RESPONSES OF PYRAMIDAL TRACT CELLS DURING A DIFFERENTIAL CLASSICAL CONDITIONING PARADIGM USING CENTRAL STIMULATION AS THE CONDITIONED AND UNCONDITIONED STIMULI

by

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The neuronal basis of associative conditioning in the cat was investigated by pairing stimulation of thalamocortical pathways as the conditioned stimulus with antidromic activation of pericruciate pyramidal tract cells as the unconditioned stimulus in a differential classical conditioning paradigm. Contrary to expectations, based both on the available literature and theoretical considerations (i.e., the pairing hypothesis), thalamic stimulation was not an effective conditioned stimulus. The response of pyramidal tract cells to thalamic stimulation did not change as a function of reinforcement with pyramidal tract stimulation. These results led to a reconsideration of the hypothesis that the simple pairing of any two neural events is the essential mechanism underlying associative conditioning changes. The results did suggest that the combined activation of specific and nonspecific thalamic nuclei may be important in producing increases in responsiveness of pyramidal tract neurons.

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TABLE OF CONTENTS

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LIST OF TABLES

LIST OF FIGURES

Page

INTRODUCTION

The search for the neurophysiological basis of learning has occupied researchers in the neurosciences for many years. As the techniques available have become more refined, approaches to the study of CNS plasticity*have become more sophisticated. The purpose of this section is to present an overall review of the work considered most relevant to this dissertation.

Neurophysiology of Learning

Olds, Disterhoft, Segal, Kornblith and Hirsh (1972) approached the problem of studying CNS plasticity with the view that learning involved changes in the flow of neural activity. That is, learning manifested itself in the CNS in the form of a change in the pathways that nerve impulses took through the brain. They felt that both before and after learning, signals might travel along common pathways until one or more key synapses were reached. At these points, modifications in the synapse, concomitant with the behavioral manifestation of learning, resulted in signals traveling along new pathways ultimately leading to different behavioral (learned) results.

These authors were very much concerned with the "local learning" issue. To determine the site of the synapse where the "switching" of activity occurred one would have to differentiate between activity changes at this site and activity changes at sites further down this new pathway. These downstream sites of change would merely reflect the actual modification that had occurred at the key synaptic locus. They reasoned that sites showing shorter latency changes in activity were

*The term "plasticity", as used in this dissertation, refers to the structural (anatomical) and functional (electrophysiological) changes which the CNS may undergo as the result of experience.

more likely to be the sites of synaptic modification than those sites showing longer latency changes.

Using chronic unit recording (about four sites per subject) in the rat, Olds et al. (1972) examined changes in unit activity before, during and after acquisition of a behavioral response. A tone of one frequency signalled activation of a food magazine, while a tone of a second frequency was without consequence. Changes in unit activity in 20 ms intervals up to 80 ms poststimulus were examined. They found evidence for short latency changes in unit activity in a variety of areas comparing pre- versus postconditioning histograms. They felt that, as a whole, the data indicated that local learning changes took place in a set of different areas (e.g., pontine reticular formation, ventral tegmentum, posterior nucleus of the thalamus). Using a similar approach, Segal and Olds (1972) and Segal (1973) presented data which indicated a functional ordering of changes in hippocampal activity during learning which seemed to parallel the known anatomical connections within the hippocampus.

Woody and coworkers have approached the problem of analyzing CNS plasticity in a slightly different manner. They selected a very specific type of learned behavioral response and concentrated their electrophysiological analysis on a specific part of the CNS. They chose to work with classical conditioning of the eye blink response. The basic paradigm involves a click CS and a glabella tap US. Most of the electrophysiological work from this laboratory has concentrated on analyzing changes in extra- and intracellular single unit activity in the pre-

cruciate cortex. This area contains motor cells innervating facial regions in the cat. As was Olds' group, Woody and coworkers were concerned with determining whether the differences in activity they found in conditioned versus control animals were generated locally in the motor cortex or were projected to this region from the "real" learning area.

Woody (1970) observed an increase in amplitude in the gross evoked potential recorded in the precruciate cortex of cats in the conditioned state, as opposed to the naive and extinguished states. He further noted that those areas with lower thresholds for eye-blink elicitation (he stimulated the precruciate cortex through the recording electrode) had higher amplitude click evoked potentials. Woody, Vassilevsky and Engel (1970) then analyzed conditioning changes in extracellularly recorded single units in the same area of cortex. They tested the units for their projection to the muscles controlling the eye blink response with low current stimulation. They reported that, in the conditioned animals, more cells projected to eye-blink muscles and these cells tended to produce EMG activity at a lower threshold than cells of a similar nature found in the naive and extinguished groups. In addition, in conditioned animals, those cells which were classified as projecting to eye-blink muscles showed greater click-elicited activity than cells in the same group which were not found to project to eye-blink muscles.

Engel and Woody (1972), based on an experiment comparing the use of two different auditory stimuli as CSs, theorized that the significance of sensory input (i.e., whether it was a CS) was represented in the

motor cortex both by an increase in unit response to the stimulus and by an increase in the total number of cells responding to that stimulus. Woody and Engel (1972) expanded on the work of Engel and Woody (1972) and Woody et al. (1970) by comparing two different types of conditioned responses: eye blink and nose twitch. In animals in which the nose twitch was the CR, more nose muscle projection areas than eye muscle projection areas were found, while in those animals in which eye blink was the CR, more eye than nose muscle projection areas were found. Similarly, in animals trained to give a nose-twitch CR, the threshold for stimulation of the nose-twitch muscles was lower than the threshold for stimulation of the eye-blink muscles. An analagous trend was observed for animals which were trained with an eye-blink CR. These data support Engel and Woody's (1972) hypothesis concerning the basis for the development of neuronal plasticity (i.e., an increase in both the response of a given cell to the stimulus and the total number of cells responding to it).

Woody and Black-Cleworth (1973) extended these analyses by recording intracellularly in cats which had undergone classical conditioning of the eye-blink response. By stimulating cells intracellularly and averaging EMG responses they were able to classify cells as projecting to eye, nose or other muscles. Following conditioning, they found a prevalence of cells projecting to eye muscles (all animals received eye blink conditioning). In addition, the threshold for producing an EMG response in eye muscles was lower than for nose muscles. These results confirm and extend the findings of Woody et al. (1970). Thus,

these studies of Woody and coworkers have resulted in an impressive body of evidence for local changes occurring in the motor cortex as the result of classical conditioning of motor activity.

Thompson and coworkers have taken a very systematic approach to analyzing the mechanisms of CNS plasticity. They have sought to develop a model learning system which has the appropriate behavioral and physiological characteristics to permit definitive research. Thompson, Berger, Cegavske, Patterson, Roemer, Teyler and Young (1976) outlined what they felt were the essential criteria for such a model system:

1. "The conditioned response (CR) should be acquired at least to a significant degree in a single training session but should require a substantial number of trials."
2. "The CS should not produce a CR ('alpha') response before training."
3. "Presentation of the CS or UCS should not yield sensitization or pseudoconditioning."
4. "The parametric features of the CR should be well characterized."
5. "The behavioral response should be robust and discrete, and the exact amplitude-time course of the response should be measurable."

Thompson et al. (1976) went on to state that the classically conditioned nictitating membrane (NM) response of the rabbit appeared to fit these criteria fairly well. Since then, a great deal of work in his laboratory has focused on this preparation.

Cegavske, Patterson and Thompson (1979) analyzed multiple unit activity in the abducens nucleus during conditioning of the NM response. The abducens nucleus is thought by some to be the final common pathway for neurons innervating muscles controlling the NM response. They found that the time pattern of increased unit activity in the abducens was virtually identical to the time course of the NM response. That is, "... the neuronal response is an essentially perfect predictor of the behavioral response."

Berger and Thompson (1978a) studied multiple unit activity in the hippocampus during classical conditioning of the rabbit NM response. Similar to the data for the abducens nucleus, the pattern of unit firing closely paralleled the amplitude-time course of the NM response. Berger and Thompson (1978b) studied unit responses in the lateral and medial septum using the same paradigm. While lateral septal units displayed a similar kind of activity during conditioning to that of hippocampal units, they felt that medial septal units were best interpreted as sensory in nature.

Berger and Thompson (1978c) analyzed in more detail which subunits of the hippocampus were particularly important in the demonstration of plasticity. They stated that the pyramidal cells (which they identified using antidromic activation), the output cells of the hippocampus, appeared to be the major contributors to the neuronal plasticity which developed during classical conditioning of the NM response. Berry and Thompson (1979) showed that lesions of the medial septum delayed learning of the conditioned NM response. Thompson and coworkers' approach

is similar to that used by Olds' group. They both are analyzing cell activity in multiple locations attempting to chart the flow of information in a learning situation. Woody's work is more geared toward discovering the membrane changes underlying the alteration of function at a single synaptic locus.

Cohen and associates have also developed a model system for studying the neurophysiology of learning. Cohen (1969) outlined what he felt were the two most essential criteria for a model preparation that is to be used for this purpose:

1. "It must have a quantifiable behavioral response, autonomic or somatic, whose probability of occurrence can be modified with the appropriate training conditions."
2. "Equally important, the neuroanatomical pathways necessary for normal development of the learned response must be specified in detail."

Cohen (1969) felt that classically conditioned cardioacceleration (light CS, foot shock US) in the pigeon was such a model preparation. His approach has been to analyze the neuroanatomy of the system as completely as possible. This has included determining the conditioned and unconditioned stimulus pathways and the efferent output pathway. One technique which Cohen (1969) felt had merit in outlining the basic cell systems involved in cardiac conditioning was analyzing the effects of brain lesions on the development of the conditioned response. His rationale was that if the lesion altered the heart rate response relative to the control in any way then the lesioned area was tentatively

considered important.

Cohen's group has been working toward this end. Leonard and Cohen (1975) studied the peripheral unconditioned stimulus pathway using both lesion and stimulation techniques. Cohen (1975), Cohen and MacDonald (1976) and Cohen and Goff (1978) have pursued the analysis of central lesion effects on heart rate conditioning. The picture which they have presented points to the importance of the archistriatal projection to the medial hypothalamus and further relay through the ventral brainstem. More recently, Cohen's group has begun to analyze the electrophysiological changes taking place along these pathways (Wall, Wild, Broyles, Gibbs & Cohen, 1980; Gibbs & Cohen, 1980). Cohen's work has highlighted the importance of understanding the neuroanatomical pathways mediating the response system under study.

O'Brien and coworkers have been systematically analyzing characteristics of changes in single cell responses in the postcruciate cortex of the cat during a classical conditioning paradigm (O'Brien & Fox, 1969 a&b). Unlike some of the other groups which always monitor the behavioral response, O'Brien's work has concentrated primarily on the analysis of single unit activity changes. O'Brien and Packham (1973) did demonstrate that leg flexion conditioning resulted using the experimental parameters of O'Brien and Fox (1969a). O'Brien's group has also carefully investigated various aspects of the electrophysiological changes which take place during the conditioning session. O'Brien and Packham (1974) found that cell activity could exhibit both increases and decreases in activity at different time intervals after a CS. O'Brien and Rosenblum (1974)

and O'Brien and Rosenblum (1975) presented evidence that a particular poststimulus period of activity in the postcruciate cortex was due to a projection from the nonspecific thalamic system.

O'Brien's group has also been concerned with determining whether the learning changes monitored at the postcruciate cortex represent local or projected changes. They have taken a variety of approaches to answering this question. Rosenblum and O'Brien (1977) studied changes in cell activity before, during and after a reversible cryogenic blockade of activity in the ventral anterior (VA) nucleus of the thalamus. They reasoned that if the learning changes observed were occurring locally in the cortex, then blockade of the thalamus should disrupt the appearance of the learning changes. However, if the learning changes were occurring at some other level and then were projected via the thalamus to the cortex then, upon termination of the blockade, one should see the projected learning changes in the cortex immediately. Rosenblum and O'Brien (1977) found that learning was disrupted by thalamic blockade, providing evidence in favor of a local learning hypothesis.

Another approach was that of O'Brien, Wilder and Stevens (1977). They used antidromic activation of pyramidal tract (PT) cells as the US and peripheral shock stimulation as the CS in a classical conditioning paradigm. They reasoned that if activation, per se, of the neuron was the critical feature of the US, rather than activation via presynaptic terminals, then antidromic firing of the neuron should be a satisfactory US. They felt that if conditioning occurred with this para-

digm, it would provide evidence toward two points: 1. That local learning had occurred in the cortex, 2. That the learning change was mediated by a postsynaptic as opposed to a presynaptic modification. O'Brien et al. (1977) did find evidence of conditioning using this paradigm.

Other groups have also used direct central activation as the CS or US in an attempt to get more information about the learning changes which occur in the CNS. Black-Cleworth, Woody and Niemann (1975) demonstrated that classically conditioned eye blink in the cat resulted using a click CS and direct electrical stimulation of a motor branch of the facial nerve as the US. Woody and Yarowsky (1971) demonstrated conditioned eye blink in the cat using glabella tap US and direct electrical stimulation of the coronal-precruciate cortex as the CS. Using an avoidance paradigm, Doty, Rutledge and Larsen (1956) were able to condition cats to avoid forelimb shock by making a leg flexion, with direct electrical stimulation of the cortex as the CS.

Baranyi and Feher (1978) studied neuronal plasticity in the motor cortex of the cat using antidromic activation of PT cells and direct stimulation of cells in the ventral lateral (VL) nucleus of the thalamus. They recorded intracellularly from motor cortex cells. Although they did not use an associative conditioning paradigm, their results are still of interest in analyzing the nature of CNS plasticity. Their "conditioning" series consisted of either paired VL and PT stimulation or just trains of PT stimulation. As a result of this stimulation, they reported a facilitation in firing of cells and a reduction in latency of

firing in response to stimulation of VL. They also applied trains of direct stimulation to the motor cortex neurons recorded from and noted enhancement of VL EPSPs following this stimulation. All these effects were short lasting, typically ending in less than 60 s. Although they did not exclude the possibility of presynaptic changes participating in the facilitation of activity, they seemed to feel that the evidence pointed toward a postsynaptic model.

Baranyi and Feher (1981) provided further evidence in support of their hypothesis. Using a stimulus presentation sequence closer to a true associative conditioning paradigm, they found changes in neuronal excitability in 27% of the cells tested. Similarly, Bindman, Lippold and Milne (1979) demonstrated long term changes in excitability of PT neurons in the cat following antidromic activation which they felt were based on a postsynaptic mechanism.

The studies reviewed above provide a general outline of the kinds of approaches that have been used in assessing CNS plasticity. The following section provides an overview of the anatomical connectivity and electrophysiological characteristics of the neural system which was the subject of the present study.

Anatomy and Physiology of Pericruciate Cortex

The structural and functional characteristics of the pericruciate cortex of the cat have been a topic of interest for many years. Architectonically, this region includes fields 4γ, 3a, 3b, 1 and 2 (proceeding caudally) (Hassler and Muhs-Clement, 1964). Buser and Imbert (1961) studied the sensory responsiveness of cells in these regions and

found an ordered progression of cells ranging from polysensory near the lip of the cruciate sulcus, to somatic-nonsomatotopic to somatotopic units as they proceeded caudally. They concluded that the region of cortex anterior to the somatotopically organized S1 possessed several unique characteristics (e.g. presence of polysensory neurons, motor representation) which indicated that it should be considered functionally as representing a site for sensorimotor integration.

The thalamocortical innervation of this region has been the subject of intense interest. There have been two basic approaches to this question: anatomical tracing (histological) and electrophysiological mapping. Historically, thalamocortical afferents to the cerebral cortex have been subdivided into two major types, due to activation of the so-called "specific" and "nonspecific" thalamic systems. The work of Morison and Dempsey in the early 1940s was instrumental in establishing this distinction. Morison and Dempsey (1942) described, in the cat, two different patterns of evoked potential activity which were elicited by repetitive stimulation of either primary thalamic nuclei (e.g., VPL or VL) or nuclei in the region of the internal medullary lamina (e.g., centromedian [CM]).

Morison and Dempsey (1943) and Dempsey and Morison (1943) went on to describe in detail, the nature of the so-called augmenting response (due to stimulation of primary thalamic nuclei) and the recruiting response (due to stimulation of intralaminar thalamic nuclei). It was in large part based on this distinction in the type of evoked activity obtained to stimulation of different thalamic nuclei that a functional

distinction was postulated between specific and nonspecific thalamic systems. One of the major cortical foci for these responses was the pericruciate region of the cat. Since the early 1940s, quite a large body of literature has been devoted to investigating in greater detail the specific anatomical and electrophysiological basis for these two thalamocortical systems.

Thus, Hanbery and Jasper (1953) demonstrated that recruiting responses persisted following lesions of specific thalamic nuclei, supporting the concept that specific and nonspecific thalamocortical systems were independent of each other. Purpura and Housepian (1961) described the relationship between pyramidal tract (PT) cell activity and thalamocortical recruiting responses. They found that recruiting responses of both long and short latency were associated with similar latency discharges in PT cells.

Purpura and Shofer (1964) described patterns of IPSPs and EPSPs in neurons of the pericruciate cortex during elicitation of augmenting and recruiting responses. Schlag and Balvin (1964) described the pattern of events in PT neurons following stimulation of specific (VL) and nonspecific (e.g., CM) thalamic nuclei. They found an initial short latency excitatory response followed by a period of inhibition which was succeeded by a period of "rebound excitation". With delivery of a train of impulses, responses correlated with recruiting or augmenting responses could be observed in the PT.

Schlag and Villablanca (1967) challenged the strict division of responses into the augmenting versus recruiting type associated with

stimulation of specific versus nonspecific thalamic nuclei. They suggested that augmenting and recruiting responses (defined according to Morison and Dempsey's criteria) were capable of being elicited in different cortical loci by stimulation of the same thalamic nucleus.

Sasaki, Staunton and Dieckmann (1970) suggested a redefinition of augmenting and recruiting responses based on laminar field potential analysis of evoked activity in the cerebral cortex. Their study indicated two distinct types of thalamocortical responses: deep and superficial, due to activity in two distinct thalamocortical projection systems. They defined augmenting responses as a succession of deep and superficial thalamocortical responses while recruiting responses were a pure form of the superficial thalamocortical response. Their studies on the patterns of activation following stimulation of certain thalamic nuclei supported the observations of Schlag and Villablanca (1967).

Sasaki, Kawaguchi, Matsuda and Mizuno (1972) and Sasaki, Matsuda, Kawaguchi and Mizuno (1972) studied further the thalamic origin for the superficial and deep thalamocortical responses and found, for the nuclei they studied, that the superficial response was mediated by neurons in the VA nucleus while the deep response was mediated by VL neurons. Continuing this line of investigation, Sasaki, Matsuda, Oka and Mizuno (1975) concluded that stimulation of various intralaminar nuclei resulted in discharges of neurons in the VA nucleus associated with superficial thalamocortical responses.

Velasco, Weinberger and Lindsley (1968) and Velasco and Lindsley (1968 a&b) performed a series of experiments demonstrating the direct

inhibitory influence of the reticular formation on recruiting responses. Similarly, Sasaki, Shimono, Oka, Yamamoto and Matsuda (1976) found that reticular formation stimulation resulted in hyperpolarization of VA neurons associated with the superficial thalamocortical responses.

In summary, Morison and Dempsey's distinction between two types of thalamocortical projection systems appears to remain valid. However, in light of more recent evidence (e.g., Sasaki and coworkers) a distinction based on augmenting versus recruiting responses appears to have less value than one based on the type of thalamocortical activation (i.e., deep or superficial) which results from stimulation of a particular thalamic locus.

