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A COMPARISON OF MULTIPLE UNIT ACTIVITY DURING CLASSICAL, INSTRUMENTAL, AND DISCRIMINATION LEARNING USING A NOXIOUS UNCONDITIONED STIMULUS

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Signature James Beardsley

Date May 20, 1968

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

ABSTRACT

a. Statement of problem.

Multiple unit recording is a relatively new electrophysiological recording technique which records spike potentials from many neurons. In the Halas laboratory and others, systematic changes have been observed during classical conditioning but none under other conditioning procedures.

Recently, some have interpreted changes in evoked potentials and multiple unit potentials as due to emotional arousal (fear) and thus related more to a conditioned emotional response (CER) than to conditioning *per se*. If a CER occurs, and not a learning modification, then during classical aversive conditioning a high sustained neuronal response ought to occur. This would reflect the high degree of emotionality displayed behaviorally by the animal throughout classical conditioning. On the other hand, during instrumental avoidance conditioning a decreasing level of neuronal response ought to occur which would reflect the reduced emotionality displayed during this kind of conditioning procedure.

b. Procedure.

Thirteen permanent macroelectrodes were implanted in each of seven cats. A total of twenty sites were implanted in the classical auditory pathways, the reticular system (myelencephalon, mesencephalon, and diencephlon), the limbic system, and the cortex. Standard electrophysiological techniques were used to display the multiple unit activity on an

oscilloscope. The tracings were photographed and then were judged as to the level of their neuronal activity. A three day running average was computed which served as the neuronal dependent variable. Counterbalanced classical aversive, instrumental avoidance, and discrimination conditioning procedures were applied. A 1500 hz tone served as the conditioned stimulus and a mild shock to the right hindpaw served as the unconditioned stimulus.

c. Results.

For all sites taken together a significant linear negative neuronal trend emerged for classical aversive conditioning, and a significant linear positive neuronal trend emerged for instrumental avoidance conditioning. Moreover, the neuronal responses for classical extinction decreased further, and the neuronal responses for instrumental extinction increased significantly. These results do not bear out the prediction stated earlier. The emotional arousal hypothesis is untenable since one would expect the opposite kinds of trends.

Inflections in the neuronal trends in the auditory sites during the two types of conditioning suggested that a neural associative process may have occurred in these sites. The neuronal activity of the reticular structures tended to follow the increasing neuronal trends found in the auditory centers during instrumental conditioning but not during classical conditioning.

Some high positive neuronal-behavioral correlations (as high as .96) occurred during instrumental avoidance conditioning and some high negative correlations (as high as -.88) occurred during classical conditioning.

Due to equipment problems little invariance across cats occurred for discrimination learning; and thus, the data was not presented.

d. Conclusion.

As the conditioned stimulus and the unconditioned stimulus for the two types of training procedures were kept as congruent as possible, the pervasive, remarkably different neuronal patterns which resulted imply that classical aversive and instrumental avoidance conditioning share very little in common in the CNS. Apparently, two quite different reactions occurred in rather widespread areas of the brain.

Clearly, a CER cannot account for the trends in the data found in this study. Nor is the explanation for these contrasting results, particularly during extinction after classical and instrumental conditioning, readily available through any other theory of learning known to the experimenter.

The inflection points in the neuronal trends in the subcortical auditory sites appeared to occur first in the lowest center (cochlear nucleus) and latest in the highest center (medial geniculate) for both types of conditioning (one exception occurs in the inferior colliculus during classical conditioning). These data suggest that the subcortical auditory nuclei function as a unit during learning. There is already considerable data showing that these same nuclei function as a unit durin the transmission of sensory information which therefore indicate that these nuclei are capable of mediating several quite different functions.

INTRODUCTION

Multiple unit recording is a relatively new neurophysiological recording technique that promises to add significantly to the very rapidly growing knowledge of the electrical events in the brain. It is particularly useful because it may be related to single unit recording which in turn is related to the considerable knowledge of neuronal spike and graded potentials.

As a new technique, the basic parameters of the measures of the method need to be explored. Almost all the handful of articles using the technique have turned to this task. The purpose of this study is to review briefly the published literature on the technique and to extend the method's application to the relationships between multiple unit activity and conditioned behavior. Pioneer observations have been made on classical aversive conditioning and it is proposed to extend these observations to include instrumental and discrimination learning.

CHAPTER I

REVIEW OF THE LITERATURE ON MULTIPLE UNIT RECORDING

Early studies used multiple unit recording as a special technique to supplement the record of gross action potentials from nerve fibers. The technique was developed to investigate the case when the nerve fiber carried an irregular distribution of impulses in response to some stimulation. Thus, the method was felt to be particularly adapted to recordings that arose from the asynchronous firing of neurons as the result of gustatory (Zotterman, 1935; Beidler, 1953, 1957; Pfaffman, 1955) or olfactory (Konishi and Zotterman, 1963) stimuli.

However, the method was of limited usefulness until the resulting increase in asynchronous firing could be measured or summarized in some way. It appears that Beidler (1953) developed the first satisfactory summarizing device while working with gross gustatory nerve action potentials. He developed an electronic "integrator" that reflected both the frequency and amplitude of the massed unit discharges. Since then, at least three other laboratories nave independently developed similar summarizing devices (Starr and Livingston, 1963; Weber and Buchwald, 1965; Arduini and Pinneo, 1962). The Arduini group extended the usefulness of the method by applying it to records of massed unit discharge in the spinal nerves and cord.

Starr and Livingston, and the Buchwald laboratories, plus a third laboratory (Schlag and Balvin, 1963), extended the application of the technique even further by recording from the grey matter in nuclei of permanently implanted cats. Curiously, all three laboratories tended to focus their attention on the auditory projection system and the diffuse reticular system.

Once the technique for recording multiple units within the CNS was developed, the type of studies in the United States took on two basic foci: Interest in ongoing activity (background activity, massed unit activity) itself; and the multiple units as a response to some explicit training procedure. The former type usually employed rather long and complex stimuli such as the observation of responses to white noise or sleep over several minutes or hours. The latter type observed neuronal responses as the result of shorter (.5 to 1.5 sec) and simpler (1500 hz tone) stimuli usually carried out in relation to some explicit training procedure (e.g., Pavlovian classical conditioning). The Buchwald and Halas laboratory have made the latter type of investigation.

One other group of workers in France (Guiot <u>et al</u>., 1962; Albe-Fessard <u>et al</u>., 1962, 1963) have utilized multiple unit records in a clinical setting to study Parkinsonism. This group did not use an averaging device since bursts of unit activity were striking enough so that amplitude changes could be observed easily. These investigators appear to have developed the technique independently since they found it impossible to record from white matter. This is surprising because the prior development of the multiple unit technique by all others grew out of records from gross nerve action potentials.

