cryogenic blockade in the nonspecific thalamus.

According to Disterhoft and Olds, the posterior thalamic nucleus receives auditory input, receiving afferents from the inferior colliculus and being reciprocally connected to the auditory cortex. The posterior nucleus appears to be an accessory thalamocortical auditory channel in addition to the better known leminscal channel through the medial geniculate. It is the purpose of the present experiment to investigate whether the centromedian region of the thalamus fulfills a special role in conditioning for the somesthetic modality (60).

Centromedian nucleus

In a previous study, the contribution of sensory evoked activity through the centromedian area of the thalamus was described (45, 51). It was concluded that this region contributed in a significant manner to sensory evoked activity of the postcruciate cortex, with a cryogenic thalamic blockade often diminishing the postcruciate unit responses by about 50%. In addition, the predominant influence of the centromedian blockade was from about 100 through 200 milliseconds poststimulus.

Other studies have shown the responsiveness of units of CM to somatic stimulation (2,3). Unlike some of the responses recorded in the ventrobasal complex, units of CM are diffusely responsive with large receptive fields to somatic stimulation often covering the entire animal. Polysensory units are common. At least one investigator has suggested that the centromedian area of the thalamus acts as a reticular core conveying information undifferentiated with regard to modality

(64, 65). However, an analysis of the influence of cryogenic blockade of CM suggests indirectly that there is a high degree of CM sensory convergence but that differential responsiveness exists (46). These conclusions were based upon the differential effects of CM blockade upon units of the postcruciate cortex, both for a light stimulus and different placement of a somatic stimulus.

Even with regard to auditory stimuli as used by Olds et al., they have described short latency conditioned changes ("learning centers") within that region most analogous to the centromedian-parafasicular complex of the cat (47). Their sites have included nucleus parafasicularis of the rat. The parafasicular nucleus is relatively better developed in lower species; however, it is often equated with the large-celled dorsomedial region of the CM-Pf complex in higher species. Thus, it was hypothesized that using a conditioning paradigm within the somesthetic modality, conditioning changes will be evident at the level of centromedian area of the thalamus. To monitor these changes a macroelectrode was placed within this region.

Postcruciate cortex

The important role of the sensori-motor cortex in associative learning has been suggested by several investigators (42, 43, 47). Olds has reported conditioned changes of less than 20 msec post-stimulus occurring in the sensori-motor cortex of the rat. Although only a small percentage of neurons sampled from the sensori-motor cortex showed conditioning, the conditioned changes were of large magnitude and stimulus specific. Thus, the cortical neurons showed differential responsiveness to the CS+ and CS- stimuli (47).

Woody using electrophysiological and lesion techniques has shown that the sensori-motor cortex of the cat is involved in acquisition of a conditioned eye blink. Although performance of the eye blink is still possible, lesioning the sensori-motor cortex impairs acquisition of this associative learning (78). Papsdorf, Longman, and Gormezano reported that the sensori-motor cortex was involved in associative learning of a nictitating membrane response in the rabbit (48).

The postcruciate cortex shows a considerable amount of sensory convergence, as shown by the large proportion of cells which respond to at least two sensory modalities. Buser described that approximately 92% of the neurons that were immediately posterior to the cruciate sulcus were polysensory (14). The proportion of polysensory cells decreased rapidly proceeding in a posterior direction from the sulcus. O'Brien and Fox further substantiated the polysensory nature of the postcruciate cortex of the cat. Using an auditory click, light flash, somatic stimulation, and brainstem stimulation, 89% of the cells of the postcruciate cortex responded to two or more stimuli. Fifty-seven percent of the cells were multisensory with respect to click, light, and somatic stimulation (43).

O'Brien and Fox reported that neurons of the postcruciate cortex showed conditioning changes in accordance with a classical conditioning procedure. Using a light flash as the conditioned stimulus and a weak somatic shock as the unconditioned stimulus, they observed neuronal changes for a conditioning group that were significantly different from that of a sensitization control group. During the pairing procedure, postcruciate neurons showed systematic response changes which varied

with the sign of the initial response and the number of trial presentations. The conditioned changes showed reversibility during repeated extinction trials in which the conditioned stimulus was not followed by the unconditioned stimulus occurrence. Additionally, the post-cruciate neuron conditioning changes showed more rapid development of response changes analogous to the phenomenon of savings for behavioral conditioning (42). The phenomenon of savings is the long lasting effect of conditioning, which is operationally observed as the more rapid re-acquisition of a conditioned response after an animal has been previously presented with a conditioning and extinction sequence.

Ventral anterior thalamic nucleus

It appears as if VA is a prime candidate for serving as the final link in the nonspecific thalamic projection. Several physiological studies indicate that the ventral anterior thalamic nucleus plays an essential role (23, 63, 69).

Starzl and Whitlock analyzed the thalamic regions which yield recruiting responses (63). They included the ventral anterior nucleus as part of a diffuse thalamic projection system, as well as centromedian and intralaminar nucleus. They noted a low threshold to ventral anterior nucleus stimulation for eliciting recruiting responses, which was applicable in both cat and monkey. Again, intranuclear effects of repetitive stimulation were most noticeable in ventral anterior nucleus. These effects could be seen when both stimulating and recording electrodes were positioned in the ventral anterior nucleus.

Hanberry and Jasper used a study of the distribution and latency of the cortical responses, intrathalamic recording, electrolytic lesions, and cortical ablation to determine the pathway of the nonspecific

thalamocortical projection system (23). These several lines of evidence led them to conclude that the pathway of the nonspecific thalamocortical projection system was independent of specific thalamic relay nuclei and association nuclei in the cat. Furthermore, it appeared from their physiological data that the final link in the multisynaptic chain of the nonspecific thalamocortical projection was within the anterior portion of the thalamus. Likely candidates for the role of the final link were considered to be the ventral medial nucleus, reticular nucleus of the thalamus, and ventral anterior nucleus.

Anatomic studies of the ventral medial nucleus seemed to suggest that the neurons are probably independent of the cortex, as judged by the limited extent of retrograde degeneration evident after decortication (23).

It had been previously suggested that the reticular thalamic nucleus was the final link in the nonspecific thalamocortical projection. However, the Golgi studies of Scheibel and Scheibel, while showing long axoned cells, demonstrated a predominant caudally directed influence. Thus, the Scheibels have concluded that the reticular thalamic nucleus does not serve as the final relay of the ascending projection, but rather that this nucleus acts to integrate thalamocortical and corticothalamic impulses via abundant collaterals which supply both specific and nonspecific thalamic nuclei (52, 53).

The importance of the ventral anterior nucleus as a final relay for the nonspecific thalamocortical projection is suggested by several lines of evidence. Several investigators using degeneration studies have confirmed that VA projects to central and precentral frontal cortex (23). Stimulation studies have shown that not only can recruiting responses be elicited by repetitive stimulation of VA but that the responses are

widespread and of short latency. Using electrolytic lesions Hanberry and Jasper traced the pathway for cortical recruiting responses as elicited by repetitive CM stimulation. They found that lesions of the dorsal and lateral aspects of ventral anterior nucleus were sufficient to reduce or abolish recruiting responses of the somatosensory cortex (23). This finding has been confirmed by Skinner using a reversible cryogenic blockade, in which the blockade of VA was shown to impair the synchronization of cortical activity as per recruiting responses or barbiturate spindling (60).

Cryogenic technique

It was deemed advantageous to use a technique for producing reversible functional blockade, rather than permanent lesioning. Such a procedure allows a comparison with controls, both before and after the cooling procedure, enabling an analysis of the effects specifically related to the experimental procedure. Various methods have been used to render neural tissue temporarily inactive, including disruptions caused by chemicals (37, 59), anoxia (37, 59), pressure (37), ultrasound (5, 37), warming (37, 59), and cooling (3, 4, 7, 8, 26, 37, 59). The cooling technique seems particularly suitable for subcortical blockade. Advantages include rapid reversibility, the localization of the functional blockade, and a minimum of permanent damage.

Previous studies have shown a restricted temperature gradient around the cryoprobe tip inserted in a brain at 37° C or in an agar medium (10). Skinner reported that cryogenic blockade of the inferior thalamic peduncle did not interfere with conduction of fibers in the

adjacent part of the internal capsule (60). Thus, the effective temperature gradient is limited to a few millimeters from the cryoprobe tip when the probe temperature is stabilized at 10° C. It is evident that the volume of the functional blockade is most directly related to probe temperature. Skinner suggested that the functional blockade extends 2-3 mm from the probe tip when the cryoprobe temperature is stabilized at 0° C (61).

The effective cooling gradient is influenced by the local cerebral vasculature, acting as a heat source. Thus, the volume of tissue subjected to blockade is dependent upon the site of cooling. It is possible that with differing vasculature the temperature gradient associated with topical cortical cooling would differ from that associated with subcortical cooling (39).

The extent of the functional blockade is not only determined by the physical temperature gradient, but by the sensitivity of the neuronal processes to disruption by cooling. It is well established that lower temperatures are required to block fiber conduction than synaptic activity. Mammalian A fibers can be reversibly blocked at temperatures of 5-6° C, while smaller fibers require lower temperatures (16). Conduction along C fibers is disrupted at 0° C (36).

Synaptic processes are more sensitive to decreased temperatures than fiber conduction. Several investigators have observed blockade of cerebral synaptic processes at temperatures in the vicinity of 20° C. Jasper reported that the local direct cortical response could be blocked by cooling to approximately 20° C, reflecting disrupted neuronal activity among the most superficial cortical synapses (26).

The blockade of cerebral synaptic processes in the vicinity of 20° C has been observed by other investigators. Dondey, using a subcortical cooling probe, reported the blockade of conduction through nucleus ventralis posterior of the thalamus (18).

Andersen reported that the cerebral cortex could be completely deafferented by cooling the thalamus to 20° C (4). Benita and Conde used the differential sensitivity to decreased temperature to selectively block synaptic processes while leaving fiber conduction intact (7).

The careful regulation of cooling has been chosen as a technique for blocking synaptic activity of the ventral anterior nucleus of the thalamus. The temperature of the cooling probe was stabilized at 5° through 10° C to achieve as large a volume of tissue blocked as possible. In assuming a temperature gradient of approximately 6° C/mm (26), it is possible that synaptic blockade, at 20° C, would extend across the ventral anterior nucleus. However, the shape of the blockade would not be a simple spheroid, but rather would be influenced by the geometric shape of the cooling probe tip. The shape of the blockade would approximate an ellipsoid, reflecting the irregular geometry of the cooling surface. Measurement of the gradient of cooling gave support to the assumptions about the shape and extent of the cooling.

Aim of study

The aim of this study has been to evaluate neural changes concomitant with a simple associative learning situation. The specific goal has been to analyze the interaction between conditioning changes of the sensorimotor cortex and nonspecific thalamus. For these purposes a reversible cryogenic blockade has been used in the region of the ventral anterior nucleus of the thalamus during the conditioning sequence.

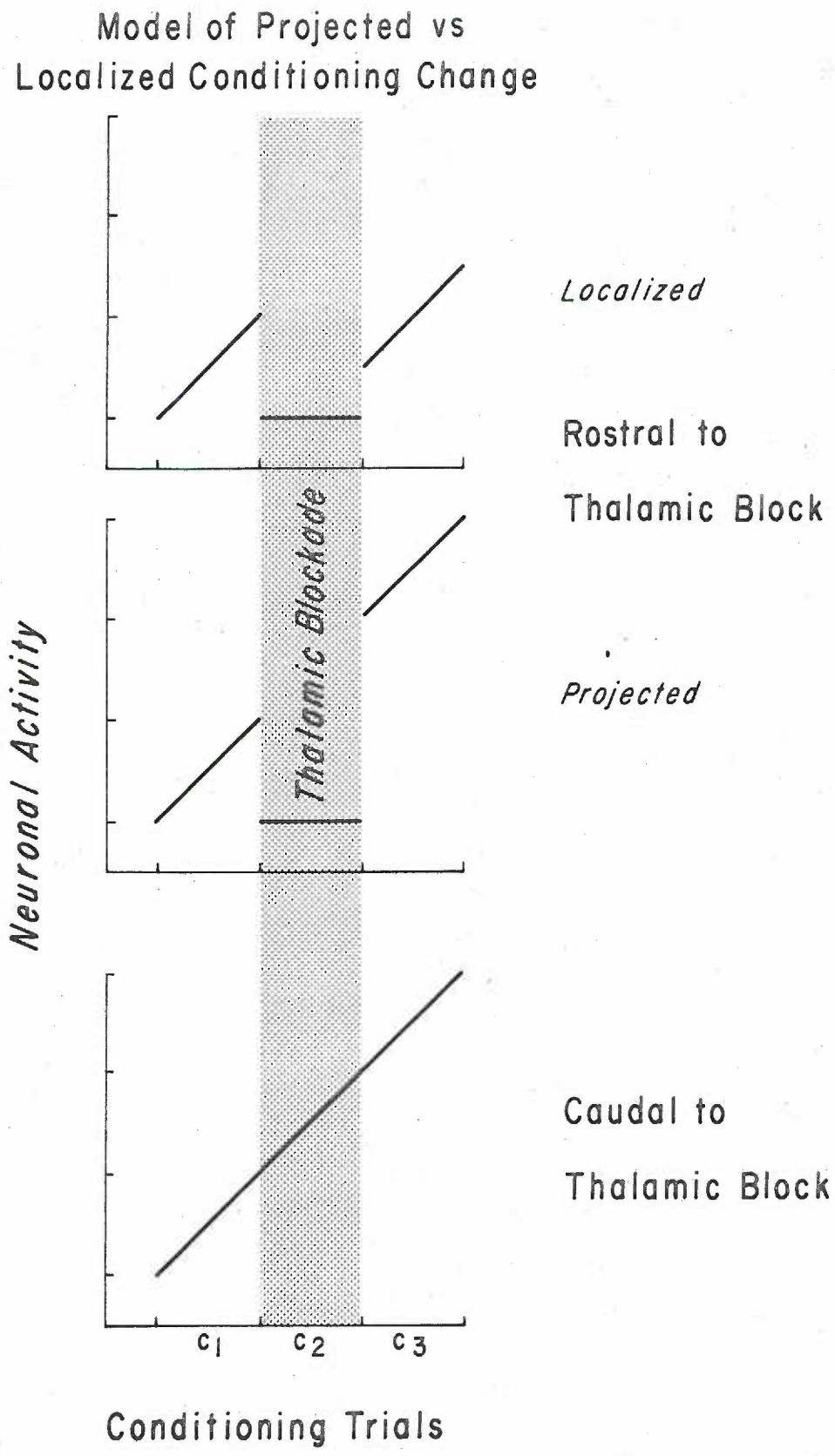
Hypotheses

1. If the functional blockade of the ventral anterior nucleus region interrupts the afferent pathway for the conditioned stimulus, then a deficit in the sensory evoked activity of the postcruciate neurons will be evident during the cooling blockade. By measuring the degree of change in the sensory evoked activity of the cortical neuron, it will be possible to evaluate the role of the ventral anterior nucleus region with regard to the conditioned stimulus afferent pathway.

2. If the conditioning changes of the cortical neurons are dependent upon afferent activity at the neuron, then the development of conditioning will be impaired during the thalamic blockade of the conditioned stimulus afferent pathway.

3a. If the conditioning changes are localized in neurons caudal to the thalamic blockade, then immediately after termination of the blockade the projected conditioned response will be observed in the cortical neurons (middle graph).

3b. If the conditioning changes are localized in neurons rostral to the thalamic blockade, then a prolonged disruption of conditioning changes will be observed in the cortical neurons (upper graph).


Model of Projected vs
Localized Conditioning Change
Localized
Rostral to
Thalamic Block
Thalamic Blockade
Projected
Neuronal Activity
Caudal to
Thalamic Block
C1
C2
C3
Conditioning Trials

METHODS

Subjects

Mature cats weighing between 2.2 and 4.0 Kg were used as subjects. Siamese cats, part Siamese cats, and those with upper respiratory ailments were excluded from subject population. A total of 45 cats was utilized in the preliminary work plus the experiment reported here. Thirty-nine subjects contributed to the data reported in this study with the procedure common to these subjects being highly standardized. All cats were obtained from the Department of Animal Care of the University of Oregon Health Sciences Center.

Animal preparation

Experiments were performed on acutely prepared cats with operative procedures performed under ether anesthesia. All subjects were anesthetized in a wooden box with a transparent glass front which permitted observation of anesthetic level. Depth of anesthesia was judged from repiratory rate, corneal reflex, and motor tone. The epiglottis and throat were topically anesthetized with Cetacaine spray to prevent reflexive gagging, and a soft endotracheal tube was inserted with the aid of a laryngoscope. The tracheal tube was secured using either an inflatable cuff or adhesive tape and Michel clips. The endotracheal tube was intermittently connected to a smaller ether bottle allowing for anesthesia during the operative procedures.

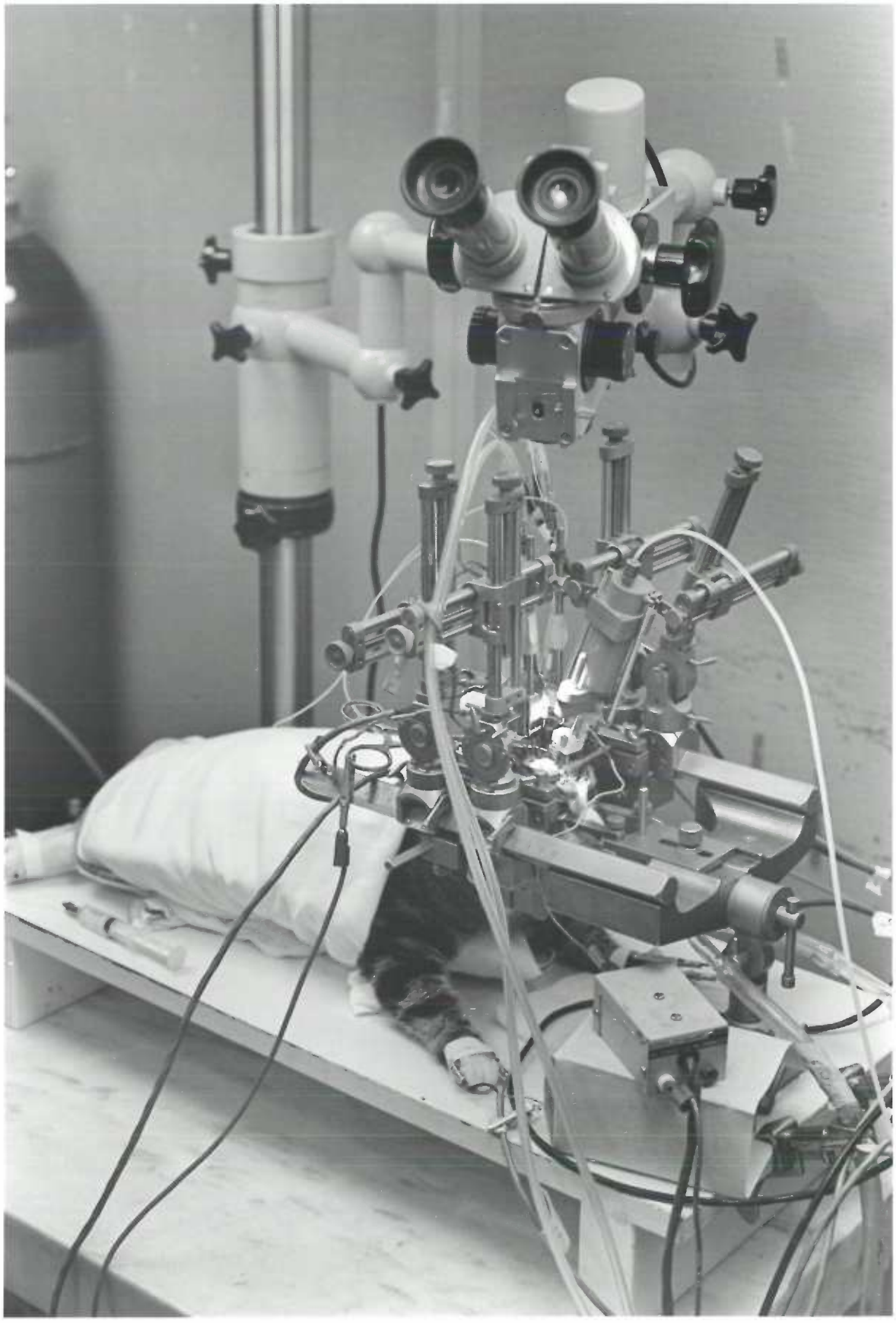
A small incision was made in the skin of the right hindlimb to allow exposure of the saphenous vein. The vein was dissected free of adjacent tissues and the distal end tied off. The vein was then cannulated with 1 mm outside diameter polyethelene tubing which was secured to the vein by tying with thread.

While under general anesthesia, the animal was mounted in a Kopf model 1204 stereotaxic apparatus using standard ear and eye bars. A longitudinal incision was next made along the midline of the skull. After retraction of the temporal muscles with a periostial elevator, all cut surfaces were infiltrated with 4% procaine HCl. The skull was cleared of periosteum and tissue, was washed with physiological saline, and was dried.

According to stereotaxic coordinates four burr holes were made in the skull using a Foredom electric dental drill. All drilling was aided by the use of a Bausch & Lomb microscope for visual guidance. In general a fine layer of bone was removed using a stainless steel micro-pick after terminating drilling. For all probes, small openings in the dura mater were established. The size of the dura openings were minimized, being just sufficient to allow probe penetration.

After the cryoprobes and the macroelectrode were positioned in their proper locations, a system allowing a less traumatic securing of the skull was initiated. For this purpose it was necessary to secure two acrylic plastic tubes to the top of the skull using stainless steel screws and cranioplastic cement. Stainless steel pins were positioned from an adapter on the stereotaxic instrument into the plastic sleeves attached to the skull. Figure 1 illustrates the experimental arrangement. The cat is positioned in the stereotaxic device with both the standard eye and ear bars in place as well as the adapter skull head-holders. The manner in which the anterior plastic sleeve has been secured to the skull is shown in Figure 1. After the dental cement had hardened it was possible to remove both the ear and eye bars,

Figure 1: Animal preparation, standard eye and ear bars.



relieving any pain produced by these pressure points. Figure 2 shows the subject secured by use of this atraumatic headholder. Since the electrodes were positioned while the standard ear and eye bars were in place, the stereotaxic accuracy was maintained.