Anatomical correlates for the distinction between specific and nonspecific thalamic systems have been more difficult to obtain. While traditional degeneration methods and more recent histochemical techniques have confirmed the projection loci of specific thalamic systems (e.g., Macchi, Angeleri & Guazzi, 1959; Hand & Morrison, 1970; Jones & Leavitt, 1973), it has been more difficult to establish the projections of the nonspecific nuclei. Only with the advent of modern anterograde and retrograde tracing techniques have the projections of the nonspecific nuclei become more clear. Thus, using retrograde transport of HRP, Jones and Leavitt (1974) examined the intralaminar nuclei of the thalamus following injections of the caudate or cerebral cortex. They found that the intralaminar nuclei project densely to the caudate while the projection to the cortex is sparse and spread over many different regions. Similarly, Ermolaeva, Brukhanskaya, Kratin and Tolchenova (1980)

using retrograde transport of HRP found stained cells in VA and a diffuse distribution of stained cells in the intralaminar nuclei following injection of HRP into the primary somatosensory cortex.

Recently, Herkenham (1980) based on anterograde transport of radiolabelled amino acids has suggested a four part classification of thalamocortical projections. He found that specific thalamic nuclei projected primarily to layers III and IV while intralaminar nuclei projected principally to layers V and VI. The third and fourth categories he proposed had major terminations in layer I. The nuclei in these latter categories included the ventroanterolateral, lateral dorsal, ventromedial and reuniens nuclei. There was a clear separation in cortical layers labelled between these nuclei and those of category 2 (intralaminar nuclei) even though many of the nuclei of categories 3 and 4 lay just lateral to the intralaminar nuclei.

Thus, the data of Herkenham (1980) on the projection of intralaminar nuclei conflict with the electrophysiological data of Sasaki and coworkers (based on the cat). The proximity of these two categories of nuclei to each other may explain the discrepancy between Herkenham's and Sasaki's conclusions.

Thus, there is strong electrophysiological data and somewhat less substantial anatomical data for projections from intralaminar nuclei (including e.g., CM) reaching the pericruciate cortex. The nature of projections from primary thalamic nuclei to this region of cortex are known in much greater detail.

For example, Jones and Powell (1969) lesioned regions of the ventro-

posterior thalamus (= ventrobasal complex [Vb] + ventromedial nucleus) in the cat and observed the pattern of fiber degeneration which occurred in the cortex. They provided confirming evidence for the somatotopic projection of the Vb complex upon area S1. Jones and Leavitt (1973) and Ralston and Sharp (1973), using retrograde transport of HRP demonstrated somatotopic projections from VPL to the postcruciate cortex (Vb = VPL + VPM).

Strick (1973) studied the functional and structural projections of the VL nucleus in the cat. He first stimulated a region of VL and noted the type of motor activity which resulted. He then placed a small electrolytic lesion at this site and analyzed the cortex for signs of fiber degeneration. On a histological basis, he was able to divide VL into a dorsal, relatively nonmyelinated zone and a ventral, more densely myelinated zone. In addition, he noted a thin transitional zone between the ventral VL and the Vb complex.

Strick (1973) found that stimulation of the ventral zone of VL produced contractions of contralateral distal somatic musculature. Lesions placed at these sites resulted in degeneration in the area of the motor cortex containing the topographic representation of these muscles. Similarly, stimulation of the dorsal zone of VL produced contractions of contralateral proximal musculature. Again, lesions placed in these areas produced cortical degeneration confined to those areas representing these muscles (primarily area 6).

In general, Strick (1973) found that the cortical projection of the ventral VL was more discretely organized than that of the dorsal VL. In

comparing degeneration patterns following VL and Vb lesions he did not find evidence of overlap between the two systems. In lesions damaging primarily the transition zone between VL and Vb, Strick (1973) noted that cortical degeneration appeared confined to area 3a on the post-cruciate gyrus. He stated that this region has been considered to be an input area for group Ia afferents. Strick and Sterling (1974) found that the pattern of cortical degeneration which developed following lesions in VL of the cat were primarily confined to layer III with some additional degeneration in layers I and VI.

Cesari, Michelini, Spidalieri and Vedovato (1979) studied single unit responses in cat motor cortex in response to stimulation of dorsal and ventral sections of VL. In contrast to the anatomical data presented above, this group reported that dorsal VL stimulation excited cells in area 4 as well as area 6 and ventral VL stimulation excited cells in area 6 as well as area 4. Asanuma, Larsen and Yumiya (1979) and Larsen and Yumiya (1979) studied the cortical projection of the VL-VPL border area following HRP injections in cortical area 3a. Asanuma et al. (1979) found cells in this border region which could be antidromically activated by discrete microstimulation of the motor cortex.

Araki and coworkers have conducted an extensive series of studies detailing the types of postsynaptic potentials in PT cells of the peri-cruciate cortex of cats resulting from stimulation of various thalamic nuclei. Araki and Endo (1976) examined EPSPs in PT cells following stimulation of CM, VA and VL thalamic nuclei. They presented evidence

which indicated that some short latency EPSPs resulting from CM and VA stimulation could be of monosynaptic origin.

Araki, Endo, Shigenaga, Kawai and Ito (1976) further studied the types of short and longer latency EPSPs in PT cells resulting from stimulation of the same three thalamic nuclei as mentioned in the preceding paragraph. They found a pattern of three EPSPs ranging in latency from 5 to 30 ms for each of the three thalamic nuclei stimulated. Endo, Araki and Ito (1977) presented evidence for a monosynaptic projection from the central lateral (CL) nucleus of the thalamus to PT cells in the pericruciate cortex. They suggested that this pathway, along with direct pathways from VA, VL and CM functioned in producing incrementory postsynaptic potentials in PT cells.

Deschenes, Labelle and Landry (1979) studied EPSPs in PT cells produced by stimulation of VL and recurrent collaterals of PT cells. Based on an analysis of the difference in rise time and peak latencies of the EPSPs for the different sites of stimulation, they proposed that the VL terminals ended on apical dendrites while recurrent collaterals synapsed on basal dendrites of the PT cells.

The effects of antidromic activation of, and recurrent collateral input to PT cells have been studied for many years. Phillips (1959) studied the effects of antidromic activation of the PT on Betz cells in the cat. He was particularly interested in the role of the recurrent collaterals which synapsed back onto cells in the motor cortex. In order to study these effects in isolation from the actual antidromic spike invasion of the Betz cell, he stimulated the PT at levels just

above and below that necessary for production of an antidromic spike. When stimulating just below threshold for antidromic activation he typically saw an initial phase of depolarization, followed by a phase of increased membrane potential. In a few experiments, only the initial phase of depolarization appeared in the record. The same patterns of polarization were also generally detectable when the PT was stimulated above threshold levels for antidromic activation.

Stefanis and Jasper (1964 a&b) presented definitive results indicating the presence of recurrent collateral inhibition in PT neurons. They described both IPSPs and EPSPs occurring in response to antidromic activation of the PT. The recurrent inhibition resulting from antidromic activation of PT cells was strong enough in some cases to block the arrival at the soma of a second antidromic spike within a specified time after the arrival of the first spike. This blocking effect on a "test" antidromic spike increased with an increase in the strength and number of the previously delivered "conditioning" antidromic spikes. Based on these and other data, Stefanis and Jasper (1964b) postulated that this pattern of recurrent collateral inhibition could provide a type of lateral or "surround" inhibition which has been observed in the function of sensory systems (e.g., the visual system of Limulus). This lateral inhibition mechanism would provide a means for increasing the contrast between a central group of active PT cells and surrounding, less active cells which would thus become inhibited. They saw a possible relationship between this mechanism and a role for the motor cortex in the control of precise voluntary movements.

Brooks and Asanuma (1965) confirmed many of Stefanis and Jasper's (1964 a&b) findings and concluded similarly that the pattern of inhibition resembled the phenomenon of afferent surround inhibition. Schlag (1966) studied the PT response to stimulation of both ipsilateral pericruciate cortex and the VL nucleus. He described a pattern of activity following stimulation of either of these areas which included early excitation, a middle latency inhibition which lasted up to 100 to 200 ms poststimulus which was followed by a period of rebound excitation. His observations meshed well with those of Stefanis and Jasper (1964 a&b) and Brooks and Asanuma (1965) as well as Schlag and Balvin (1964).

Schlag (1966) presented a model for PT cell function which included the concept of lateral inhibition, first postulated by Stefanis and Jasper (1964b) as well as lending insight into how augmenting and/or recruiting responses could arise as a result of the pattern of activity generated in PT cells. One general concept which these studies on PT cell activity repeatedly confirm is that the pattern of activation of PT cells, by whatever mechanism initiated, is typically an early (<10 ms) excitation, middle period of inhibition (up to 100 to 200 ms) followed by a period of post-inhibitory rebound. These observations made at the level of evoked potential analysis (Schlag, 1966) seem to have a cellular basis in the pattern of IPSPs and EPSPs generated in PT cells.

More recently, Renaud, Kelley and Provini (1974) have shown that recurrent IPSPs of PT cells can be potentiated by cortical surface stimulation. Takahashi, Kubota and Uno (1967) also studied activity in recurrent collaterals of cat PT cells. Given that PT cells can be divided

into fast and slow conducting types, they presented evidence indicating that facilitation via recurrent collaterals occurred only in the fast conducting type PT cell while the facilitation itself appeared to be mediated by axons of the slow conducting type. Thus, the postsynaptic facilitation was definitely not mediated by axon collaterals of the PT cell being recorded from. Tan, Marangoz and Senyuva (1979) presented evidence that PT cells could be divided into three populations, as opposed to two, based on axonal conduction velocities.

Landry, Labelle and Deschenes (1980), using intracellular injection of HRP into electrophysiologically identified PT cells, described the pattern of branching of PT recurrent collaterals within the motor cortex. They observed that the collaterals ended primarily in layers V and VI of cortex and consisted of a field localized to the vicinity of the cell body and a second set of longer branches extending for a distance of a few millimeters beyond the vicinity of the cell body.

Blum (1971) and Endo, Araki and Yagi (1973) both presented electrophysiological evidence that some axon collaterals of PT cells end in subcortical structures. In particular, Endo et al. (1973) demonstrated axon collaterals in specific and nonspecific thalamic nuclei and also structures associated with the extrapyramidal system, suggesting a much more complex picture of the role of the PT system in the control of behavior.

Statement of Problem

In review, much histological and electrophysiological data has been accumulated detailing the nature of the thalamic afferents to PT cells,

their efferent pathways and the patterns of activity which result in PT cells when these pathways are activated. In addition, there is much electrophysiological data indicating that both the spike pattern and membrane properties of neurons show changes as the result of experience. However, the characterization of these changes is far from complete. Especially with regards to plasticity at the cortical level, questions concerning which types of neurons show modification in activity, and which afferent pathways are important for demonstrating these modifications, are largely unanswered.

As has been previously mentioned, a major interpretational issue in studying changes in neuronal responsiveness is determining when one is dealing with change taking place at the level of the membrane of the neuron being recorded from, as opposed to changes which take place at a different neuron and are then projected to the neuron being studied. The laboratories of O'Brien, Olds and Woody have offered several different approaches to solving this problem.

Black-Cleworth et al. (1975) and O'Brien et al. (1977) lend support for a model of neuronal classical conditioning in which the adequate US is a generalized activation of the postsynaptic cell membrane. The fact that O'Brien et al. (1977) were able to demonstrate differential classical conditioning using the antidromic activation of the neuron as the US indicates that different CS afferent inputs to the neuron are distinguishable. The question then becomes that of determining the nature of the differentiation process.

A first step in this direction is to determine more precisely the

afferent pathways involved in the development of neuronal plasticity. The major source of input to the PT cells in the O'Brien et al. (1977) study probably was via the thalamus. As reviewed above, one can divide this input into two types: from specific and nonspecific thalamic systems. The question then becomes: Is one or the other thalamic system particularly important in mediating the plasticity observed in the O'Brien et al. (1977) study?

In dealing with a neocortical conditioning model, activation of discrete thalamic nuclei appears to be a logical choice for use as the CS. Thalamic activation of PT cells produces a complex but stereotyped response pattern (see studies reviewed above) which would be amenable to precise analysis. Thus classical conditioning of PT cell activity using thalamic stimulation as the CS and antidromic activation of the PT cell as the US would seem a logical experiment to perform in order to further deliniate the mechanisms responsible for neuronal plasticity at the cortical level.

Aim of This Study

The primary purpose of this study was to determine whether activation of thalamic nuclei as the CS+/CS- could support differential classical conditioning of PT cell activity using antidromic activation of the PT cell as the US. A second purpose was to investigate whether different thalamocortical systems differ in their ability to serve as an effective CS+ in this experimental paradigm. In Experiment 1, stimulation of two different thalamic nuclei (VPL and VL) served as the CS+ and CS-. In Experiment 2, stimulation of a nonspecific thalamic nucleus (CM) was used

as the CS+ and stimulation of VPL was used as the CS-. The firing patterns of PT cells following delivery of the CS and US were analyzed in order to assess the effects of the conditioning paradigm. Experiment 3 provided information primarily concerning the unconditioned response characteristics of the PT cells under different stimulation conditions.

METHODS

Ethical Considerations

The data were collected using an awake, paralyzed preparation. For ethical reasons, this type of procedure should be used only when necessary, and then only if it can be assured, as fully as possible, that the animal will not be subjected to excessive stress or to pain.

The use of an unanesthetized preparation is important when conducting an experiment dealing with the neurophysiology of learning. There has been a great deal of evidence collected demonstrating alteration in CNS activity as a function of the anesthetic used. Thompson, Johnson and Hoopes (1963) mention that chloralose versus barbiturate anesthetics differentially enhance components of the cortical evoked response (e.g., chloralose enhances the "association" components of an evoked response). From the very purpose for which they are employed, it is obvious that any type of anesthetic has definite effects on the pattern and level of CNS activity. In experiments dealing with conditioning of cortical activity, these modifications of neural activity pose serious methodological and interpretational problems for the investigator. For these reasons, it was felt that the use of the unanesthetized, paralyzed preparation was necessary for the success of this experiment.

The safety and comfort of the animal was controlled for adequately during the experiment. From the surgical procedures to the experimental design, the issue of animal comfort always was kept in mind.

Subjects

Fourty-eight adult cats, weighing between 2.0 and 4.0 kg, obtained through the University of Oregon Health Sciences Center Animal Care Department were used. No Siamese or part-Siamese cats were included in the study.

Surgical Procedures

Animals were prepared for data collection in a two-stage sequence. All surgical procedures were performed approximately 7 days prior to the day on which the conditioning paradigm was administered.

Pre-Experimental

The cats were initially anesthetized using ether and an endotracheal tube was inserted. The cat was then placed in a walk-in, sound-attenuated room (Industrial Acoustics Company). At this point, the animal was taken off ether and switched to halothane anesthesia, using an Ohio Medical Products Vaporizer set at 1.5% - 2.5% concentration with a flow rate of approximately 700 ml/min (oxygen). A Godart capnograph was used to monitor the CO_2 level. The cat then was mounted in a Kopf model 1204 stereotaxic apparatus, using standard eye and ear bars.

A strict antibiotic regimen was begun at this point based on the data of Malis (1979). All skin areas were first scrubbed with an iodine (Betadine) solution. Tobramycin (Nebcin) was injected IM (0.1 ml, 40 mg/ml). The left saphenous vein was cannulated and a vancomycin (Vancocin) solution (1.0 ml of stock vancomycin in 15 ml dextrose [5% in H_2O]) was infused over a two to three hour period during surgery using a Holter model 903 infusion pump. A streptomycin solution (.05 ml of stock streptomycin in 200 ml of saline) was used for irrigation.

When the vancomycin infusion was finished, the cannula was plugged and remained chronically implanted in the vein for use during the data collection phase of the experiment.

The scalp was incised and the temporal muscles were retracted, exposing the skull surface. Using a hand drill, holes were made in the skull to permit the insertion of two thalamic electrodes, a PT electrode and two skull bolts to help anchor the dental acrylic to the bone. A small bone flap overlying the frontal sinus was removed and two additional holes were drilled in a region overlying the pericruciate cortex (approximate stereotaxic coordinates: AP +26.0). During surgery, cortical evoked potentials (EPs) were recorded from this area. Following surgery, stainless steel bolts were screwed into these holes.

Implantation of thalamic and PT electrodes was accomplished under physiological control. Final vertical placement of the stimulation electrodes was determined by monitoring evoked activity. For the VPL electrode, the peripheral limbs were electrically stimulated while recording EPs from the tip of the electrode. Stimulation also was applied through the VPL electrode and EPs recorded at the pericruciate cortex. Stimulation was applied through the VL, CM and PT electrodes and EPs were recorded at the pericruciate site. In addition, evoked activity at the PT electrode was sometimes monitored while stimulation was applied to the pericruciate cortex. As a general rule, there was a clear-cut onset of a characteristic EP waveform which indicated the arrival of the electrode in the desired location. At this point, the electrode was bonded to the skull with dental acrylic.

Two small plastic tubes also were attached to the skull using dental acrylic. These tubes permitted the animal to be held in a Kopf semi-chronic headholder, without using standard eye and ear bars. A stainless steel ground wire was inserted into the temporal muscle. The leads from the stimulation and ground wires were attached to amphenol plugs which were embedded along with the plastic tubes and stainless steel bolts in a mound of dental acrylic. The two bolts at the pericruciate site were not embedded in the dental acrylic. A local antiseptic (Bactofura Wound Powder) was applied to the wound margins and the incision was closed with suture. Approximately one week for recovery following surgery was allowed before the start of data collection.

Experimental

On the day on which the actual data collection took place, the animal was briefly anesthetized with ether and an endotracheal tube was inserted. The animal then was placed in a Kopf stereotaxic apparatus using the semichronic headholder, thus eliminating pressure points caused by the use of standard eye and ear bars. Supplemental ether administration ceased at this point and gallamine triethiodide (Flaxedil, 20 mg/ml/hr) was infused (Holter pump, model 903) through the chronically implanted saphenous vein cannula to provide a block of neuromuscular activity. Thus, the animals were under ether anesthetic for less than 10 minutes. Sixty to 90 minutes were allowed from time of cessation of ether administration until the beginning of data collection. It should be stressed that no surgical procedures were per-

formed on the day of data collection. An artificial tears solution (Tears Naturale) was applied to the cornea periodically throughout the experiment to prevent drying and irritation of the cornea.

Artificial ventilation was maintained with a Harvard pump (model 606). Frequency was set at 16 strokes/min, with the stroke volume varied to maintain the tracheal CO_2 levels at 3.6 to 4.0% as monitored on a Godart Capnograph. Following the onset of gallamine triethiodide administration, the bolts were removed from the holes overlying the peri-cruciate cortex, a small hole was poked in the dura and the microelectrode inserted into the cortex. Liquid agar (3% in saline) at body temperature was applied to the area surrounding the microelectrode to help maintain the cortical temperature, to prevent drying of the cortex and to help control cortical pulsations.

Recording and Stimulating Electrodes

In each animal, side by side bipolar stainless steel stimulation electrodes (each pole 280 um diameter - 0.5 mm intertip distance) were introduced into two of three selected thalamic nuclei and into the PT. Thus, a total of three stimulation electrodes (two thalamic and one pyramidal) were implanted in each animal. The stereotaxic coordinates for the VPL electrode were: AP +9.0, L 6.5, V +1.5. The coordinates for the VL electrode were: AP +11.0, L 4.5, V +2.0. The coordinates for the CM electrode were: AP +7.3, L 3.0, V +0.5. The coordinates for the PT electrode were: AP +6.0, L 5.5, V -4.5. The final vertical placement of these electrodes was determined by monitoring evoked activity at both the thalamic and cortical level (see above for details). All

stimulation electrodes were implanted ipsilateral to the cortical microelectrode site.

The recording electrode was a stainless steel enamel-coated wire (tip diameter of 38 um) coated with additional layers of epoxylite insulation.

Recording Apparatus

EPs collected during placement of the stimulation electrodes were led through a Tektronix 122 preamplifier, with the filter set at 0.2 Hz - 250 Hz. The EPs then were displayed on a Tektronix 5111 storage oscilloscope and photographed using a Tektronix C-5B oscilloscope camera.