A description of the method in detail and a schematic of the electronic averaging device (integrator) has been reported by Weber and Buchwald (1965). Recently, more refined devices have been installed in the Halas laboratory and the Buchwald laboratory.

The name "multiple unit activity", which has been adopted here as a general descriptive term, arises because it appears that the macroelectrode employed yields a record of the action potentials of an undetermined number of neurons. These neurons are spontaneously firing (usually called ongoing or background activity) or are induced to fire by some stimulus (Buchwald, Halas, and Schramm, 1965; Schlag and Balvin, 1963; Winters _et al_., 1967). A recent report (Halas and Beardsley, 1968) indicates that the method records rapid voltage changes from spikes near the tip of the electrode ranging from a distance of .5 to 1 mm. What exactly contributes to an overall increase or decrease in activity, reflected by changes in the integrator line, cannot be ascertained at the present time. Perhaps the same units may fire more rapidly during the stimulation period, or possibly other previously silent units may begin to fire. From the results of microelectrode studies both could occur in any combination. Alterations in the rate of firing of the same neurons (Morrell, 1960) possibly accounts for the greater part of the change.

By studying photographs of the oscilloscopic tracing and using a high speed movie camera, it is possible to count the distinct spikes that occur. Such a count for any amount of data is extremely tedious but reveals that the larger spikes seen on some records are usually not composed of a number of units simultaneously firing as some investigators have thought (cf. Winters _et al_., 1967). By utilizing electronic counting devices, one ought to be able to count the spikes over larger amounts of data. Amplitudes could be measured as well. The best that can be said at the present time is that an overall change in responsiveness has occurred in the population of neurons sampled by the electrode. It is hoped that further research will shed light on this process.

As a result, the present usefulness of the technique revolves around the degree of sensitivity and specificity of responses to discrete stimuli that are obtainable by the method. For instance, does the overall activity in an auditory, but not a visual site, change as a function of an auditory stimulus, or vice versa? Are behavioral changes reflected in specific multiple unit changes that are not discriminable by other macroelectrode techniques? The answer to these questions is "yes", conclusively.

Halas and Beardsley (1968) showed that a response to a 1500 hz tone occurred in the inferior colliculus (IC) which is an auditory nucleus, but did not occur to light. Also, they demonstrated that the superior colliculus (visual nucleus) responded to light but not to tones. Podvoll and Goodman (1967) showed that thalamic and medial reticular sites showed activity levels closely correlated with the subject's observed behavior even under conditions when the EEG appeared dissociated from behavior (also see Buchwald, Halas, and Schramm, 1965_a, 1966_b). Albe-Fessard (et al., 1967) indicated that multiple unit activity has different characteristics from one nucleus to another and that as an electrode was lowered into the brain its progress could be "tracked" by characteristic changes occurring from one location to another. The latter has been routinely observed in the Halas laboratory and in others (Schlag and Balvin, 1963). Goodman and Mann (1967) and Podvoll and Goodman (1967) reported detectable differences in multiple unit activity under different depths of anaesthesia: "Recording sensitivity was sufficient to permit detection of slight variations in depth (under ether) produced by reflex testing or reduction of vapor concentration. The intensity of behavioral response was consistently matched by the average multiple unit activity rise." Furthermore, "neither systemic blood pressure nor arterial PCO_2 seemed important determinents of multiple unit activity within the range observed."

Others have explored multiple unit responsiveness to other behavioral conditions. Galin (1965) found high variability to tones and white noise in the inferior colliculus (IC). Tones (presented 10-18 times at 500 hz) in the IC usually inhibited unit activity whereas white noise increased them. Units in the cochlear nucleus (CN) increased under both conditions when an appropriate tone was used (some sites did not respond). The presence of electric shock during sustained noise altered ongoing activity significantly in the IC and medial geniculate (GM). Podvoll and Goodman (1967) found that multiple unit responses to white noise habituated at thalamic levels (medial geniculate, centrum medianum) but not below in the auditory system. Initial responses in the mesencephalic reticular formation also habituated. Starr and Livingston (1963) documented decreasing neuronal responsiveness to white noise as they recorded from lower to higher CNS centers.

Explorations of the relationship between EEG and multiple units has been done by utilizing the same recording electrode simultaneously (Schlag and Balvin, 1963; Buchwald, Halas, and Schramm, 1965_a, 1966_b; Winters _et al_., 1967). Buchwald _et al_. found that the usual relationship was one of independence between the EEG and multiple unit response, especially in subcortical sites. The technique showed some dependence in cortical sites as very large spikes observable in the multiple unit record appeared to follow high amplitude slow wave activity (EEG). Similar dependence has been found in the cortex utilizing single unit recording (e.g., Frost and Alexander, 1966). However, Schlag and Balvin, using a different method of displaying the EEG data, found definite dependence depending upon location of the electrode and the degree of alertness of the animal. In the mesencephalic reticular formation Schlag and Balvin showed that when large waves (alpha waves) were preceded and accompanied by increased multiple unit activity, the units appeared to be active in inhibiting the EEG activity. Further, a negative linear relationship was found between the degree of multiple unit activity and the amplitude of the EEG under the conditions of paradoxical sleep (multiple unit activity greatest), alertness (multiple unit activity moderate), and deep sleep (multiple unit activity least). In the sigmoid cortex, on the other hand, spindling was accompanied often by multiple unit bursts. Thus, multiple unit firing seems to appear prior to the presence of EEG waves and may be seen as inhibitory or excitatory of other electrical events in the brain.

Winters _et al_. extended the work of Schlag and Balvin and found that multiple units in the mesencephalic RF regularly accompany changes in evoked potentials in the mesencephalic RF and cochlear nucleus. They conclude that the RF has a modulating effect "at or before the first synaptic relay of the auditory afferent system."

One of the most interesting applications of the multiple unit technique has been to record changes occurring during behavioral conditioning (Buchwald, Halas, and Schramm, 1965_b, 1966_a, 1966_b; Sandlie and Halas, 1966). During the development of a classically conditioned behavioral response to tone and shock, regular and systematic conditioned changes occurred in multiple unit activity in the primary projection system of the conditioned stimulus (tone) and non-specific reticular system. Conditioned changes in the unconditioned stimulus (US) pathway were not seen. This was contrasted to a decrease observed in multiple unit activity during non-reinforced trials both prior to or after behavioral conditioning to only the tone. These observations have been duplicated in the Halas laboratory (Sandlie and Halas, 1966) in paralyzed as well as in freely moving cats.