Subsequent to removal of the ear bars and eye bars, ether anesthesia was terminated and gallamine triethiodide (Flaxedil) was administered for immobilization. Initially a 20 mg dosage of gallamine triethiodide was manually injected via the cannulated saphenous vein. After this initial manual injection, a Sage model 216 DC infusion pump was used to administer the drug. This pump allowed the continuous influsion of gallamine triethiodide at the rate of 10 mg per hour for a maximum of ten hours.

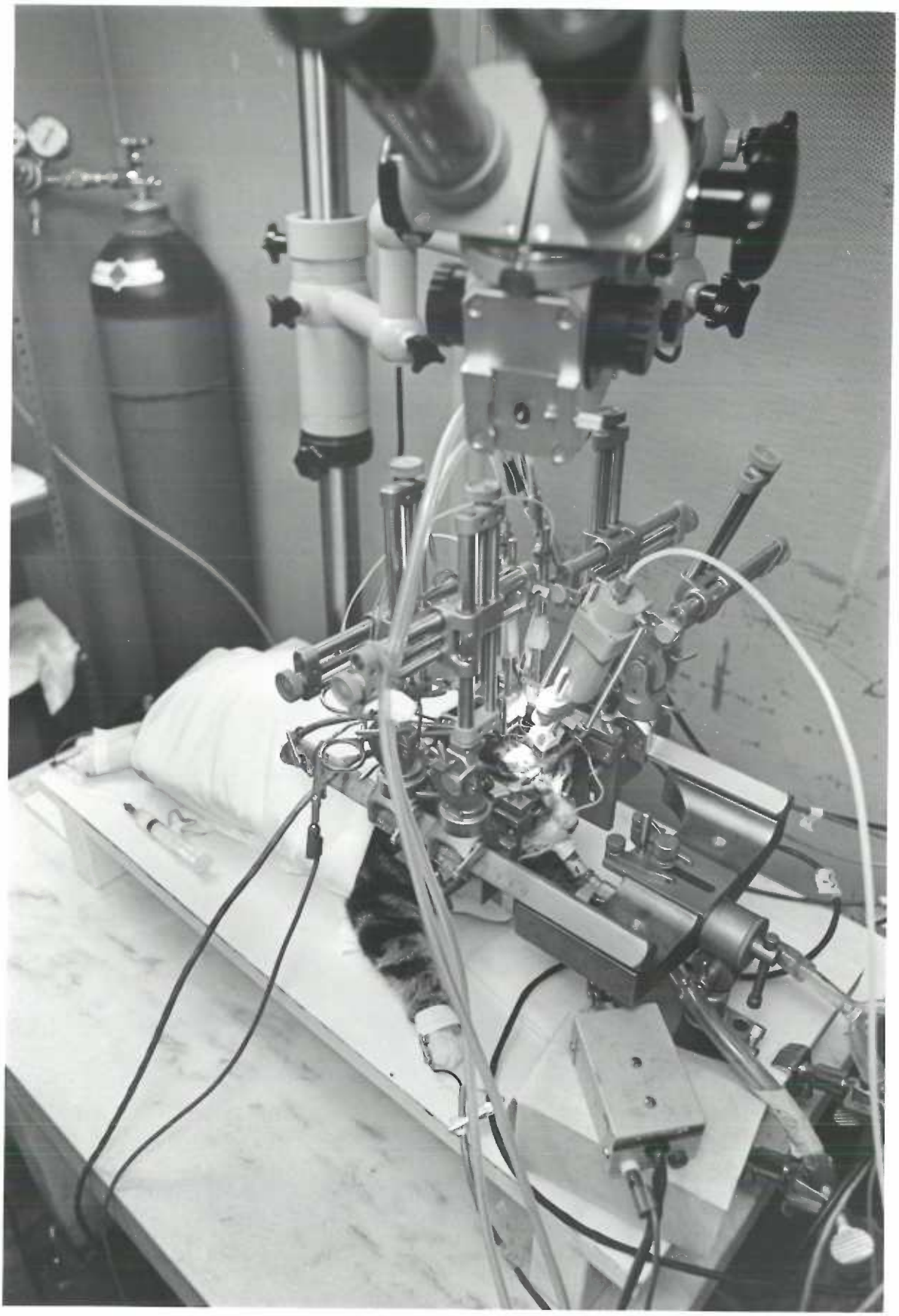
Artificial ventilation was maintained with a Harvard pump. Frequency was set at 26 strokes per minutes with stroke volume varied to maintain 3.6-4.0% tracheal CO_2 levels as monitored on a Godart capnograph. Body temperature was monitored and maintained near 37° C with a heating pad and hot water bottles. All precautions were taken to insure the comfort of the subject with local anesthesia being maintained on a 4 hour schedule.

Recording electrodes

Evoked potential data were recorded from the centromedian nucleus of the thalamus. The stereotaxic coordinates were AP+7.5, lateral 2.5, and vertical+0.5, with a unilateral electrode placement in the right hemisphere.

The macroelectrodes utilized in this study were bipolar with either concentric or parallel design. The concentric bipolar electrodes

Figure 2: Animal preparation, skull headholder adapter.



were constructed of .64 mm O.D. (22 gauge) tubing with an inserted insulated stainless steel wire of .25 mm diameter. The center wire extended beyond the end of the stainless steel tube by 1.0 mm with only the cross section of its cut end being free of insulation. The tube was coated with Epoxylite such that only the cross sectional end remained exposed.

Alternatively, bipolar macroelectrodes were made of two pieces of .25 mm stainless steel insulated wire. One wire extended below the other wire, allotting a 0.5 mm bared recording tip, then a 0.5 mm insulated region, followed by a 0.5 mm bared tip of the second wire.

The largest burr hole exposed the peri-cruciate cortex, using stereotaxic coordinates of AP +22 and lateral 2.0. The approach through the skull was made via the frontal sinus with a large opening drilled in the region of the posterior juncture of the frontal sinus. The microelectrode was positioned at approximately 2 mm lateral from midline and 1 through 2 mm posterior to the cruciate sulcus. The microelectrode was positioned to be perpendicular to the cortical surface at the point of entry.

The microelectrodes used were either 25 micron diameter wires or 2-4 micron diameter etched stainless steel pins. The 25 micron wire electrodes were a glass coated platinum-iridium alloy. These small wires were placed inside of a .41 mm O.D. (27 gauge) stainless steel tube and fused to the tube with 1 cm of the wire tip extending beyond the tube. The tube was insulated with Epoxylite. The effective recording surface was the cross sectional area of the 25 micron

diameter wire tip. These wires exhibited some flexibility to movement but did not have sufficient strength to penetrate the pia membrane.

In most cases, etched insect pins were utilized as recording microelectrodes. Size 000 stainless steel insect pins were electrolytically etched to a 2-4 micron tip. The insect pins were coated with several coats of an Epoxylite insulation. These microelectrodes provided an excellent ratio of signal to noise with the ratio sometimes being as high as 20 to 1.

Recording procedure

The signal from the microelectrode was led through a Bak cathode follower, amplified by Tektronix 122 amplifiers, and filtered. The subject, the cathode follower, and the preamplifiers were all located inside a walk-in double walled, electrically shielded sound-proof room. A marble table supported the animal and stereotaxic instrument which was firmly mounted to reduce vibratory effects on recording and electrode stability.

The recording from the microelectrode was monopolar, using either the temporal muscle of the scalp or the frontal sinus as the reference for the microelectrode recording.

The signal was passed from the cathode follower and was divided into two parallel preamplifiers with different frequency pass settings. The frequencies between 0.2 and 250 Hz comprise the evoked potential data, while the frequencies between 500 Hz and 3 kHz comprise the single cell data. Additionally, the bipolar macroelectrode record was amplified by a Tektronix 122 amplifier and filtered. Both the single cell data

and the evoked potential data were led into amplifiers with adjustable gain (Tektronix 2A60, 3A72, or 2A63). These signals were continuously monitored on an oscilloscope. The evoked potential data were amplified to 1 v peak-to-peak, stored on a Sangamo FM magnetic tape recorder, and maintained as the data record. In addition, two channels of the tape record were reserved for storing event marker pulses. This facilitated the sbusequent off-line analysis of the evoked potential data by means of a Digital Equipment Corporation PDP-12 computer.

After the single cell recording was suitably amplified, it was led through a Krohn-Hite filter allowing differential filtering of the signal from the noise, effectively enhancing the data record. The single cell record was led to a Schmitt trigger on the PDP-12 computer for on-line analysis. The Schmitt trigger level and single cell trace were continuously monitored to assure that only spikes were counted and noise was reliably excluded.

Cooling blockade procedure

A description of the cooling probe (cryoprobe) has been published (58). The cryoprobe system enables localized cooling at the tip of the probe. Briefly, the cryoprobe functioned by the regulated flow of a coolant, cold ethyl alcohol, under pressure through a "U-tube" cryoprobe. A DC heater wire is wrapped around the shaft of the probe, leaving 2 mm bare at the tip. The DC current was supplied by two automobile storage batteries. It was found beneficial to isolate the heater current circuit from the temperature monitoring circuit. The DC current did not interfere with the electrophysiological recording. The amount of current supplied to the heater

circuit was regulated using a power transistor, and directly read from an ammeter. The drawing of approximately one through two amperes of heater current through the low resistance circuit maintained the shaft near body temperature. The temperature of the shaft was maintained between 30 and 36° C. A comparator circuit was utilized to terminate heating if the temperature exceeded 36° C, which prevented irreversible coagulation. In practice, the heater current and alcohol flow were exceedingly stable, and once established remained constant for the experiment's duration.

The temperature of the tip and shaft of the cryoprobe was continuously monitored via appropriately placed microthermocouples (0.12 mm in diameter). Temperature measurements were accomplished using two copper-constantan junctions. Maintaining one junction at a known reference temperature, the temperature of the second junction could then be determined. The small voltage generated by the opposed junctions was approximately a linear function (40 u V/C) of the temperature difference between the monitoring and reference junctions. It had been found most convenient to use room temperature as the reference temperature. By calibrating the deflection of a meter with respect to the voltage generated by the difference of the two junctions, the temperature could be directly read.

To avoid irreversible damage, cooling below 0° C was prevented through the use of a comparator circuit. The tip temperature was maintained at 5° C during cooling.

Cooling blockade sites

Two cryoprobes were placed in the region of the ventral anterior nucleus of the thalamus with one cryoprobe in each hemisphere. The stereotaxic coordinates used were AP: +11.5 mm, lateral: 4.5, vertical: +4.0 according to Jasper and Ajmone Marsan. The locations were verified histologically.

Experimental design

The basic design was to divide the subjects into two groups, those undergoing the experimental intervention, and those serving as a procedural control. The experimental intervention was the cryogenic blockade of neural activity in the vicinity of the ventral anterior nucleus of the thalamus. This functional blockade was in effect during a 75 trial block of the stimulus pairings that constitute the necessary condition for classical conditioning.

The procedural control group evaluated the effect of implantation and operation of the cryoprobes. For these purposes the control group was surgically prepared in an identical fashion to that of the experimental group. The cryoprobes were placed bilaterally into the region of the ventral anterior nucleus of the thalamus. During the appropriate 75 trial block of the conditioning sequence, the cryoprobes were operated and alcohol was circulated. For the procedural control group, a dry ice bath was not used so that the circulated alcohol was at room temperature (approximately 24° C) rather than being cold. To duplicate the operation of the cryoprobe system in the procedural control group, the heater circuit was supplied with a low DC current thus warming the cryoprobe toward body temperature while the probe temperature was monitored.

In summary, the experimental design involved two groups, an experimental group which underwent thalamic cooling, and a procedural control group. Cats were randomly assigned to either group. Nineteen cats were assigned to the experimental group and 20 cats were assigned to the procedural control group.

Stimulation apparatus

Weak electrical shock was the stimulus used in all experiments. In all cases the stimulus was delivered by means of 21 gauge hypodermic needles placed subcutaneously in the paw pad region. The isolated stimulators were used to deliver: (1) the stimulation to one forepaw, (2) stimulation to the other forepaw, and (3) stimulation of both hindpaws simultaneously. Stimulation of the forepaw served as the conditioned stimulus (CS) and stimulation of both hindpaws served as the unconditioned stimulus (US).

In all cases Devices Mark IV isolated stimulators were utilized. The stimulus consisted of four square wave pulses of 0.5 msec duration at a frequency of 280 Hz. The intensity of the forepaw shock (CS) was always less than half of the intensity of the hindpaw shock (US). The forepaw shock was less than 10 volts and always below a minimum value necessary to produce a perceptible muscle twitch.

The occurrence of a trial was regulated by the closure of a microswitch using a mechanical film programmer, and the presentation of the stimuli during the trial was controlled by a Devices Digitimer.

Conditioning paradigm

A differential classical conditioning paradigm was utilized. The standard classical conditioning paradigm is to pair a conditioned

stimulus (CS) with an unconditioned stimulus (US), establishing an explicit contingency between the occurrence of the CS and the US occurrence. The differential classical conditioning paradigm includes a CS+ stimulus and a CS- stimulus. The CS+ stimulus is explicitly paired with the US presentation while the CS- is never paired with the US. Thus, this conditioning paradigm measures a differentiation process, in which the organism is able to differentiate a paired stimulus from an unpaired stimulus. In these experiments the CS+ was weak shock of one forepaw and the CS- was weak shock of the other forepaw. The CS+ was assigned in a random manner for any individual cat and remained the same for the subject. The stimulus parameters of the CS+ and CS- were equated. In all cases the CS+ and CS- were not presented as separate and distinct blocks of trials, but rather were interdigitated and randomized, one amongst the other. The actual sequence of CS+ and CS- presentations is contained in Appendix A.

The time between CS presentations, the intertrial interval, was randomized around a mean. The mean intertrial interval was 14.5 seconds with a range from 11.5 through 18.5 seconds. The list of intertrial intervals is contained in Appendix A.

Both the sequence of CS+ and CS- presentations and the intertrial intervals were determined by a mechanical film program. Within one circuit of the film program there were 25 CS+ presentations and 25 CS- presentations. Thus, for 25 trial blocks sequentially there was an identical distribution of intertrial intervals and the CS+ and CS- sequence.

The stimulus presentation sequence was divided into three parts: (1) habituation, (2) conditioning, and (3) extinction. The inset of Figure 3 shows the details of the stimulus presentation sequence.

During habituation baseline data were gathered, which provided a measure of the initial responsiveness to the CS alone stimuli. For this purpose, 75 CS+ and 75 CS- trials were presented during habituation.

The conditioning sequence immediately followed habituation. The occurrence of the CS+ was followed 550 msec later by the occurrence of the US and 225 CS+ and 225 CS- trials were presented. In contrast to the CS+, the CS- was never paired with the US.

The extinction sequence of 75 CS+ and 75 CS- trials immediately followed the conditioning. During extinction the US was never presented, and the extinction and habituation sequences were identical.

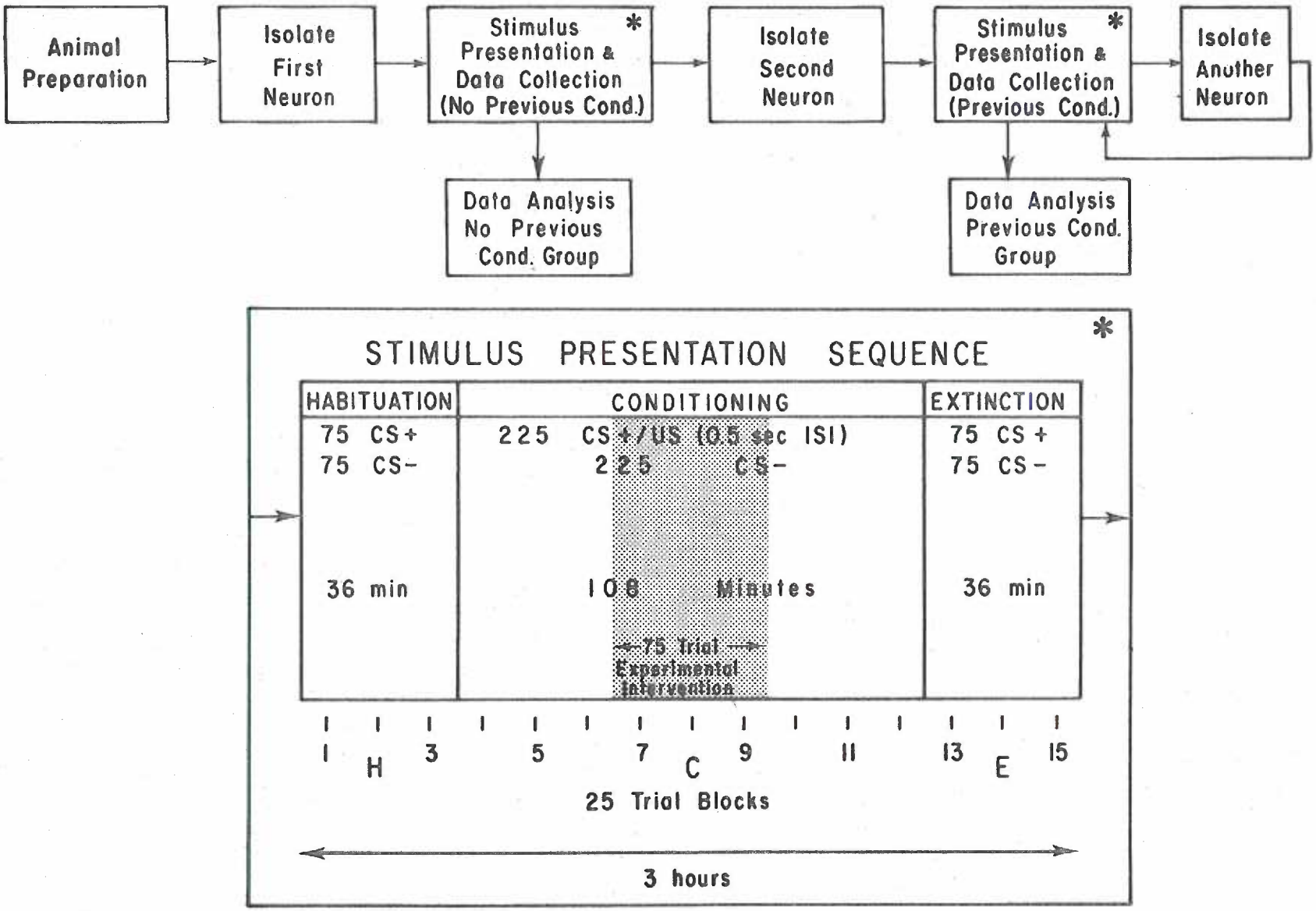
The entire stimulus presentation sequence lasted three hours, with a total of 375 CS+ presentations and 375 CS- presentations. Of this total, 225 of the CS+ presentations were followed by the occurrence of the US. The duration of the habituation, conditioning, and extinction sequences are shown in the inset of Figure 3.

Experimental procedure

After the animal was surgically prepared, a code list was checked. According to this list the CS+ was assigned to either the right or left forepaw in a random manner, and the subject was assigned to either the experimental group, which underwent thalamic cooling, or to the procedural control group.

Under visual guidance the microelectrode was lowered until it penetrated the cortex. With this accomplished the experimental chamber

Figure 3: Flow chart of the sequence of experimental procedures and the stimulus presentation sequence. (description in text)


Animal Preparation
Isolate First Neuron
Stimulus Presentation & Data Collection (No Previous Cond.) *
Isolate Second Neuron
Stimulus Presentation & Data Collection (Previous Cond.) *
Isolate Another Neuron
Data Analysis No Previous Cond. Group
Data Analysis Previous Cond. Group
STIMULUS PRESENTATION SEQUENCE *
HABITUATION
CONDITIONING
EXTINCTION
75 CS+
75 CS-
225 CS+/US (0.5 sec ISI)
225 CS-
75 CS+
75 CS-
36 min
108 Minutes
36 min
←75 Trial→
Experimental Intervention
1 H 3 5 7 C 9 11 13 E 15
25 Trial Blocks
3 hours

was vacated and darkened. Further adjustments of the microelectrode were achieved using a Kopf hydraulic drive controlled from the adjoining room. The hydraulic microelectrode drive allowed measurement of depth in microns. The microelectrode was slowly lowered while the recording was observed using audio and visual monitors. The electrode was lowered in a step-wise fashion and allowed to stabilize at any particular depth. Randomly selected test stimuli of the CS+, CS-, and US were presented using the internal circuitry of the Digitimer.

The bias in selecting a neuron for inclusion into the experimental sample was according to the suitability of the signal to noise ratio and the apparent stability of the recording. Recordings which showed fluctuation in spike amplitude or high bursts of activity were not selected for prolonged study and only those neurons that could be clearly differentiated from the noise level were selected. This differentiation was achieved according to voltage amplitude by means of a Schmitt trigger and the separation of the single neuron trace was monitored on the oscilloscope of the PDP-12 computer. Occasionally, a Krohn-Hite filter was used to enhance the differentiation of the signal from the noise. The filter was used in a band pass fashion in which a frequency window for the neuron action potential was selected. The signal to noise ratio ranged from approximately 2 to 1 through 20 to 1.

After the first neuron was isolated, the stimulus presentation sequence was started. The flow chart of Figure 3 shows the procedural order. If the data were successfully recorded during the entire three hour stimulus presentation sequence, then the data from this neuron

would be placed into the no previous conditioning group, this being the naive animal's first exposure to the conditioning paradigm. Since learning is dependent upon the exposure to a conditioning paradigm, the naive reaction was separated from that of previous exposure. On numerous occasions the isolated neuron could not be recorded for the three hour duration. In the event this occurred the stimulus presentation was halted and the microelectrode was moved until another neuron could be isolated. If another neuron was isolated, the stimulus presentation sequence was set back to the beginning, trial 1 of habituation.