Signals from the microelectrode were led through a Bak cathode follower and then amplified and filtered (500 Hz - 3 kHz) by a Tektronix 122 preamplifier. The signal was further amplified by a series of Tektronix amplifiers (AF501, 2A63, AF501) and displayed on a Tektronix R561B oscilloscope. The signal then was converted into digital form by a Schmitt trigger and stored on-line by a PDP-12 computer.

Stimulation Apparatus

Pre-Experimental

Peripheral somatic stimulation (applied during the stereotaxic placement of stimulation electrodes during surgery) was delivered to contralateral and ipsilateral (in reference to recording site) limbs by means of 21 gauge hypodermic needles placed subcutaneously in the paw pad region. The stimulus was delivered by a Devices Mk IV isolated stimulator as three pulses of 0.1 ms duration at a frequency of 250 Hz. Stimulation of cortical or subcortical sites during surgery also used

a Devices Mk IV stimulator (one or three pulses, 0.1 ms duration).

Experimental

Stimulation of thalamic and pyramidal sites during the experimental phase was accomplished using Devices Mk IV isolated stimulators. Thalamic stimulation consisted of either one or three pulses (0.1 ms duration, 250 Hz) while the PT stimulus was a 100 ms pulse train (0.1 ms duration, 250 Hz). The voltage level of the Mk IVs was adjusted on an individual neuron basis. In the case of the PT electrode, the voltage level was always just above threshold for elicitation of an antidromic spike.

Collection of Data

Classical conditioning (in the form of a differential trace conditioning paradigm) was administered following isolation of a neuron which met the appropriate criteria (see Experimental Protocol). For each animal, stimulation of one thalamic nucleus was designated as the CS+, stimulation of the other nucleus was designated the CS-. The CS+ was paired, during the conditioning phase, with the US at a CS-US interval of 550 ms. The CS- was never paired with the US. PT stimulation served as the US.

For each unit isolated in each animal, there were 75 habituation trials each for the CS+ and CS-. Then, there were 225 paired CS+ - US presentations and 225 unpaired CS- presentations. Following the conditioning trials, there were 75 extinction trials each for the CS+ and CS-. The CS+ and CS- trials were interspersed with each other in a sequence randomized within a block of 50 trials. The intertrial interval was ran-

domized with a mean of 11 s and a range of 8 - 15 s.

Both the sequence of CS+s and CS-s and the intertrial intervals were controlled by a mechanical film programmer. Closure of a microswitch on the film programmer triggered a Devices Digitimer D4030 which generated a time zero pulse signalling the beginning of a trial to the PDP-12 computer. A second microswitch on the film programmer determined whether the stimulus to be presented was a CS+ or a CS-. A CS was presented 1.0 s after the time zero pulse and, on CS+ trials only, during conditioning, was followed after 550 ms by the US. The PDP-12 computer controlled the sequence of trial presentations (habituation, conditioning and extinction).

Experimental Protocol

For each animal, the microelectrode was lowered slowly through the cortex while monitoring the electrical activity, until a spontaneously active single unit was isolated. The unit then was tested for antidromic activation by PT stimulation and for responses to stimulation of the thalamic electrodes. Only cells showing responses for all types of stimulation were used for conditioning. If a given unit did not meet these criteria, another cell was isolated and tested.

Criteria for defining a cell as showing an antidromic discharge were stringent. The cell had to show: 1) discharge following PT stimulation at a fixed, relatively short latency (< 4 ms); 2) a well-defined threshold, below which spike discharge failed; 3) collision of an antidromic spike with an orthodromic spike (Schlag, 1978). This is considered the most definitive test of antidromic activation; 4) the ability to

"follow" PT stimulation with antidromic spikes, at a stimulation frequency of 250 Hz.

If the neuron met all criteria, then, depending on the particular group that the animal had been assigned to, one of a number of conditioning procedures was followed (see Table 1). Following completion of the conditioning paradigm, an attempt was made to isolate another cell, and the same procedures followed as outlined above. Figure 1 outlines the complete protocol.

At the end of the experiment, the animal was deeply anesthetized with Pentobarbital Sodium (Nembutal, IV, 40 mg/kg). A cardiac perfusion was performed using 10% buffered formol-saline. The brain was blocked to contain the stimulation electrode sites and stored in a solution of 30% sucrose in 10% buffered formol-saline for later histological processing (frozen sections and Nissl staining). The stereotaxic atlas of Jasper and Ajmone-Marsan (1954) was consulted in determining the actual location of the electrodes.

Data Analysis

Occurrences of spikes from single units were recorded on a trial by trial basis in reference to the Digitimer time zero pulse, by setting the Schmitt trigger at an appropriate voltage level. The PDP-12 counted spike occurrences for a total of 3.5 s following the time zero pulse (see Figure 2).

Unit data were analyzed by accumulating the spike activity into consecutive bins each of 1 or 5 ms width, beginning at time zero, to form stimulus histograms for selected blocks of trials. The 1 s period preceding CS occurrence was included in order to determine the background rate

Table 1: Experimental Design

Experiment 1	CS: Single Pulse		US: 250 Hz, 100 ms
	CS+	CS-	
N = 7	VPL	VL	suprathreshold PT stimulation
N = 8	VL	VPL	suprathreshold PT stimulation
Experiment 2	CS: Single Pulse		US: 250 Hz, 100 ms
	CS+	CS-	
N = 9	CM	VPL	suprathreshold PT stimulation
Experiment 3	CS: Triple Pulse		US: 250 Hz, 100 ms
	CS+	CS-	
N = 3	CM	VPL	suprathreshold PT stimulation
N = 3	VPL	CM	suprathreshold PT stimulation

Total N = 30

Figure 1: Flow chart of basic experimental protocol.

EXPERIMENTAL PROTOCOL

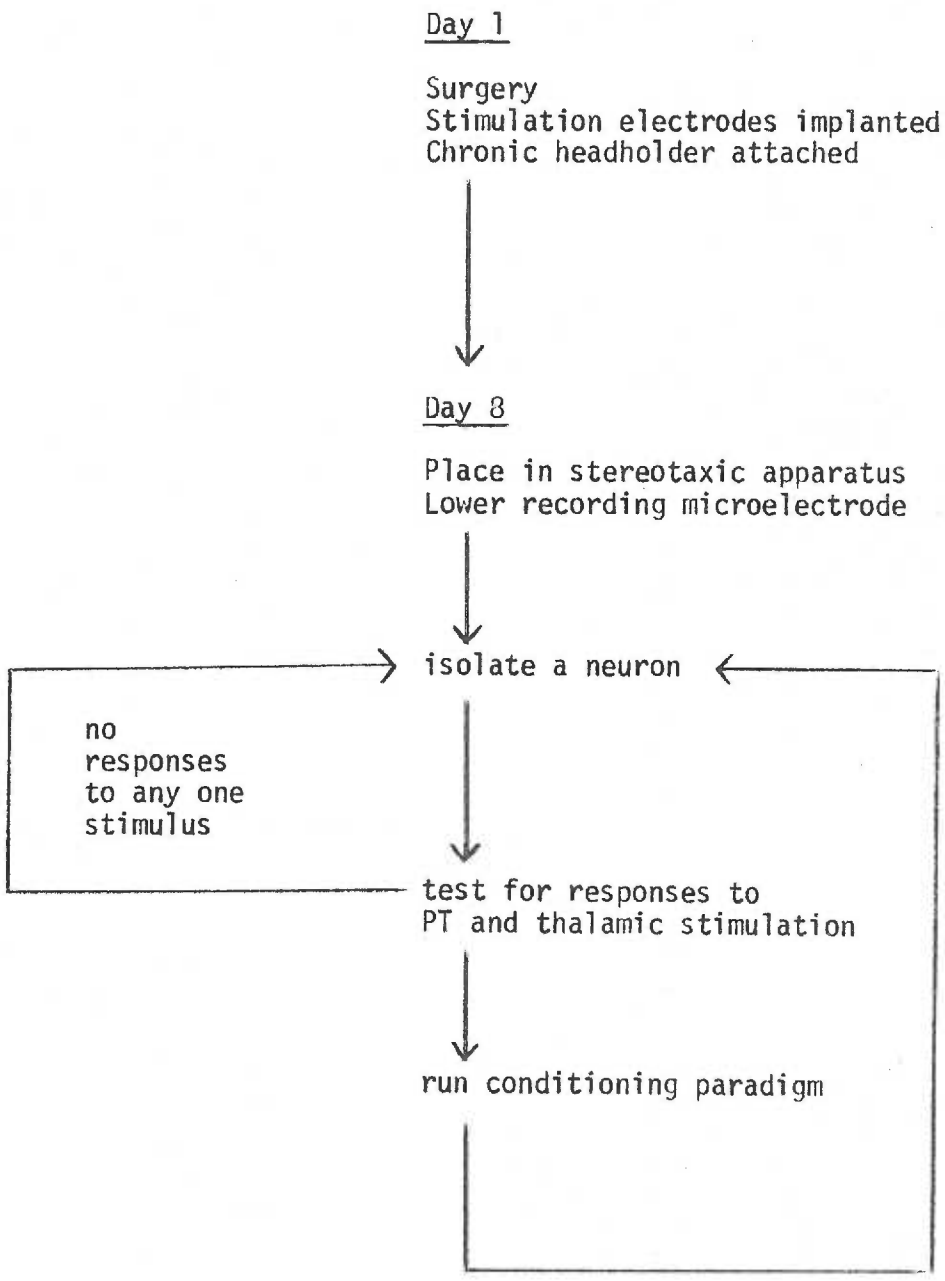
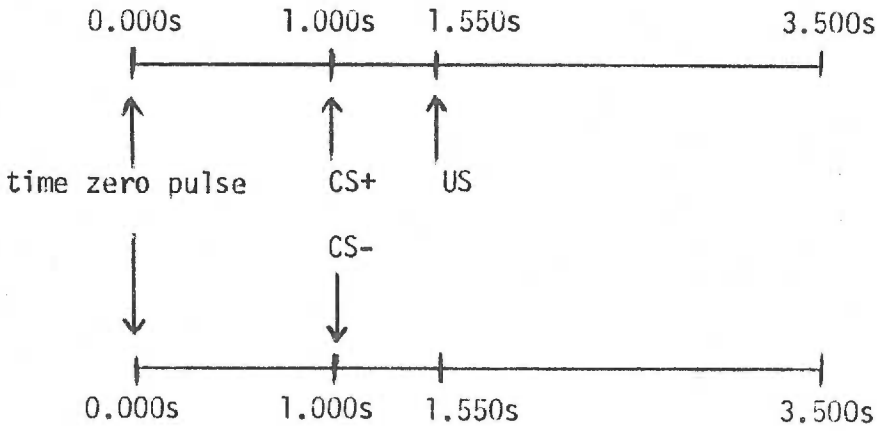


Figure 2: Basic stimulus presentation sequence and time base used by computer for sampling unit activity.

STIMULUS PRESENTATION SEQUENCE



of activity. For preliminary analysis, two histograms were formed, one of the entire 225 trial conditioning sequence, another for the 75 trial habituation sequence. These histograms were visually displayed on the CRT of the PDP-12. Movable cursors were adjusted by the experimenter so that they coincided with the first bin of the principal rising phase and the last bin of the principal declining phase of the response period of interest. The exact position of each cursor was recorded and this time interval then was used to compute the spike rate changes during the different phases of the paradigm. This procedure was followed for all response periods present in a given cell, for both the CS+ and CS-. In most cells there were two principal response periods following thalamic stimulation (see RESULTS section for details).

Statistical Analysis

This study was divided into three experiments. In experiment 1, single pulse stimulation of VPL and VL served as the CSs. In experiment 2, single pulse stimulation of CM and VPL served as the CSs. In experiment 3, triple pulse stimulation of CM and VPL served as the CSs.

The basic datum in the design of each experiment was the spikes per second (evoked minus background rate), for a selected interval of the poststimulus histogram (as determined by the method outlined above), averaged over a 25 trial block. Thus there were three blocks of habituation, nine of conditioning and three of extinction for a total of 15 blocks of data (total of 15 X 25 = 375 trials). For the determination of the significance of changes in firing rate related to presentation of the conditioning paradigm for experiment 1, a three-way analysis of

variance (ANOVA), with repeated measures on two factors, was used. The factors were as follows:

Factor A: Blocks of trials (15 levels)

Factor B: Differential conditioning (2 levels, CS+, CS-)

Factor C: Thalamic nucleus selected as CS+/CS- (2 levels)

Since the blocks of trials from habituation through conditioning and extinction were all collected from each cell and since each cell received both the CS+ and CS-, factors A and B were the within-subject factors. In any given animal, a particular thalamic nucleus served only as a CS+ or CS-, so factor C was the between-subject factor.

For experiment 2, a two-way ANOVA, with repeated measures on two factors, was used. Factor A and factor B were the same as described above. Since CM always served as the CS+ there was no factor C. The data from experiment 3 were not analyzed as a separate group due to the low "N", however for some of the analyses described in the RESULTS section the data from experiments 1, 2 and 3 were pooled. It is specifically stated in the RESULTS section when an analysis is based on pooled versus unpooled data.

It was considered desirable to be able to rate the response change during conditioning which a cell showed to a given stimulus with a single score value. For this purpose, the "grand t score" analysis (t_g) has been derived. The t_g score has been used previously in the preliminary analysis of results by Rosenblum and O'Brien (1977) and O'Brien, Wilder and Stevens (1977). The t_g score has the advantage over a simple difference score measure because it takes into account the variance as well

as the difference between evoked and background firing rate during different phases of the paradigm. The t_g score was not used to make a statistical inference concerning the significance of the change in firing pattern of a particular cell. It was meant to provide an index of change which could be compared with other cells and related to various neuronal parameters (e.g. conduction velocity, baseline firing rate, etc.). In a general sense, the larger the value of the t_g score, the more reliable was the difference in firing pattern during conditioning as compared with habituation and extinction. The formula used to derive this score was as follows:

$$t_g = \frac{\frac{\Sigma x_c}{N_1} - \frac{\Sigma x_{h+e}}{N_2}}{\sqrt{\frac{(N_1 + N_2)(\Sigma x^2_{h+c+e}) - (\Sigma x_{h+c+e})^2}{(N_1 + N_2)(N_1)(N_2)}}}$$

Where:

Σx_c = sum of the differences between evoked and background rate during conditioning trials

Σx_{h+e} = sum of the differences between evoked and background rate during habituation and extinction trials.

Σx^2_{h+c+e} = sum of the squared differences between evoked and background rate for all trials

Σx_{h+c+e} = sum of the differences between evoked and background rate for all trials

$N_1 = 225$ (conditioning trials)

$N_2 = 150$ (habituation + extinction trials)

RESULTS

Electrode Placement

Accurate placement of the stimulation electrodes was a necessary condition for the success of this study. During surgery, the final position of the electrodes was monitored electrophysiologically. Following data collection, electrode placements were confirmed histologically.

Electrophysiological Confirmation

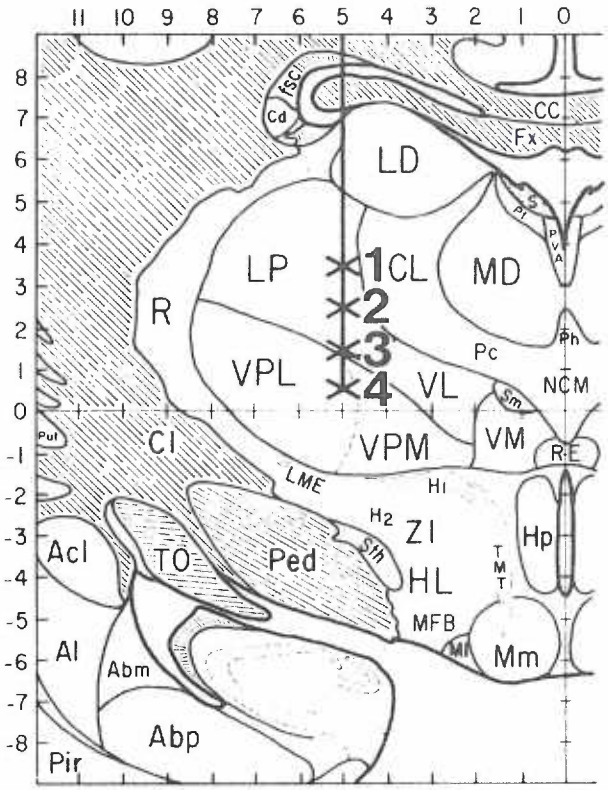
Final placement of the VPL stimulation electrode in the vertical stereotaxic plane was monitored by stimulating a contralateral peripheral limb of the animal and recording the evoked activity obtained at the electrode tip. Figure 3 is an example of the type of response obtained to stimulation of the periphery as the electrode was lowered in the vicinity of VPL. As indicated in Figure 3, there was a rather abrupt onset of evoked activity at the tip of the electrode as it reached the vertical placement corresponding to the location of VPL. This figure represents the typical sequence of events for placement of the VPL electrode. The VPL electrode was located in an area which was responsive predominantly to stimulation of the contralateral forepaw. There was minimal or no responsiveness to stimulation of the contralateral hindpaw.

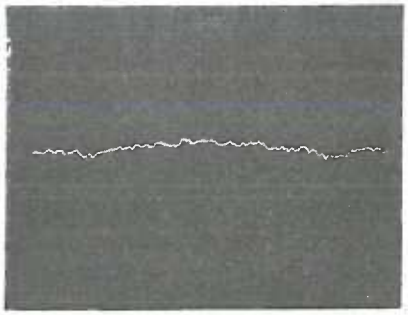
The evoked response in CM and VL to peripheral somatic stimulation was of a very low magnitude compared to that obtained in VPL. A more reliable method of determining the vertical placement of the CM and VL electrodes was to record evoked activity from the surface of the

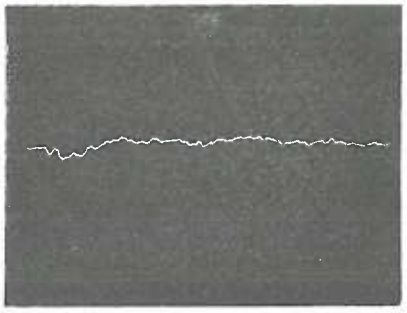
Figure 3: Placement of VPL electrode under electrophysiological control. Each photograph represents the response to contralateral forepaw stimulation at the point indicated on the schematic brain section.

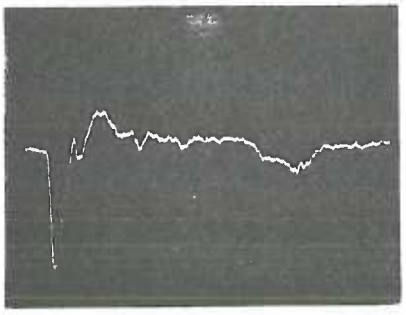
VPL Electrode
Contra Forepaw Stimulation

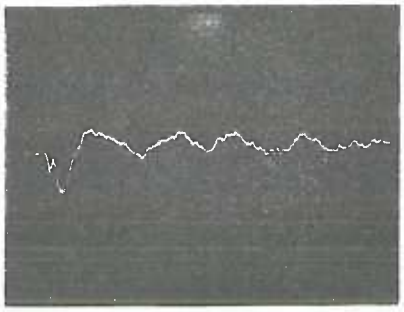
AP : 9.5 Cat no. 68











pericruciate cortex in response to stimulation through these electrodes. As with the placement of the VPL electrode, there was a characteristic pattern of evoked activity associated with stimulation of the VL and CM thalamic regions which marked the arrival of the electrode at the proper vertical placement. Figure 4 displays examples of the types of cortical responses obtained with stimulation of VL and CM as well as with stimulation of VPL. Note that VL and VPL showed a characteristic short latency response which was, however, different for the two nuclei, while the response to CM stimulation showed a longer latency and broader waveform.