Laboratories using single unit recording have also found what appeared to be conditioning in thalamic (Kamikura, 1964; Yoshi and Ogura, 1960; Burešova and Bureš, 1965; Kamikura, McIlwain, and Adey, 1963) and cortical sites (Jasper, Ricci, and Doane, 1960). Single units may do one of several things during the conditioning period (tone or light as the CS; peripheral or central shock as the US): they may increase; decrease; or show no change. Multiple unit recording appears to tap what is happening in an overall sense; it reflects what a majority of the neurons are doing at that time. Ordinarily, under peripheral stimulation the majority of neurons appear to increase in activity. However, there are several notable exceptions. Under pure tone stimulation (1.5 sec in duration) the usual overall neuronal response in the IC is to decrease (Galin, 1966). This is often true in the GM and even more so in certain cortical sites (unpublished observations). Some investigators (Bureš and Burešova, 1967) have raised serious questions as to whether the changes observed in single unit during classical training procedures in the thalamus can be considered true conditioned changes. The changes could just as well be attributed to pseudoconditioning, reflex sensitization, or dominant focus (Bureš and Burešova, 1967). As a result, these authors preferred to think of the results as plastic changes (a more general term) rather than conditioned changes.

The same kind of interpretation is applicable to multiple unit records when classical conditioning under noxious stimulation is used. One must eliminate the possibility of pseudoconditioning, reflex sensitization, etc. Experience with EEG and evoked potentials under classical aversive conditioning (Galambos, 1962) has demonstrated that it is wise to use pseudoconditioning controls routinely. Another criticism of the classical aversive conditioning procedure has been made by Mark and Hall (1967) and Hall and Mark (1967). They pointed out that the observed changes under noxious stimulation may reflect emotional arousal instead of conditioned changes since the same behavioral operations are employed to create both effects. However, they have proposed that under the conditions of instrumental conditioning neuronal responses indicating emotional arousal should decline as an avoidance response is mastered.

A third criticism has been that the observed conditioned responses may reflect only the training of the middle ear muscles (Eliasson and Gisselsson, 1955; Galambos and Rupert, 1959; Galambos, 1960). Simons _et al_. (1966; cf. Starr, 1964) appear to have answered this question with respect to EEG recording, and at least tangentially for multiple units. They found in cats without the middle ear muscles, habituation of responses in the auditory cortex, medial geniculate, inferior colliculus, and cochlear nucleus occurred indistinguishably from those whose muscles were intact. In addition, they found that habituation was the greatest in higher centers (GM, IC) and least in lower centers (CN) which is precisely what Podvoll and Goodman (1967) found using multiple units.

This review indicates that one important step in expanding the usefulness of multiple unit recording is to extend the work on classical conditioning to include other kinds of learning. Extension to instrumental avoidance learning would not only reply to the important criticisms of Mark and Hall (1967) and Hall and Mark (1967) but may yield empirical information about systematic neuronal changes which occur under instrumental conditioning procedures. In addition, discrimination learning ought to yield interesting comparisons.

CHAPTER II

STATEMENT OF THE PROBLEM

Since all the work on learning using multiple unit recording has focused on classical conditioning (Galin, 1964; Buchwald, Halas, and Schramm, 1965_b; 1966_a; Halas and Sandlie, 1966) it is the purpose of this study to extend the method further to include its application to instrumental and discrimination conditioning. In order to facilitate comparison with the work done on classical conditioning, noxious stimuli will be employed as the reinforcer and a 1500 hz tone as the conditioned stimulus. In addition, the use of instrumental conditioning will serve as a means of resolving the issue raised by Hall and Mark (1967) and Mark and Hall (1967). These authors suggested that the neuronal changes observed during the pairing of auditory and shock stimuli may only reflect the emotional arousal of the animal and not associative factors (learning) as claimed by others.

Hall and Mark reviewed some twenty nine published articles on the conditioning of neuronal responses. They reviewed articles with evidence pertinent to classical (Pavlovian) aversive conditioning, instrumental avoidance conditioning, and appetitive conditioning (positive reinforcement). The review covered mostly EEG and evoked potentials, but one multiple unit study was included (Buchwald, Halas, and Schramm, 1965_b). They found that the only consistent results involved changes occurring under the classical paradigm. This suggested to them that the factor responsible for changes might be emotional arousal rather than a factor(s) relating to the neural substrate of conditioning. Using the work of Mowrer (1940) and Miller (1948) as a theoretical base, they postulated that conditioned fear instead of some associative process could account for the changes seen under classical conditioning.

The present study attempts to resolve the issue raised by Hall and Mark by answering the following questions. Are the changes in multiple unit activity observed during instrumental avoidance conditioning the same as those in classical aversive conditioning? Hall and Mark felt that classical aversive conditioning procedures would result in sustained emotional arousal whereas instrumental conditioning procedures would allow emotional arousal to dissipate gradually as the behavioral task is mastered. If changes in multiple unit activity were a product of emotional arousal, then during classical aversive conditioning a sustained high level of multiple unit activity should be observed but during instrumental avoidance conditioning, after an initial increase, a decrease should be observed. The decrease should reflect the gradual dissipation of emotional arousal.

A second relevant question concerns the correlation between the conditioned behavioral response and the neural event. If the neural changes are due to emotional arousal, Hall and Mark would predict a high correlation between the two for classical conditioning but a low correlation for instrumental conditioning. It is hypothesized in this study that the neuronal changes are due to learning and not emotional arousal. Therefore a high correlation is predicted between behavioral and neural changes during instrumental as well as classical conditioning.

Unfortunately, it was not the purpose of Hall and Mark to test these hypotheses directly, but to determine if the observed changes in the reticular formation (RF) and other sites were due to cessation of movement, and thus to the contraction of inner ear muscles. They found that movement changes were uncorrelated with neuronal changes, but they also found that increased amplitudes of some evoked potentials were more closely related to the strength of a conditioned emotional response (CER) than to conditioned behavior. They state quite correctly, "together these findings point to the emotional response itself, fear, as the factor responsible for the observed changes in evoked potentials during conditioning" (p. 906).

CHAPTER III

METHOD AND PROCEDURE

Each of seven adult cats was implanted with thirteen permanent electrodes. Sites were chosen in the primary auditory system (cochlear nucleus, CN; inferior colliculus, IC; medial geniculate, GM; and the auditory cortex), the diffuse reticular system (the medullary reticular formation, MRF; the mesencephalic reticular formation, RF; the central lateral thalamic nucleus, CL; the centrum medianum, CM; the nucleus centralis medialis of the thalamus, NCM; and the nucleus reticularis, RN), the limbic system (amygdaloideus lateralis nucleus, Al; the hippocampus, Hipp), the postectosylvian and ectosylvian cortex, and one site in the hypothalamus (area hypothalamica dorsalis, aHd). The atlases of Jasper and Ajmone-Marson (1960) and Snider and Niemer (1961) were used to direct the placement of the electrodes using a Kopf sterotaxic instrument. The electrode placements for each cat are given in Table 1, and Appendix A reports the Horsley-Clarke coordinates.