After successfully recording during the course of the entire stimulus presentation sequence, an attempt was made to isolate a second neuron, and to repeat the entire stimulus presentation sequence. Since on these occasions the cat had already been exposed to the conditioning paradigm, these data have been grouped and analyzed separately. As illustrated in Figure 3, this procedure could continue indefinitely; however, the upper limit to the number of neurons recorded from any one animal was limited by the physiological state of the animal. In practice it was very difficult to gather data from more than two complete stimulus presentation sequences for any individual cat.

During the stimulus presentation sequence, the shielded room in which the cat was located was not entered. Flaxedil was administered by means of an infusion pump. Between recording sessions the experimental chamber was entered to administer procaine, check the room and body temperatures and maintain the cryoprobe system.

Immediately after terminating the final stimulus presentation sequence, the cat was sacrificed using an overdose of sodium pentobarbital (Nembutal).

Histology

After the cat had been sacrificed, the recording electrodes and cryoprobes were withdrawn. To facilitate the eventual brain sectioning, the stereotaxic ear bars and eye bars were reinserted into the animal. With the ear bars and eye bars in place, it was possible to remove the stereotaxic adapter, which had been cemented to the skull, and sufficient bone to permit exposure of the brain. A large scalpel blade was mounted in an electrode holder and zeroed in the same plane as the cryoprobes. A plane of reference was established by sectioning the brain with the stereotaxically mounted scalpel blade. The single section was done in a transverse fashion, 10 mm posterior to the cryoprobe tracts.

The anterior brain section was placed into buffered 15% formalin solution. The thalamic area was prepared with the right hemisphere identified, and was frozen with carbonic ice. Fifty micron thick sections were prepared using an American Optical Company, Model 860 microtome. Every serial section of the probe tracts was placed into water and mounted, being mounted two sections per slide. Staining was accomplished using toluidine blue. Occasionally luxol fast blue was utilized to aid fiber identification.

Data analysis: Evoked potential (EP)

The original data were stored on a Sangamo FM tape recorder in analogue form. The electrocortical potentials were led from variable gain amplifiers into A/D converters of the PDP-12 computer, and a time zero event marker signal was led to a Schmitt trigger. The time zero marker initiated computer sampling. The amplitude of the potential resulting from the CS was sampled every 2 msec starting 10 msec after

the CS occurrence and for 256 consecutive sampling addresses. This resulted in an evoked potential of 512 msec during the CS-US interval. Similarly, the US evoked potential amplitude was sampled every 2 msec for 512 msec following the US occurrence.

The measurements from 25 trials were added algebraically and the averaged evoked potential (AEP) was stored on LINC tape in digital form. An averaged evoked potential was similarly formed from three 25 trial averages sequentially, yielding a 75 trial averaged evoked potential. It was thus possible to compare the averaged evoked potentials for the habituation, conditioning, and extinction conditions.

One method of analysis was a comparison of the peak-to-peak amplitudes. The computer was programmed to display the ordinate (voltage) value of any selected abscissa (time) value of the AEP. Thus, it was possible to place the cursor on the peak positive and negative deflections and obtain the quantitative difference in peak-to-peak amplitude. The peak-to-peak amplitude was computed using the absolute difference. The abscissa values were selected using the 75 trial averaged evoked potential from habituation and selecting prominent components. Using the same abscissa values, the conditioning and extinction AEPs were quantified for peak-to-peak amplitude. Since shifts in the latencies of the wave forms did not result from conditioning, the same abscissa values could be used to measure the peak-to-peak amplitude across conditions. If more than one component was measured for peak-to-peak amplitude, then that component which was more dynamic across conditions was selected. The same procedures were performed for both the CS+ and CS- elicited AEP.

The measure of peak-to-peak amplitude assumes that the majority of significant information is conveyed in the peak response. However, a second method for examining the AEP data was to integrate the AEP waveform, quantifying the area described by the AEP. This permits an assessment of the overall neural activity which comprises the waveform. The AEP in its original form was transformed, without changing the shape, to provide equal negative and positive area around a zero baseline. The AEP waveform was then rectified, giving all positive values, and subsequently integrated. The integrated waveform represents all the area described by the AEP waveform which deviates from the mean in either the positive or negative direction.

A third method of AEP analysis examined that interval of the AEP which was most affected by the cooling procedure. By superimposing the integrated AEP of the pre-cool condition with that of the experimental condition, it was possible to define the interval of the response influenced by cooling. There were typically two portions of the integrated AEPs which could be virtually superimposed, with an aberrant portion between the two.

Data analysis: Single cell data

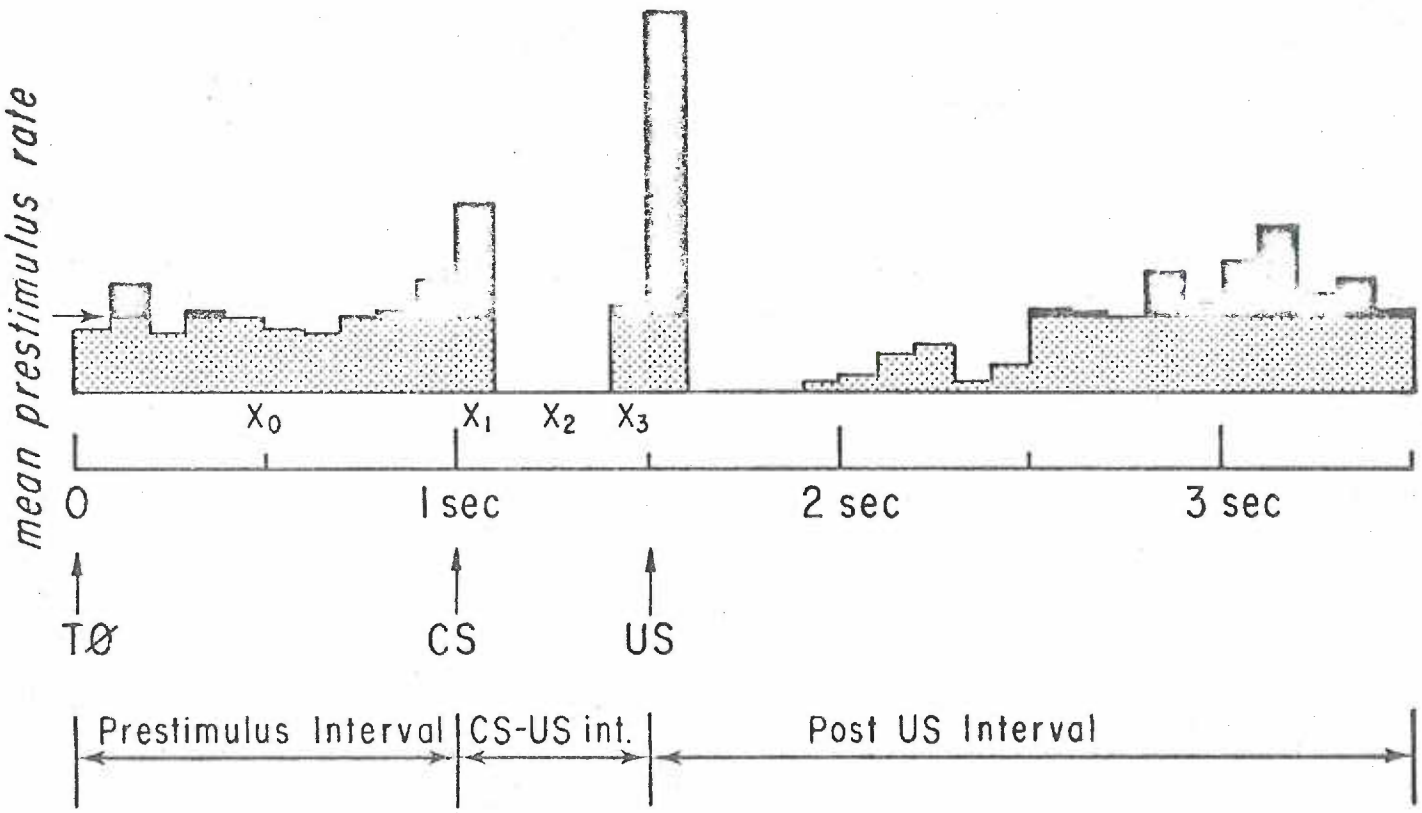
The PDP-12 computer was used to accumulate the single cell data in an on-line fashion. The computer program allowed for three simultaneous signals into Schmitt triggers for detecting voltage amplitude. The time zero (TØ) was led to one of the Schmitt triggers. Identical signals from the microelectrode were led on parallel lines to the other two Schmitt triggers. This dual input allowed the selection of an amplitude window, setting the minimum voltage and maximum voltage to

be considered as the positive occurrence of an action potential. The time zero occurrence initiated the sampling of unit activity received on the signal channels. The relationship of the data epoch with respect to the time zero occurrence is shown in Figure 4. The data sampling lasted for 3.5 sec following the time zero. This could be divided into three parts: a prestimulus interval of 1.0 sec, an interval following the CS occurrence and prior to the US occurrence (CS-US interval) of about 0.5 sec, and a 2.0 sec interval following the US occurrence. Throughout this data epoch, the occurrence of an action potential that fell within the voltage window was digitized and stored with respect to its latency following the time zero. The data was stored on LINC tape in a continuous trial series. Both the CS+ and CS- data were handled in an identical fashion with the data being stored on separate LINC tapes.

The PDP-12 computer was used to further analyze the single cell data in an off-line manner. The standard procedure was to form a histogram by superimposing the time zero markers. As shown in Figure 4, the histogram extended for the 3.5 sec data epoch. The raw data values showed the number of spikes per bin for the summed number of trials using a 100 msec bin width. It was then possible to consider the mean spike rate during any one trial and during any portion of the response. Thus, the poststimulus histogram was meaningful as a statement of the probability of the firing of a neuron on any one trial at a particular latency.

Typically, most cells showed complex poststimulus histograms and it was necessary to consider individually certain periods of the response. As in Figure 4, the CS elicited activity showed both

Figure 4: Data sampling epoch. (explanation in text)



X=Difference Scores= $X_1 - X_0$, $X_2 - X_0$, $X_3 - X_0$

$$\sigma = \sqrt{\frac{N\Sigma x^2 - (\Sigma x)^2}{N^2}}$$

N=no. of trials

$$t = \frac{\bar{X} \text{ grand diff. score}}{\sigma}$$

excitatory and inhibitory components. Excitatory components are defined as those which exceed the prestimulus rate, and inhibitory components as those below the prestimulus rate. In Figure 4, the height of the stippled area corresponds to the mean prestimulus rate; the darkened areas, exceeding the mean prestimulus rate are excitatory components, and the areas below the mean prestimulus level are inhibitory response components. Rather than use an a priori fixed interval for analysis of the poststimulus response, it seemed preferable to select an interval individualized for each cell. The technique of individualizing the interval for each neuron is more sensitive to suble changes since a fixed interval analysis might misrepresent the data, such as in the overlap of an excitatory and inhibitory response within the same interval being averaged as the absence of a response.

The disadvantage of an individualized selection of response intervals is the difficulty of replication across neurons. To facilitate the replicability, a standardized and systematic procedure was used for response interval selection. Additionally, the same procedures were used for both the CS+ and CS- data.

A computer program allowed the visual display of the histograms of the PDP-12 oscilloscope. Standard procedure was to display the histogram with the histogram starting at time zero and being shown in 10 msec bins. Using a histogram comprising the 75 trial habituation block, response components were visually selected with the criterion of selecting as long an interval as possible without combining both excitatory and inhibitory components. The response components selected were chosen from the half-second period between the CS occurrence and

the US occurrence (CS-US interval). Thus, the analyzed neuronal activity always preceded the US occurrence.

In general, three response components were selected from the half-second following the CS occurrence and each had a duration ranging from 70-300 msec. After response components were selected using the histogram comprised of the habituation trials, the same intervals were used to analyze the neuronal activity during conditioning and extinction.

A computer statistical program was used to quantify the neuronal spike activity during the selected response intervals. As shown in Figure 4, the spike frequency was recorded both before and after the CS occurrence. The response was expressed with respect to the prestimulus rate, and a difference score was used. The mean rate for the prestimulus interval was calculated using an interval extending from the time zero through 980 msec. The upper limit was chosen to definitely exclude any CS artefact. The frequency was converted into spikes per second and printed. Similarly, the computer program enabled a spike frequency calculation during the selected response intervals. With the earlier and later latencies specified, the equivalent number of spikes per second for each of the three response intervals was printed. The difference score was derived by subtracting the mean prestimulus rate from the mean response rate for each selected interval, with a positive sign denoting an excitatory response and negative sign denoting an inhibitory response.

These computations were derived for blocks of 25 trials in sequence. By utilizing 25 trial blocks it was possible to obtain 15 difference score values for the entire stimulus presentation sequence, each value corresponding to a mean difference score for 25 trials.

When it was necessary to choose a response interval for inclusion into the grouped data, the interval exhibiting the greatest conditioning change was selected. To evaluate the change across conditions a conditioning t-score was used. Basically, this measure compared the CS elicited activity during habituation and extinction against that of conditioning. Of the 15 mean difference scores, there were three for habituation, nine for conditioning, and three for extinction. The difference scores for habituation and extinction were pooled to form an overall mean difference score. A similar procedure was followed for the nine conditioning difference scores. A grand mean difference score was derived by comparing the overall habituation and extinction mean difference score with the overall conditioning mean difference score. In addition the t-score took into account the variance of the difference scores. The formulas are shown in Figure 4. Essentially, the conditioning t-score was a quantitative statement of the amount of change in response during conditioning trials as compared with the mean response activity of habituation and extinction. The interval with the largest absolute t-score value was chosen to be included into the grouped data. Identical procedures were followed for both the CS+ and the CS- data.

TABLE 1: CRYOPROBE PLACEMENTS

	Left Probe			Right Probe		
Cat #	frontal	lateral	vertical	frontal	lateral	vertical
54	11.5	5.5	4.0	11.0	5.5	4.0
57	15.0	6.0	4.0	15.5	3.5	4.0
58	13.0	6.0	5.0	13.0	3.5	4.0
61	11.5	4.5	4.0	11.5	6.0	5.0
62	14.0	3.0	4.0	14.0	4.0	4.0
63	11.5	5.0	5.0	11.5	5.0	5.0
64	11.5	5.0	4.0	11.5	4.0	4.0
65	11.5	4.5	4.5	11.0	5.0	4.5
66	12.0	5.5	4.5	11.5	4.0	5.0
68	11.5	4.5	4.5	11.5	4.5	4.5
70	11.5	4.5	4.5	11.5	5.0	5.0
71	11.5	4.5	5.0	11.5	4.0	5.0
72	10.0	4.5	5.0	10.0	4.0	5.0
76	12.0	4.0	6.0	11.5	4.0	5.0
77	12.0	4.5	5.0	12.0	4.5	6.0
78	11.5	4.0	3.0	11.5	4.0	3.0
80	11.5	4.5	5.5	12.0	4.5	6.0
$\bar{X}$	11.9	4.7	4.6	11.9	4.4	4.6
sd	1.1	0.8	0.7	1.2	0.7	0.8

RESULTS

Histology

Frozen serial sections were used to examine the placement of the cryoprobes. Histological verification was performed for twelve experimental and five control animals and the placements in the two groups did not differ significantly. The placements of the right and left probes for each cat were determined by comparing light projected serial sections with photographed Nissl stained sections of the atlas of Jasper and Amjone-Marsan (25). Convenient lankmarks used in judging the probe placements included the caudate nucleus, stria terminalis, and the third ventricle.

The following anterior and lateral coordinate values describe the center of the probe tract; the vertical coordinate value describes the tip of the probe. The right hemisphere cryoprobe placement ranged from A 10.0 mm through A 15.0 mm, with a mean of 11.9 mm and a variance of 1.1 mm. The left hemisphere probe placement ranged from A 10.0 mm through A 15.5 mm, with a mean value of 11.9 mm and variance of 1.2 mm. The lateral placements ranged from 3.5 through 6.0 mm, with a mean value of 4.6 mm, and a variance of 0.7 mm. The vertical placements ranged from H +4.0 through H +6.0 mm, with a mean value of 4.6 mm and a variance of 0.8 mm. A list of the probe placements is in Table 1.

Figure 5 shows photomicrographs of four brain sections using a three power magnification. The cryoprobe tracts are shown for cats 63, 68, and 71. The irreversible damage associated with the probe tract can be seen, including cortical damage as well as damage to the lateral

aspect of the third ventricle and the caudate. The vertical coordinate was chosen to allow placement of the cryoprobe tip adjacent to the dorsal aspect of the ventral anterior nucleus of the thalamus; in general, the cryoprobe tract did not extend into the ventral anterior nucleus.

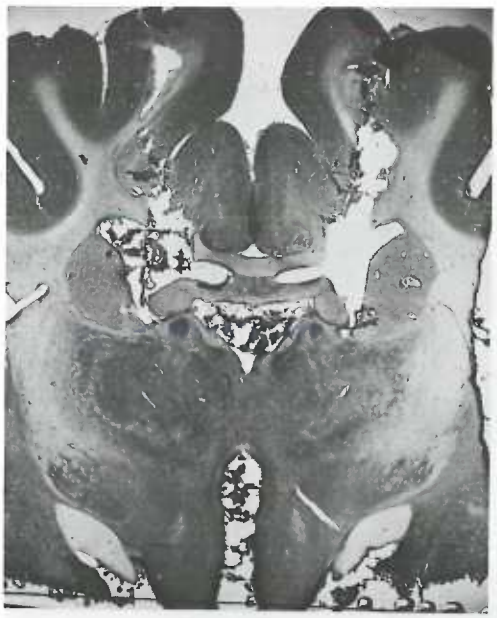
Histological verification was occasionally performed for the macroelectrode placement. Figure 5 shows the macroelectrode placement in the region of centromedian nucleus of the thalamus for cat 63. All the macroelectrode placements analyzed were similar to this example.

The effect of thalamic cooling on cortical sensory evoked activity

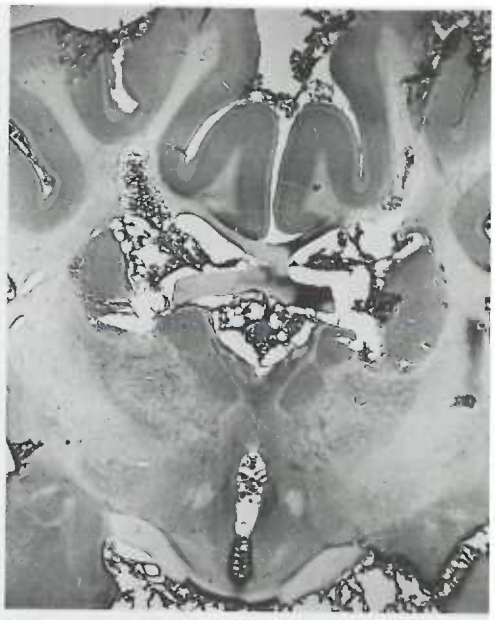
The effect of bilateral cooling of the area of the ventral anterior nucleus of the thalamus was a reduction in the sensory evoked activity recorded from neurons of the postcruciate cortex. A similar reduction was seen for both excitatory and inhibitory response components. The excitatory responses were defined as those exceeding the mean prestimulus level while the inhibitory responses were below the mean prestimulus spike activity level. The effect of thalamic cooling on cortical sensory evoked activity was analyzed by comparing the 75 trial presentations before, during, and after thalamic cooling for the unpaired forepaw stimulation. Thalamic cooling tended to maintain the response activity near the prestimulus level. Thus, the difference score (mean response rate minus mean prestimulus rate) had a smaller absolute value during thalamic cooling for both excitatory and inhibitory response components. Typically, thalamic cooling had less effect on the initial response immediately following the stimulus occurrence than on the longer latency responses.

Figure 6 shows the effect of thalamic cooling on neuron 63B. Each histogram was derived from 75 trial presentations and has been plotted in 20 msec bins starting at the stimulus occurrence. The histogram has

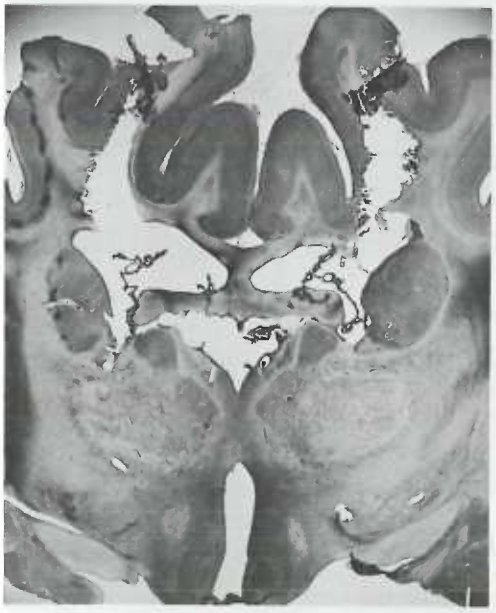
Figure 5: Photomicrographs of brain sections.