Final vertical placement of the PT electrode was determined by recording evoked activity at the pericruciate cortex in response to stimulation at the electrode tip. Figure 5 depicts a typical example. Figure 5(1) and 5(2) show the cortical response to stimulation as the electrode passes through part of the Vb complex (VPM) and the external medullary lamina (LME). Note the large amplitude evoked activity, especially in Figure 5(2), which resembles the typical response obtained to VPL stimulation (see Figure 4). Figure 5(3a) shows the cortical response after the electrode was lowered into the PT (Ped). Note that the large amplitude activity in Figure 5(2) has been replaced by a shorter latency, smaller amplitude evoked waveform.

Figure 5 indicates that within the voltage levels used in this experiment, there was little likelihood for significant voltage spread from the final PT placement to the closest afferent pathway contributing directly to activity in the pericruciate cortex (i.e., medial

Figure 4: Evoked responses in pericruciate cortex to stimulation of VPL, VL and CM nuclei of the thalamus.

Pericruciate Cortex
Response to Thalamic Stimulation

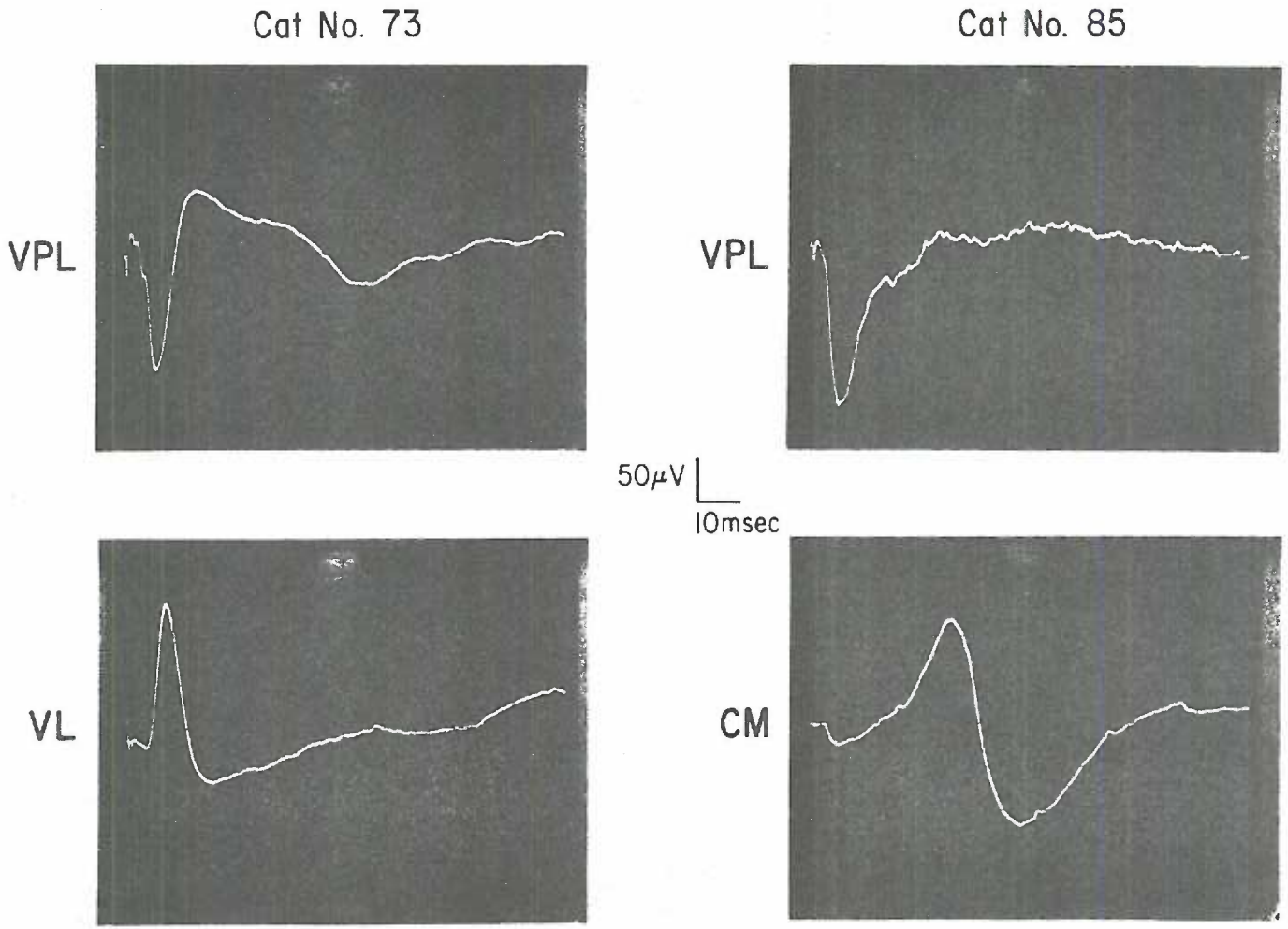
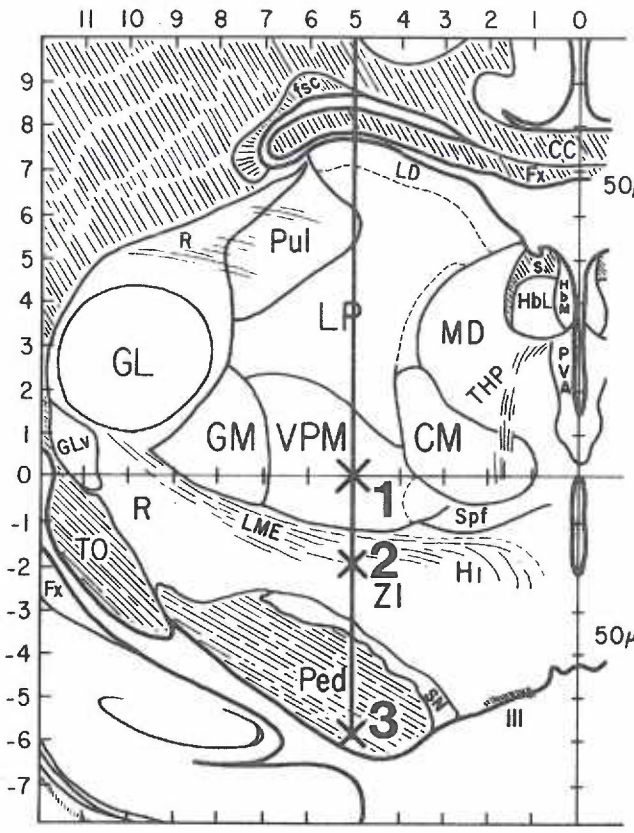


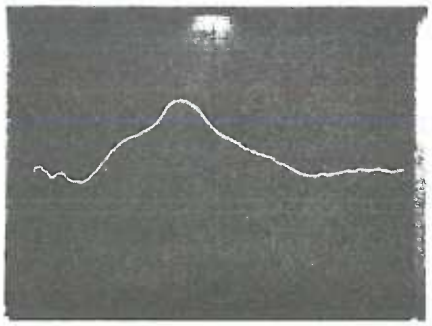
Figure 5: Placement of PT electrode under electrophysiological control. Photographs (1) - (3a) represent the evoked response in peri-cruciate cortex to stimulation at the points indicated on the schematic brain section. Photograph (3b) represents the response at (3) on the schematic to stimulation of the peri-cruciate cortex. Note difference in time base between (1) - (3a) and (3b).

Pyramidal Tract Electrode Placement

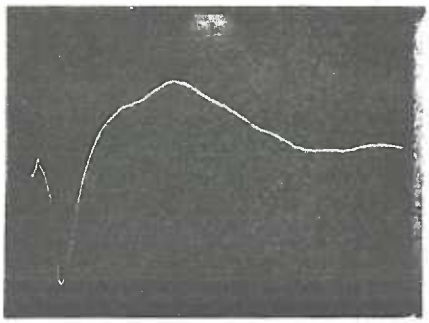
AP: 7.5 Cat no. 62



stim. PT/record cortex

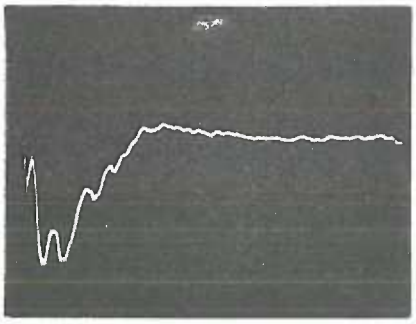


(1)
50μV
10msec

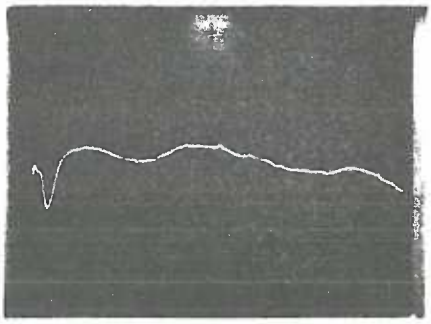


(2)
50μV
10msec

stim. cortex/record PT



(3b)
50μV
2msec



(3a)
50μV
10msec

lemniscus-ventrobasal complex). On occasion, following final placement of the PT electrode, the cortex was stimulated and evoked activity was recorded at the PT electrode. Figure 5(3b) is an example of the type of activity obtained in response to such stimulation.

In summary, the electrophysiological data collected during surgery provided strong evidence that: 1) the respective electrodes were in the desired locations; and 2) stimulation of a given locus produced activity which was well differentiated from activity due to stimulation of the other loci.

Histological Confirmation

All animals in which single cells were recorded successfully throughout a conditioning session were perfused and the electrode sites histologically verified using frozen sections and Nissl staining. Thus, a total of 20 animals were processed. In all twenty animals, the PT electrode was found in the PT. In 19 of 20 animals, the VPL electrode was found within the Vb complex. In one animal, the VPL electrode was on the border between the lateral posterior nucleus and VPL. The placements of the VL and CM electrodes were more variable. The details concerning their placement is given in Table 2. Table 5 in the APPENDIX details the precise locations of all electrode placements in stereotaxic coordinates. The data in this study were grouped and analyzed on the basis of the target nuclei selected as detailed in the METHODS section.

Table 2: Histological analysis of VL and CM electrode placements, based on atlas of Jasper and Ajmone-Marsan (1954).

Cat #	Target: VL	Cat #	Target: CM
67	VPL-VL	81	CM-Pf
68	VA-Ext Ret	82	CM
69	VL	84	MD-CM
70	VL-VPL	85	VPM-CM-PC
71	VL-VPL	86	CM
73	VA-VL-VPL	87	CM-NR
75	VL	88	*
76	Ext Ret	89	CL
77	VA	90	CM
80	VA	94	CM

Abbreviations: CL = central lateral, CM = centromedian, Ext Ret = external reticular, MD = medial dorsal, NR = red nucleus, PC = paracentral, Pf = parafascicular, VA = ventral anterior, VL = ventral lateral, VPL = ventral posterior lateral, VPM = ventral posterior medial.

*tissue sections lost during processing, unable to verify placement.

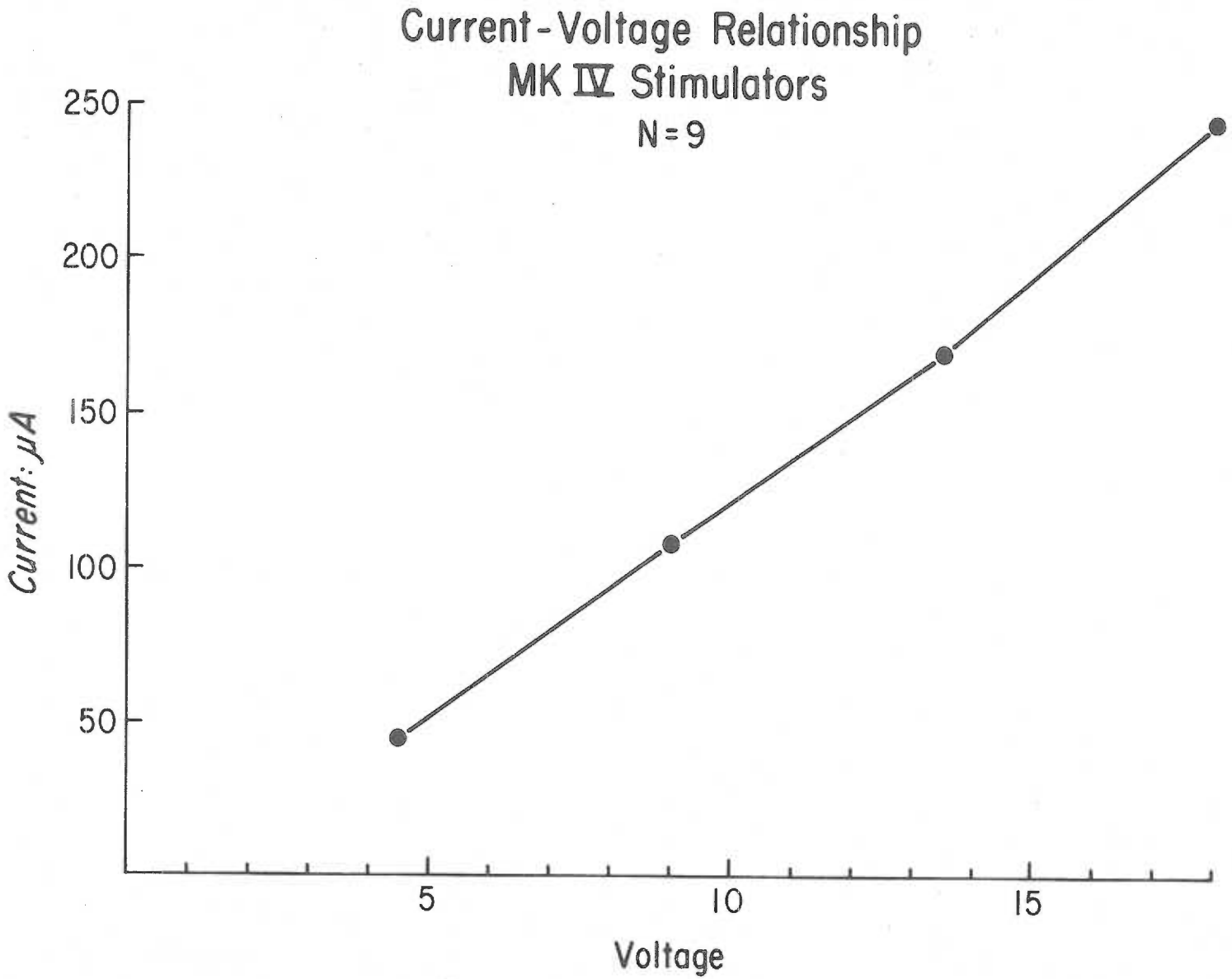
Stimulation Parameters

The Mk IV stimulators used in this study were constant voltage as opposed to constant current devices. A potential problem with constant voltage devices is the possibility of electrode polarization leading to a reduction in the effectiveness of the stimulation. This possibility was investigated in the present study in several ways and was not found to be a problem.

First of all, polarization typically occurs under long term chronic stimulation conditions such as exist in intracranial self-stimulation experiments. This was not the type of stimulation employed in the present study. Secondly, the accepted method of preventing polarization is to use biphasic pulse stimulation (i.e., instead of delivering a single pulse of a particular polarity, whenever stimulation is administered, a double pulse consisting of one normal and one reversed polarity is used). The Mk IVs are not equipped for this type of stimulation but Grass S 9 stimulators can be modified to provide this type of pulse. When Grass S 9 stimulators were compared with Mk IVs no differences were found in their stimulation characteristics under identical conditions.

Thirdly, in nine implanted electrodes, the relationship between the voltage output of the Mk IV and the current passed through the electrode as measured by the IR drop across a series resistor, was determined. The results are displayed in Figure 6. As can be seen, there was a linear relationship between Mk IV voltage output and current passed by the stimulation electrodes. These considerations indicated that, within the stimulation parameters selected for this study, there

Figure 6: Relationship between voltage applied by Mk IV stimulator and current passed by the stimulating electrodes, in vivo, as measured across a series 2 kΩ resistor.


Current-Voltage Relationship
MK IV Stimulators
N=9
250
200
150
100
50
Current: μA
5
10
15
Voltage

was no serious problem with using constant voltage devices.

Cell Population Sampled

A total of 48 cats were used in this study. The first 20 animals provided pilot data concerning the proper stimulation parameters and the most appropriate type of microelectrode to use. Twenty-eight animals were used during the data collection phase of the experiment. Of these cats, a total of 20 had one or more cells which were recorded from for an entire session (habituation, conditioning & extinction). Table 3 presents the breakdown on the total number and types of neurons recorded. As can be seen from the table, only 14% of the total sample failed the collision test. This outcome indicated a strong microelectrode bias towards sampling PT neurons. It should be noted that cells which failed the collision test could have been either non-PT cells or PT cells whose axons were not close enough to the PT electrode to be activated by the stimulation.

Response of PT Cells to Stimulation

The data derived from the single unit recording will be presented in two phases. In the first phase, general characteristics of the PT cells recorded in this study will be described. In the second phase, the response plasticity of these cells will be described.

After a single unit had been isolated in any given experiment, the cell was tested to determine if it responded to PT stimulation with an antidromic spike. As discussed above, four criteria were used: 1) a fixed short-latency discharge following stimulation; 2) a well-defined threshold; 3) collision of the presumed antidromic spike with a spon-

Table 3: Sampling Distribution of Neurons

Classification	# of Cells	% of Total
Failed collision test	19	14
Passed collision test		
recorded for entire session	30	22
recorded for part of session	17	12
non-frequency following	38	27
unsatisfactory signal/noise ratio	35	25
Totals	139	100

taneous orthodromic spike; 4) the ability to "follow" PT stimulation with antidromic spikes, at a stimulation frequency of 250 Hz.

The collision test, as described by Schlag (1978) is perhaps the most definitive measure of antidromic activation. In conducting this test, the PT was stimulated at particular times following the occurrence of a spontaneous orthodromic spike. If the latency between occurrence of an orthodromic spike and stimulation of the PT was less than the conduction time from the PT electrode to the cell body being recorded from, and if that cell was capable of responding antidromically, then the orthodromic spike would collide with the antidromically produced spike and the latter would not reach the vicinity of the cell body. Figure 7 is an example of a positive outcome. In conducting this test, the occurrence of the orthodromic spike triggered the oscilloscope trace. Note that at a certain critical interval between initiation of an orthodromic spike and stimulation of the PT, the stimulus-evoked spike did not appear. This indicated that the axon of the cell was activated by the PT stimulation.

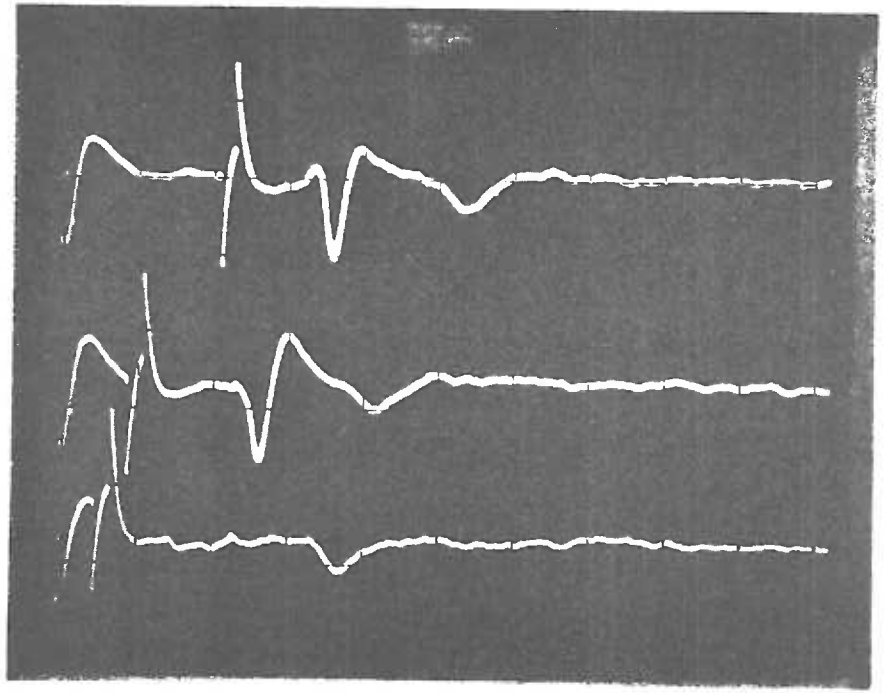
It is known from previous anatomical work that PT cells send collaterals into the thalamus. This possibility was tested for in the present study using the criteria for antidromic activation outlined above. In 3 of 30 cells examined, there was evidence that stimulation of a thalamic structure resulted in antidromic activation of a PT cell. However, as Figure 8 indicates, there are two alternative interpretations of the result of obtaining antidromic activation from PT and thalamic stimulation (Schlag, 1978). In Figure 8A, one is stimulating

Figure 7: Collision of an antidromic spike with a spontaneous orthodromic spike. Oscilloscope was set to trigger on occurrence of spontaneous orthodromic spike. Large amplitude, sharp deflections between the orthodromic and antidromic spikes are the stimulus artifacts from PT stimulation. Top two traces show lack of collision. Bottom trace shows successful collision of orthodromic and antidromic spike (antidromic spike failed to reach vicinity of recording electrode).