At the beginning of surgery each cat was placed under a general anesthetic (ether). While the cat was anesthetized, an acute tracheotomy was performed so that later the cat could be artificially ventilated. An intravenous canula for drug administration was inserted into a vein of the right foreleg. After placement in the Kopf sterotaxic instrument, a longitudinal incision was made on the scalp and the cranium cleaned of muscle and connective tissue. Then burr holes for

TABLE 1

ELECTRODE PLACEMENT IN SEVEN CHRONIC CATS

SITES	CATS						
	A	B	C	D	E	F	G
Auditory System:							
Dorsal Cochlear Nucleus	x	x	x	x	x	x	x
Inferior Colliculus	x	x	x	x	x	x	x
Medial Geniculate	x	x	x	x	x	x	x
Brachium of the Inferior Colliculus	x	x		x			
Reticular System:							
Mesencephalic Reticular Nucleus	x	x	x	x	x	x	x
Medullary Reticular Nucleus						x	x
Centram Medianum	x	x		x		x	x
Central Lateral Thalamic	x		x	x	x	x	x
Nucleus Centralis Medialis			x		x		
Nucleus Reticularis (Anterior)		x					
Nucleus Reticularis (Posterior)				x			
Cortex:							
Right Anterior Ectosylvian	x		x		x	x	x
Right Posterior Ectosylvian	x	x	x	x	x	x	
Left Anterior Ectosylvian		x	x	x	x		
Left Posterior Ectosylvian	x	x		x	x	x	x
Miscellaneous Sites:							
Nucleus Ventralis Postero-medialis	x	x	x	x	x		
Area Hypothalamica Dorsalis		x					
Nucleus Amygdaloideus Lateralis	x	x		x		x	x
Hippocampus						x	x
Caudate Nucleus	x		x		x	x	x

the electrodes were drilled through the cranium using a dental drill.

After all surgery was completed, the ether anesthetic was terminated, and the animal was paralyzed with a neuromuscular blocking agent (gallamine). The cat was artificially respired while paralyzed. The reason for removing the cat from general anesthesia was to allow the investigator the opportunity of monitoring the multiple unit activity while the electrode was being lowered into the brain. As the electrode passed through the various nuclei, changes in multiple unit activity were very obvious especially when different stimuli were presented to the animal. The multiple unit activity was monitored visually on the oscilloscope as well as audibly on a loudspeaker system. After the electrode reached its designated site, it was cemented to the skull with cadon.

When the last electrode was cemented to the skull, the cat was again placed under a general anesthetic (sodium pentobarbital) and stainless steel screws were screwed into the skull to provide stability for the head cap and also for use as an indifferent lead. The electrodes were soldered to a Cannon plug and cadon was used to cement the plug to the skull. The electrodes were isolated from possible EMG potentials by a covering of dental cement. The cats were given at least two months to recover from surgery before being started on the behavioral experiments.

The electrodes were made of size 00, stainless steel insect pins which were insulated with Epoxylite. About 0.1 - 0.5 mm of the electrode tip was bared. The electrodes were referenced to the several stainless steel screws mentioned above.

The recording method has been detailed elsewhere (Weber and Buchwald, 1965). The multiple unit activity was fed into a field effect amplifier, to a Tektronix 122 preamplifier, through high pass filters, into an "integrating" circuit (averaging device), and finally to a Tektronix 565 oscilloscope which displayed the signal. Simultaneous integration (averaging) of the signal was displayed immediately below the signal carrying multiple units. A rise in the integrator line indicated an increase in neuronal activity whereas a drop indicated a decrease in activity. The entire display was then photographed by a Grass C4 camera during the manipulations of the stimuli.

To provide noxious stimulation an isolated 60 cycle shock was applied to the right hind paw. The current was made just strong enough to motivate the cat to lift his paw sharply. The current necessary varied from 2 - 10 ma depending upon the cat and the length of training time in the harness since the animals tended to grow accustomed to the shock and failed to lift their paws vigorously.

Prior to training all animals were handled by the laboratory personnel. However, one person was designated responsible for carrying, harnessing, and training. Each was fitted into a harness which restricted its gross bodily and head movements but allowed its legs to be free to touch the floor.

The animals were individually trained in a sound deadened room large enough for the animal, his trainer, and a 3' x 8' table. The speaker for the tone was mounted 1 meter directly in front and above the animal.

Sessions were 39 trials long with 3 trials for each of 13 electrodes. Usually one session per day was given. The training schedule consisted of habituation, classical aversive conditioning, extinction, retraining, extinction II, instrumental avoidance conditioning, extinction III, habituation for discrimination learning, discrimination learning I with a 1500 hz tone as the positive CS, and discrimination learning II with a 500 hz tone as the positive CS. Two cats began with instrumental conditioning and ended with classical conditioning, and five cats began with classical conditioning and ended with discrimination learning. One cat died of an infection half-way through the training process. A more detailed explanation of the training conditions follows:

Training Conditions

Habituation

Usually 10 - 15 sessions (39 trials each) of only the CS resulted in the diminution of any neuronal response. Many sites needed no habituation trials but others, especially in the auditory system, needed several more sessions. Occasionally, a site did not habituate. In this case, conditioning trials were begun anyway. The specific number of habituation trials given are denoted in the results chapter.

Classical Aversive Conditioning

A tone CS of 1500 hz was paired each time with a shock sufficiently strong to flex the right hind paw. The CS lasted 1.5 sec and a .5 sec shock overlapped the latter part of the tone. As the tone and shock stimulated the animal, pinlights automatically displayed the time periods on the oscilloscope for photographing. Ordinarily, 15 - 25 training sessions were employed.

Extinction

The CS was presented alone. The behavioral response ceased within 1 - 2 sessions but the neural responses required up to 5 or 6 sessions. Some neuronal responses did not extinguish.

Reconditioning

Three to six sessions were usually required to re-establish the behavioral and neuronal responses. The procedure followed exactly the classical aversive conditioning condition.

Extinction II

These procedures were the same as those presented under "Extinction."

Instrumental Avoidance Conditioning

No shock was given to the animal if he would lift his paw after the onset of the tone (1500 hz) but before one second had elapsed. Although the task was usually learned in 6 - 8 sessions, the training was continued for 20 - 30 sessions. This continuation allowed observation of the neuronal responses as the behavioral response tended to deteriorate.

Extinction

Extinction after instrumental avoidance conditioning ordinarily lasted longer than after classical conditioning since the behavioral response tended to persist. Up to 10 sessions were ordinarily given.

Discrimination Habituation

A CS of 1500 hz and a CS of 500 hz were given with no shock. Four or five sessions were given.

Discrimination Conditioning I

The animal was required to lift his paw to the positive CS of 1500 hz but not to the negative CS 500 hz tone. Shock was given for failure to lift to the positive CS. No shock was given should the animal lift to the negative CS.