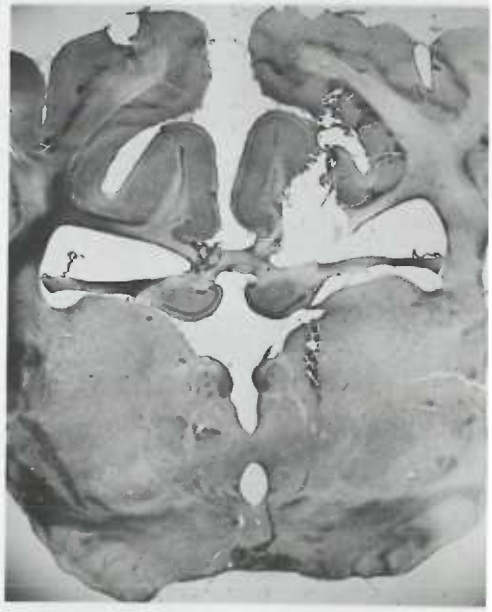
Cryoprobe Placement
Cat 63



Cryoprobe Placement
Cat 68

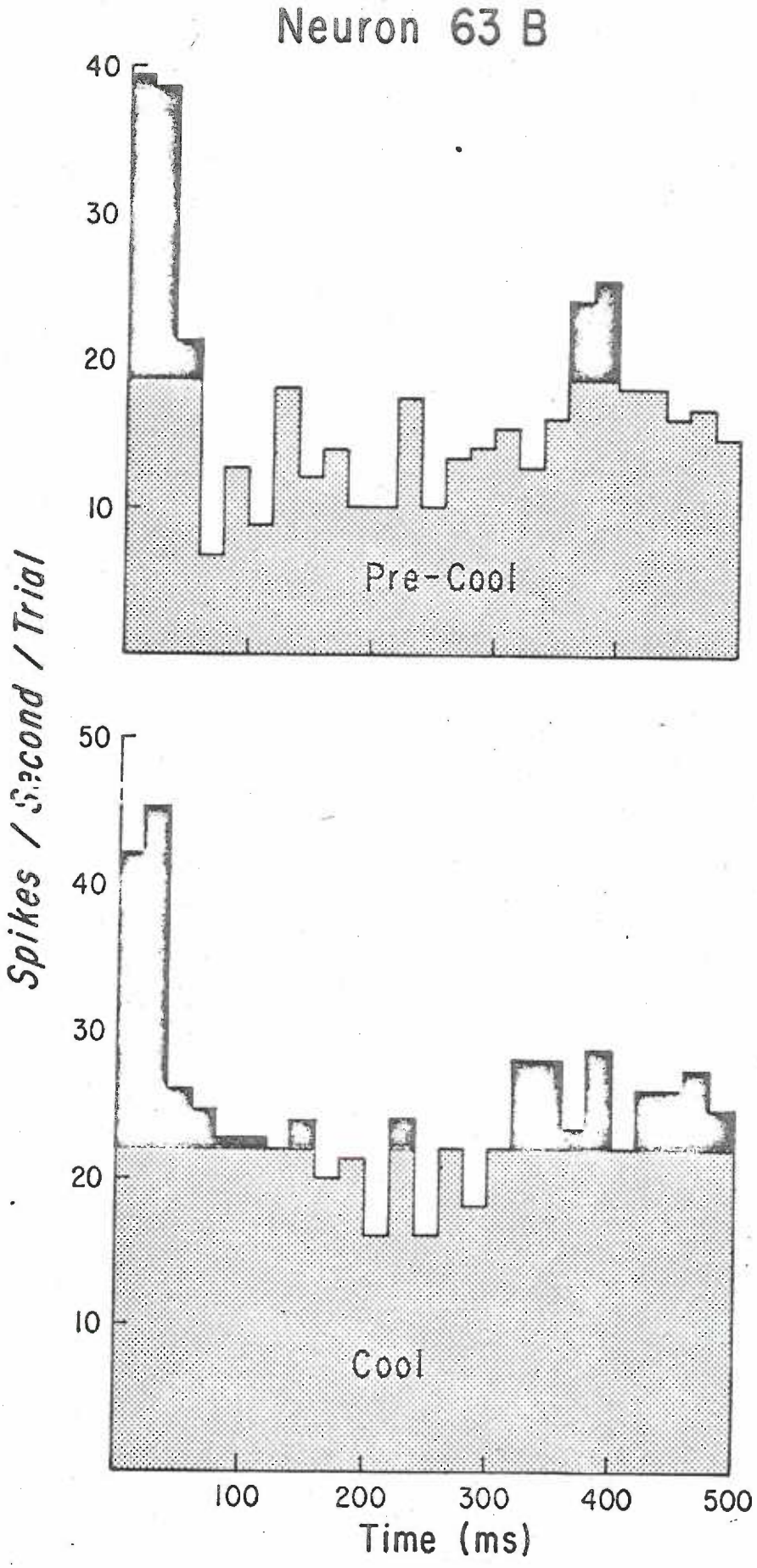


Cryoprobe Placement
Cat 71



Macroelectrode Placement
Cat 63

Figure 6: The effect of thalamic cooling on neuron 63B.

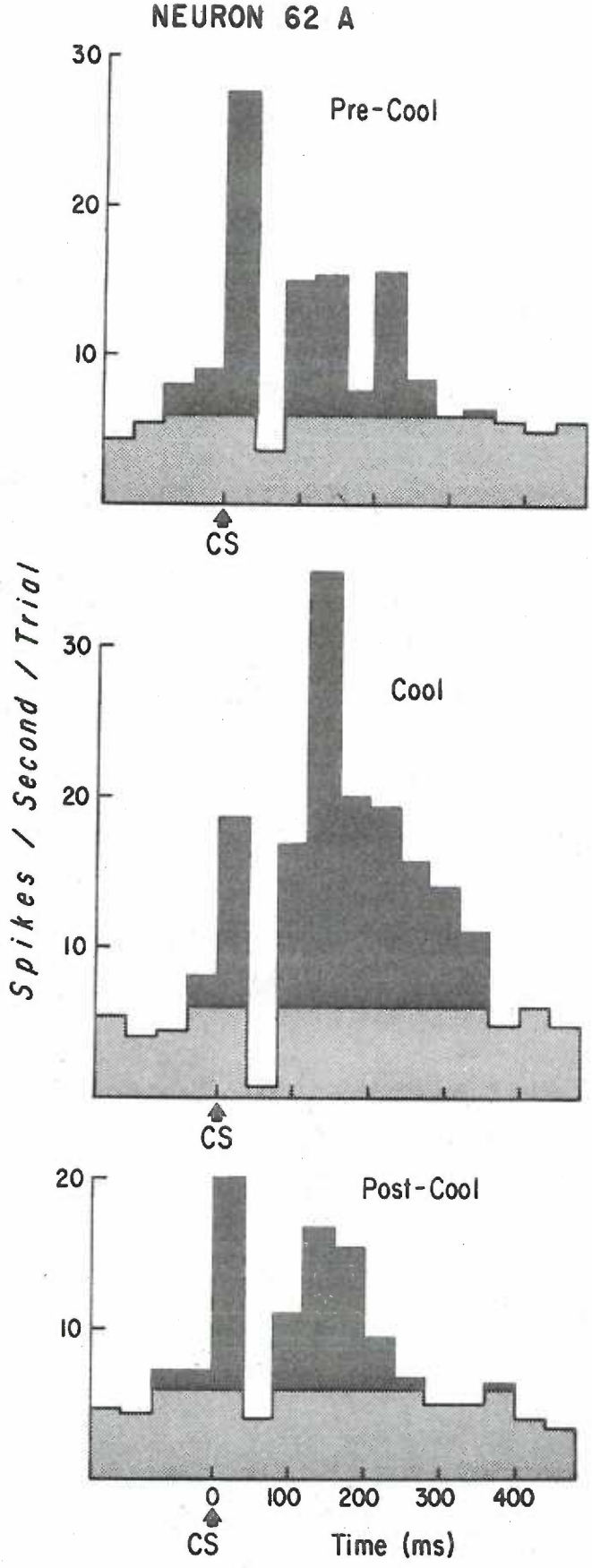

Neuron 63 B
40
30
20
10
Pre-Cool
50
40
30
20
10
Cool
Spikes / Second / Trial
100
200
300
400
500
Time (ms)

been composed with the stimulus artefact omitted. The histogram for the 75 trials prior to thalamic cooling (pre-cool) shows a complex response with an initially excitatory response component and an inhibitory response component starting at approximately 60 msec poststimulus. The highest stippled value represents the prestimulus (baseline) spike rate. During thalamic cooling there was slight change in the initial excitatory response, yet substantial reduction of the inhibitory response, the spike rate being maintained near the baseline level. The difference score for the inhibitory response changed from -6.4 spikes/sec/trial prior to cooling to a value of -0.9 spikes/sec/trial during thalamic cooling.

The neuronal responses were analyzed by segregating the excitatory and inhibitory response components; since similar results were observed for both components, the results were grouped. Of 40 neurons analyzed during thalamic cooling, 92.5% showed a change in response similar to that of neuron 63B, with smaller difference scores during cooling. These changes ranged from a 97.8% through 5.0% reduction in the response difference score. In all instances, these values reflect the histogram mean difference scores derived from 75 trial presentations prior to cooling as compared with 75 trial presentations during cooling.

Of the 40 neurons analyzed during thalamic cooling, 7.5% showed increased absolute difference scores. The largest response increase seen was a change from less than 0.5 spike/sec/trial above baseline to more than 4.3 spikes/sec/trial during thalamic cooling. An example is neuron 62A (Figure 7). Each histogram represents 75 trial presentations of the unpaired forepaw stimulation. The histogram is plotted

Figure 7: The effect of thalamic cooling on neuron 62A. Each histogram is averaged for 75 trial presentations.


NEURON 62 A
Pre-Cool
Cool
Post-Cool
Spikes / Second / Trial
30
20
10
CS
0
100
200
300
400
Time (ms)

in 40 msec bins with the stimulus artefact omitted. During thalamic cooling there was a substantial increase in both the early inhibitory component and the longer latency excitatory component. The inhibitory response was initially 2.5 spikes/sec/trial below the baseline rate; during thalamic cooling this value was 5.0 spikes/sec/trial below the baseline, representing a doubling of the response. The late excitatory component showed an even greater increase in response during thalamic cooling.

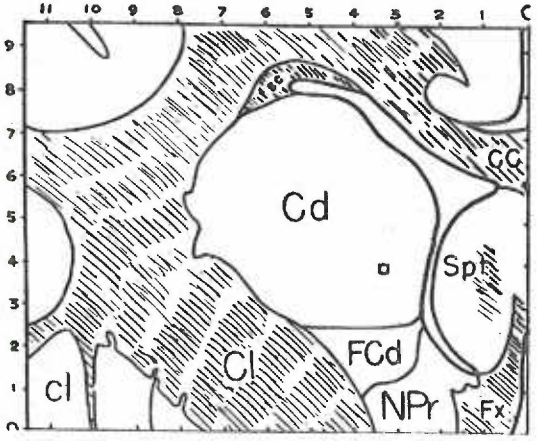
The cryoprobe location was examined as a factor in accounting for the magnitude and direction of the thalamic cooling effect. For the 12 animals for which the cryoprobe locations were known, the cooling effect was categorized into one of three groups: (1) a decrease in the sensory evoked activity during thalamic cooling ranging from 50% through 100%; (2) a smaller change ranging from a slight decrease through a 50% decrease; (3) no decrease or increased sensory evoked activity. Symbols corresponding to these three categories were placed on appropriate atlas sections to show the anatomical location of the cryoprobes which produced the respective effects. Figure 8 shows that the increased sensory evoked activity during cooling was associated with either cooling of the ventromedial caudate nucleus or the boundary of nucleus lateralis dorsalis and centralis lateralis. The symbols placed on Figure 8 represent the cryoprobe tip location. The location in the caudate nucleus in the frontal plane of 14.0 resulted in the cooling effect of increased response shown in Figure 7 (neuron 62A).

Interval analysis

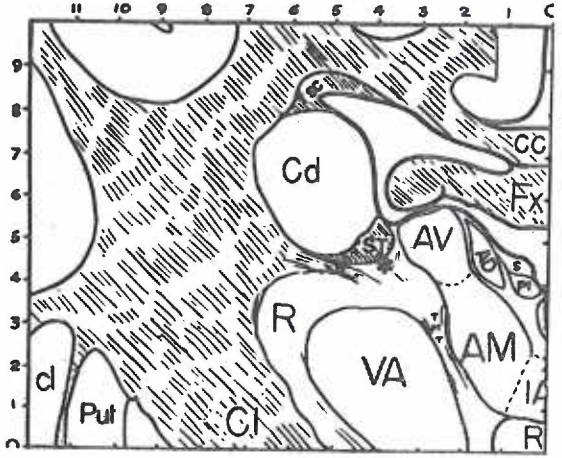
In a previous study a cryogenic blockade was used in the centromedian thalamic region (51). It was found that the effect of the

Figure 8: Distribution of cryoprobe placements and cooling effects. The symbols show the magnitude of cortical spike activity change corresponding to the indicated cryoprobe placement.

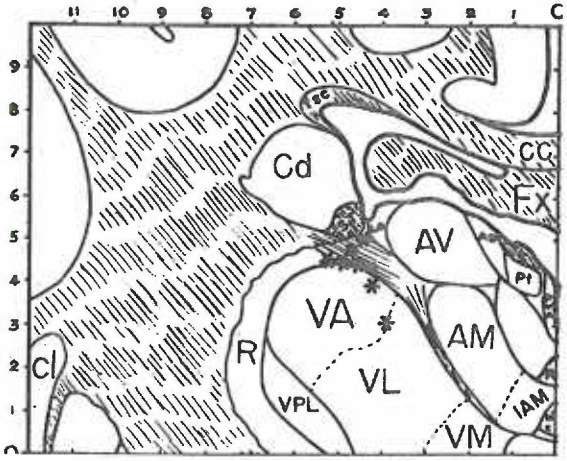
CRYOPROBE PLACEMENT



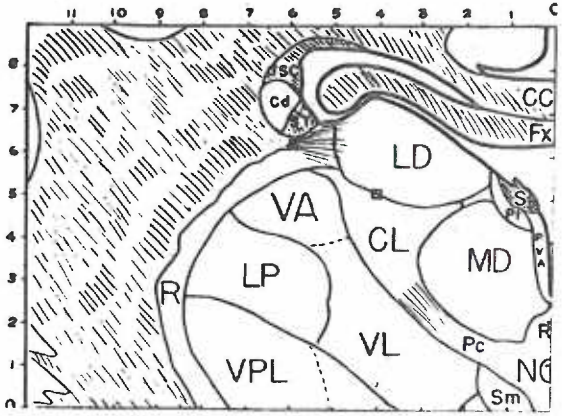
Fr. 14.0



Fr. 12.0



Fr. 11.5



Fr. 10.0

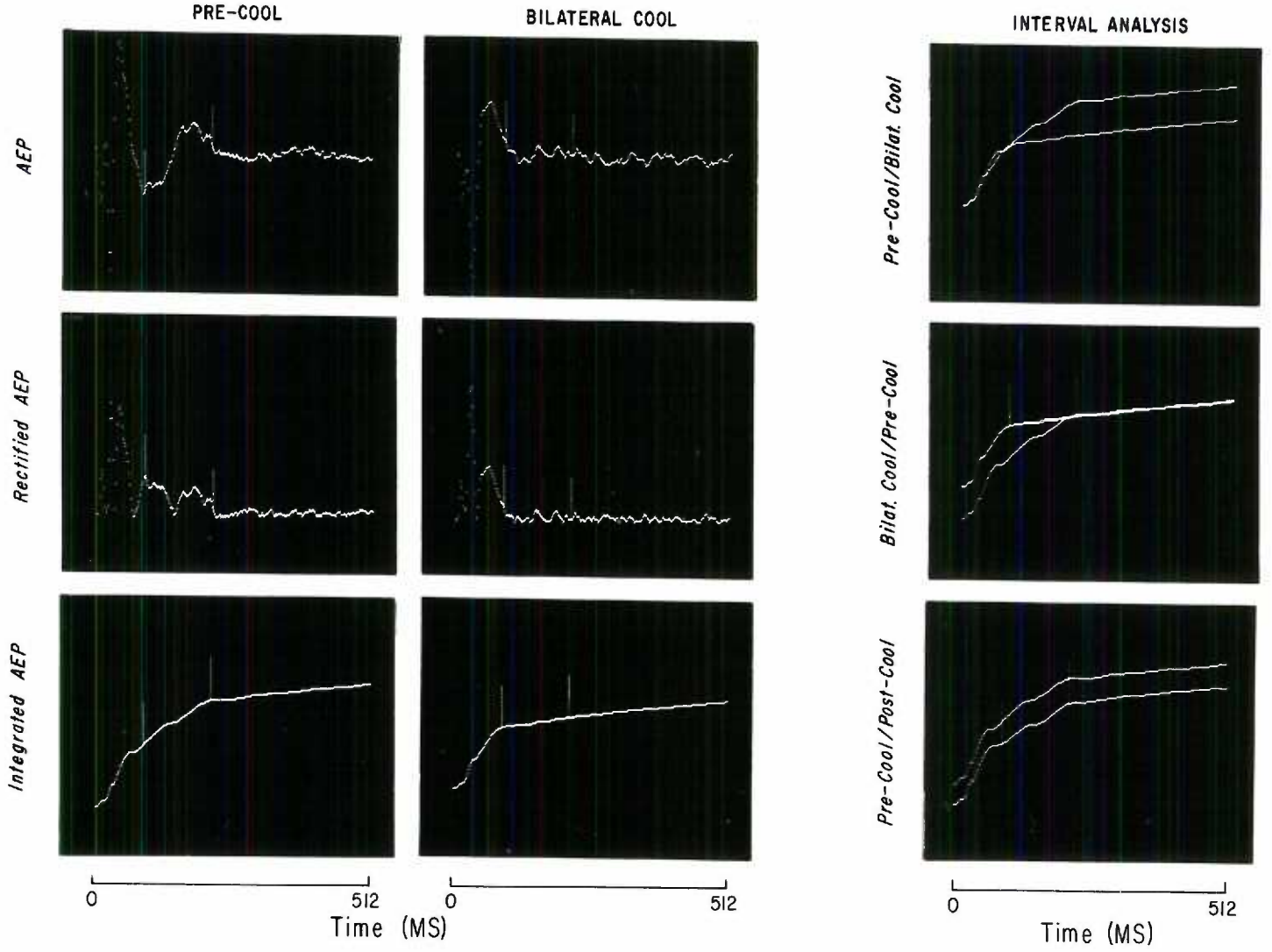
* 100–50% Decrease • 50–0% Decrease ▫ No Decrease

blockade could be described in terms of the poststimulus period which showed the greatest reduction in the response. To determine the response intervals most affected by thalamic cooling the integrated averaged evoked potentials were used. The sequence of data transformations is shown in Figure 9. The 75 trial presentations of the unpaired forepaw shock (CS-) were used to derive a "pre-cool" averaged evoked potential (shown as upper left oscillogram). The AEP was rectified so that all deviations from the baseline were given positive values (middle left). The final transformation was the integration of the AEP, in which the cummulative values of the AEP were summed to yield the area under the curve (lower left).

The interval analysis involved comparing two integrated AEPs, a "pre-cool" AEP derived from trial presentations prior to thalamic cooling, and a "bilateral cool" AEP derived from trial presentations during thalamic cooling. The right column of Figure 9 shows the manner in which the interval most affected by thalamic cooling has been described. Both integrated AEPs are displayed simultaneously on the PDP-12 oscilloscope. The traces are aligned at time zero, and with the initial portions of the integrated waveforms superimposed, a cursor (visible marker) was set at the point of deviation (shown in upper right oscillogram). The cursor time base value was recorded as the lower limit of the interval predominantly altered by thalamic cooling. The upper limit was established by superimposing the later portions of the integrated AEP pair (middle right oscillogram), and a second cursor was placed at the point of convergence. These temporal limits were recorded for each paired comparison of the AEPs.

Figure 9: Sequence of data transformations of the averaged evoked potentials.

AREA and INTERVAL ANALYSIS
Cat 39A, Ipsilateral Paw Stimulus



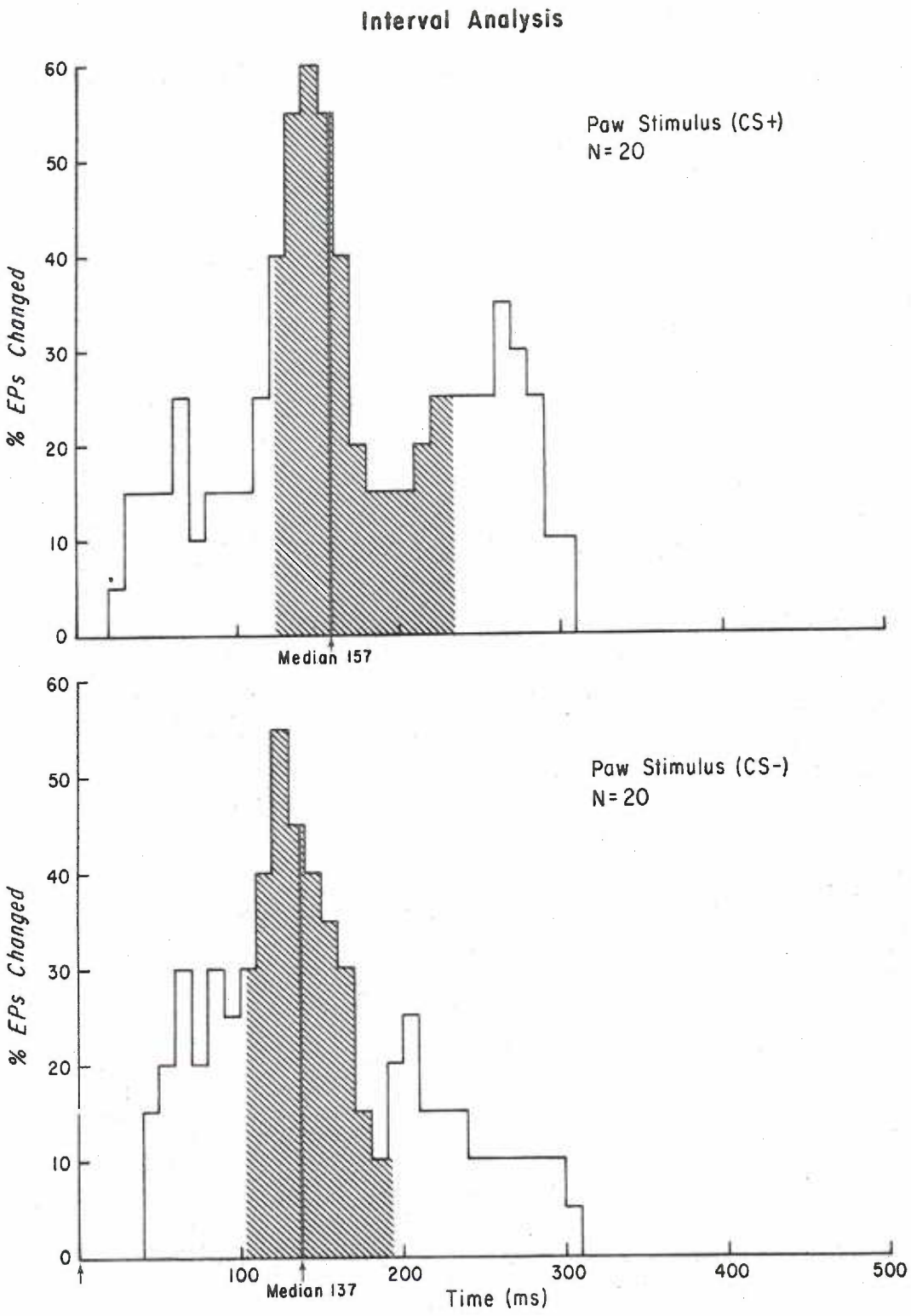
The limits describing the interval most affected by thalamic cooling were compiled into a grouped histogram. The group histogram shows the relative proportion of AEP pairs showing response change during any given interval (Figure 10). The central tendency of the distribution is shown using the median with one quartile on either side of the median shaded. This analysis was performed for both the CS+, the stimulus paired with the US, and the CS-, the unpaired stimulus. The quartile measure showed that the predominant cooling effect occurred between 125 msec and 234 msec for the CS+ and 103 msec and 191 msec for the CS-. The respective medians were 157 and 137 msec poststimulus.