Pyramidal Tract Cell

Collision of Orthodromic and Antidromic Spikes

Cell No. 76C

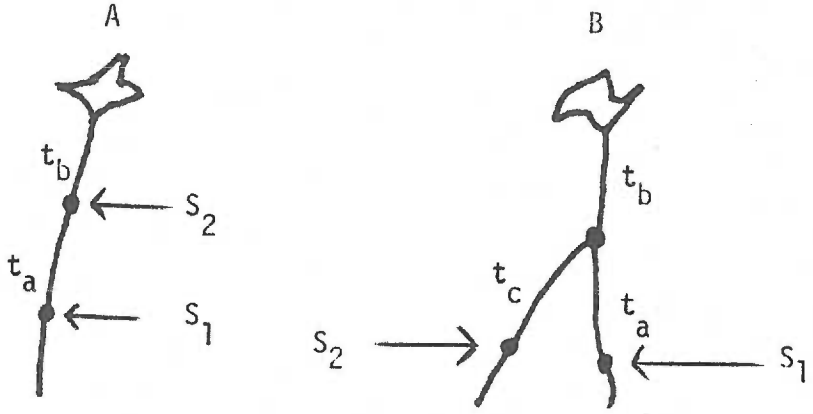


1msec

Figure 8: Evidence for branching of a PT cell axon. Data necessary to evaluate branching shown in upper part of figure. Schematic of alternatives distinguished and mathematical proof of branching in lower part of figure.

AXONAL BRANCHING IN PYRAMIDAL TRACT FIBERS

	PT	CM
Threshold	8.1V	6.3V
Latency	1.4 ms	2.1 ms
$c_{PT,CM}$	2.5 ms	
r	0.7 ms	



$l_1 = t_a + t_b$

$l_2 = t_b$

$c_{1,2} = t_a + r$

$l_1 = t_a + t_b$

$l_2 = t_c + t_b$

$c_{1,2} = t_a + t_c + r$

$$t_b = 1/2(l_1 + l_2 - c_{1,2} + r)$$

$t_b = .85$ ms

$l_2 = 1.4$ ms

if case A then $l_2 = t_b$

if case B then $l_2 > t_b$

c = interstimulus collision interval
r = refractory period

the same axon at two different locations. In Figure 8B, one is stimulating two separate branches. By collecting the proper type of data, it is possible to distinguish these possibilities.

The top half of Figure 8 details the type of data which must be collected in order to distinguish between alternatives A and B. The latency data were collected by measuring the time interval between electrode stimulation (PT or CM) and appearance of the antidromic spike at the PT cell. The interstimulus collision interval "c" was determined by sequentially stimulating the PT and CM electrodes at shorter and shorter intervals until collision was obtained (i.e., the antidromic spike from the first stimulus collided with the antidromic spike from the second stimulus). The refractory period "r" was determined by subtracting the antidromic spike latency from the collision interval (the collision interval was determined by the sum of the antidromic spike latency and the refractory period of the axon).

The key to deciding between alternatives A and B involves calculating the value t_b. As can be seen in Figure 8, both alternative A and B reduce to the same formula for calculating t_b. The decision is then based on determining whether the antidromic spike latency for S_2 is the same as (A) or greater than (B) the value for t_b. In the case of the cell depicted in Figure 8, the results indicated that the PT cell axon branched, sending a collateral into the vicinity of CM as well as into the PT. In the other two cells examined which showed antidromic activation to thalamic stimulation, insufficient data were collected to distinguish these alternative explanations.

The fourth criterion used for determining antidromic activation was the frequency following characteristics of the cell. This is not as stringent a criterion as the collision test since Stefanis and Jasper (1964a) have shown lack of frequency following in confirmed PT cells and Morin and Steriade (1981) and Steriade and Yossif (1977) demonstrated that certain cells could follow high frequency (100 - 700 Hz) orthodromic Vb stimulation.

It was observed in the present study that there was a distinct subpopulation of neurons which fulfilled the first three criteria for demonstrating antidromic PT activation but failed the frequency following test. Figure 9 shows an example of a frequency following and a non-frequency following cell. In both cell responses depicted in this figure, one has to distinguish between the stimulus artifacts and the spikes. In the frequency following cell (Figure 9, top) both artifacts and spikes have positive/negative deflections, although the spikes have a much larger amplitude than the artifacts. The spike in each case is the second of the two closely paired deflections. In the non-frequency following cell (Figure 9, bottom), the spikes are small negative deflections below baseline. The artifact is completely above baseline (positive). The non-frequency following cells typically gave from 1 to 12 out of a possible 25 antidromic spikes (100 ms pulse train at 250 Hz = 25 stimulus pulses).

It was determined that the frequency and non-frequency following PT cells examined in the present study could be separated into two populations based on their conduction velocity (i.e., latency of anti-

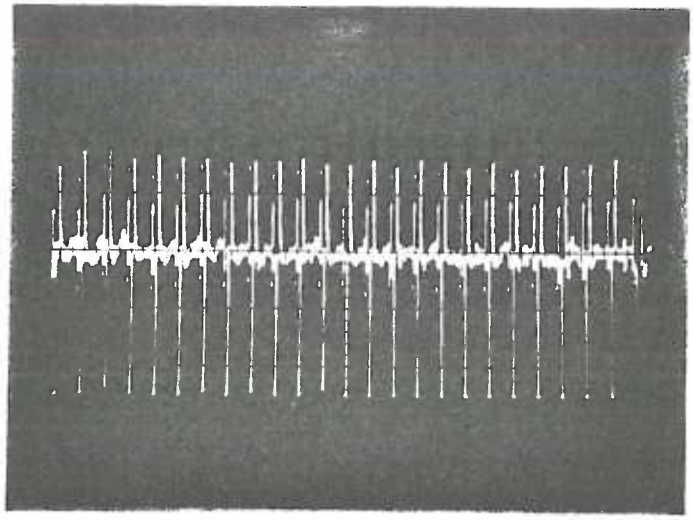
Figure 9: Response of PT cells to high frequency (100 ms, 250 Hz) stimulation of the pyramidal tract. Upper photograph - stimulus artifacts are the first and cell spikes, the second, of the two closely spaced deflections in oscilloscope trace. Lower photograph - stimulus artifacts are positive going (above baseline) and neuronal spikes, negative going (below baseline).

Pyramidal Tract Cells

PT Stimulation (100 msec, 250 Hz)

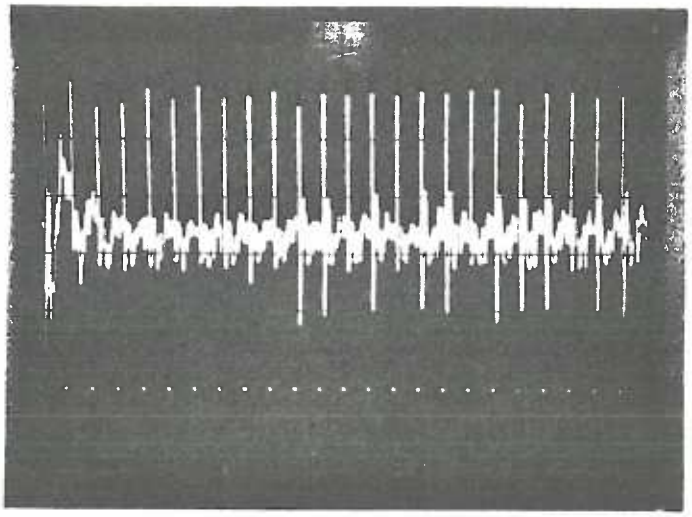
Cell No. 76 A

frequency following



Cell No. 68 D

non-frequency following



dromic spike). Figure 10 demonstrates this relationship. The average antidromic spike latency for frequency following PT cells was 0.9 ms and for non-frequency following cells, 1.5 ms. A t test performed on These data indicated a significant difference in latency between the two groups ($t(66) = 4.24$, $p < .01$). There was no difference in stimulus voltage required for antidromic activation for frequency versus non-frequency following cells. Since for the purposes of this study frequency following was considered an essential feature of the cell (in order to provide an adequate US), all further data discussed pertain to frequency following PT cells.

In addition to the antidromic spikes, the PT cells responded at offset of the stimulus pulse train as well. This response was designated the unconditioned response (UR) in the present study although technically the UR should be considered as the antidromic spikes plus the response following pulse train offset. The UR in the present study had one of two distinct patterns: 1) a complete inhibition of cell firing followed by a gradual return to baseline rate, "I-UR"; 2) a marked excitatory response superimposed on a background of inhibition, "E-UR". Figure 11 gives an example of each pattern observed. There was no statistically significant difference in antidromic spike latency for E-UR versus I-UR cells. However, cells which had an E-UR had a higher threshold for antidromic spike elicitation than cells which had an I-UR (E-UR = 9.7 V, I-UR = 3.0 V, $t(66) = 4.2$, $p < .01$).

PT cells showed a stereotyped response pattern to thalamic stimulation. Figure 12 shows the response histograms of PT cells to single

Figure 10: Histograms showing number of cells which responded with an antidromic spike at a particular latency following stimulation of the pyramidal tract. Upper histogram - cells which showed frequency following to the PT stimulation. Lower histogram - cells which did not show frequency following to the PT stimulation.

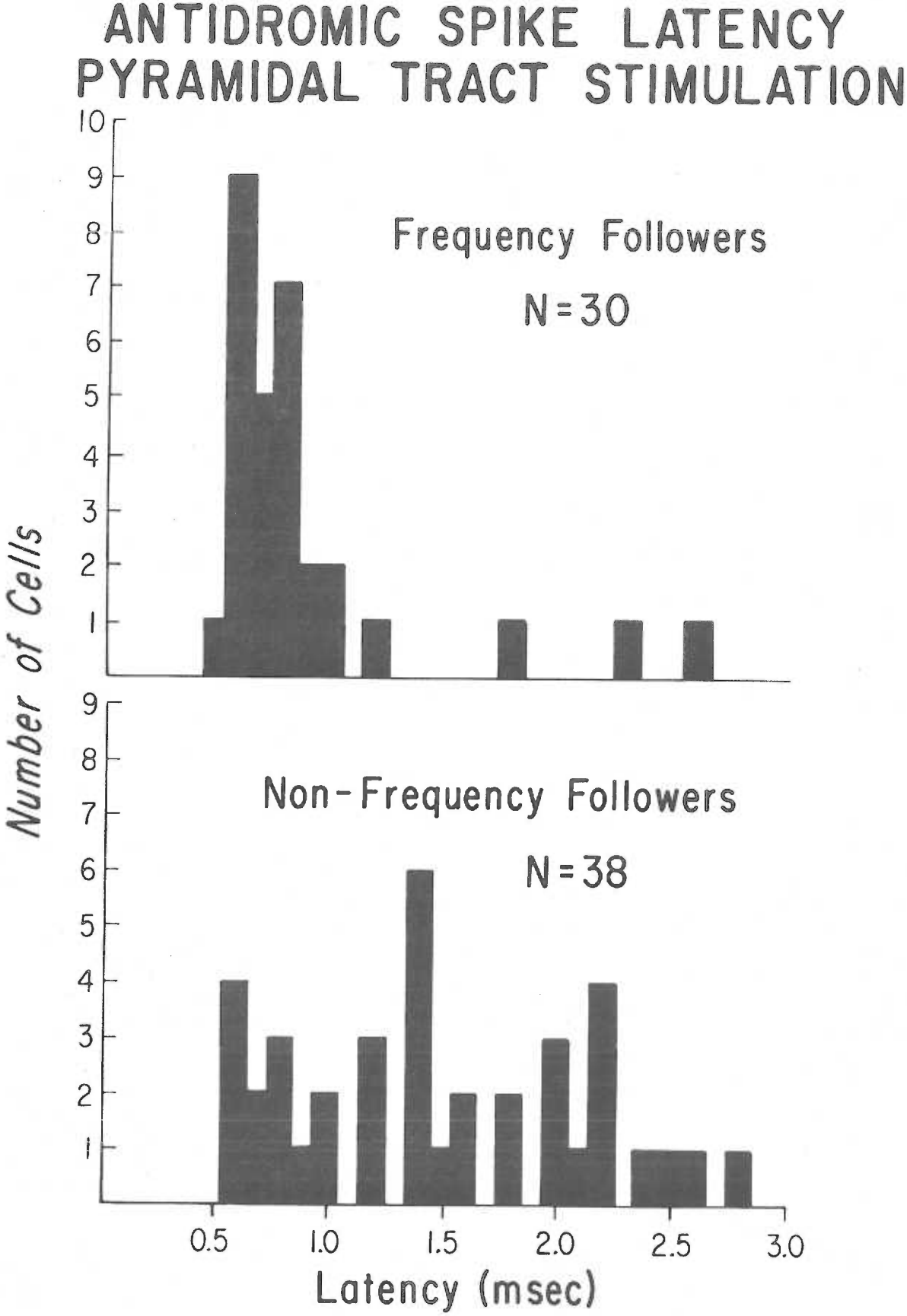

ANTIDROMIC SPIKE LATENCY
PYRAMIDAL TRACT STIMULATION
Number of Cells
Frequency Followers
N=30
Non-Frequency Followers
N=38
Latency (msec)
0.5
1.0
1.5
2.0
2.5
3.0

Figure 11: Poststimulus response histograms of PT cells for thalamic (CS) and pyramidal tract (US) stimulation. Stippled region in both histograms represent responses obscured by the stimulus artifact. Upper histogram - note absence of evoked response following offset of the US. Lower histogram - note presence of a large excitatory response following US offset.

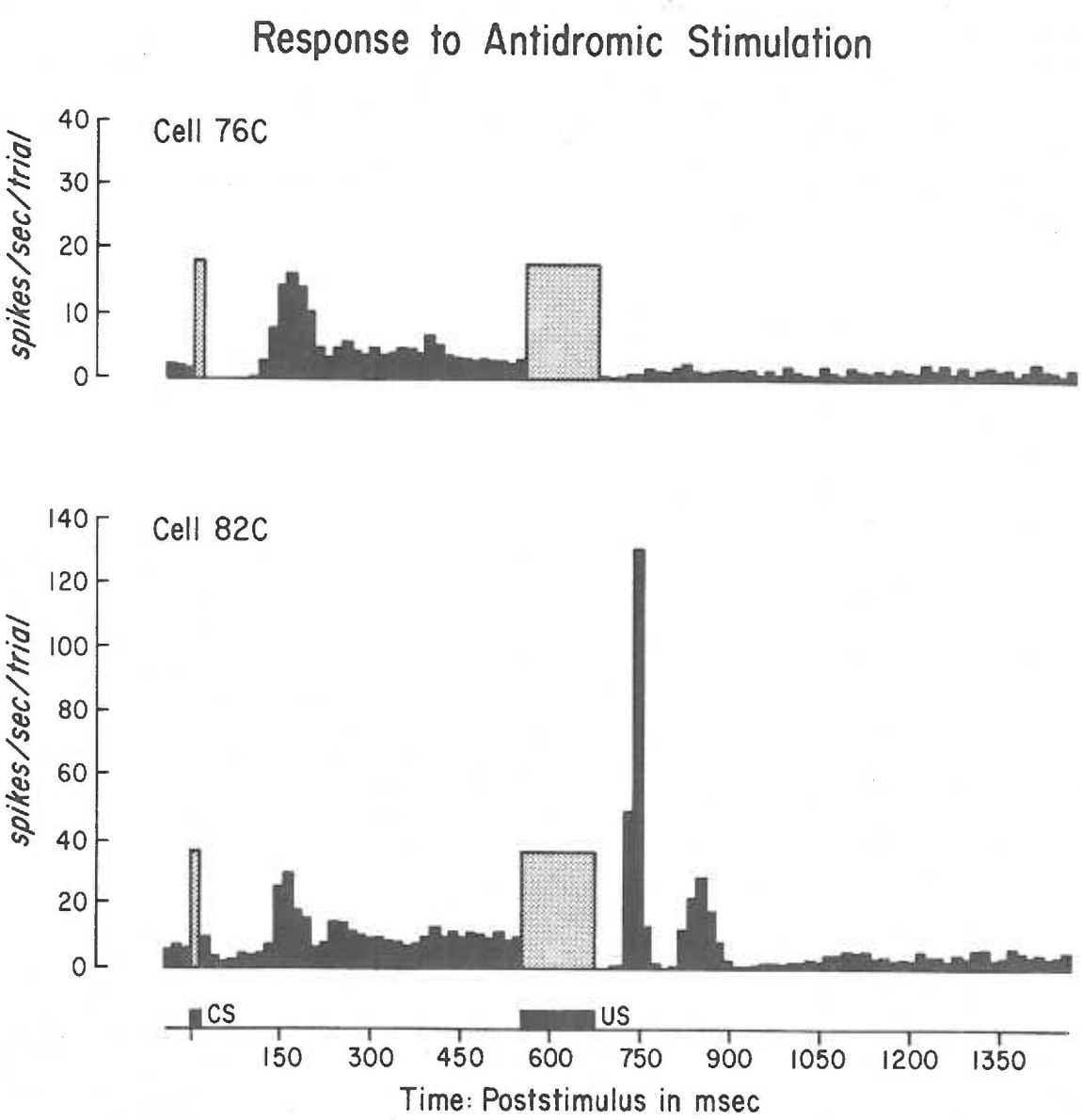
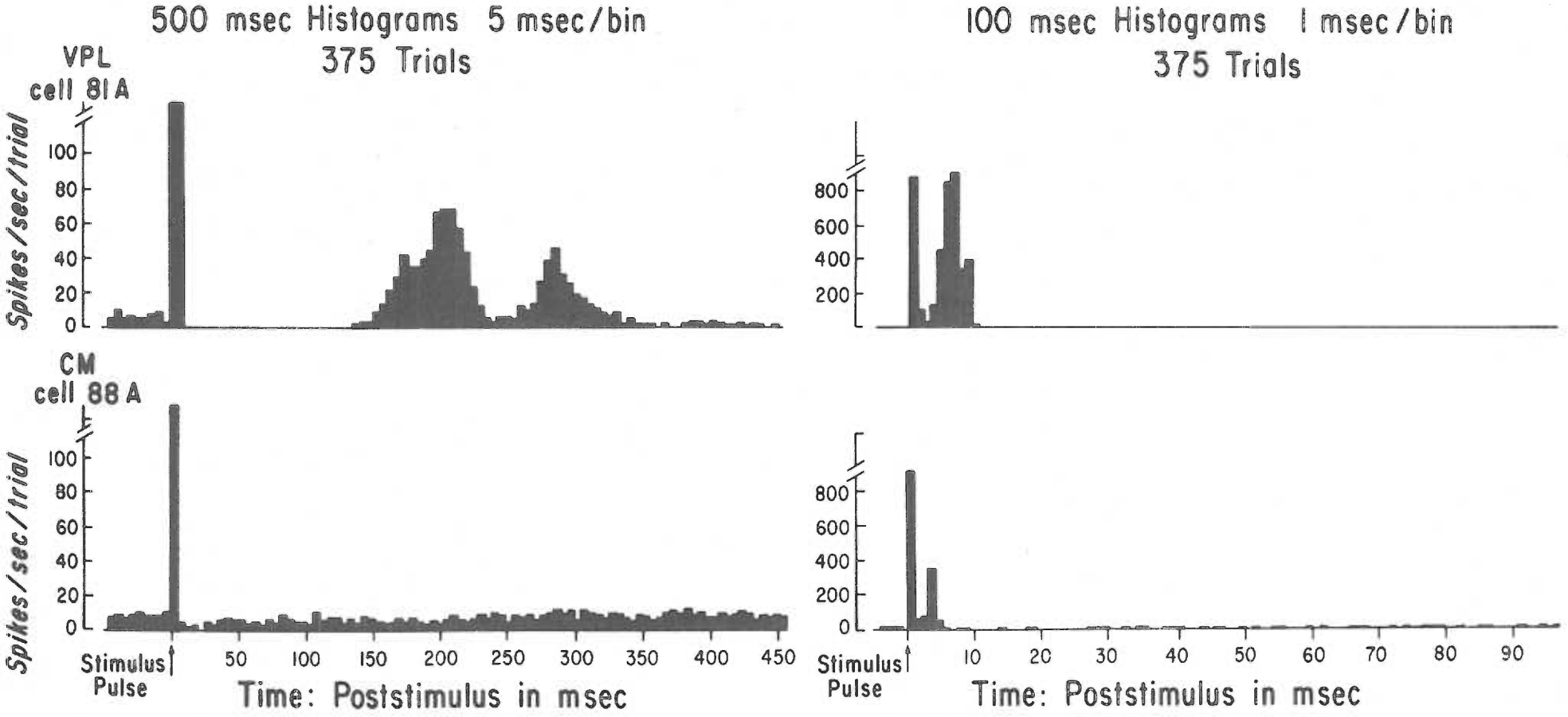

Response to Antidromic Stimulation
Cell 76C
spikes/sec/trial
40
30
20
10
0
Cell 82C
spikes/sec/trial
140
120
100
80
60
40
20
0
CS
US
150
300
450
600
750
900
1050
1200
1350
Time: Poststimulus in msec

Figure 12: Poststimulus response histograms of PT cells for single pulse thalamic stimulation. Each histogram on the right is an expanded time scale representation of the identical data on the left. Upper histograms - response to stimulation of VPL. Lower histograms - response to stimulation of CM. Stimulus artifact is part of the first large amplitude bin in each histogram.