Discrimination Conditioning II

With no intervening extinction period the positive CS became the 500 hz tone and the negative CS became the 1500 hz tone. All tones and shock in each condition were presented randomly on the average of one presentation every thirty-five seconds. A table of random permutations was used.

The Dependent Variable

Changes in multiple unit response produced a fluctuation in the integrator line. The fluctuations and the multiple unit activity were then photographed.

The resulting strips of film were mounted on 27" x 45" pieces of cardboard side by side and in sequence. Three judges made independent decisions as to whether the integrator line rose slightly (+1), rose greatly (+2), remained the same (0), declined slightly (-1), or declined greatly (-2) on each trial relative to the ongoing neuronal activity one second prior to CS stimulation and/or CS - US stimulation. Agreement of at least two of three judges constituted the rule for adopting one measure or the other. Amost always two judges would agree. If an increase occurred at any time during the CS period (but before the shock onset) it was scored regular, slight, great, etc.

Retest reliability in most sites was consistently above .90. To further reduce the bias, two of the three judges did not know the purpose of the experiment.

Figure 1 illustrates the type of multiple unit integrator increase scored slight, great, no response, slight decrease, great decrease.

The judgement of the animal trainer determined whether a positive behavioral response had occurred. A positive response was not counted until the animal's paw had actually raised from the table. If the animal had executed a sharp rapid flexion, it was scored (+2); if a minimal flexion, it was scored (+1). During discrimination learning a positive score was given if the animal responded correctly, i.e., flexion to the negative tone was scored (0) but no flexion to the negative tone was scored (+2). Flexion to the positive CS was, of course, scored (+1) or (+2).

After the judges had scored the neurological and behavioral data, a three-session (9 responses) running average was computed. The running average is essentially a curve smoothing technique especially suited to display the trends in the data.

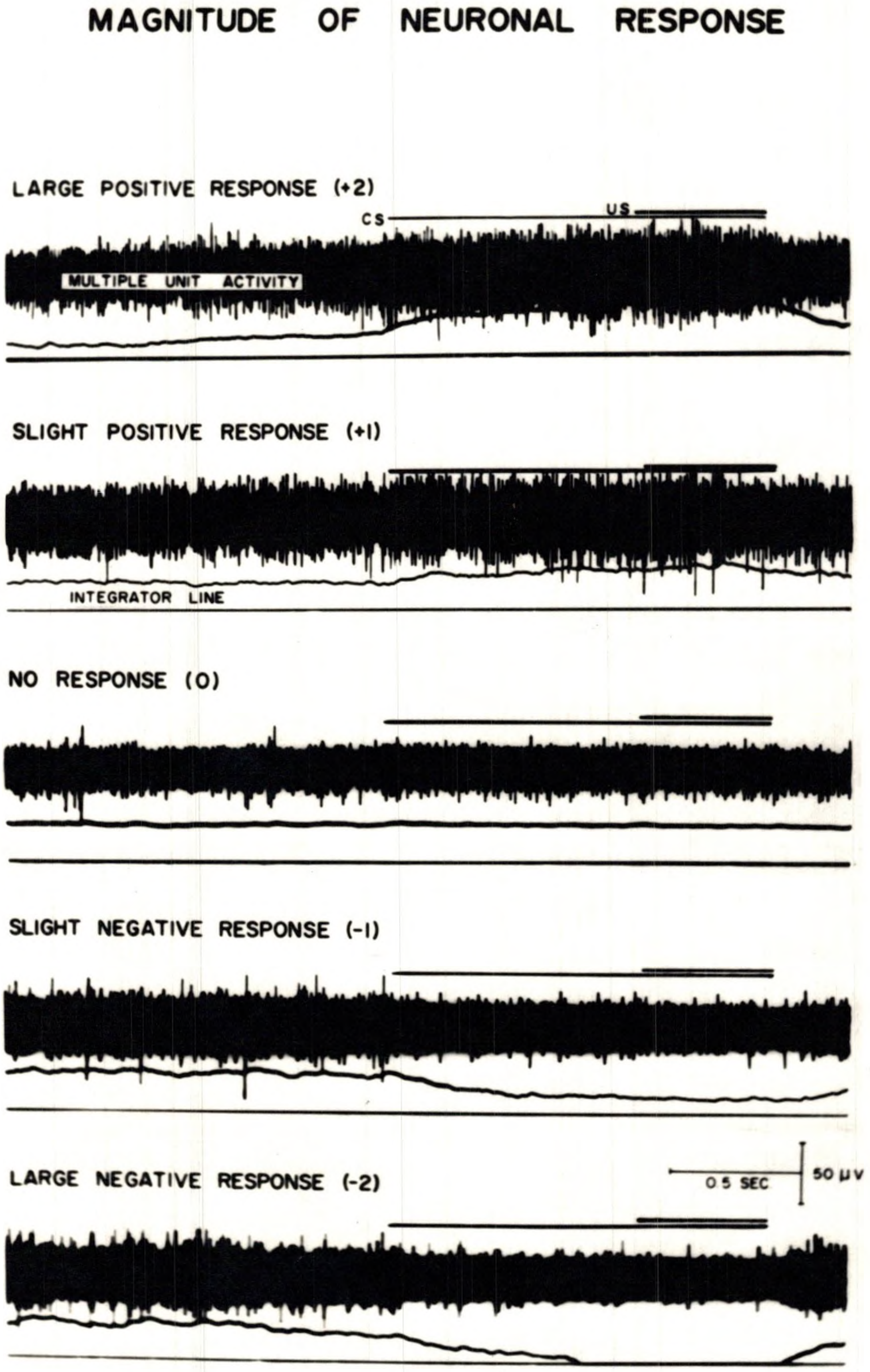

Figure 1. Examples of integrator line deflection scored as large positive response (+2), slight positive response (+1), no response (0), slight negative response (-1), and large negative response (-2). The time sequence of the figure should be read from left to right.

CHAPTER IV

RESULTS[1]

Introduction

The level and trend of the neuronal multiple unit activity differed markedly between classical aversive conditioning and instrumental avoidance conditioning. Most frequently, the neuronal trend between the two conditions was a contrary one. If the trend in classical conditioning would be downward, the trend during instrumental conditioning would be upward.

The same contrariness between trends in the neuronal activity was also reflected in the neuronal-behavioral correlations. That is, if instrumental neuronal activity were positively correlated with behavior, then classical neuronal activity would be negatively correlated. Unfortunately, equipment and training problems rendered almost all the results of discrimination conditioning useless.

Another general feature of the results was the overwhelming predominance of positive neuronal responses whenever a stimulus was presented. In only a few isolated sites, and then only in particular cats, was the overall multiple unit activity negative. Similarly, positive responsiveness was typically obtained in evoked potential

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[1] The neuronal and behavioral scores for the three-day running average for each cat during the training sessions may be obtained by writing the Archives Division of the Chester Fritz Library, the University of North Dakota, Grand Forks, North Dakota 58201.

records. However, unlike evoked potential records, multiple unit responses at the termination of habituation and extinction periods in this study often yielded no response at all (i.e., no relative deflection of the integrator line when the CS was presented).