Procedural control group: Heart rate measure

Since the cats were immobilized during the experiment, it was not possible to measure the conditioned reflex movements of the leg. For the purpose of including another estimate of conditioning, heart rate was measured during the experiment. Additionally, the heart rate recording helped assess the stability of the experimental preparation.

Heart rate was recorded from 13 cats before, during, and after the differential classical conditioning. An attempt was made to analyze the heart rate changes during conditioning by examining the change in response to the conditioned stimulus (CS); however, the short duration of the CS-US interval, a half-second, did not allow meaningful measurement during conditioning. If the heart rate measure extended beyond the half-second CS-US interval, then the occurrence of the US would obscure the measurement of the conditioned heart rate change. During the CS-US interval, approximately one heart beat occurred since the average

Figure 10: The proportion of averaged evoked potentials showing thalamic cooling effects with respect to time poststimulus. Separate histograms have been compiled for the CS+ and CS- presentations. In both cases 10 msec bin widths have been used.

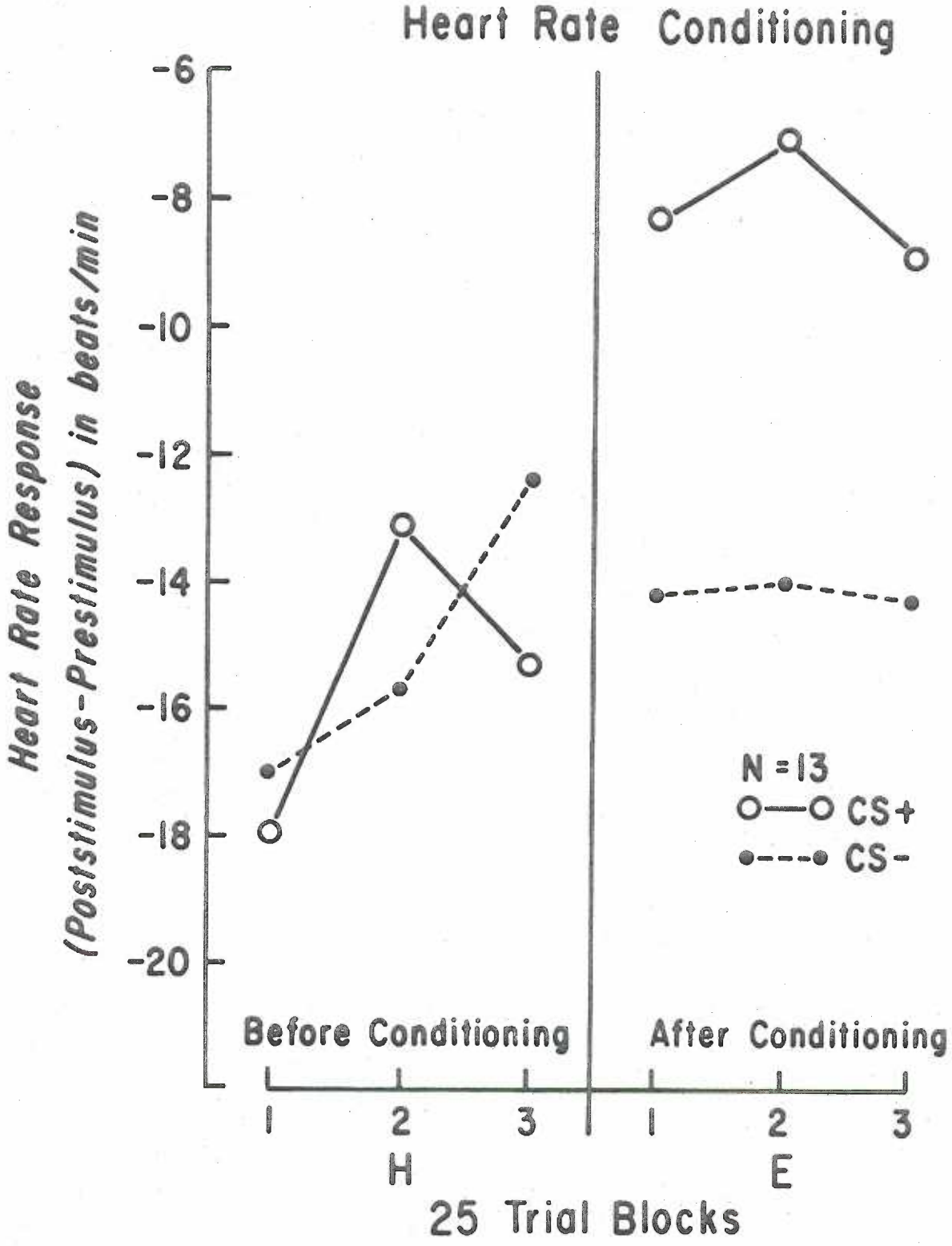

Interval Analysis
Paw Stimulus (CS+)
N= 20
% EPs Changed
60
50
40
30
20
10
0
Median 157
Paw Stimulus (CS-)
N= 20
% EPs Changed
60
50
40
30
20
10
0
100
200
300
400
500
Median 137
Time (ms)

background heart rate was about 90 beats-per-minute. Thus, a change within the first interbeat interval was required, and no such change was found.

Since the US presentations did not occur during habituation or extinction, it was possible to analyze the entire 3.5 sec post CS data epoch. The heart rate response before conditioning was compared to that after conditioning with the CS-US pairing intervening in the case of the CS+ stimulus. The mean heart rate response, described as a difference score of the response rate minus the prestimulus rate, is shown in Figure 11. During the habituation trials, the response to the CS alone presentation was a deceleration of the heart rate, shown as a minus value difference score. Both the CS+ and CS- initially showed comparable heart rate decreases of 15 beats-per-minute averaged for the 75 trial habituation sequence. Heart rate was then measured after the conditioning sequence, during the 75 trial extinction sequence, and there was a significant level difference between the CS+ and CS-. The CS- heart rate response was maintained at approximately the same level as that prior to conditioning. In comparison, the CS+ heart rate response was a much smaller deceleration. There is a significant difference between the 25 trial block just before and immediately after conditioning for the CS+ stimulus (paired t value 3.31, df 12, $p<.01$).

Procedural control group: Thalamic evoked potentials

To better analyze the conditioning changes in the thalamo-cortical system, it was deemed beneficial to record simultaneously from both the thalamic and cortical levels. To analyze the modification of the ascending afferent activity, electrophysiological data were gathered

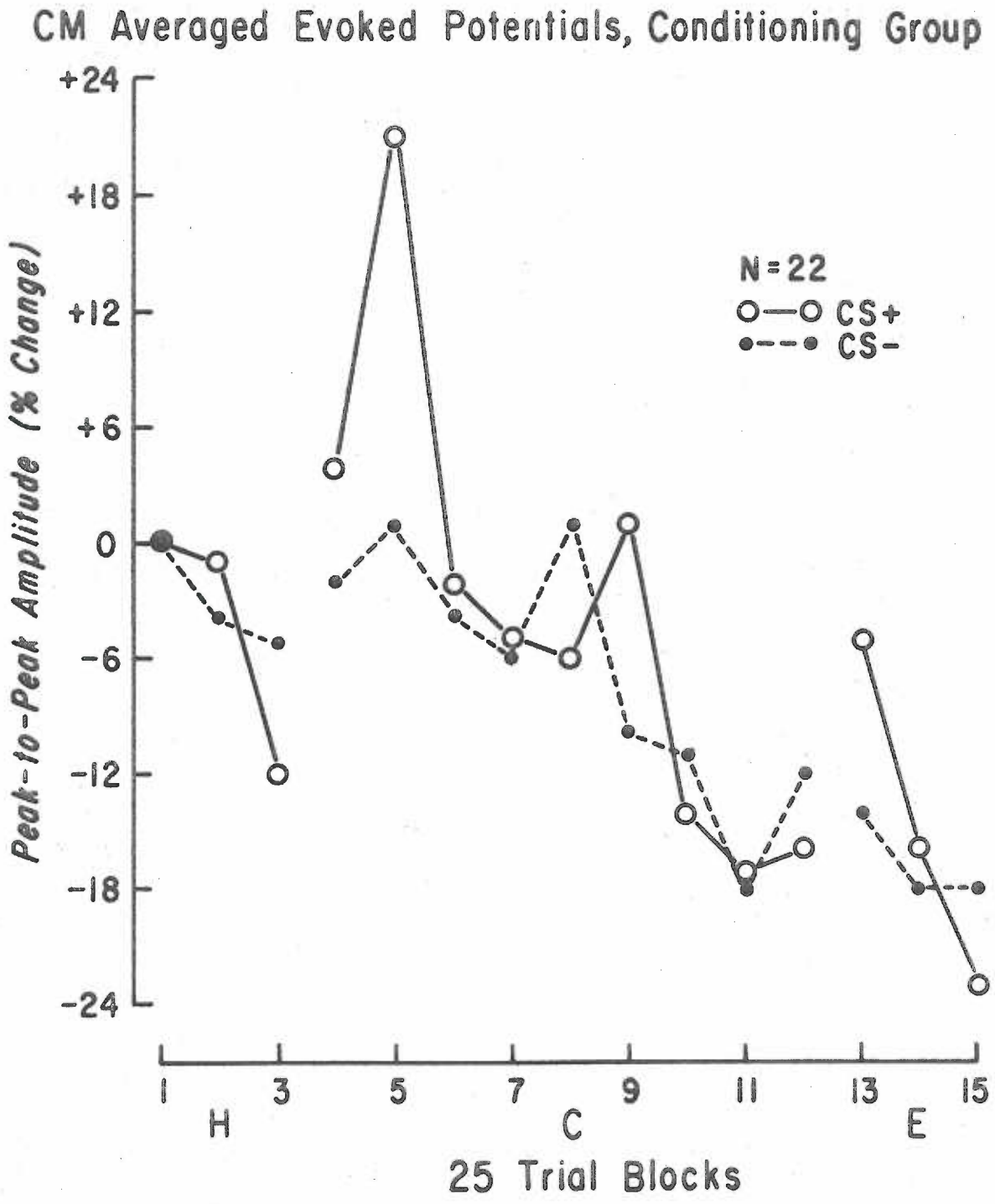

Heart Rate Conditioning
Heart Rate Response
(Poststimulus-Prestimulus) in beats/min
-6
-8
-10
-12
-14
-16
-18
-20
N = 13
CS+
CS-
Before Conditioning
After Conditioning
1
2
3
1
2
3
H
E
25 Trial Blocks

from a site caudal to the thalamic cooling blockade. For this purpose, evoked potential activity was recorded from the centromedian nucleus region of the thalamus before, during, and after differential classical conditioning. The centromedian site was chosen because the importance of this region with regard to sensory evoked activity of the postcruciate cortex had already been demonstrated.

The evoked potentials were analyzed by summing the 75 habituation trials to form a single averaged evoked potential for the entire habituation sequence. The latencies of the components of the habituation averaged evoked potential were determined using the visual display of the PDP-12 oscilloscope. Using these determined latencies, the peak-to-peak amplitudes of the waveforms were measured for 25 trial averaged evoked potentials during habituation, conditioning, and extinction. The peak-to-peak amplitude was scored as relative change expressed in percent with respect to the first 25 trial block during habituation. Figure 12 shows the change in AEP values across trials, averaged over 22 conditioning series from 14 animals. Both the initial CS+ and CS- values have been arbitrarily defined as the 100% value, which does not allow an absolute comparison of the CS+ and CS-. However, the absolute values of the CS+ and CS- peak-to-peak amplitudes for the first 25 trial block were within 3%.

During habituation, the response to both the CS+ and CS- showed a decrease in peak-to-peak amplitude. From the last habituation block to the first conditioning block, the CS+ showed an average 15% increase in peak-to-peak amplitude. Moreover, between trials 25 through 50 (5th trial block) the CS+ evoked potential showed a continued decrease to +20%.

Figure 12: Thalamic evoked potentials recorded during conditioning. The initial 25 trial habituation block has been defined as 100% of peak-to-peak amplitude with any subsequent changes compared with the initial values.


CM Averaged Evoked Potentials, Conditioning Group
+24
+18
+12
+6
0
-6
-12
-18
-24
Peak-to-Peak Amplitude (% Change)
N=22
CS+
CS-
1
3
5
7
9
11
13
15
H
C
E
25 Trial Blocks

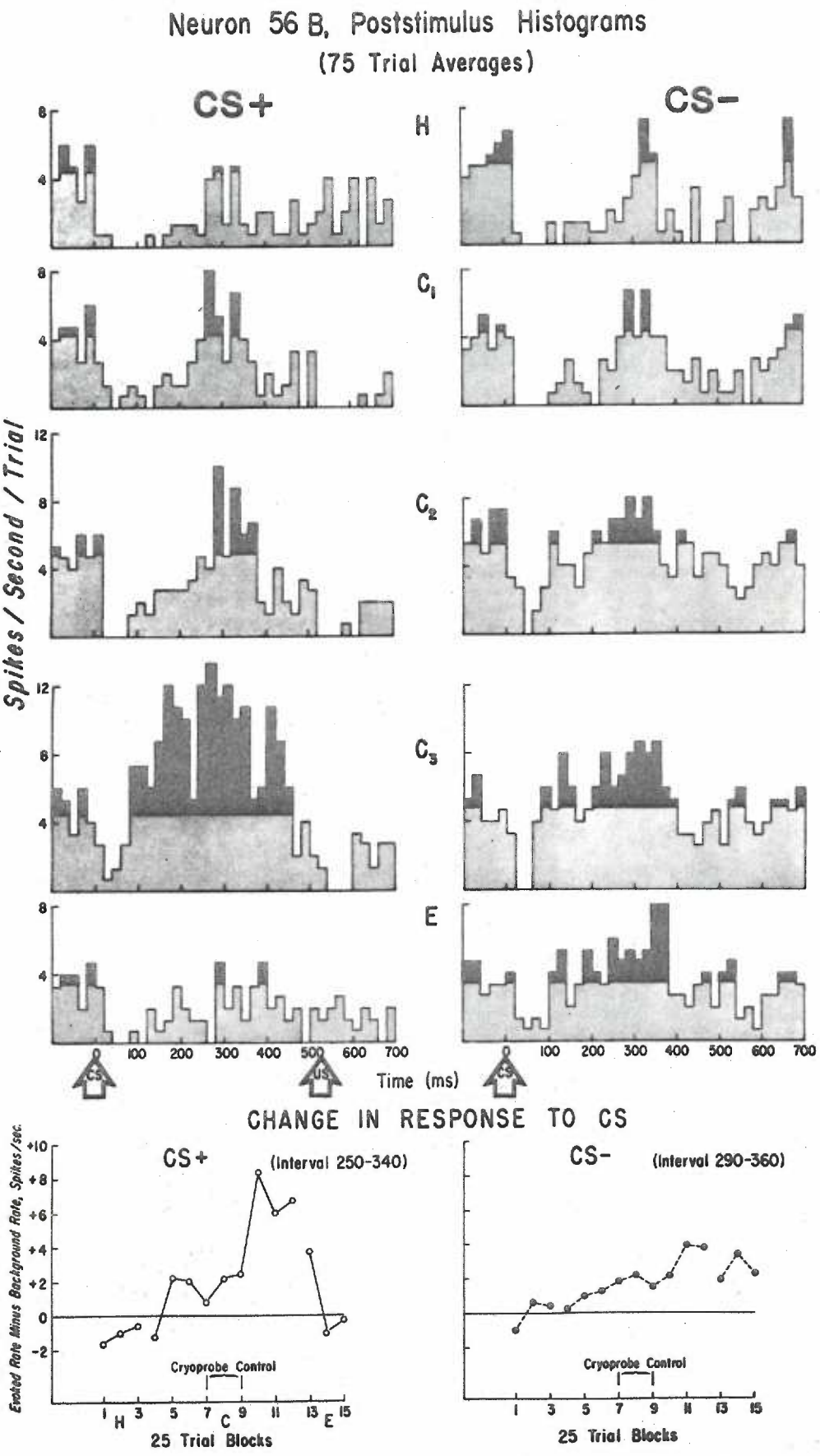
In contrast, the response to the CS- was relatively unchanged from the initial habituation level during the first 50 trials of conditioning. The statistical analysis of the data showed that the CS+ and CS- values significantly differed on conditioning trials 25 through 50 (paired t value 3.76, df 21, $p < .01$). During the remainder of the conditioning sequence, the changes taking place in the CS+ and CS- AEPs were statistically comparable, with a few points virtually superimposed. The overall trend from conditioning trial 50 through 225 was a decrease in the peak-to-peak amplitude for both the CS+ and CS-, a trend similar to that seen during habituation. The CS- AEPs maintained this decremental trend during extinction, while the CS+ AEPs showed an initial increase immediately after conditioning which was then followed by a large decrease in amplitude.

Procedural control group: Cortical unit data

A total of 33 neurons from 20 cats were satisfactorily recorded for the entire three hour habituation-conditioning-extinction sequence. The neuronal spike activity elicited by the conditioned stimulus (CS) was analyzed before, during, and after conditioning and was represented in spikes per second per trial.

An example of changes in spike activity with conditioning is shown in the poststimulus histograms of Figure 13. The 75 trial histograms were separated into response components based upon the histograms formed during the habituation sequence (uppermost histograms). For the CS+ the initial histogram was broken into the following three components: (1) an inhibitory response interval, extending from 10

Figure 13: Change in spike activity with conditioning of neuron 56B.


Neuron 56 B, Poststimulus Histograms
(75 Trial Averages)
CS+
CS−
H
C1
C2
C3
E
Spikes / Second / Trial
Time (ms)
CS
US
CHANGE IN RESPONSE TO CS
CS+ (Interval 250-340)
CS− (Interval 290-360)
Evoked Rate Minus Background Rate, Spikes/sec.
Cryoprobe Control
25 Trial Blocks

msec poststimulus through 250 msec poststimulus; (2) an interval extending from 250 msec poststimulus through 340 msec poststimulus; (3) an inhibitory interval extending from 340 msec poststimulus through 510 msec poststimulus. The upper limit of 510 msec poststimulus was established so that the US occurrence and US elicited activity would be excluded from analysis. The initial histogram derived for the CS- stimulus was similarly divided into three parts, but with slightly different lower and upper limits corresponding to the response components.

The mean spike rate was computed for the 1 sec prestimulus period and during the selected response intervals. Neuron 56B had a prestimulus spike rate of about 4 spikes per second which was relatively stable throughout the experimental sequence. As one might expect, the prestimulus rate was quite comparable for the CS+ and the CS- since the stimuli were presented in a randomized and interdigitated fashion. In contrast to the stability of the prestimulus rate, the CS elicited activity showed modification during conditioning. The poststimulus histograms C1, C2, and C3 of Figure 13 show the sequential 75 trial blocks during conditioning for both the CS+ and CS-.

During conditioning the response to the CS showed an increase in excitatory activity and a slight decrease in the early inhibitory responses. For both the CS+ and CS- the largest response modification, as measured with the overall t-score, occurred in the intermediate latency response components. It is apparent that for the CS+ and CS- little short latency response modification took place and the portion

of the inhibitory response extending from approximately 10 through 60 msec poststimulus was relatively unaltered during the conditioning sequence.

During C1 and C2 there was a small, but consistent increase in spike rate for both the CS+ and CS-. By the end of C2 it was apparent that the CS+ excitatory response exceeded that of the CS- and that there was clear differentiation between the CS+ and CS- responses. The differential responsiveness was maintained during extinction. The CS+ response showed a reversal during extinction, exhibiting a histogram pattern similar to that during habituation, while the CS- extinction histogram differed from the CS- habituation histogram.

The increased excitatory activity was not due to sensitization caused by the US presentation. The US elicited a strong inhibition of neuronal activity as evident in histograms C1, C2, and C3.

These response modifications have also been presented graphically as acquisition curves for the CS+ and the CS- (Figure 13, bottom). The curves show the mean evoked spike rate during the selected response interval minus the prestimulus spike rate plotted against trials. Of the three response intervals derived from the habituation sequence, the interval which showed the largest and most consistent response modification for both the CS+ and CS- was chosen for graphical analysis. For neuron 56B the intermediate latency response component was chosen from the three response components analyzed for both the CS+ and CS-. The duration of the selected intervals is shown on the acquisition curve graphs.

To allow a finer grain analysis of the response modification as it developed across trials, a 25 trial block was used. The number of

trials grouped into a block was a compromise between trying to have the maximum number of data points while attempting to average large numbers of trials to minimize variability. In Figure 13 each histogram is represented by three data points of the acquisition curve.