SINGLE PULSE THALAMIC STIMULATION



pulse stimulation of a specific (VPL) and nonspecific (CM) thalamic nucleus. As can be seen in this figure, stimulation of specific and non-specific thalamic nuclei both resulted in short latency responses in PT cells. However, stimulation of the specific thalamic nucleus also produced a characteristic strong inhibitory period followed by a rebound excitatory period. This pattern of early excitation, inhibition and later rebound excitation was observed in response to single pulse stimulation of both VPL and VL. Single pulse stimulation of a nonspecific thalamic nucleus did not produce this pattern of activity. Table 4 presents the average values for the excitatory and inhibitory response intervals for single pulse stimulation of VPL, VL and CM.

Figure 13 shows the response histograms of PT cells to triple pulse (250 Hz) stimulation of specific and nonspecific thalamic nuclei. As can be seen, the response of the cell to specific thalamic (VPL) stimulation was modified. The early response was buried in the stimulus artifact. The characteristic period of inhibition and rebound excitation remained but a new excitatory response period appeared during the period of inhibition. Triple pulse stimulation of the nonspecific thalamus (CM) produced a more dramatic change in the response pattern. The period of inhibition and rebound excitation seen for specific thalamic stimulation was present. In addition, there was a strong early excitatory response as well.

The pattern of early excitation, inhibition and rebound excitation was a highly reproducible phenomenon. In an attempt to investigate some of the characteristics of this phenomenon, the following experi-

Table 4: Response intervals for single pulse thalamic stimulation in ms poststimulus.*

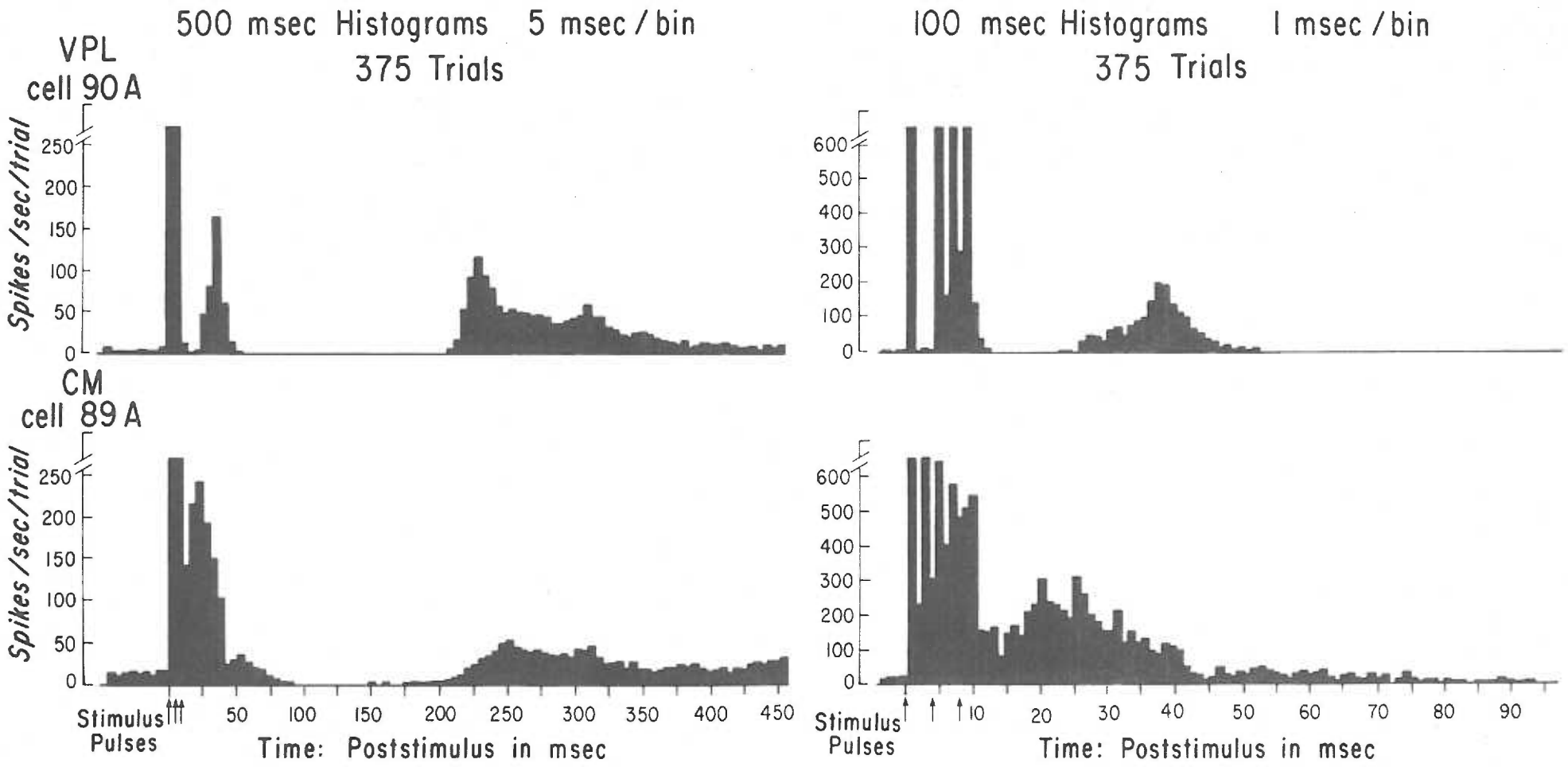
Nucleus	early excitatory	inhibitory	rebound excitatory
VPL	2-11	13-132	137-276
VL	3-9	7-137	140-264
CM	2-10	12-144	**

*discrepancies between end of one interval and beginning of the next due to unequal N for different intervals

**only one animal had a rebound excitatory response to CM stimulation

Figure 13: Poststimulus response histograms of PT cells for triple pulse thalamic stimulation. Each histogram on the right is an expanded time scale representation of the identical data on the left. Upper histograms - response to stimulation of VPL. Lower histograms - response to stimulation of CM. Note stimulus artifact contribution to the large amplitude early latency bins in all histograms.

TRIPLE PULSE THALAMIC STIMULATION



ment was performed in one animal. The results are depicted in Figure 14. The top histogram shows the response of a PT cell to triple pulse stimulation of CM paired with PT activation at the normal CS-US interval of 550 ms. The characteristic early excitatory, inhibitory and rebound excitatory periods were present in the response. Note as well the large E-UR in response to the PT stimulation. After this cell had been run through the complete conditioning paradigm, the CS-US interval was shortened to 60 ms so that the PT stimulation would occur during the inhibitory response period for the CM stimulation.

As can be noted in the figure, there was a marked change in the response pattern. On the basis of latency, the first excitatory period following US offset resembled the E-UR. The second excitatory period, which followed a complete inhibition of activity, resembled the previous CM response with a 550 ms CS-US interval except that the onset of this response was shifted an additional 165 ms further poststimulus. It would appear that for the most part, the systems responsible for the generation of the E-UR and the rebound excitatory response period are segregated from each other in separate pathways.

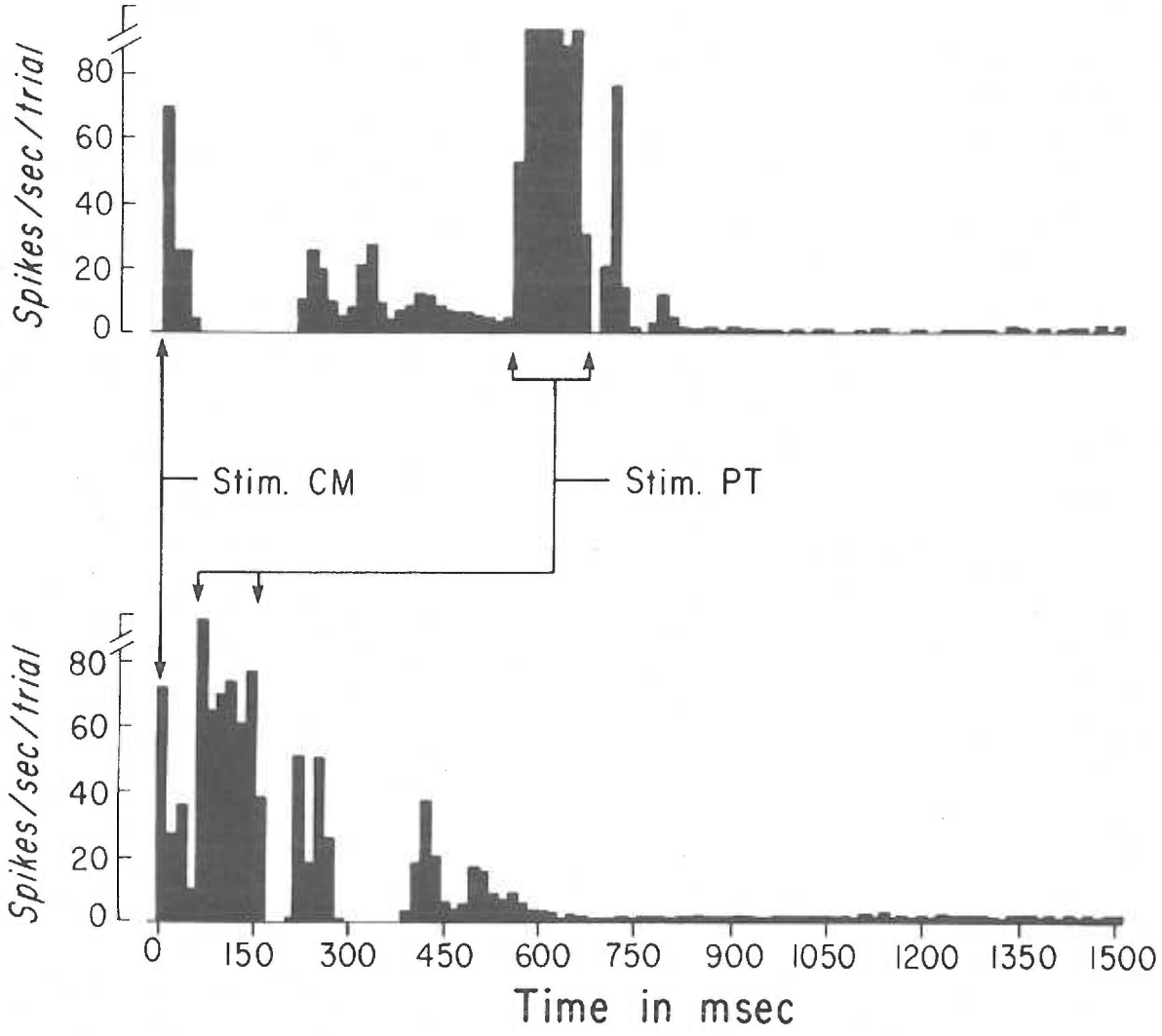
Response Plasticity of PT Cells

In experiments 1 and 2, single pulse thalamic stimulation was used as the CS. The results of these experiments are displayed in Figures 15 and 16 respectively. Because single pulse stimulation of CM did not result in a rebound excitatory response (see Figure 12), experiment 3 was run in which triple pulse stimulation was applied. As Figure 13 demonstrates, triple pulse stimulation of CM did result in a rebound excita-

Figure 14: Poststimulus response histograms of PT cell for triple pulse thalamic and high frequency pyramidal tract stimulation. Upper histogram - response of cell with a 550 ms interval between thalamic and pyramidal tract stimulation. Lower histogram - response of same cell with a 60 ms interval between thalamic and pyramidal tract stimulation.

PAIRED STIMULATION

Cell 94-225 Trials



tory response in PT cells. Because of the low N (CS+: VPL, N = 3; CS+: CM, N = 3), the results of experiment 3 were not analyzed as a separate group to determine whether differential conditioning occurred. However, the data from experiment 3 were pooled with the data from experiments 1 and 2 to run the analysis displayed in Figure 18.

In experiment 1, VPL and VL stimulation served as the CS+ and CS- (counterbalanced) in the conditioning paradigm. The change in response of these neurons across trials is depicted in Figure 15, for both the early and rebound excitatory response period. The total N for the early excitatory response was less than that for the rebound excitatory response because some cells showed a short latency response only to VPL or VL. A three-way analysis of variance (ANOVA) (repeated on two) was used to test each set of data (early and rebound). Since the analysis in both cases revealed no statistically significant differences in the way the cell responded to VPL versus VL, the data were collapsed across this factor and graphed as CS+ versus CS-. In neither case did the ANOVA indicate any statistically significant trends in the data. There was no overall difference in the responses of cells to the CS+ versus CS- and the response of cells did not change across trials.

In experiment 2, stimulation of CM served as the CS+ and stimulation of VPL as the CS-. As mentioned above, single pulse stimulation of CM did not produce a rebound excitatory response in the neuron. Thus, Figure 16 depicts the change in response of cells for the early excitatory response period only. A two-way ANOVA (repeated on two) was run on the data and indicated that there was a significant overall difference in the response of the neurons to CM versus VPL stimulation

Figure 15: Response of PT cells to thalamic stimulation during administration of conditioning paradigm. Upper graph - early excitatory response period. Lower graph - rebound excitatory response period. H = blocks of habituation trials, C = blocks of conditioning trials, E = blocks of extinction trials.

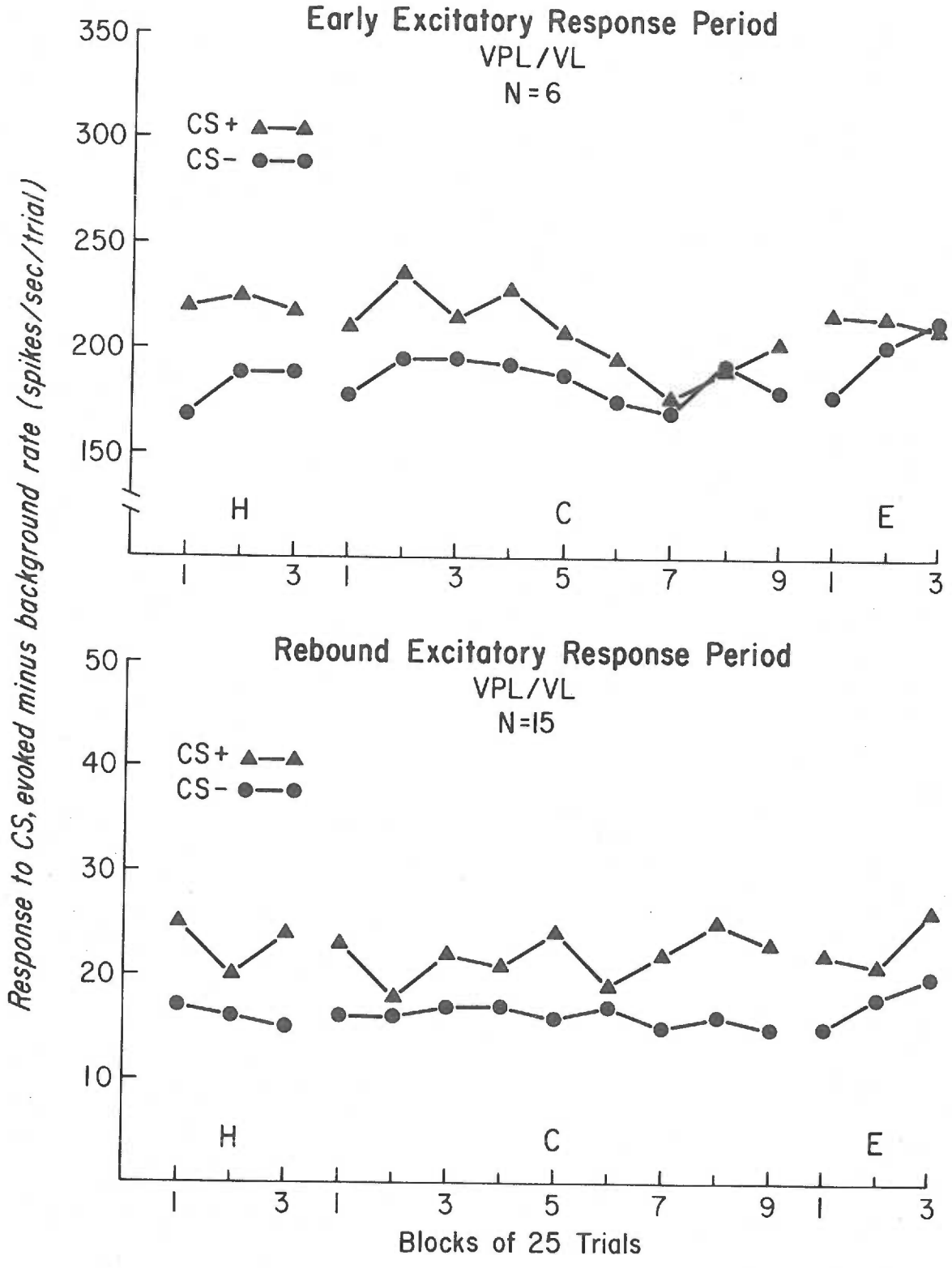
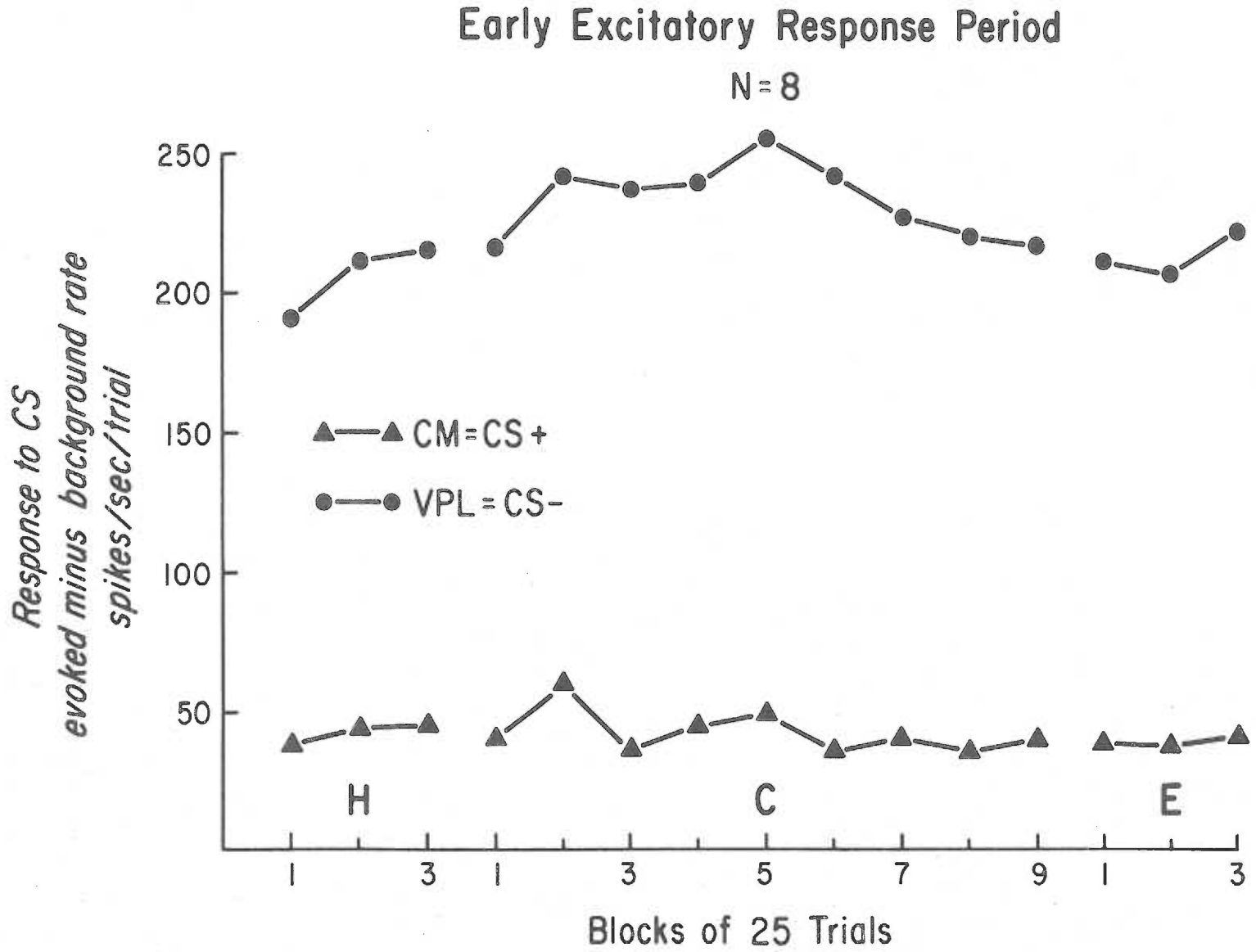