Using multiple unit responsiveness as the dependent variable, the prediction of Hall and Mark (1967) that neuronal activity levels should remain at high levels during classical aversive conditioning and decrease in the latter stages of instrumental avoidance conditioning was conclusively contradicted in most of the sites sampled in this study--especially in the mesencephalic and thalamic reticular systems. In this study, the typical result was one in which classically conditioned neuronal responses began at a high level and then dissipated, whereas the instrumental neuronal responses continued to increase. This is the opposite result from that predicted by Hall and Mark.

Concerning the question of the neuronal-behavioral correlations for the two types of conditioning, the prediction made in Chapter II is only half-fulfilled.

In Chapter II the prediction was made that neuronal-behavioral correlations for classical and as well for instrumental conditioning ought to be highly positive. Inspection of Table 5 indicates that the general pattern was one of no or negative correlation for classical conditioning and a positive correlation for instrumental conditioning. This is especially true for the auditory and reticular systems. The latter system is, of course, highly implicated in the mediation of emotional arousal. Further comment is reserved for discussion in Chapter V.

The results for discrimination learning are not presented since almost no neuronal invariance across cats emerged. This was probably due to serious equipment problems. Moreover, the cats generally failed to learn the discrimination task.

Finally, a certain degree of tentativeness to this study must be advanced in view of the fact that histological vertification of electrode locations has not been made. For anticipated published material, site verification will be made. The decision to delay histological verification was made for two reasons: (1) since the value of each cat is many thousands of dollars, the cats are being used for additional experiments; and, (2) past experience from earlier implantations in our laboratory has shown that site locations have been accurate. As electrodes are inserted it has proven relatively simple to follow its progression due to the marked changes in neuronal activity levels which accompany movement from nucleus to nucleus. The kind of activity changes detectable from nucleus to nucleus have been reported by others (Halas and Beardsley, 1968; Albe-Fessard, et. al., 1967; Schlag and Balvin, 1963).

General Presentation of the Data

The large amount of data gathered in this study mandated a considerable amount of summarizing. Tables 2 through 5 present the overall results of the experiment. Following the tables, a site by site presentation will be made.

A summary of the mean levels of the multiple unit activity for habituation, three levels of conditioning, and extinction are given in Table 2. Tests for linearity were performed on the overall site means for classical and instrumental conditioning. In classical conditioning a significant ($p \leq .01$) overall negative trend was determined and in instrumental conditioning an overall significant ($p \leq .01$) positive trend was determined. Higher order polynomials proved insignificant. Specific trends found in particular sites and in some systems are presented later. Table 3 gives the analysis of variance utilized in the orthogonal polynomial tests for trends (Winer, 1962).

Another feature of Table 2 which was quite remarkable was the systematic decrease in multiple unit activity which occurred in the extinction period following classical conditioning. This result was not unexpected. However, in extinction after instrumental conditioning a pervasive increase in activity occurred. The latter was unexpected and is very difficult to interpret. Table 2 indicates that of the twenty sites denoted none completely reversed the order (although some data is missing in the posterior RN and the Hipp). Only four of twenty sites in any way contradicted the overall pattern.

A summary of the standard deviation for each site is found in Table 4. Since each cat in a site tended to emit a pattern similar to that of the other cats but at a higher or lower overall activity level, a mean constant difference over all training sessions for that cat was subtracted from each training session observation. The standard deviations in Table 4 were then computed from the adjusted observations. Thus, if the same pattern of activity among cats in a given site and training procedure tended to occur together, the variability would be reduced. If no common pattern existed, the variability would not be reduced.

Appendix B contains the standard deviations computed on the original observations.

TABLE 2

MEAN LEVEL OF MULTIPLE UNIT ACTIVITY FOR THE THREE DAY RUNNING AVERAGE FOR EACH SITE DURING HABITUATION, CLASSICAL CONDITIONING, INSTRUMENTAL CONDITIONING AND EXTINCTION

			Conditioning			Total		Number	
		Habituation	1/3	1/3	1/3	Average	Extinction	of Cats	Figure
AUDITORY SYSTEM									
Dorsal Cochlear Nucleus (CN)	Class.	.67	1.12	.89	.90	.96	.34	4 of 6	2
	Instr.	.62	1.47	1.96	1.88	1.77	2.00	4 of 6	2
Inferior Colliculus (IC)	Class.	.69	1.35	1.11	.99	1.17	.83	4 of 5	3
	Instr.	-.26	1.22	1.49	1.97	1.59	1.97	3 of 6	5
Medial Geniculate (GM)	Class.	.07	.64	.67	.60	.64	.18	5 of 6	6
	Instr.	.34	.69	.71	.85	.77	1.01	6 of 6	6
Brachium of the Inferior Colliculus (BCI)	Class.	.43	1.37	1.54	1.53	1.48	.78	1 of 2	8
	Instr.	.22	.50	.20	.26	.31	.72	1 of 1	8
RETICULAR SYSTEM									
Mesencephalic Reticular Nucleus (RF)	Class.	.64	1.34	1.24	1.10	1.30	.52	4 of 7	10
	Instr.	.40	.91	1.31	1.23	1.15	1.22	4 of 6	10
Medullary Reticular Nucleus (MRF)	Class.	-	1.00	1.00	.83	.96	-	1 of 1	11
	Instr.	.53	1.18	1.50	1.69	1.46	1.84	2 of 2	11
Centram Medianum (CM)	Class.	.42	.52	.38	.29	.41	.41	3 of 3	12
	Instr.	.44	.33	.71	.98	.71	.67	3 of 4	12
Central Lateral Thalamic Nucleus (CL)	Class.	.64	1.15	1.08	1.26	1.18	.66	4 of 5	13
	Instr.	.45	.72	.86	1.12	.91	1.31	5 of 5	13