The CS curve showed a slight trend toward the baseline during the 75 trial habituation sequence (Figure 13, lower left). Early in conditioning, the CS+ response was virtually identical with that during habituation. However, after 25-50 presentations of the CS+ followed by pairing with the US, the CS+ response became excitatory. The acquisition curve shows that the mean CS+ response was a consistent excitatory response through 150 CS+/US pairings. The final 75 conditioning trials showed a spike rate exceeding the prestimulus level by approximately 7 spikes per second per trial. During extinction the CS+ response remained excitatory for the first 25 extinction trials and then decreased to below the baseline level.

The initial response to the CS- during the selected interval was slightly below the baseline. During habituation a small average increase in the spike activity was apparent. This trend continued in a fairly constant fashion throughout subsequent trial blocks, appearing to approximate a linear function. There was no dramatic increase in spike activity level, and the CS- excitatory response was approximately half that of the CS+. It is noteworthy that the excitatory change was maintained during extinction in the case of the CS-.

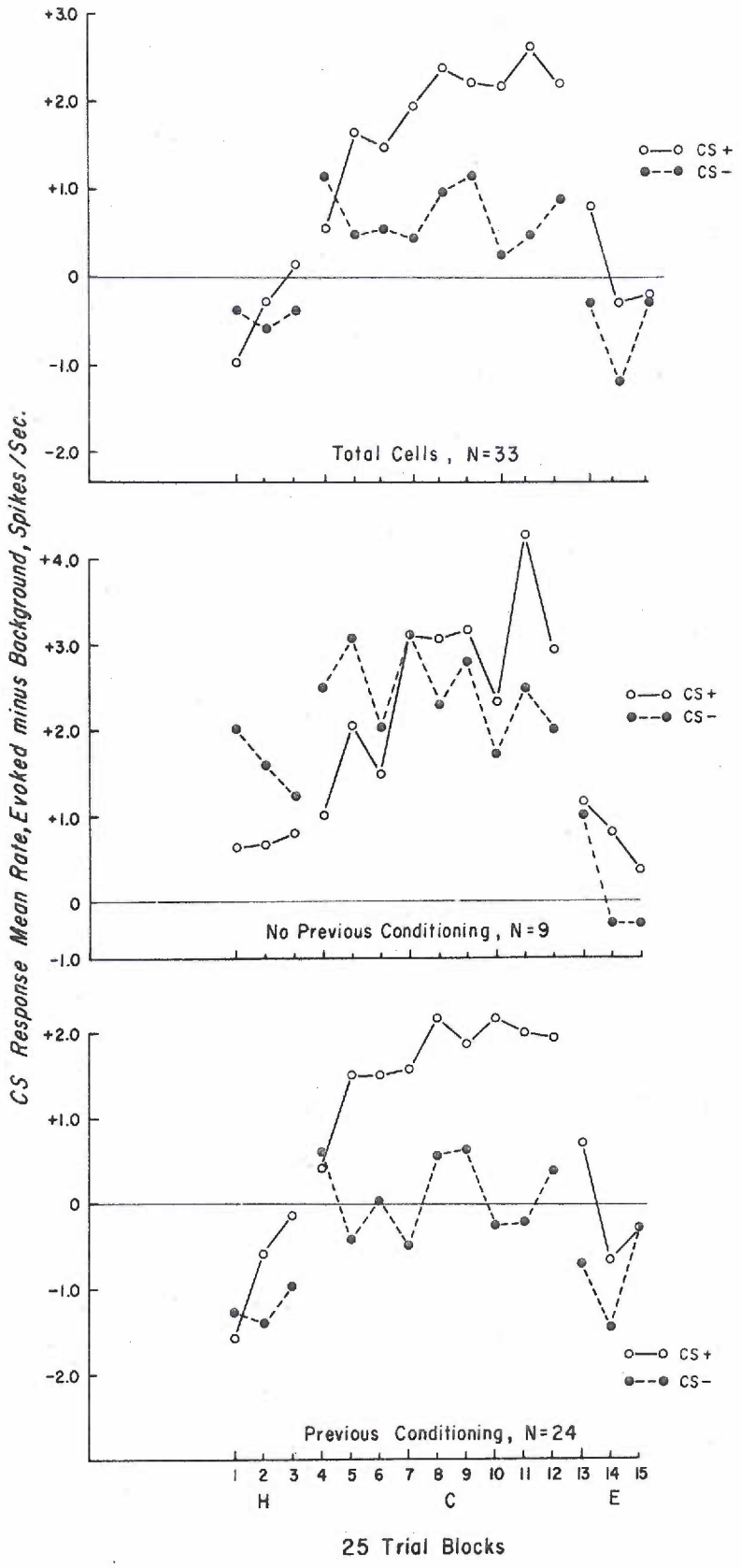
Procedural control group: Grouped cortical unit data

Single unit data were successfully obtained for the complete trial sequence for 33 neurons from 20 cats. The data from this sample of neurons were grouped to examine the overall tendency of the differential classical conditioning paradigm to produce response modification. In addition, the factor of whether the animal was naive to the conditioning paradigm was examined as a group variable.

To group together the individual data, an acquisition curve as shown in Figure 13 was formed for each neuron. Approximately 58 percent of the neurons sampled showed initial inhibitory responses which, during the conditioning procedure, reversed sign and became excitatory. Thirty-four percent of the neurons sampled had excitatory responses which tended to decrease during conditioning and some became inhibitory.

The group that showed decreases in spike activity during conditioning were initially analyzed separately from the group showing increases. Subsequently, both groups were combined since no differences in time-course or magnitude of response change were apparent. For this purpose, all difference scores were inverted in sign for neurons with conditioned decreases in response so that the direction of change in response would be consistent when combined with excitatory changes.

The group acquisition curve for the 33 neurons is shown in Figure 14 (upper graph). Each data point represents a 25 trial average, with the open circles showing the CS+ response. Since the response measure is a difference score, the zero line represents the baseline (prestimulus) rate. The CS+ response was initially slightly below the baseline level.


CONDITIONING GROUP
+3.0
+2.0
+1.0
0
-1.0
-2.0
CS +
CS -
Total Cells , N=33
+4.0
+3.0
+2.0
+1.0
0
-1.0
CS +
CS -
No Previous Conditioning , N=9
+2.0
+1.0
0
-1.0
-2.0
CS +
CS -
Previous Conditioning, N=24
CS Response Mean Rate, Evoked minus Background, Spikes/Sec.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
H
C
E
25 Trial Blocks

During habituation the response decreased toward the prestimulus level with the final habituation showing virtually no response.

The group curve shows a steadily increasing change in response. This trend continued during the 225 conditioning trials and was uninterrupted during the procedural control period, trial blocks seven, eight, and nine. It was during this 75 trial block that the cryogenic system was operated, although for this control group no thalamic cooling was produced. The continuity of the CS+ acquisition curve indicates that simply the operation of the cryogenic apparatus, without functional blockade, did not impair the response modification during the conditioning sequence.

Subsequent to completing the conditioning trials, the CS+ was presented for 75 extinction trials. The initial response during extinction was similar to that during conditioning and additional presentations of the CS+ alone showed the reversibility of the response toward the habituated level. This overall CS+ acquisition pattern is similar to that described for neuron 56B, although the averaged group change is smaller.

The group curves for total cells showed a prominent difference in the CS+ and CS- pattern. As shown in Figure 14, the CS- response modification was of smaller magnitude and showed a different trend across trials (upper graph filled circles). The initial habituation level for the CS- was comparable with that of the CS+, both being slightly inhibitory. During conditioning the CS- showed an initial increase in excitatory activity, exceeding that of the CS+. However, during the remaining conditioning trials, there was an enlarging divergence

between the CS+ and CS- response levels. An analysis of variance of the CS+ versus CS- data showed a significant level difference during the conditioning trials, even though no statistical difference existed prior to conditioning ($F = 36.6$, df 1/544, $p < .01$). Furthermore, a significant F value ($F = 2.20$, df 8/544, $p < .05$) was found for the interaction term of the CS+ versus CS- factor with trials, indicating a different change across trials for the CS+ as compared with the CS-. The analysis of variance values are in Appendix B.

Since the factor of exposure to conditioning affects the rate of learning and differentiation, the "no previous conditioning" group was separated from the "previous conditioning" group. The no previous conditioning group comprised data from animals naive to the conditioning paradigm, while the previous conditioning group consisted of data from animals who had experienced the learning situation at least once before. This distinction has been described in the experimental procedure section and was depicted in Figure 3.

The no previous conditioning group curve was derived from data of nine neurons (middle graph, Figure 14). The overall change during conditioning was similar to that for the total conditioning group, an upward trend during the 225 conditioning trials. The CS+ response showed a slow increase in magnitude throughout the 225 conditioning trials. In contrast, the CS- response which initially exceeded that of the CS+ showed a decrease in response during the conditioning sequence. The no previous conditioning group showed poor differentiation in that there was no clear separation of the CS+ and CS- response

level. However, during the latter half of the conditioning sequence, there was increased CS+/CS- differentiation, suggesting that approximately 100 trial presentations are necessary for successful differentiation in the case of this particular conditioning paradigm.

The no previous conditioning group should be contrasted with the previous conditioning group (lower graph of Figure 14). The clearest differences are the rate of response modification during conditioning and the level of differentiation. During the habituation sequence, there was an apparent decrease in the response magnitude toward the baseline level for both the CS+ and CS-. The initial data point during conditioning shows close equivalence between the CS+ and CS-. Between trials 25-50, there was a marked increase in the CS+ response with an opposite effect for the CS-. Eight of the nine data points during conditioning showed a level difference in which the CS+ response exceeded that of the CS-. The CS+ response peaked in the middle of the conditioning sequence, having plateaued at an average level of about 2 spikes/sec/trial above the prestimulus spike rate. In contrast, the CS- spike rate varied around the baseline level, with the mean response during conditioning closely approximately the prestimulus level.

It is apparent that operation of the cryogenic apparatus without cooling did not interfere with sensory evoked activity recorded during trial blocks seven, eight, and nine. During this procedural control period the response magnitude was comparable with that both before and after this 75 trial period.

The experimental group: Single cell data, interval analysis

Nineteen cats composed the experimental group. These animals had cryoprobes bilaterally positioned with the tips in the area of the ventral anterior nucleus of the thalamus. During the middle 75 conditioning trials a functional thalamic blockade was established by circulating cold ethanol through the implanted cryoprobes. In all other respects the experimental group and procedural control group are comparable, and the identical stimulus presentation sequence has been utilized for both.

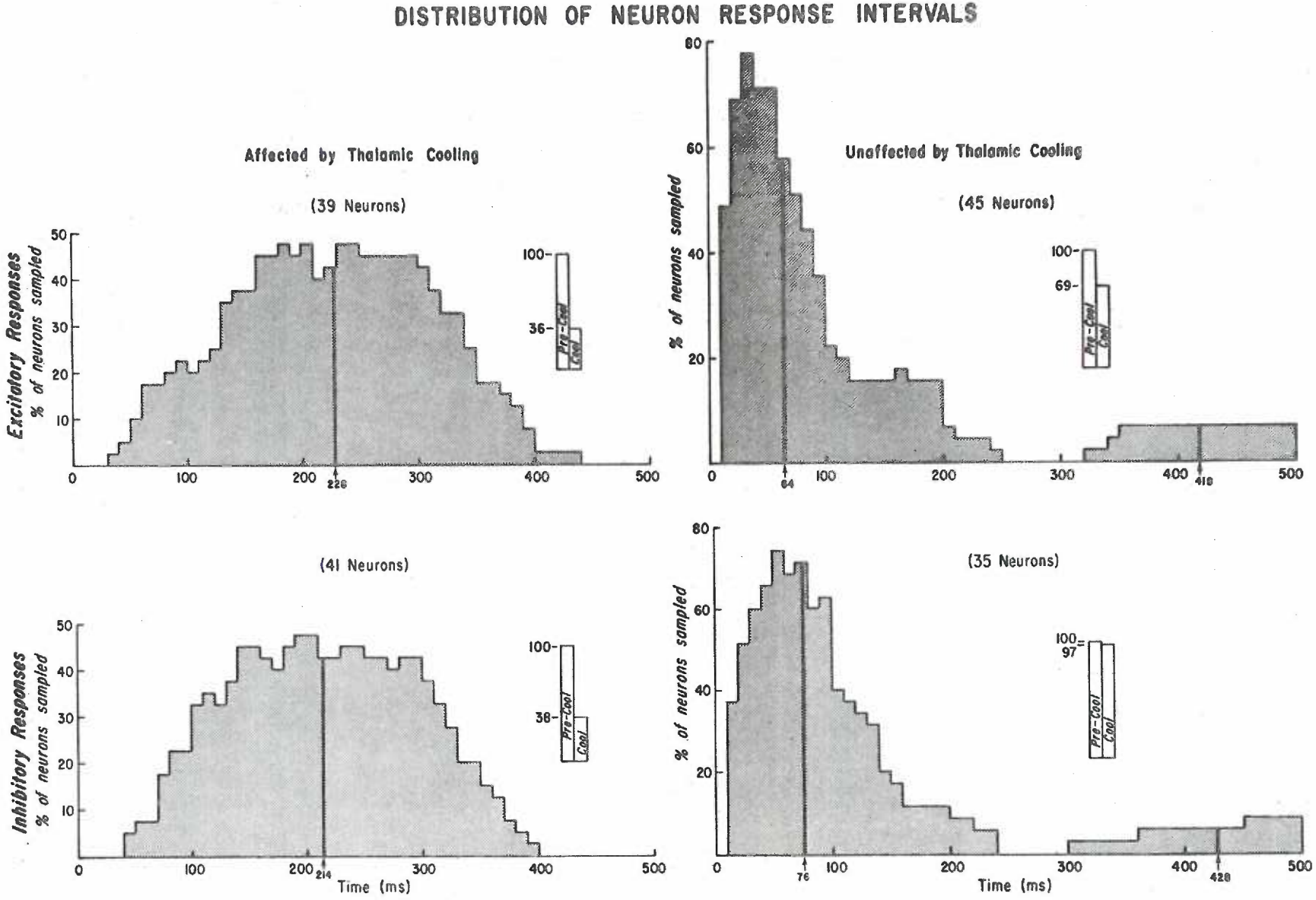
An analysis of the evoked potential data suggested that the thalamic cooling effect was not uniform across all response intervals, but rather that certain response intervals were disproportionately influenced. Since this finding was based upon analysis of the evoked potential data, it was important to know whether the thalamic blockade similarly affected the neuronal response elicited by paw stimulation during particular portions of the response. For these purposes, a critical interval, defined as the AEP response interval predominantly affected, was used as an independent measure to test whether this applied for the single cell data. In accordance with the AEP-derived critical interval for the influence of thalamic cooling, neuronal response components were assigned as either including the critical interval or excluding the interval for both the CS+ and CS- stimuli. Those neuronal response components which included over half of the AEP defined critical interval, from 121 msec poststimulus through 227 msec poststimulus, were designated as covering the critical interval. Any neuronal response component which included less than half of the AEP defined critical interval was designated as excluding the critical interval.

After neuronal response components were assigned to either category, the distributions of the intervals were computed, as shown in Figure 15. In the event that the excitatory and inhibitory response might be differentially influenced by thalamic cooling, the data were separated according to the sign of the response. However, the distributions for the excitatory and inhibitory responses were quite comparable (compare upper and lower distributions). The distributions of the neuronal response components designated as covering the critical interval are shown on the left side of Figure 15, labelled as "affected by thalamic cooling". These distributions are broader than the AEP derived critical interval and reflect the bias for choosing as long a neuronal response of the same sign as possible.

In contrast, the neuronal response intervals which did not cover the critical intervals were distributed as shown in Figure 15, labelled "unaffected by thalamic cooling". The medians for the excitatory and inhibitory responses were 64 msec and 76 msec poststimulus respectively, and both showed a positively skewed distribution.

Whether these intermediate latency neuronal response components were preferentially affected by thalamic cooling was measured. This was accomplished by comparing the difference scores of the response magnitudes before and during thalamic cooling. Thalamic cooling yielded decreases in the unit responses recorded in the postcruciate cortex, and the intermediate latency response components were more radically altered during the cooling. The inset bar graphs of Figure 15 show the corresponding response decrement for each distribution. For example, the excitatory and inhibitory response components which

Figure 15: The distribution of neuron response intervals. The inset bar graphs show the magnitude of thalamic cooling effect upon the neuronal spike activity for the corresponding response intervals.


DISTRIBUTION OF NEURON RESPONSE INTERVALS
Affected by Thalamic Cooling
(39 Neurons)
Excitatory Responses
% of neurons sampled
Pre-Cool
Cool
100
36
228
Unaffected by Thalamic Cooling
(45 Neurons)
% of neurons sampled
100
69
64
416
(41 Neurons)
Inhibitory Responses
% of neurons sampled
100
38
214
Time (ms)
(35 Neurons)
% of neurons sampled
100
97
76
428
Time (ms)

included most of the AEP defined critical interval showed an averaged 63% decrease in response magnitude during the 75 trial cooling blockade.

In contrast, those neuronal response intervals which did not correspond to the AEP intervals most affected by cooling showed smaller decreases in response magnitude during thalamic cooling. For these neuronal response intervals, the average decrease during thalamic cooling was only 19% of the response prior to cooling. The magnitude of the decrease was 31% for the excitatory response components and 3% for the inhibitory response components. Thus, as in the case for the cortical averaged evoked potential data, the neuronal recordings showed certain response intervals predominantly affected by the thalamic blockade. Those neuronal response intervals which include at least half of the AEP defined critical response interval were affected more than three times as much as neuronal response intervals differing from that of the critical interval.

Experimental group: Conditioning and the critical response interval

Since it was apparent that the effect of thalamic cooling was interval dependent, the analysis of the functional blockade upon conditioning included a consideration of the critical interval. The response interval separation that yielded the distributions shown in Figure 15 has been maintained in analyzing the conditioning changes. Thus, the data have been grouped with two response components selected from each single cell response, one response component including the critical interval most affected by thalamic cooling, and the other primarily excluding the critical interval. These response components were then grouped across cells, in the identical manner in which the conditioning data for the procedural control group were grouped.

The grouped data for the response intervals affected by cooling has been presented in Figure 16. The group curves were collected from 19 cats and were composed from the data of 40 neurons, which were successfully recorded during the entire trial sequence. As in the case of the control group, a distinction was maintained with regard to exposure to the conditioning paradigm, segregating the naive from the previously exposed. Consequently, the group acquisition curves have been separated into groupings of all cells, first cells recorded from an animal, and subsequent cells.

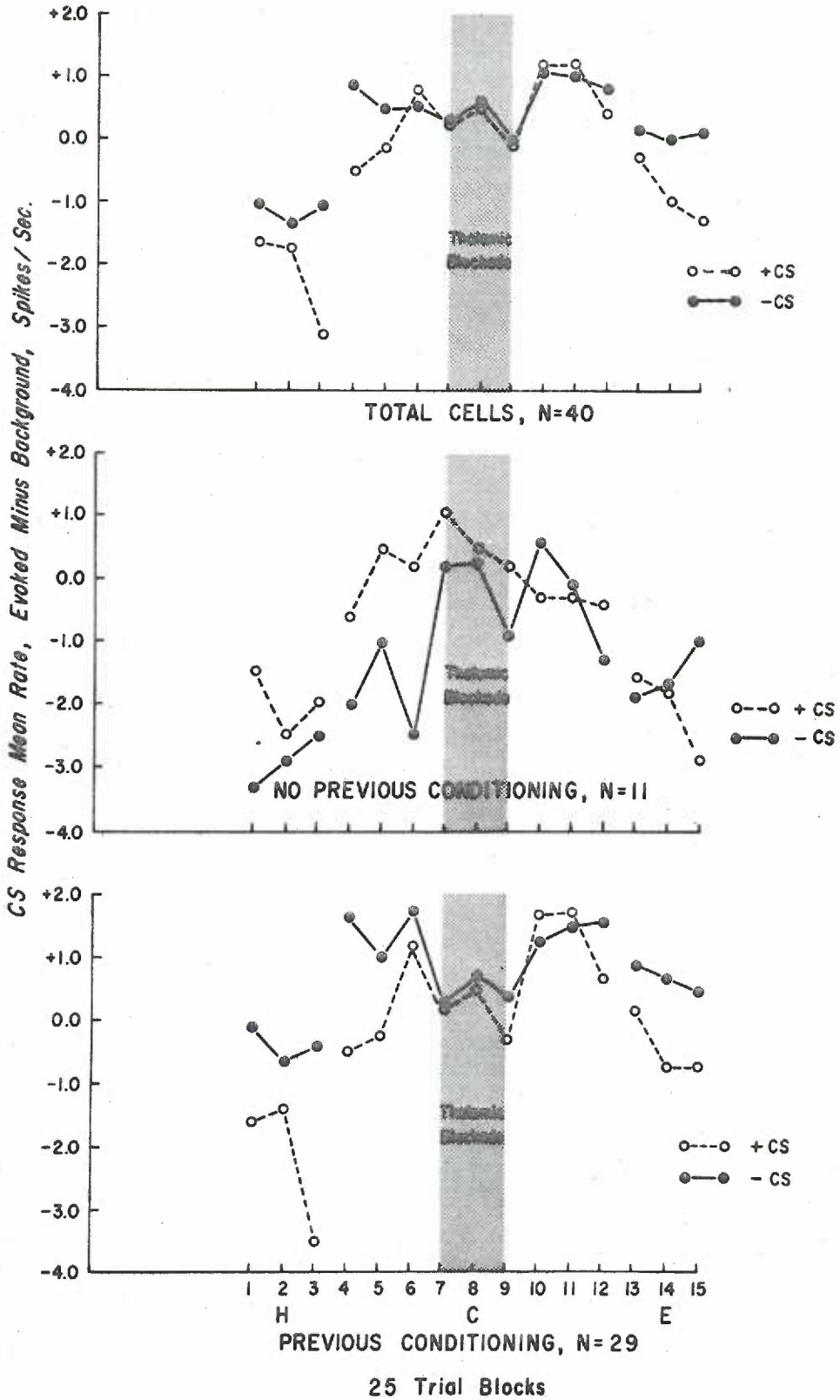
The top graph of Figure 16 is that for the total group. The CS+ (open circles) and CS- (filled circles) both show an inhibitory response during the 75 habituation trials.