Early Excitatory Response Period
VPL/VL
N=6
CS+
CS-
350
300
250
200
150
H
C
E
1
3
1
3
5
7
9
1
3
Rebound Excitatory Response Period
VPL/VL
N=15
CS+
CS-
50
40
30
20
10
H
C
E
1
3
1
3
5
7
9
1
3
Blocks of 25 Trials
Response to CS, evoked minus background rate (spikes/sec/trial)

Figure 16: Response of PT cells to thalamic stimulation during administration of conditioning paradigm. Early excitatory response period for CM and VPL stimulation. H = blocks of habituation trials, C = blocks of conditioning trials, E = blocks of extinction trials.


Early Excitatory Response Period
N = 8
Response to CS
evoked minus background rate
spikes/sec/trial
250
200
150
100
50
CM = CS +
VPL = CS-
H
C
E
1
3
1
3
5
7
9
1
3
Blocks of 25 Trials

($F(1,7) = 28.4$, $p < .005$). There was also a significant change in response of the neurons across conditioning trials only ($F(8,56) = 2.61$, $p < .05$). However, the analysis did not reveal any differential effect of CS+ versus CS- stimulation as a function of trials. Inspection of Figure 16 suggested that the response of the cells to the CS-(VPL) increased during conditioning while the response to CM did not show any systematic change as a function of trials.

As indicated above, neurons showed one of two different types of UR, a complete inhibition of firing (I-UR) or a pattern of inhibition with marked excitation (E-UR). The possible relationship of the type of UR and the change in response of neurons during conditioning was investigated. The "grand t score" (t_g) (see METHODS) was used as a single value indication of the overall response change during conditioning versus habituation and extinction. A t test was run comparing the overall t_g for cells from experiments 1, 2 and 3 which had an E-UR versus an I-UR. The t_g scores were collapsed across CS+, CS- and all available response periods to yield a single score for each cell. The result of this analysis indicated that cells with an E-UR tended to show an increase in response during conditioning while cells which had an I-UR tended to show a decrease in response during conditioning (E-UR = +.98, I-UR = -.35; $t(27) = 3.29$, $p < .01$).

This result suggested that the type of UR was important in determining the nature of the conditioning process. To assess this possibility more accurately, the responses of cells in experiment 1 during the rebound excitatory response period were reanalyzed with an additional

factor added - type of UR. The response of these cells is displayed in Figure 17. As suggested by the analysis of the t_g scores, the response of cells with an E-UR was on the average, higher than cells which had an I-UR. However, the ANOVA did not reveal any statistically significant trends in any aspect of the data.

The above results indicated that the cells in experiment 1 and 2 did not show differential conditioning. However, the results of the analysis of experiment 2 and the t_g analysis on experiments 1, 2 and 3 suggested that the presence of nonspecific thalamic (CM) stimulation and an E-UR tended to increase the reactivity of the neuron to specific thalamic (VPL) stimulation. In order to examine this possibility more systematically, the following analysis was performed. The rebound excitatory response period for VPL stimulation was separated into four subgroups, collapsed across the CS+/CS- factor for experiments 1, 2 and 3. The response of the cells to VPL stimulation across blocks of trials was grouped for cells which were: 1) presented with CM stimulation and had an E-UR; 2) presented with CM stimulation and had an I-UR; 3) not presented with CM stimulation and had an E-UR; 4) not presented with CM stimulation and had an I-UR.

The data are depicted in Figure 18. There was a significant effect of the presence of CM stimulation as part of the paradigm on the overall response of the cells to stimulation of VPL ($F(1,25) = 10.4$, $p < .01$). That is, with CM stimulation present, the response of the cells to VPL stimulation was greater than when CM stimulation was not present. There was also a significant effect of the presence of an E-UR versus

Figure 17: Response of PT cells to thalamic stimulation during administration of conditioning paradigm. Same data as portrayed in lower graph of Figure 15 but grouped according to type of response (E-UR or I-UR) following US offset. H = blocks of habituation trials, C = blocks of conditioning trials, E = blocks of extinction trials.

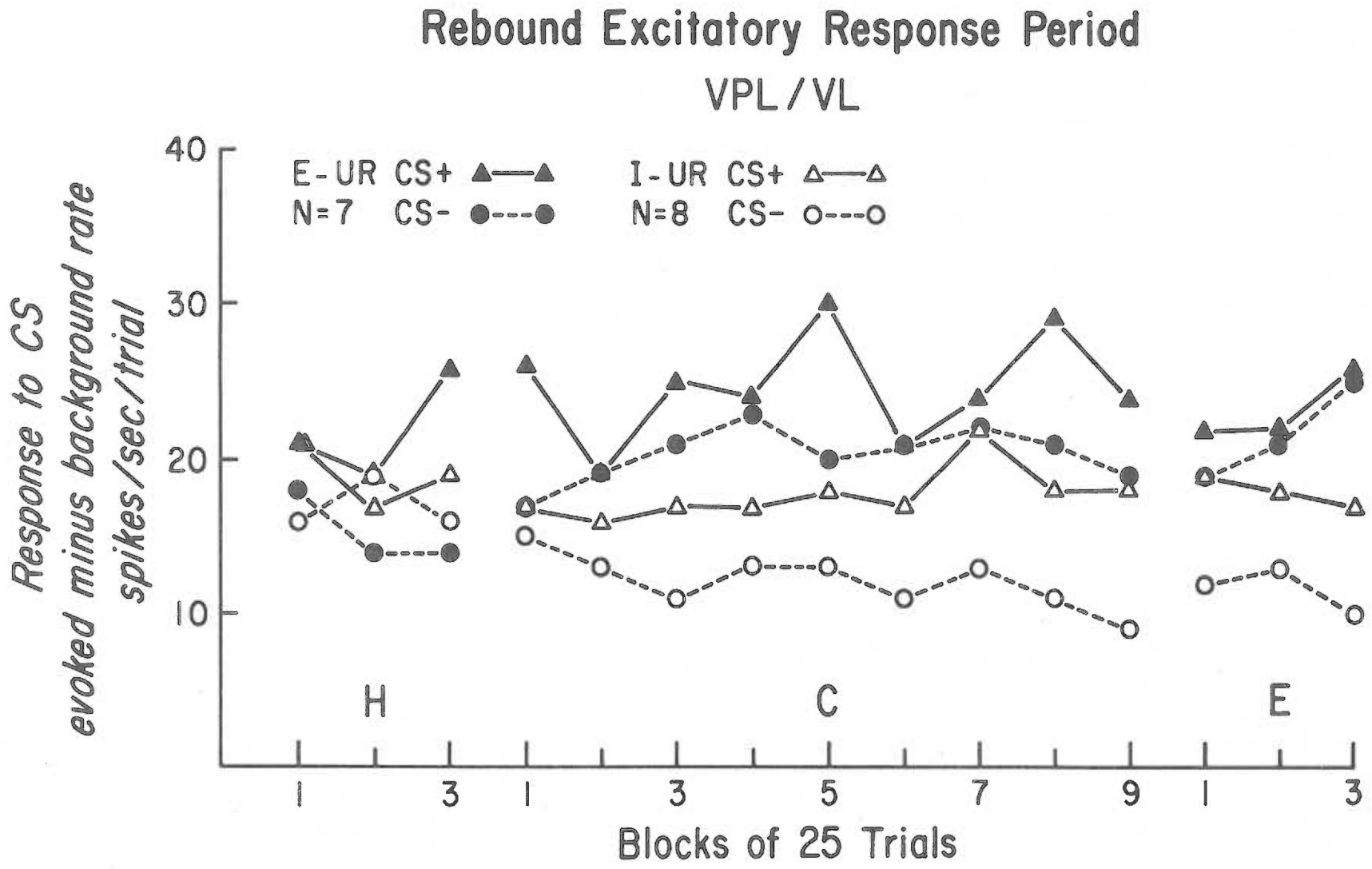
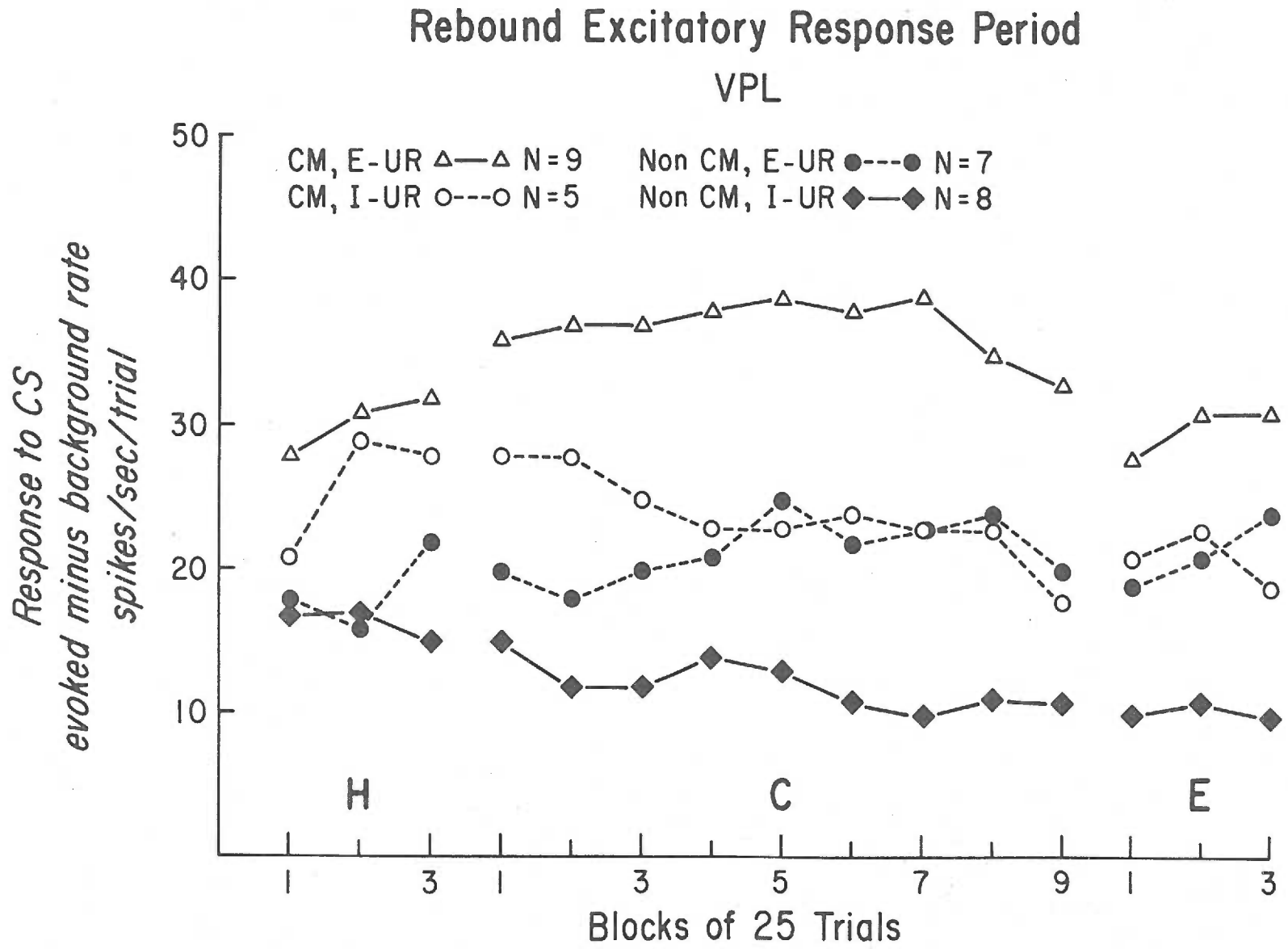

Rebound Excitatory Response Period
VPL / VL
E-UR CS+
N=7 CS-
I-UR CS+
N=8 CS-
Response to CS
evoked minus background rate
spikes/sec/trial
40
30
20
10
H
C
E
1
3
1
3
5
7
9
1
3
Blocks of 25 Trials

Figure 18: Response of PT cells to thalamic stimulation during administration of conditioning paradigm. Rebound excitatory response to stimulation of VPL. Data grouped according to presence or absence of CM stimulation as the other CS and presence of response (E-UR vs I-UR) following US offset. H = blocks of habituation trials, C = blocks of conditioning trials, E = blocks of extinction trials.


Rebound Excitatory Response Period
VPL
CM, E-UR △—△ N=9
CM, I-UR ○---○ N=5
Non CM, E-UR ●---● N=7
Non CM, I-UR ◆—◆ N=8
Response to CS
evoked minus background rate
spikes/sec/trial
50
40
30
20
10
H
C
E
1
3
1
3
5
7
9
1
3
Blocks of 25 Trials

an I-UR on the overall response of the cells to stimulation of VPL ($F(1,25) = 6.09$, $p < .05$). That is, with an E-UR present, the response of the cells to VPL stimulation was stronger than when an I-UR was present.

In addition, there was a significant effect of blocks of trials on the response of the cells ($F(14,350) = 1.79$, $p < .05$), and a significant interaction between the presence of an E-UR versus an I-UR and blocks of trials ($F(14,350) = 2.81$, $p < .01$). That is, the response of the cells to VPL stimulation changed across trials in a manner that was different for those cells which had an E-UR versus an I-UR. When the subgroup analyses were performed, it was determined that only the "CM, E-UR" cells showed a significant change in response over trials ($F(14,112) = 2.60$, $p < .01$). That is, the response of the cells to VPL stimulation changed across trials only when CM was the other nucleus stimulated and only when the cells had an E-UR.

DISCUSSION

Electrode Placement

The position of the stimulation electrodes and, more importantly, the effective spread of the current from the electrodes are critical issues in the interpretation of the results of this experiment. As described in the RESULTS section, the electrophysiological and histological analysis indicated that the stimulating electrodes were positioned in the desired locations. However, the amount of current spread due to stimulation is more difficult to ascertain. In this study, the two most closely positioned electrodes were the VPL and VL electrodes in experiment 1. On the average, they were 2.7 mm apart.

It was felt that these electrodes stimulated two separate populations of thalamic neurons. There has not been a great deal of research investigating the effective spread of stimulating current from electrodes in the CNS. Ranck (1975) presented a comprehensive review but ignored the work using bipolar electrodes, citing only monopolar studies. Fuller (1975) presented one of the few estimates for current spread for bipolar electrode stimulation. He used concentric electrodes with a tip-barrel distance of 750 um and estimated that the spread of effective current for axonal stimulation was approximately 300 um for current intensities in the 100 to 400 uA range. Fuller's electrode construction parameters (amount of tip bared, tip-barrel separation) match fairly well the ones used in the present experiment (except for the use of concentric vs. double-barrel construction). The current range for the present experiment (see Figure 6) also falls well within the range he

studied.

It thus seems reasonable to conclude that Fuller's estimate is a good approximation for the present experiment. Allowing for an increased spread in the present experiment of even 100% (Fuller bared .25 mm of the tip vs. .5 mm in the present experiment) to 600 um still indicates that the current spread in the present experiment most likely was limited to the region immediately surrounding each electrode, and thus it was concluded that no two electrodes directly activated a common pool of neurons.

Responses of PT Cells

Response to Thalamic Stimulation

As described in the RESULTS section (see Figures 12 & 13) PT cells had a stereotyped response to thalamic stimulation. It consisted of a short latency excitatory response followed by an inhibitory period of approximately 130 ms, and ended in a rebound excitatory response. This pattern could be observed for both specific (VPL or VL) and nonspecific (CM) thalamic stimulation. Using triple pulse stimulation, a fourth response period appeared immediately following the short latency excitatory response (Figure 13).

These findings are in agreement with many previous studies documenting the responses of cortical neurons to thalamic stimulation. Schlag and Balvin (1964) divided the response of PT cells to thalamic stimulation into five distinct phases: 1) short latency excitation (1-6 ms); 2) & 3) a period of disinhibition and potentiation (6-25 ms); 4) inhibition (25-100 ms); and 5) a period of rebound excitation at the

end of the inhibitory phase.

They found that the development of augmenting and recruiting responses depended on the reoccurrence of stimulation during a particular phase of the response to previous thalamic stimulation. This led to the development of responses during phases 2 and 3 which, under single stimulus situations, were not present. The results of Schlag and Balvin (1964) agree quite well with the findings of the present study. Phases 1, 4 and 5 were seen with single pulse stimulation of VL and VPL (Figure 12). With triple pulse stimulation, there was an excitatory response whose latency corresponded with that described as phases 2 and 3 by Schlag and Balvin (1964).

The major question of interest with regard to these findings concerns the neural mechanisms underlying the phasic response pattern seen. Several alternatives exist. The cortical responses could be driven by an intrathalamic generator, by a cortical generator or by a combination of these two general systems. These possibilities have been studied since Morison and Dempsey first described the development of augmenting and recruiting responses. The results have not been clear cut.