TABLE 2--Continued

		Habituation	Conditioning			Total Average	Extinction	Number of Cats	Figure
			1/3	1/3	1/3				
Nucleus Centralis Medialis (NCM)	Class.	.86	1.56	1.33	1.22	1.34	.58	2 of 2	14
	Instr.	.29	.51	.40	.48	.47	1.11	2 of 2	14
Nucleus Reticularis Anterior (RN)	Class.	1.10	1.24	.74	.94	.97	.16	1 of 1	15
	Instr.	.09	.48	.23	.81	.51	.89	1 of 1	15
Nucleus Reticularis Posterior (RN)	Class.	.33	1.94	1.78	1.46	1.74	-	1 of 1	16
	Instr.	1.04	1.81	1.92	2.00	1.90	1.33	1 of 1	16
CORTEX									
Right Anterior Ectosylvian	Class.	.55	.87	.73	.89	.81	.75	4 of 4	20
	Instr.	.63	.70	.76	.98	.83	1.33	4 of 4	20
Right Posterior Ectosylvian	Class.	.70	1.33	1.31	.99	1.21	.72	5 of 5	18
	Instr.	.38	.20	.07	.06	.11	.65	4 of 5	18
Left Anterior Ectosylvian	Class.	.63	1.13	.65	.44	.74	.41	3 of 4	19
	Instr.	.25	.10	.07	.53	.24	1.02	4 of 4	19
Left Posterior Ectosylvian	Class.	.85	1.30	.84	.76	.97	.41	3 of 5	17
	Instr.	.27	.60	.63	.88	.71	1.08	5 of 5	17
MISCELLANEOUS SITES									
Nucleus Ventralis Postero-medialis (VPL)	Class.	.65	.67	.82	.88	.77	.17	4 of 5	21
	Instr.	.32	.54	.73	.67	.65	.62	3 of 4	21
Area Hypothalamica Dorsalis (aHd)	Class.	.75	1.12	1.88	1.85	1.61	.29	1 of 1	-
	Instr.	.00	1.13	1.01	.78	.97	1.21	1 of 1	-

TABLE 2--Continued

		Habituation	Conditioning			Total Average	Extinction	Number of Cats	Figure
			1/3	1/3	1/3				
Nucleus Amygdaloideus Lateralis (Al)	Class.	.68	1.38	1.38	1.51	1.42	.82	2 of 4	22
	Instr.	.94	.62	.67	1.13	.80	.75	3 of 4	22
Hippocampus (Hipp)	Class.	-	.28	.17	.06	.18	-	1 of 1	23
	Instr.	1.47	1.15	.93	1.33	1.15	1.15	2 of 2	23
Caudate Nucleus (CdN)	Class.	.74	1.12	.83	.86	.94	.44	3 of 4	24
	Instr.	.54	.41	.38	.36	.38	.70	3 of 3	24
Total Mean of Means	Class.	.63	1.12	1.02	.97	1.04	.50		
	Instr.	.45	.76	.83	1.00	.84	1.08		

To reduce extraneous variability even further, the results of all cats in a given site were not always used. Typically, if all six cats were implanted in a given site, one or two would yield markedly different patterns from the other five cats. These two cats were arbitrarily omitted. The column next to the far right-hand column of Table 4 gives the number of cats utilized out of the total number of cats implanted.

Slight differences in electrode placement may account for the atypical cats. Along with the presentation of the Horsley-Clarke coordinates in Appendix A, relevant histological comments are made.

Finally, some electrode placements yielded no data due to technical difficulties. One cat died during classical extinction and the film was inadvertently destroyed in two sites in the remaining cats.

The orthogonal test for trends was also made on the standard deviations found in Table 4, but no significant trends for classical or instrumental conditioning were found. This indicates that the overall variability of the neuronal activity level was about the same across both kinds of conditioning.

A summary of the neuronal-behavioral correlations during classical and instrumental conditioning for each site may be found in Table 5. Table 5 highlights two important overall observations: (1) higher correlation coefficients generally occur in instrumental conditioning; and, (2) most of the positive coefficients occur in instrumental conditioning and most of the negative coefficients occur in classical conditioning.

The three highest correlation coefficients occurred in the IC,

TABLE 3

SUMMARIES OF ANALYSES OF VARIANCE FOR THE OVERALL TRENDS OCCURRING IN CLASSICAL AVERSIVE AND INSTRUMENTAL AVOIDANCE CONDITIONING.

Summary of Analysis of Variance for Classical Aversive Conditioning

Source of variation	SS	df	MS	F
Classical Conditioning	2248	2	1224.0	5.52*
Experimental Error	12636	57	221.7	
Total	15084	59		

$^*p \leq .01$

Tests for Trend for Classical Aversive Conditioning

T_j	(1/3) 2246	(1/3) 2040	(1/3) 1939	c^2	C	D	C^2/D
Linear	-1	0	+1	2	-307	40	2356
Quadratic	+1	-2	+1	6	105	120	91.87

Test for linear trend: 2356/221.7 = 106.3, $p \leq .01$
Test for quadratic trend: 91.87/221.7, N.S.

Summary of Analysis of Variance for Instrumental Conditioning

Source of variation	SS	df	MS	F
Instrumental Conditioning	5924	2	2962.0	14.5*
Experimental Error	11622	57	203.9	
Total	17546	59		

$^*p \leq .01$

Tests for Trend for Instrumental Avoidance Conditioning

T_j	(1/3) 1527	(1/3) 1656	(1/3) 1998	c^2	C	D	C^2/D
Linear	-1	0	+1	2	471	40	5546
Quadratic	+1	-2	+1	6	213	120	378

Test for linear trend: 5546/203.9 = 27.19, $p \leq .01$
Test for quadratic trend: 378/203.9 = 1.85, N.S.

TABLE 4

TRANSFORMED STANDARD DEVIATIONS OF MULTIPLE UNIT ACTIVITY FOR THE THREE DAY RUNNING AVERAGE FOR EACH SITE DURING HABITUATION, CLASSICAL CONDITIONING, INSTRUMENTAL CONDITIONING, AND EXTINCTION

			Conditioning						
		Habituation	1/3	1/3	1/3	Total Cond.	Extinction	Number of Cats	% of reduction due to transformation
AUDITORY SYSTEM									
Dorsal Cochlear	Class.	.12	.18	.32	.17	.35	.18	4 of 6	12
Nucleus (CN)	Instr.	.19	.18	.03	.19	.20	.00	4 of 6	20
Inferior Colliculus	Class.	.22	.17	.15	.12	.23	.08	4 of 5	38
(IC)	Instr.	.11	.08	.05	.03	.14	.05	3 of 6	18
Medial Geniculate	Class.	.26	.29	.09	.09	.33	.06	5 of 6	13
(GM)	Instr.	.14	.14	.18	.22	.20	.17	6 of 6	43
Brachium of the Inferior	Class.	-	-	-	-	-	-	1 of 2	-
Colliculus (BCI)	Instr.	-	-	-	-	-	-	1 of 1	-
RETICULAR SYSTEM									
Mesencephalic Reticular	Class.	.15	.17	.20	.10	.18	.19	4 of 7	25
Nucleus (RF)	Instr.	.11	.18	.18	.15	.23	.24	4 of 6	41
Medullary Reticular	Class.	-	-	-	-	-	-	1 of 1	-
Nucleus (MRF)	Instr.	.15	.24	.11	.08	.18	.06	2 of 2	54
Centram Medianum	Class.	.09	.10	.07	.04	.14	.08	3 of 3	39
(CM)	Instr.	.15	.10	.20	.25	.20	.15	3 of 4	31
Central Lateral Thalamic	Class.	.24	.22	.32	.38	.36	.09	4 of 5	08
Nucleus (CL)	Instr.	.24	.15	.15	.12	.24	.20	5 of 5	58