The conditioning sequence for the experimental group can be divided into three portions: (1) preceding, (2) during, and (3) after the thalamic cooling. The group acquisition curve shows a gradual reversal of response sign during the first 75 conditioning trials for the CS+ response. This trend is comparable to that seen for the control group. During the thalamic blockade the response difference score was decreased and maintained near zero (0.2 spikes/sec/trial). There was, therefore, an interruption of the continued growth of the acquisition curve. In contrast with the control group, during the thalamic blockade the response magnitude was below the level of the immediately preceding trials.

Following termination of the cooling blockade, the response magnitude showed a level approximating that of the 25 trial block immediately preceding the cooling blockade (data points 6 and 10). The remaining

Figure 16: Grouped single neuron conditioning data for those response intervals most affected by thalamic cooling. The duration of the thalamic cooling is shown with shading. The upper graph includes the total cell data. The total cell data have been separated into those cells in which the cat was naive to the conditioning paradigm, the no previous conditioning group, and those cells in which the cat had exposure to the conditioning paradigm, the previous conditioning group.

THALAMIC BLOCKADE DURING CONDITIONING:
Response Intervals Affected By Cooling



conditioning trials did not show a resumption of the acquisition curve, as shown for the control group. Instead, the CS+ response showed a decrease in magnitude, even with the additional conditioning trials (data points 11 and 12).

It is apparent that the differentiation of response has not developed for this experimental group. Instead of the clear divergence in responsiveness to the CS+ and CS- exhibited in the control group, there was a close correspondence between the CS+ and CS- response for the thalamic cooling group. As shown in Figure 16, the CS- (filled circles) initially approximated the CS+. During conditioning there was an initial level difference which was followed by convergence of the CS+ and CS- response. During thalamic cooling, the CS- response was virtually identical with that of the CS+. During the final 75 conditioning trials, after terminating the cooling, the CS- continued to closely approximate the CS+.

An analysis of variance was used to directly compare the control and experimental groups. Since the middle 75 conditioning trials of the experimental group were disrupted by thalamic cooling, these trials were deleted from both groups for the purpose of statistical analysis. The analysis of variance was achieved by using difference scores of the CS+ values minus the CS- values over 6 conditioning trial blocks. The analysis showed significant F values for the control versus experimental factor ($F = 5.32$, df 1/64, $p < .05$) and the interaction term of the trend across trials for the two groups ($F = 4.29$, df 5/320, $p < .001$).

The data were grouped for those neuronal recordings taken from animals that were naive to the conditioning paradigm. This grouping, that of "no previous conditioning" is shown in the middle graph of Figure 16. The group curves have been derived from 11 neurons in this instance. Both the CS+ and CS- showed an initial inhibitory habituation level. During the initial phase of conditioning, prior to thalamic cooling, there was a slight excitatory trend for the CS+ response, and the trend for the development of differentiation. Thalamic cooling maintained the average response level for both the CS+ and CS- near zero and negated the differential responsiveness. It is interesting to note that subsequent to termination of the cooling, there was a downward trend in the response. Furthermore, there was no evidence for differentiation taking place in this experimental group during the final 75 trials of conditioning. As seen from the control group, approximately 150 uninterrupted CS-US pairings are necessary for the development of differentiation.

The lower graph of Figure 16 presents the group curves for recordings from cats that had already been exposed to the conditioning paradigm at least once. The data from 29 neurons composed these group curves. For the CS+ curve, the 75 conditioning trials prior to thalamic cooling showed an orderly increase in spike rate. During cooling the influence of the thalamic blockade was to reduce the spike rate to approximately the prestimulus level which was maintained at about the baseline level for the entire 75 trial cooling period. During the final 75 conditioning trials, following the thalamic cooling, the spike rate resumed at approximately the precool level. However, during subsequent trials, the spike

rate showed a decline rather than the continued excitatory direction of response modification found for the control group.

The grouped previous conditioning acquisition curves do not demonstrate differential conditioning. In the control group, there was a significant differentiation of the response elicited by the CS+ and CS- which was apparent after 25 conditioning trials. Since this differentiation was only evident in the previous conditioning group, it was apparently the result of savings from exposure to the conditioning paradigm. However, when the thalamic blockade was instituted during the conditioning sequence, these savings effects were not evidenced.

Experimental group: Conditioning and the non-critical response intervals

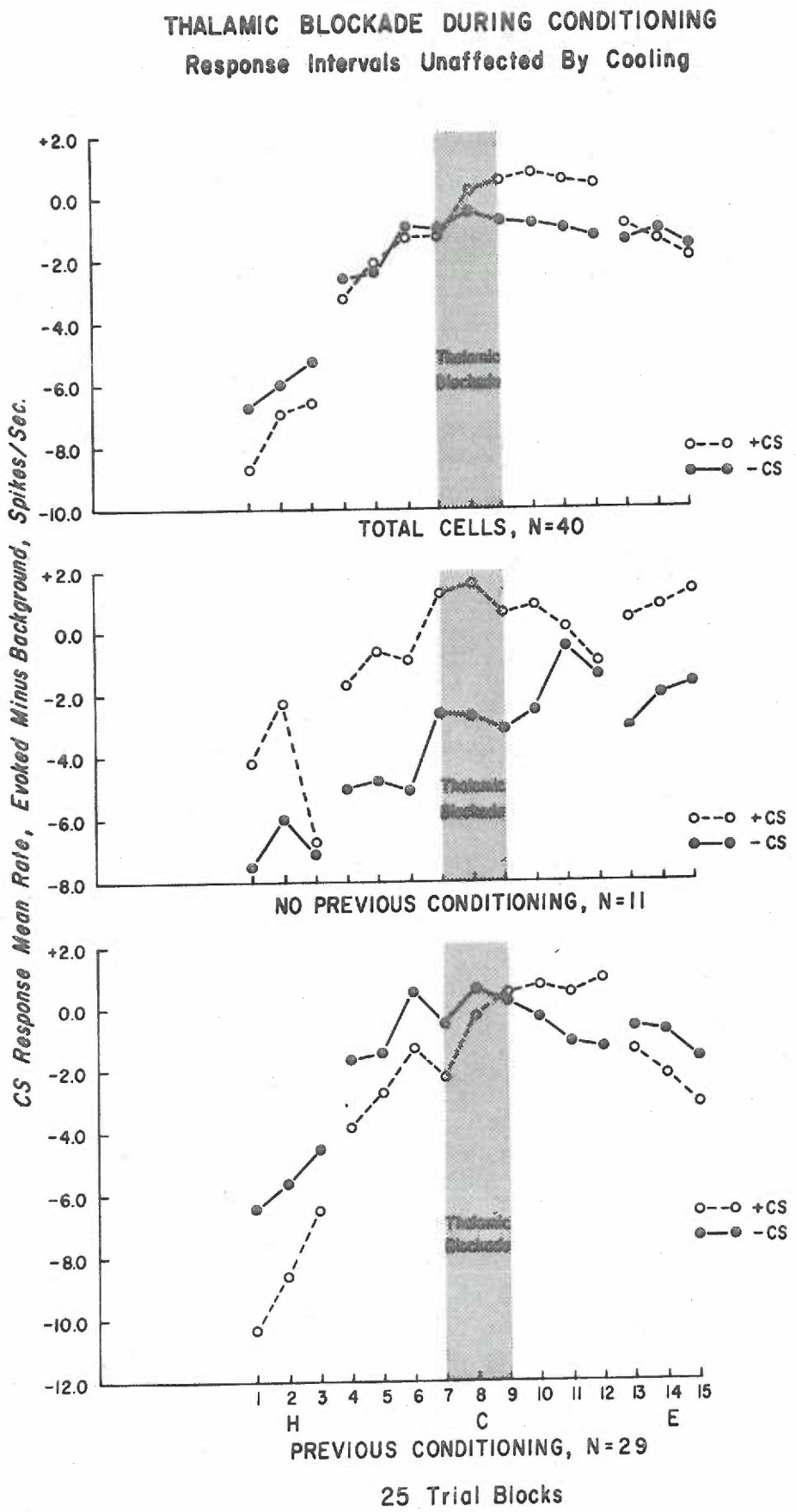
The effect of thalamic cooling is best described as being interval dependent. The preceding analysis examined the response modification during the AEP-defined critical interval during conditioning. This section will detail the response modification of the non-critical intervals concomitant with conditioning. The data presented are gathered from the identical neuronal sample as that presented for the critical intervals, the only difference being the portions of the poststimulus histograms incorporated into each grouping. Furthermore, the response components were analyzed in an identical fashion. Figure 17 shows the group acquisition curves divided into total cells, no previous conditioning, and previous conditioning.

The total group curves show a greater magnitude of response plasticity than that for the response intervals more affected by thalamic cooling considered previously. Marked changes are apparent during the conditioning sequence, the CS+ elicited activity being

altered by an absolute difference of over 8 spikes/sec. At the beginning of conditioning the CS+ and CS- values were exceedingly close. As conditioning continued, there was a trend toward successful differentiation of the CS+ and CS-, the separation becoming significant during the thalamic cooling sequence ($F = 2.03$, df 8/616, $p < .05$). It is noteworthy that in the case for the response intervals relatively unaffected by cooling, there was continuous response modification, uninterrupted by the thalamic cooling sequence. During the extinction sequence the CS+ elicited activity showed a decrease in the spike rate, while the CS- elicited activity was virtually unaltered ($t = 1.78$, df 39, $p < .05$).

The group curves for the no previous conditioning data suggest a convergence of response activity. Unlike the control group, there is no evidence of successful differentiation with repeated conditioning trials. If it were the case that additional conditioning trials are necessary for differentiation, then differentiation might only be apparent in the previous conditioning group. This, in fact, appears to be the situation. The lower graph of Figure 17 shows the previous conditioning group. There was a large magnitude change in spike rate during the conditioning sequence for the CS+. The response modification during conditioning was apparently uninterrupted during the thalamic cooling sequence. Subsequent to termination of the thalamic cooling, there was a maintained CS+ response which was differentiated from the CS- response.

Figure 17: Grouped single neuron conditioning data for those response intervals least affected by thalamic cooling. The duration of the thalamic cooling is shown with shading. The upper graph includes the total cell data. The total cell data have been separated into those cells in which the cat was naive to the conditioning paradigm, the no previous conditioning group, and those cells in which the cat had exposure to the conditioning paradigm, the previous conditioning group.


THALAMIC BLOCKADE DURING CONDITIONING
Response Intervals Unaffected By Cooling
CS Response Mean Rate, Evoked Minus Background, Spikes/Sec.
+2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
Thalamic Blockade
+CS
-CS
TOTAL CELLS, N=40
NO PREVIOUS CONDITIONING, N=11
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
H
C
E
PREVIOUS CONDITIONING, N=29
25 Trial Blocks

Experimental group: The effect of decreased cortical afferent activity upon conditioning changes

The conditioning changes observed for the postcruciate neuronal responses were related to the effectiveness of the thalamic blockade. Evidence for this is suggested by the finding that those grouped response intervals which were more influenced by thalamic cooling showed the least differentiation of the CS+ and CS- response. It was, therefore, hypothesized that the magnitude of the afferent block of the cortical responses caused by thalamic cooling would correlate with the degree of cortical conditioning changes. If this were true, then the cortical neuronal responses which were most influenced by thalamic blockade would show little response modification during conditioning. To test this hypothesis, a linear regression analysis was performed. For these purposes the magnitude of the afferent block of the cortical responses was compared against a measure of the conditioning change.

The magnitude of the cortical afferent blockade was evaluated as the difference score of the response level prior to thalamic cooling minus the response level during thalamic cooling, in which a large positive value indicated a large afferent block. However, a sufficiently large neuronal response prior to cooling was needed in order to evaluate the afferent block during thalamic cooling. To evaluate the afferent block, the difference score prior to thalamic cooling needed to exceed one spike per sec to be meaningfully analyzed.

The conditioning change was quantified by obtaining the difference score of the mean response level during the final 75 trials of conditioning minus the response level immediately preceding thalamic cooling,

conditioning trials 50 through 75. The actual values for the afferent block and conditioning changes are included in Appendix C.

A correlation coefficient of -0.42 was derived using 52 data points ($p < .005$, $df = 50$). The degree of afferent block was a significant predictor of the amount of conditioned change in response. The regression equation was found to be: $Y = 2.19 + (0.98) X$ in which X was the measure of the afferent block and Y was the measure of the conditioning change. These variables show a significant inverse relationship such that the larger the afferent block, the less the conditioning change.

DISCUSSION

Somatic responses of the postcruciate cortex

It is evident that the non-specific thalamus contributes to sensory responses of neurons in the postcruciate cortex. Although a mapping study was not done, this study suggests that the ventral anterior nucleus appears to be an important accessory sensory nucleus via which somesthetic afferent activity reaches the sensori-motor cortex. A measure of the change in sensory evoked activity of the cortical neurons showed that some response intervals were decreased an average of 63% during the functional blockade of the ventral anterior nucleus. This finding is similar to that which O'Brien and Rosenblum found for the centromedian nucleus of the thalamus (45, 51). In this study they observed that sampled postcruciate neurons showed decreased responses to somesthetic and visual stimuli during functional blockade of the centromedian region. Although the magnitude of the change in response varied across neurons, an average decrease of 50% was found. As in the case for the ventral anterior nucleus blockade, the decrease in sensory evoked activity applied for both excitatory and inhibitory responses. Thus, using the measure of evoked spike activity of postcruciate neurons, it is apparent that both the centromedian and ventral anterior nuclei are part of the afferent pathways for the somatic conditioned stimuli used in this experiment. However, a detailed neuroanatomical analysis would be beneficial in describing other nuclei of the afferent pathways for the somatic conditioned stimuli.

The effect of the functional blockade of the centromedian nucleus was shown to be interval dependent (45, 51). Using integrated averaged evoked potentials recorded from the cortex, it was determined that the predominant effect of the thalamic blockade was evident between

approximately 100 msec and 210 msec poststimulus. This compared with the finding that the response interval most affected by blockade of the ventral anterior nucleus extended from 120 msec through 230 msec poststimulus. The distributions of the critical intervals are quite comparable for the centromedian and ventral anterior nuclei blockades. The median values for the distributions are within 10 msec of each other and the modal values show that in both cases about 60% of integrated averaged evoked potentials are affected.

The critical interval analysis was based upon the cortical evoked potentials. However, it is apparent that the poststimulus histograms of the postcruciate neurons show the effect of thalamic blockade during particular response intervals. In the case of the centromedian blockade, this finding was suggested by viewing the histograms. For the ventral anterior blockade, this question has been analyzed by using the critical response interval derived from the averaged evoked potentials as an independent measure. It was confirmed that neuronal responses during the critical interval showed a much greater decrease in sensory activity than those neuronal responses occurring outside the critical time limits. This supports the concept that the somatic sensory evoked activity recorded in the sensori-motor cortex from approximately 100 msec through 200 msec poststimulus reflects a predominant nonspecific thalamic pathway contribution (34, 35, 36). In that the neuronal responses exhibit the same phenomenon as that described for the cortical evoked potentials, we can be certain that the finding is not based upon volume conduction which might be included in the monopolar evoked potential record, but rather that the observed cooling effect is localized in the cortex.

As in the case for the centromedian blockade, it is apparent that the short latency somatic responses are unimpaired. The interval analysis for the ventral anterior blockade did not show any effect less than 20 msec poststimulus. The poststimulus histogram of neuron 63B (Figure 6) showed, in typical fashion, that the first bin poststimulus was unaffected by thalamic cooling. In contrast, it is noteworthy that during the thalamic blockade the inhibitory response from 60 msec through 150 msec poststimulus was abolished.

While there was close correspondence between the effect of functional blockade of either centromedian or ventral anterior nuclei thalamic sites, it is evident that the effects differ across anatomical boundaries. It was found that blockade of the ventro-medial caudate nucleus resulted in enhanced sensory evoked activity recorded from postcruciate neurons. Although the sample size was small, all neurons recorded during ventromedial caudate blockade consistently showed characteristic changes. Unlike the ventral anterior nucleus site, blockade of ventromedial caudate nucleus affected short latency somatic responses; the first data bin poststimulus showed decreased spike activity, while the longer latency responses showed substantially increased responses. The predominant effect of increased sensori-motor cortical activity during blockade is consistent with positing an inhibitory role for the ventromedial caudate (28).

Neuronal response plasticity

It is apparent that the postcruciate neuronal responses were not static and invariant. In contrast, it was found that the response elicited by a stimulus was susceptible to modification by establishing

a temporal contingency in which the conditioned stimulus was followed by the presentation of the unconditioned stimulus. It was observed that the poststimulus histogram as a representation of a neuronal response was subject to modification, and thus was neither invariant nor simply related to stimulus parameters (Figure 13).

The neuronal response modifications which were studied in this investigation were concomitant with the presentation of stimuli in the manner of a learning paradigm.in which the occurrence of the conditioned stimulus was followed by the occurrence of the unconditioned stimulus with a short fixed interstimulus interval. Procedurally, the stimulus presentation manner clearly falls within that genre of learning entitled classical conditioning. However, it is of considable interest to evaluate whether the neuronal response modifications seen in this study are specifically in accordance with the parameters of classical conditioning or whether the response modifications belong to the larger class of neuronal plasticity.

Several characteristics of the response changes suggest that the recorded modifications are classical conditioning changes rather than nonspecific effects of sensory stimulation, adaptation, habituation, or fatigue. The following characteristics are consistent with that observed while measuring behavioral conditioned responses:

1. The reinformcement of the conditioned stimulus (CS) yielded a different response than that from the CS alone. The conditioned response was often opposite in sign and greater in magnitude than the CS alone presentations, and thus could be differentiated from an habituation phenomenon.

2. The CS-US pairing resulted in an orderly, incremental development of the conditioned response (Figure 14). In distinction with sensitization effects, there was not an immediate modification in the response, but rather the change was apparent only after a number of trials.

3. Once the change in response occurred during conditioning, the change was maintained throughout the pairing sequence.

4. The form of the acquisition curve is comparable to that for behavioral measures of conditioning; for example, the grouped neuronal changes parallel the measure of conditioned leg flexion in a similar experimental preparation (44). The particular behavioral experiment used massed trials, a weak US, and a 0.5 sec CS-US interval in a trace conditioning manner, all factors in common with the present experiment.

5. In addition to the clear development of the conditioned response during the pairing sequence, there was a reversibility of the response during an extinction condition of CS alone presentations. Given sufficient unreinforced trials following the conditioning, the response was altered and approximated that of the habituated state. The extinction process was evidently influenced by the number of unreinforced trials (Figure 14).

6. It is characteristic of genuine learning situations that the previous exposure to the task facilitates learning. The grouped data, divided into the no previous conditioning situation and the previous conditioning situation, shows a slower rate of acquisition and greater variability for the naive animals. The situation in which the animal had already been exposed to the learning paradigm yielded more rapid and less variable conditioning changes (Figure 14).

7. The finding of differential responsiveness is sufficient evidence to demonstrate bona fide conditioning rather that sensitization or pseudoconditioning, especially if the two stimuli excite the same sensory modality (30, 56). In establishing the differential conditioning it was necessary to present two stimuli of which only one was followed by the US occurrence. In the present experiment both the CS+ and CS- stimuli were somatic forepaw stimulation with the only difference being the place of stimulation, specifically the right versus the left forepaw. After sufficient conditioning trials, the CS+ and CS- elicited different responses. In naive cats, differentiation was apparent after approximately 150 trials. However, the rate of differentiation was influenced by exposure to the conditioning paradigm; those animals previously exposed to the conditioning paradigm showed differential responsiveness after 25 trials.

8. In addition to the neuronal activity showing differential responsiveness to the CS+ and CS- presentations, the measure of heart rate showed differential responsiveness as well, another indication of learning taking place (38).

It is evident that the neuronal changes of the postcruciate cortex parallel a number of characteristics of classical conditioning. Thus, this form of neuronal plasticity should be equated with a bona fide learning phenomenon rather than nonspecific stimulation effects.

Subcortical conditioning

Conditioning changes were evidenced at both the thalamic and cortical levels. Numerous studies have shown evidence for conditioning changes at the thalamic level. Kamikawa et al. (29) reported

conditioning changes in thalamic neurons occurring at around trial 50. Disterhoft and Olds (17) found thalamic response changes to the CS presentation at around trial 40. Using evoked potential activity recorded bipolarly from the centromedian nucleus of the thalamus, conditioning changes were evidenced in this study early in the pairing sequence, after 25 through 50 trials. It has been hypothesized that very rapid conditioning changes take place in the reticular formation (usually within 25 trials) followed by conditioning changes in the thalamus (about trial 50), and subsequently in the cortex (42, 79). The present experiment offers partial support for this hypothesis by confirming thalamic changes early in the trial sequence and cortical changes later in the trial sequence.

The thalamic evoked potentials showed a transient conditioning change which was prominent for only a portion of the total conditioning sequence. This phenomenon has been reported when learning has been measured by EEG, evoked potentials, and D-C responses (27). In contrast, the learning changes measured by single neuron activity showed longer duration response modification throughout the conditioning sequence. It is possible that the differences reported are dependent upon the measures used, in that the evoked potential was measured in peak-to-peak amplitude while the single cell response was reported as a difference score of elicited activity minus background activity.

Olds et al. (47) reported that a relatively high proportion of the neurons sampled from the posterior thalamus (53%) showed criterion learning changes. Of these thalamic neurons sampled, most showed a similar pattern of conditioning change. Neurons of the posterior

thalamus showed similar responses during habituation, the conditioned response was larger in magnitude but in the same direction as the habituation response, and there was considerable generalization of the response to the CS-. These characteristics led Olds to consider the thalamic site an area of nonspecific learned responses.