Purpura and Cohen (1962) studied recruiting activity in thalamic neurons and concluded that an intrathalamic mechanism probably was responsible for generation of recruiting responses. Similarly, Schlag and Villablanca (1968) concluded that the potentiation of thalamic neuronal responses they observed with repeated thalamic stimulation (in VL) had a thalamic origin since they obtained identical results in decorticate preparations. The assumption that both studies make is that the

pattern (excitation, inhibition, excitation) and potentiation of thalamic responses upon repeated stimulation cause the pattern and potentiation of the cortical responses. This is not necessarily so.

Steriade and Yossif (1977) and Morin and Steriade (1981) concluded that the phasic response to Vb stimulation was a cortical phenomenon. In particular, Steriade and Yossif (1977) found that the pattern of inhibition and rebound excitation was observed with white-matter stimulation following Vb lesions, suggesting that the pattern depended primarily on cortical circuitry.

Thus there does not appear to be a straightforward answer to the question concerning the mechanisms responsible for generation of the pattern of PT cell response observed. It would appear that there are both intrathalamic and intracortical mechanisms which are capable of producing the pattern of neuronal response observed. When both systems are intact, they presumably reinforce each other. Steriade and Yossif (1977) did state that although it was possible to observe the phasic response pattern in cortical neurons with lesions of the Vb complex, the response pattern was stronger with the thalamus intact.

Response to PT Stimulation

The lack of frequency following to PT stimulation in a subgroup of PT cells was an unexpected finding (Figure 10), since frequency following had been considered by many as an adequate test for antidromic activation. A careful review of the literature revealed, however, that the finding in the present study was not without precedence. As mentioned in the INTRODUCTION, Stefanis and Jasper (1964b) reported failure of PT

cells to show frequency following to PT stimulation. They conducted a systematic study of the pattern of antidromic spike failure in PT cells by applying short trains of antidromic stimulation and measuring the tendency for test antidromic spikes to invade the cell body at various intervals following train offset. They concluded that the failure of test antidromic spikes to invade the cell body was due to recurrent collateral inhibition set up by the conditioning antidromic spike train.

Thus it would seem reasonable to conclude that the lack of frequency following in the present experiment was due to the same phenomenon. The fact that fast PT cells tended to follow PT activation while slow PT cells did not suggests that the pattern of recurrent collateral inhibition has a stronger effect on the slow PT cells. Takahashi et al. (1967) concluded that facilitation via recurrent collaterals occurred only in fast PT cells. This facilitation, however, was mediated by activity in slow PT cells. Taken together, these data suggest the existence of a complex feedback control system which features positive feedback on fast PT cells and negative feedback on slow PT cells.

It was also noted in the present study that PT cells exhibited one of two response patterns following offset of the antidromic pulse train: an excitatory or inhibitory UR (E-UR, I-UR). An important question concerns the site of origin of this effect. That is, were the post-pulse train alterations in PT firing rate due to activation of an intra- or extra-pyramidal system? The fact that cells which had an E-UR had a significantly higher threshold for antidromic activation suggests that the development of an E-UR could be due to spread of stim-

ulus current outside the PT to adjacent structures.

However, it should be noted that relatively low stimulus voltages were applied for both E-UR and I-UR cells. According to Figure 6, the average voltage level for E-UR cells corresponded to an applied current of approximately 120 uA. This current value is at the lower end of the range which Fuller (1975) used in estimating an effective current spread of 0.3 mm from his stimulating electrodes. The differences in electrode design between Fuller's and the present study have been noted. It still seems reasonable to conclude that the spread of effective current in the present study, based on Fuller's data, was insufficient to activate orthodromic pathways outside the PT.

However, the problem of explaining the voltage difference between E-UR and I-UR cells still remains. The average stimulus voltage for I-UR cells was 3 V. Extrapolating from the data of Figure 6, one can estimate that this corresponds to a current level of 25 uA. It would appear that for PT cells having such a low threshold voltage level for antidromic activation, the spread of current was very limited. If one makes the assumption that PT cells with higher threshold levels for antidromic activation (and E-URs) were further from the PT stimulating electrode and PT cells with lower thresholds for antidromic activation (and I-URs) were closer to the stimulation electrode, then one can conclude that the major difference between E-UR and I-UR cells was the total volume of PT tissue activated.

Stefanis and Jasper (1964b) described firing patterns in PT cells similar to the E-UR observed in the present study. They attributed it

to a rebound effect from the collateral inhibition produced by the antidromic stimulation (note the similarity to the mechanism of rebound excitation for thalamic activation proposed by Schlag and Balvin [1964]). Stefanis and Jasper (1964b) also noted that the collateral inhibition could be increased by increasing the stimulus voltage applied to the PT. Taking these data into account, one can conclude that the E-UR in the present study was due to a rebound excitation of the PT cells following recovery from collateral inhibition.

Presumably, a certain minimal amount of collateral inhibition is required before the rebound excitation can take place. In cells with an I-UR, there is minimal current spread at the stimulation site and only a limited number of PT fibers are activated. These are sufficient to produce a certain level of collateral inhibition but only when additional fibers are activated by a stronger stimulus is the inhibition deep enough to trigger the rebound excitatory effect, resulting in the E-UR. Admittedly, this explanation requires several assumptions, but the alternative explanation, spread of stimulus current to an extrapyramidal structure, seems unlikely in light of the data on current spread.

A first step towards investigating the mechanisms underlying the E-UR as it relates to the generation of the response to thalamic stimulation was described in Figure 14. As pointed out in the RESULTS section, with a 60 ms CS-US interval, a response analagous to the thalamic rebound excitatory response appeared but with a latency delayed (by 165 ms) relative to the response seen with a 550 ms CS-US interval.

These data seem to reinforce the hypothesis that the rebound excitatory response was indeed exactly that, that is, a rebound effect from inhibition and not the result of an excitatory synaptic barrage. In this framework, the determining synaptic events from the thalamic input are the inhibitory ones which cause the long period of inhibition following the initial excitatory drive to the PT cells.

It would appear from Figure 14 that the interpolation of the PT pulse train into the inhibitory response period for the thalamic stimulus caused a "resetting" of the neuronal response. By activating the neuron during a period in which it would otherwise have been inhibited, the PT pulse train reset the circuitry determining the inhibitory response and its concomitant rebound excitation. The period of inhibition for thalamic stimulation with a 550 ms CS-US interval was 165 ms, this was precisely the time of delay for the rebound excitation to occur with the 60 ms CS-US interval.

To summarize the conclusions which Figure 14 suggest: 1) the data reinforce the idea that the rebound excitatory response is a function of inhibition and not of a separate excitatory synaptic drive since it is almost impossible to conceive of a mechanism whereby an excitatory response could be "held" at a synapse while a separate excitatory drive (in this case PT activation) caused neuronal activation, and then have the "hold" released and the neuron driven by the delayed excitatory input; 2) the data lend support to the hypothesis that it is cortical as opposed to thalamic circuitry which is responsible for the inhibition and rebound excitation. The major effect of PT stimulation is the

antidromic firing of the neuron and the orthodromic activation of the cortical recurrent collateral system. The fact that the PT pulse train was able to reset the thalamic response indicated that the two systems were able to interact extensively. The most logical site for this interaction, considering the systems involved, is the cortex. However, it must be pointed out that the known PT collaterals to the thalamus provide an alternate site of interaction.

Response Plasticity of PT Cells

The inability to obtain differential classical conditioning in the present study (see Figures 15, 16, & 17) was a surprising result. There appeared to be ample precedence in the literature both for the development of plasticity in neurons in the pericruciate cortex (O'Brien & Fox, 1969 a&b) and the ability of antidromic activation of the neuron to serve as an effective US (O'Brien et al. 1977). Furthermore, Baranyi and Feher (1978 & 1981) demonstrated response plasticity in PT neurons to electrical stimulation of VL by pairing VL stimulation with antidromic activation of the PT cell.

Black-Cleworth et al. (1975) were able to condition a behavioral response (eye blink) using electrical stimulation of the motor branch of the facial nerve as the US. In related work, Mis, Gormezano and Harvey (1979), Martin, Land and Thompson (1980) and Powell and Moore (1980) all reported some measure of success in obtaining conditioning of nictitating membrane extension using electrical stimulation of the abducens nucleus as the US. Taken as a whole, these data weigh heavily in favor of the ability of central stimulation of motor output systems to

serve as effective USs.

The major question for the present study is why PT neurons failed to exhibit plasticity in their firing pattern as a consequence of the administration of the differential classical conditioning paradigm. One can divide the potential causes for this failure into two broad categories: 1) problems related to the parameters selected for the conditioning paradigm; 2) problems related to the functional characteristics of the neural system studied.

Parametric Features

One can subdivide this category into three areas. The first possible cause was selection of the wrong type of CS. That is, insufficient activation of a pathway could have resulted in a lack of conditioning. In the present study, both single and triple pulse stimulation were used. Both types produced a very definite alteration in the firing pattern of the PT cell. Thus it would seem that this possible cause was unlikely. As reviewed by Doty (1969), central stimulation as a CS has a long history of being effective in producing behavioral conditioning.

A second possibility was the selection of the wrong type of US. This too is an unlikely possibility. O'Brien et al. (1977) obtained conditioning using the exact same US as the present study employed and Baranyi and Feher (1981) were able to obtain conditioning using single pulse stimulation of the PT as the US. Bindman et al. (1979) demonstrated long term (up to 3 hrs) increases in the excitability of PT cells following continuous stimulation of the PT (100 Hz) for periods

of 5 to 10 minutes. They attributed this effect to a postsynaptic mechanism. Tzebelikos and Woody (1979) also found changes in the excitability of PT cells (measured intracellularly) as a function of low frequency (1-6 Hz, 5-30 min) antidromic stimulation.

These data indicate that antidromic activation of the PT cells can produce sensitization or associative conditioning changes in neurons, depending on the paradigm employed.

A third possibility was the selection of an incorrect CS-US interval. In the present study, the CS-US interval was 550 ms which was the interval used successfully by O'Brien et al. (1977). However, Baranyi and Feher (1981) stated that they were only successful in obtaining conditioning when using a CS-US interval of less than 100 ms. They used an extremely short (1-3 s) intertrial interval (ITI). Voronin (1980) reported that use of a short (1-5 s) versus a long (12 s - 2 min) ITI tended to result in nonassociative changes in neuronal firing patterns during conditioning experiments in which central stimulation was used as the CS and US. In spite of the differences in ITI between Baranyi and Feher's and the present study, their finding concerning the optimal interval could be considered more relevant to the present study than that of O'Brien et al. (1977) since the former study used central stimulation as the CS whereas the latter used peripheral stimulation as the CS. However, the data from the present study indicated that forepaw stimulation required only 7 ms to reach the thalamus. Thus there was a minimal difference between the two studies in the time it took for input to reach initially the PT cell. There are obviously some major

differences between central and peripheral activation of thalamocortical pathways. Whether this could have contributed to the failure to obtain conditioning will be discussed below.

In summary, it would appear that the parameters selected for use in the present study should have been sufficient to support conditioning.

Functional Features

There are two interrelated considerations which are relevant to the outcome of the present study. First, as reviewed in the INTRODUCTION, there is an extensive body of literature detailing the differences between specific and nonspecific thalamic systems. Conceivably, neuronal plasticity could be a function of one system and not both. Swett and Bourassa (1980) demonstrated that the threshold for behavioral detection of Vb stimulation in the cat was much higher than the threshold for detection of stimulation of a peripheral nerve. These considerations led to the running of experiment 2 in which stimulation of a nonspecific nucleus (CM) was used as a CS+. However, both specific and nonspecific thalamic stimulation were ineffective CSs.

A second and interrelated possibility has to do with the overall pattern of activation in the PT neuron. The major theoretical basis for this study was the "pairing hypothesis" for neural models of associative conditioning. Briefly put, this hypothesis states that when synapse A is repeatedly activated in conjunction (i.e., paired) with separate activation of the postsynaptic membrane then the tendency for activity in synapse A to produce a stronger response in the postsynaptic membrane is increased.

In contrast to some traditional theories of behavioral learning, this neural pairing hypothesis does not have a requirement for the neural equivalent of a particular motivational state. All that is necessary is the activation of one synapse paired with the activation of the postsynaptic membrane by some other means. Kandel and Tauc (1965) tested this hypothesis in the relatively simple nervous system of the aplysia. They demonstrated that the amplitude of the postsynaptic potential produced by a weak stimulus to one pathway was capable of being facilitated when it was paired with a more effective activation of a second pathway. Thus, the pairing hypothesis, in its broadest interpretation, implies that synaptic reinforcement via pairing is a basic property of all neurons. If this is true, and if the proper parameters were selected for use in the present study, then one should have observed the development of a neuronal conditioned response. The failure of the present experiment to demonstrate conditioning undermines support for the simple hypothesis of pairing.

The only parametric difference between the present study and O'Brien et al. (1977) was the method of activation of the neuron for the CS+ and CS- (central vs. peripheral). However, the CS input to the PT cells in the latter study, in all likelihood, passed through the thalamus, with a major relay in VPL and associated input from the nonspecific thalamus. The critical difference between the two studies must have been the pattern of synaptic bombardment of the PT cell which would be quite different for thalamic versus peripheral stimulation.

One can distinguish two general ways in which the pattern of activ-

ity produced by peripheral stimulation would differ from the pattern produced by central, thalamic stimulation. First, peripheral limb stimulation activates a set of different types of sensory afferents: tactile, pressure, pain, temperature, etc. The particular input pathways activated also depend on which limb (fore- or hindlimb, ipsi- or contralateral) is stimulated. Input from one or more of these systems could have been the key element in the O'Brien et al. (1977) study which was responsible for the development of plasticity in the PT cell. Thalamic stimulation would not produce the same pattern of activation of different sensory afferents as peripheral stimulation would. Swett and Bourassa's (1980) finding that the threshold for detection of Vb stimulation was much higher than that for peripheral stimulation is relevant to this point.

The second general way in which peripheral and thalamic stimulation would differ in their effects on PT cells is in the temporal pattern of activity in the PT cell which would result from the stimulation. It should be noted that this possibility is intimately related to the first one. Thalamic and peripheral stimulation produce very different response patterns in PT cells. As noted in the present study, the response of PT cells to thalamic stimulation is a stereotypic one, varying little across animals. The response to peripheral stimulation has considerable variability. The same temporal component of the poststimulus histogram can be inhibitory for one PT cell, excitatory for the next.

One should also consider that peripheral stimulation results in the sequential activation of particular thalamic and other subcortical

structures. Perhaps it is the ordered activation of specific and nonspecific nuclei as well as other subcortical structures which is essential for the development of neuronal plasticity.

The implication of the work of O'Brien et al. (1977) and the present study, taken together, is that a simple pairing of neural events is not sufficient to produce neuronal plasticity.

Thus the pattern and type of synaptic bombardment which serve as the CS could be key elements in producing neuronal associative conditioning. The data displayed in Figure 18 are particularly interesting in this light. The data indicated that activation of a particular type of pathway (nonspecific thalamocortical fibers - CM) was a factor in producing a response change in a second type of pathway (specific thalamocortical fibers - VPL). Rosenblum and O'Brien (1977) provide further evidence for the importance of coactivation of the specific and nonspecific thalamic systems in contributing to the development of neuronal conditioning. They found that localized cooling and thus blockade of function in the VA region during the administration of a conditioning paradigm inhibited the development of conditioned responses in neurons.

The data in Figure 18 suggested that the pattern of activation in the US pathway also was important. Thus, the combination of ongoing stimulation of CM combined with an E-UR to the PT pulse train produced an increase in the response of neurons to VPL stimulation over trials. This effect of the US would seem to be due to activation of orthodromic as opposed to antidromic pathways. The O'Brien et al. (1977) study included a control group in which the PT stimulation produced only orthodromic effects on the PT neuron. They observed an undifferentiated in-

crease in response to the CS+ and CS- over trials in this group. It would appear that under these conditions, the results of the present study and that of O'Brien et al. (1977) are in agreement.

In summary, these considerations suggest that, at least in the PT cell system of the cat, a simple pairing hypothesis is not valid. The activation of different types of thalamocortical input pathways in a particular pattern appears to be a necessary feature for the demonstration of neuronal plasticity with an associative conditioning paradigm.

SUMMARY AND CONCLUSIONS

The neuronal basis of associative conditioning in the cat was investigated by pairing stimulation of thalamocortical pathways as the conditioned stimulus with antidromic activation of pericruciate pyramidal tract cells as the unconditioned stimulus in a differential classical conditioning paradigm. Stimulation of both specific and nonspecific thalamic nuclei was used, resulting in a complex but highly stereotyped pattern of activation of the pyramidal tract cell.

Contrary to expectations based both on the available literature and theoretical considerations (i.e., the pairing hypothesis) thalamic stimulation was not an effective conditioned stimulus. The response of pyramidal tract cells to thalamic stimulation did not change as a function of reinforcement with pyramidal tract stimulation. These results led to a reconsideration of the validity of the hypothesis that the simple pairing of any two neural events is the essential mechanism underlying associative conditioning changes. Neither specific nor nonspecific thalamic stimulation by themselves were adequate CSs; however, when stimulation of these two types of thalamic nuclei were included in the same conditioning paradigm (as opposed to using two specific thalamic nuclei), the responsiveness of pyramidal tract cells to specific thalamic stimulation was increased.

These results suggest the possibility that the effective conditioned stimulus in this system may involve a complex, patterned bombardment of the pyramidal tract cell initiated by both specific and nonspecific thalamocortical afferents.

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APPENDIX

Table 5 : Summary of histological results based on atlas of Jasper and Ajmone-Marsan (1954).

	VPL			VL			CM			PT		
Cat	AP	L	V	AP	L	V	AP	L	V	AP	L	V
67	9.5	7.0	0.5	11.5	5.5	1.5				6.5	6.0	-5.0
68	9.5	5.0	0.5	12.0	4.5	0.5				7.0	5.0	-5.0
69	8.5	7.0	2.5	11.0	4.0	1.5				6.0	5.5	-5.0
70	9.5	7.0	0.0	10.5	5.0	1.0				6.0	6.0	-5.5
71	8.0	6.0	-.5	10.5	4.5	1.0				5.0	5.0	-5.5
73	10.0	6.5	2.0	11.5	5.5	2.5				6.0	6.5	-5.0
75	9.5	6.0	0.5	11.0	4.0	1.5				6.0	5.0	-6.0
76	10.5	7.0	1.0	12.5	5.5	2.5				6.5	5.5	-6.0
77	10.0	6.0	-.5	12.0	4.0	1.0				6.0	5.5	-5.0
80	9.5	5.5	-.5	12.0	3.5	0.5				6.5	4.0	-6.0
81	9.5	6.0	1.0				7.0	2.0	0.0	5.0	5.5	-5.0
82	9.5	6.0	-1				7.0	3.0	0.5	6.0	5.0	-5.5
84	9.5	6.0	1.5				7.5	2.0	1.5	5.0	4.0	-6.5
85	10.0	7.0	0.5				8.5	2.0	0.0	7.0	5.5	-5.5
86	10.0	6.5	0.5				7.0	3.0	0.0	6.0	6.0	-5.0
87	9.5	6.5	1.0				7.0	3.0	-1	6.0	5.5	-5.0
88		*						*		6.0	5.0	-6.0
89	11.0	6.5	1.0				9.0	2.5	1.0	7.5	6.0	-5.0
90	10.0	7.0	0.5				7.5	3.0	0.5	6.5	6.0	-5.0
94	10.0	7.0	1.0				7.5	2.5	0.5	5.0	4.5	-6.0
$\bar{X}$	9.7	6.4	0.6	11.5	4.6	1.4	7.6	2.6	0.3	6.1	5.4	-5.4
SD	0.6	0.6	0.9	0.7	0.7	0.7	0.7	0.5	0.7	0.7	0.7	0.5
N		19			10			9			20	

* unable to confirm electrode position due to loss of tissue sections.