In contrast to the findings of Olds and his associates, the present study found evidence for differential conditioning at the thalamic level. Using a measure of evoked potential activity, it was apparent that early in the conditioning sequence (25 through 50 trials) there was differential responsiveness to the presentation of the CS+ and CS-. Thus, according to the evoked potential measure, there is conditioning with stimulus specificity at the thalamic level.

Conditioning during afferent block

This study demonstrates that merely operating the bilaterally implanted cryoprobes without functional blockade did not interfere with conditioning. In the control group, the cryoprobes were operated with the full circulation of room temperature ethanol through the apparatus, yet conditioning changes were apparent during this procedure. Thus, the functional blockade caused by cooling the neural tissue was the critical factor in impairing cortical conditioning.

It had been hypothesized that during blockade of the conditioned stimulus afferent pathway there would be a cessation of conditioning changes. It was reasoned that the conditioning measure of CS elicited activity was dependent upon successful transmission of the CS occurrence along sensory pathways. However, it was inferred that in the present experiment sensory input was only partially limited. For example, it was demonstrated in a previous study that the sensory pathway through

the ventral posterior lateral nucleus of the thalamus was unimpaired during a blockade of the centromedian nucleus. Similarly, it appears from this study that the shortest latency responses of postcruciate neuronal activity are unimpaired during ventral anterior nucleus blockade. Even though substantial afferent pathways to these cortical neurons remain, no significant differential conditioning changes took place during the 75 trial thalamic blockade. This lack of differential conditioning is most evident for the intermediate latency response intervals of 121 msec through 227 msec (Figure 16).

In general, the data support the idea that learning changes are taking place in the cortical neurons. When the cortical neuron is deprived of CS afferent input via the VA nucleus, no learned change in response develops. However, when there is minimal block of transmission through the VA nucleus and afferent activity reaches the cortical neuron, then a learned change in response does develop. This basic finding suggests that the underlying learning process occurs in cortical neurons and the changes in response of cortical neurons do not simply reflect projection of activity from learning changes elsewhere in the brain.

Neuronal plasticity subsequent to thalamic blockade

It had been hypothesized that an analysis of the conditioned response after termination the thalamic blockade would enable a localization of the active site of change. If the conditioned response showed an immediate rebound, the active site of change would be at the thalamic level or at least peripheral to the blockade of the ventral anterior nucleus. For example, if the conditioning changes of the

centromedian nucleus were projected to the cortical level, then the thalamic blockade would only interrupt the projected conditioned response and immediately after the blockade the projected change would be present. This hypothesis was not upheld in the experiment; rather it was apparent that the thalamic blockade had lasting effects on the conditioning changes.

The finding of prolonged impairment in conditioning subsequent to the cooling blockade supports the hypothesis that the neuronal conditioning changes are occurring at the cortical level. If the active conditioning change is dependent upon afferent activity elicited by the CS and US, then the disruption of the afferent activity effectively reaching the postcruciate neurons would interfere with the ongoing conditioning process. The finding that conditioning is impaired during the 75 trials following the thalamic blockade supports the idea of a necessary patterning of afferent activity for a repeated number of trials for conditioning to develop.

The finding that the active conditioning process is dependent upon intact afferent pathways is supported by the linear regression analysis. This analysis showed a significant relationship between the magnitude of the blockade of the afferent activity and the disruption of conditioning changes subsequent to the blockade. Thus, the degree of conditioned response change in cortical neurons is inversely proportional to the disruption of the CS elicited activity reaching the cortical neuron.

Previous studies support the idea that neuronal activity elicited by the CS is the sine qua non of neuronal conditioning. O'Brien and

Fox (43) found that neurons which showed the most prominent conditioning changes were those that initially exhibited the largest response to the CS. In a functional study of various neuronal characteristics which might be predictive of conditioning changes, O'Brien and Fox (43) found that the magnitude of the response to the CS was significantly related to the occurrence of conditioning change. Of nine cell variables examined, the initial response to the CS was one of the two variables significantly related to conditioning changes.

Bures, Buresova, and Gerbrandt (21, 22) showed that establishing a contingency between the CS and electrical stimulation through the recording electrode is sufficient for neuronal plasticity. In these experiments, they used such small currents (15-30 nA) that the excitation was expected to be localized to the site of application. The experimenters concluded from their findings "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".(13).

Although Bures, Buresova, and Gerbrandt (21, 22) have demonstrated that pairing CS and electrical stimulation through the recording electrode is sufficient for neuronal changes, the changes that occurred cannot be unequivocally ascribed to conditioning since the necessary sensitization controls were lacking. However, Wilder (70) was able to demonstrate bona fide conditioning changes resulting from orthodromic and antidromic stimulation of postcruciate neurons. In this experiment, mild somatic stimulation (CS) was paired with pyramidal tract stimulation (US) and was shown to yield differential conditioning. Since

the CS- response was not altered during conditioning it was clear that the neuronal changes were not due to sensitization.

A recent study from Woody and his associates (9) has similarly shown that simple pairing of neuronal activity is a sufficient condition for the development of a conditional eyeblink. In this case the click CS was followed at a suitable interval by stimulation of facial motoneurons. The conditioned eyeblink which resulted from this orthodromic-antidromic neural activation pairing was essentially that of classical conditioning. The blink conditioned response showed extinction using a reverse US-CS pairing, learned savings, and discriminative learning.

These experiments suggest that establishing a pairing of CS elicited activity followed by US elicited activity is a sufficient condition for neuronal conditioning to occur. Various experimenters have simplified the US by using intracerebral electrical stimulation and have found evidence for the occurrence of conditioning (24). In the present experiment the CS elicited activity was interfered with through use of a cryogenic blockade and the conditioning process was impaired, yielding a deficiency which outlasted the cryogenic blockade. Both lines of investigation, however, suggest a model for conditioning based upon simple contiguity of neuronal patterns of activation. Furthermore, it appears that the critical feature of conditioning can be resolved at the single cell level and that large neuronal populations are not necessary for the study of neuronal conditioning.

SUMMARY AND CONCLUSIONS

This study was undertaken to analyze the thalamo-cortical system during classical conditioning. For these purposes electrophysiological data were accumulated from both the thalamic and cortical levels during an habituation, conditioning, and extinction sequence. The particular classical conditioning paradigm utilized was that of differential somatic conditioning in which a CS+ forepaw shock was explicitly followed by hindpaw shock and a CS- shock to the other forepaw was not paired with hindpaw shock. The hindpaw shock served as the unconditioned stimulus (US) in this experiment.

The electrophysiological data included single neuron activity recorded extracellularly in the postcruciate cortex and thalamic evoked potentials recorded from the centromedian nucleus region. Both the evoked potential and single neuron data were analyzed with regard to differential changes during the habituation, conditioning, and extinction sequence. A procedural control group was used to determine the neural changes concomitant with this particular conditioning paradigm.

An experimental group, utilizing a reversible cryogenic lesion in the anterior thalamus, was contrasted with the procedural control group. The two groups only differed for the single aspect of the cooling of the tissue in the vicinity of the ventral anterior nucleus of the thalamus. In all cases cryoprobes were bilaterally implanted into each animal, controlling for surgical procedures. The ventral anterior nucleus of the thalamus was chosen as the blockade site in order to disrupt the final thalamic nucleus of the nonspecific thalamo-cortical

pathway. This reversible blockade was used as a technique to examine the thalamic and cortical changes during conditioning. The functional blockade allowed an analysis of the role of the ventral anterior nucleus in the sensory pathway of the conditioned stimulus, in the cortical conditioning changes during the blockade, and in the cortical conditioning changes subsequent to the blockade. For these purposes the cryogenic blockade was activated during the middle third of the conditioning sequence, with conditioning trials preceding, during, and after the blockade. By disrupting the integrity of the nonspecific thalamo-cortical system, it was possible to examine whether neuronal conditioning changes of the cortex were dependent upon conditioning changes taking place in the thalamus.

The results have shown that electrophysiological changes are concomitant with the presentation of this particular differential classical conditioning sequence. The electrophysiological data evidenced changes at both the thalamic and cortical levels. Averaged evoked potentials recorded from the centromedian nucleus region of the right hemisphere showed changes in the peak-to-peak amplitude which were differential according to stimulus. There was an overall decrease in peak-to-peak magnitude of the thalamic evoked potentials during the habituation, conditioning, and extinction sequence. In contrast, an increase in the peak-to-peak measure was evidenced for the CS+ trials early in the conditioning sequence, trials 25 through 50. During these trials the CS+ elicited a significantly larger magnitude peak-to-peak waveform than the CS- presentations.

Neuronal activity was simultaneously recorded from neurons of the postcruciate cortex. Although there was considerable variation among the individual neurons sampled, it was evident that the CS elicited neuronal activity underwent modification during the presentation of the conditioning paradigm. The overall tendency was that of reversal of spike activity level, such that a response component which was initially below the prestimulus rate, inhibitory, increased spike activity as conditioning trials were presented. Some poststimulus histograms revealed a reversal in which an initially inhibitory response component became excitatory, and conversely, excitatory response components became inhibitory during the conditioning sequence. In all cases, the neuronal activity was contrasted between the CS+ and CS- presentations, controlling for generalized changes which might be caused by the numerous factors affecting neuronal activity. Conditioning changes per se were identified by showing changes in response to the CS+ presentations which differed from the CS- presentations. This was evidenced by a significant F score in the analysis of variance of the postcruciate neuronal data.

Functional blockade in the area of the ventral anterior nucleus of the thalamas yielded a substantial decrease in sensory evoked activity of the postcruciate neurons. These changes ranged from the extremes of a 98% reduction through a 5% reduction. The reversible cooling blockade did not simply reduce the excitatory drive since both excitatory and inhibitory response components were modified toward the baseline level during thalamic cooling.

The sensory effects of the functional blockade upon the postcruciate neurons was dependent upon cryoprobe placement. In cases in which ventromedial caudate nucleus was cooled, an increase in the sensory evoked activity recorded in the postcruciate cortex was observed. Cryoprobe placement was thus analyzed as an experimental variable.

As had been previous observed, the effect of the functional blockade was dependent upon the poststimulus response interval analyzed. It was determined through use of a focal evoked potential measure that functional blockade of ventral anterior nucleus site resulted in a cortical sensory deficit predominantly during 114 msec through 212 msec poststimulus.

Since the thalamic cooling effect was interval dependent, the analysis of the conditioning changes was undertaken with respect to poststimulus response intervals. The results showed that imposing a thalamic blockade during the conditioning sequence impaired the cortical conditioning changes. Cortical conditioning was impaired both during the thalamic blockade and after the blockade. Those response intervals which were more affected by the thalamic blockade showed a prolonged impairment of conditioning changes, while those less affected response intervals showed conditioning changes subsequent to terminating the thalamic blockade. A linear regression analysis of these results showed that those response intervals which were most affected by thalamic cooling showed impairment of conditioning changes. Conversely, those neuronal response intervals which were least affected by thalamic blockade showed the greatest conditioning changes.

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APPENDIX A: CS Presentations

I. Sequence of CS+ and CS- presentations

1.	+	26.	-
2.	+	27.	-
3.	-	28.	-
4.	-	29.	+
5.	+	30.	+
6.	+	31.	-
7.	+	32.	+
8.	-	33.	+
9.	+	34.	+
10.	-	35.	+
11.	+	36.	-
12.	+	37.	-
13.	-	38.	+
14.	-	39.	+
15.	-	40.	-
16.	+	41.	+
17.	+	42.	-
18.	-	43.	-
19.	-	44.	+
20.	+	45.	-
21.	+	46.	-
22.	+	47.	-
23.	+	48.	-
24.	-	49.	-
25.	+	50.	-

II. Intertrial Intervals (in seconds)

1.	17.5	26.	13.5
2.	17.5	27.	15.5
3.	16.5	28.	12.5
4.	11.5	29.	13.5
5.	17.5	30.	11.5
6.	15.5	31.	17.5
7.	15.5	32.	12.5
8.	16.5	33.	12.5
9.	15.5	34.	16.5
10.	13.5	35.	11.5
11.	12.5	36.	13.5
12.	16.5	37.	11.5
13.	12.5	38.	14.5
14.	16.5	39.	18.5
15.	17.5	40.	15.5
16.	11.5	41.	13.5
17.	13.5	42.	14.5
18.	12.5	43.	15.5
19.	16.5	44.	11.5
20.	15.5	45.	18.5
21.	13.5	46.	15.5
22.	16.5	47.	11.5
23.	17.5	48.	12.5
24.	17.5	49.	12.5
25.	11.5	50.	11.5

APPENDIX B: Analysis of variance of conditioning (CS+ values)

TWO-WAY ANALYSIS OF VARIANCE (Repeated measures on A and B

Key number of levels (A) and key go. 2
Key number of levels (B) and key go. 9
Key number of subjs/grp and key go. 33

Key values by rows keying go after each.

1	3.2	.3	2.5	2.6	2.7	2.7	-.7	4.7	1.7	2.1	-3.1
2	-2.3	.1	-1.5	-3.5	-.6	-1.2	3.7	3.6	3.2	.1	-2.8
3	-1.2	-.9	-.6	-1.7	6.1	3.5	.3	6.8	-1.1	1.2	-1.6
4	-5.3	-1.4	-3.0	-3.1	1.6	.0	-1.7	3.4	1.0	-.9	5.9
5	4.1	3.3	2.8	4.3	3.7	5.4	1.8	5.2	6.0	4.0	.6
6	5.4	5.6	4.2	4.1	3.2	2.3	3.2	6.4	6.4	4.5	-1.4
7	-11.0	-3.5	-4.9	-2.3	-2.4	-2.7	-.6	-.2	-.3	-3.1	2.7
8	-2.6	-1.8	.1	-.8	-2.0	-4.0	-4.1	-4.3	-3.0	-2.5	2.7
9	5.4	5.3	1.4	6.8	2.1	7.8	3.2	4.9	2.9	4.4	5.7
10	1.2	.4	3.5	-.6	.6	1.7	5.8	-.2	-1.9	1.1	-3.2
11	-1.4	-1.5	-3.4	-2.8	-3.2	-1.8	3.3	4.3	3.6	-.3	-1.4
12	1.2	10.7	11.0	10.0	14.4	8.7	8.2	12.3	12.4	9.8	-6.3
13	1.2	6.4	5.2	5.5	8.6	4.9	2.8	2.1	3.3	4.4	.1
14	-.4	-5.8	2.9	2.9	10.4	9.8	13.7	9.6	11.7	6.0	6.7
15	7.4	7.6	1.5	4.3	6.2	-.2	3.9	5.2	6.9	4.7	2.7
16	-2.5	-.3	-1.8	-2.3	-2.0	.1	-1.1	.8	-1.2	-1.1	-.1
17	-.6	.7	-.4	-2.1	-.2	-.9	-.1	.4	1.9	-.1	3.7
18	8.3	7.9	11.2	12.8	8.3	10.6	12.0	7.7	7.8	9.6	12.6
19	4.0	4.0	5.8	5.4	3.1	4.5	7.2	9.9	7.3	5.6	6.4
20	-.8	-2.6	.4	1.6	-2.3	-.6	.6	1.8	.7	-.1	-.7
21	-.3	.1	-1.9	-2.8	-.4	1.8	.1	.7	1.6	-.1	-1.7
22	-2.1	-2.8	-2.6	-2.8	-2.6	-1.9	-2.5	-1.0	-.9	-2.1	.7
23	.1	.9	.8	1.0	.4	.0	.7	.5	1.4	.6	.5
24	-1.3	-.5	-.1	.0	-1.2	-.3	-1.0	-1.6	-.7	-.7	-1.3
25	-1.9	-1.7	-1.0	-.6	-1.6	-1.3	2.4	3.1	3.1	.0	-3.4
26	-.5	-.5	-.5	-1.1	-.9	-1.0	.6	1.3	1.5	-.1	1.7
27	-.4	-.7	-.6	.5	-1.6	.8	.5	1.1	.7	.0	1.4
28	3.4	8.7	4.7	11.2	4.0	8.3	6.2	6.1	6.1	6.5	4.2
29	3.1	8.4	9.0	11.2	20.3	10.3	11.4	22.8	15.7	12.4	3.5
30	-2.2	-3.5	-3.7	-2.3	-2.8	-2.2	-1.7	-4.2	-5.8	-3.1	-2.6
31	6.4	7.1	6.7	6.5	6.4	7.0	5.3	6.3	6.7	6.4	6.1
32	.9	4.3	1.9	3.1	1.4	1.7	3.2	7.1	4.2	3.0	-.9
33	-.4	-.1	-.3	.0	-.2	-.2	-.7	-.3	-.8	-.3	.4

Analysis of variance of conditioning (CS- values)

-1.9	-3.1	-.7	-2.8	2.6	-1.5	-2.0	-.2	-1.4	.3
-5.0	-1.4	-3.8	-.2	-.4	-3.6	-.9	.7	-1.9	-.8
-2.9	-1.3	-5.7	6.3	-3.7	-.5	-4.5	-2.4	-1.8	-.2
8.1	5.2	5.4	2.3	.7	2.3	12.9	8.8	5.7	2.3
-.8	.0	-.5	-1.2	2.2	-.9	-2.9	-.5	-.4	1.8
-3.2	-2.4	-2.9	-3.6	-1.9	-3.6	-3.2	-3.8	-2.8	.8
-.7	-1.1	-1.2	2.3	-1.5	-2.3	.4	-.3	-.1	-1.6
1.9	3.1	.3	4.2	4.4	3.4	5.3	2.8	3.1	.3
6.9	5.9	8.1	5.3	7.4	4.0	5.2	6.6	6.1	5.2
-4.3	2.1	-1.5	-2.0	3.9	-3.7	-2.5	1.6	-1.0	.0
-1.1	-1.7	-2.4	-.9	-1.1	-3.7	-1.6	-2.5	-1.8	-1.0
-9.9	-10.9	-4.9	-7.3	-4.6	-2.2	-3.0	-3.1	-5.6	2.0
-2.8	-5.8	-2.0	3.7	.1	.0	-2.0	-2.4	-1.2	1.6
8.9	8.0	2.9	4.7	4.0	4.1	-2.5	-.5	3.9	5.0
-2.4	-1.1	-3.8	-8.3	-.4	-4.1	-1.3	2.9	-1.7	1.5
-1.3	-2.5	-.2	-.6	-1.0	-2.0	-1.1	-.9	-1.0	-1.1
.8	.5	-1.0	-.3	-1.1	-1.0	.9	-1.4	.1	-.0
10.0	11.0	9.9	11.7	10.4	11.5	12.8	9.3	11.0	10.3
6.3	1.2	5.7	4.6	1.9	.5	.2	-.3	2.9	4.3
2.1	.1	.9	2.9	1.6	.1	.4	-2.7	.5	.1
-4.2	-3.1	-3.1	-3.0	-2.3	-2.6	-2.2	-2.0	-2.6	-1.4
2.8	2.7	2.7	3.0	2.2	2.0	1.2	1.4	2.0	-.0
-.3	.9	.9	-.5	-.1	.4	1.3	-.8	.2	.4
-.9	-.1	-.3	-1.6	-1.2	-1.7	-1.4	-.6	-1.0	-.8
-.8	-2.6	-1.3	-2.3	-.8	-2.4	-2.9	-2.4	-2.1	-1.0
.0	1.2	.0	.7	-.4	1.4	-.9	.1	.4	.1
.4	2.2	1.9	-.1	1.5	.9	-.2	1.8	1.0	.5
6.2	5.1	6.1	5.1	4.9	3.1	6.5	7.3	5.3	5.9
5.3	4.7	3.1	5.9	6.4	6.1	4.0	1.0	4.4	8.4
-6.2	-4.5	-2.7	-3.3	-1.4	-2.2	-4.5	-6.9	-3.8	-3.4
6.6	7.4	6.4	6.8	6.3	4.2	5.1	6.1	6.1	6.3
-.9	-.9	-.7	.2	-.2	3.3	1.4	7.4	.9	2.0
.0	.0	.0	-.4	-.2	-.3	.0	-.2	-.0	-.2

	SS	DF	MS	F
WITH SUBJ	5571.888	561		
A	334.425	1	334.425	36.58
B	103.946	8	12.993	1.42
AB	161.282	8	20.160	2.20
ERROR	4972.234	544	9.140	

A factor = CS+ vs. CS-

B factor = conditioning trials in nine 25 trial blocks

APPENDIX C: Linear regression analysis

$$y = a(0) + a(1)x$$

X	Y	X	Y
8.7	-9.0	.1	1.3
3.5	-15.3	.4	4.9
1.2	-1.5	-4.6	39.5
7.0	2.0	5.7	.2
1.6	4.2	1.5	-9.5
.5	-1.1	-13.8	6.2
5.0	-40.2	-3.7	17.0
2.0	4.3	2.3	7.2
1.6	2.2	-.3	2.5
25.2	2.3	-12.3	28.2
2.0	20.1	7.3	7.3
2.9	-7.3	-1.3	-3.3
2.4	.7	.4	9.6
10.0	-1.1	1.6	-1.5
3.4	-5.9	-.8	-15.9
.7	16.8	2.9	-7.3
3.4	-6.4	.3	4.8
5.7	-24.6	-2.7	12.6
1.8	6.7	-2.7	1.4
5.1	.5	11.3	-2.9
2.3	-7.2	.7	-2.1
.6	2.1	-2.3	-15.5
5.0	-6.5	-1.6	12.1
12.9	-34.7	3.3	-6.6
1.6	6.6	3.8	-4.2
-1.0	8.7	2.7	.3

52 = no. of (X, Y) data points
113.300000000 = sum of X's
2.700000000 = sum of Y's
1878.870000000 = sum of X squares
8840.230000000 = sum of Y squares
-1596.830000000 = sum of X times Y
-.421955271 = sample correlation coefficient
2.191659843 = A(0)
-.982050413 = A(1)
11.820894926 = standard error of estimate

x = cooling block measure:
$(C_{123}) - (C_{456})$
in which C_n =
n block of 25 conditioning trials

y = conditioning change measure:
$(C_{789}) - (3C_3)$