TABLE 4--Continued

		Habituation	Conditioning			Total Cond.	Extinction	Number of Cats	% of Reduction Due to Transformation
			1/3	1/3	1/3				
Nucleus Centralis Medialis (NCM)	Class.	.21	.07	.08	.10	.17	.13	2 of 2	72
	Instr.	.07	.23	.15	.27	.22	.05	2 of 2	41
Nucleus Reticularis Anterior (RN)	Class.	-	-	-	-	-	-	1 of 1	-
	Instr.	-	-	-	-	-	-	1 of 1	-
Nucleus Reticularis Posterior (RN)	Class.	-	-	-	-	-	-	1 of 1	-
	Instr.	-	-	-	-	-	-	1 of 1	-
CORTEX									
Right Anterior Ectosylvian	Class.	.18	.15	.21	.19	.31	.14	4 of 4	-
	Instr.	.30	.18	.21	.15	.22	.14	4 of 4	60
Right Posterior Ectosylvian	Class.	.21	.19	.23	.13	.24	.19	5 of 5	43
	Instr.	.15	.19	.08	.06	.14	.17	4 of 5	12
Left Anterior Ectosylvian	Class.	.39	.24	.23	.12	.22	.16	3 of 4	04
	Instr.	.09	.29	.15	.38	.31	.21	4 of 4	09
Left Posterior Ectosylvian	Class.	.20	.13	.22	.21	.22	.26	3 of 5	04
	Instr.	.19	.14	.15	.17	.20	.18	5 of 5	59
MISCELLANEOUS SITES									
Nucleus Ventralis Posteromedialis (VPL)	Class.	.18	.21	.25	.23	.31	.06	4 of 5	16
	Instr.	.07	.21	.14	.35	.24	.09	3 of 4	46
Area Hypothalamica Dorsalis (aHd)	Class.	-	-	-	-	-	-	1 of 1	-
	Instr.	-	-	-	-	-	-	1 of 1	-

TABLE 4--Continued

		Habituation	Conditioning			Total Cond.	Extinction	Number of Cats	% of Reduction Due to Transformation
			1/3	1/3	1/3				
Nucleus Amygdaloideus Lateralis (Al)	Class.	.24	.12	.10	.12	.14	.13	2 of 4	00
	Instr.	.20	.18	.13	.26	.26	.24	3 of 4	32
Hippocampus (Hipp)	Class.	-	-	-	-	-	-	1 of 1	-
	Instr.	-	.08	.12	.09	.16	.08	2 of 2	11
Caudate Nucleus (CdN)	Class.	.24	.18	.29	.18	.24	.25	3 of 4	43
	Instr.	.38	.13	.07	.10	.12	.14	3 of 3	70
Total Standard Deviation	Class.	.21	.17	.20	.16	.25	.14		-
	Instr.	.17	.17	.13	.18	.20	.13		-

TABLE 5

PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENTS BETWEEN NEURONAL AND BEHAVIORAL RESPONSES FOR ALL SITES DURING CLASSICAL AND INSTRUMENTAL CONDITIONING

Site	Classical	Instrumental
AUDITORY SYSTEM		
Dorsal Cochlear Nucleus (CN)	-.12	.77
Inferior Colliculus (IC)	-.74	.96
Medial Geniculate (GM)	.42	.76
Brachium of the Inferior Colliculus (BCI)	.58	-.37
RETICULAR SYSTEM		
Mesencephalic Reticular Nucleus (RF)	.03	.84
Medullary Reticular Nucleus (MRF)	.16	.67
Centram Medianum (CM)	-.88	.90
Central Lateral Thalamic Nucleus (CL)	-.06	.90
Nucleus Centralis Medialis (NCM) Right	-.70	-.46
Nucleus Centralis Medialis (NCM) Left	-.22	.44
Nucleus Reticularis Anterior (RN)	-.29	.22
Nucleus Reticularis Posterior (RN)	-.43	.80
CORTEX		
Right Anterior Ectosylvian	-.31	.80
Right Posterior Ectosylvian	.16	-.71
Left Anterior Ectosylvian	-.68	.31
Left Posterior Ectosylvian	-.57	.81
MISCELLANEOUS SITES		
Nucleus Ventralis Postero-medialis (VPL)	.22	.16
Area Hypothalamica Dorsalis (aHd)	.44	-.20
Nucleus Amygdaloideus Lateralis (A1)	.58	.37
Hippocampus (Hipp)	.15	.53
Caudate Nucleus (CdN)	-.77	-.23

CM, and the CL; all occurred in instrumental conditioning and all were positive. Interestingly, two of the highest negative correlations occurred in classical conditioning in two of the same three sites (CM and IC). Clearly, if the same sites acted so differentially, more than a generalized emotional arousal ("fear" as hypothesized by Mark and Hall) was involved. A much more complicated picture implying either a differential motivational pattern under the two conditions or some associative (learning) process may have occurred.

Individual Site Presentation

Dorsal Cochlear Nucleus (CN)

Inspection of Figure 2 will reveal that the neurological responses between classical and instrumental conditioning differed markedly in the CN. After a slight initial rise during the behavioral acquisition phase of classical conditioning, the neurological activity began to decline. In addition, a rather marked inhibition of activity occurred after the behavioral acquisition phase was completed. This is in contrast to the activity displayed in instrumental conditioning where the neurological activity increased consistently with the acquisition responses and then continued at a rather high level.

Along with these overall differences, inhibition of activity relative to habituation at the outset of classical conditioning and facilitation of activity at the outset of instrumental conditioning indicate that quite different types of neurological responses occurred in the same electrode site when the training problem was changed from an aversive one to an avoidance one.

Another feature of the effect of the training procedures in this site was the marked difference in neuronal activity which occurred during the extinction periods. During classical conditioning the neuronal responses declined even further but during instrumental conditioning the neuronal responses reached the maximum and were sustained at that high level.

As a result of the two unlike trends exhibited in the training periods, the neuronal-behavioral correlation coefficient during classical conditioning was slightly negative (-.12) and the neuronal-behavioral coefficient during instrumental conditioning was positive (.77).

During discrimination training I and II no consistent results among the cats could be discerned.

Inferior Colliculus (IC)

The activity of this site was marked by two contrasting types of neuronal responses during classical conditioning; one set of four cats (Figure 3) whose habituation and training periods were made up of positive responses; and two cats (Figure 4) whose activity remained consistently negative (i.e., all responses caused the integrator line to decrease). However, one should also note that after an initial rise in activity for both types of cats a decline in activity level took place as training progressed. This is similar to what happened in the CN.

The results for instrumental training were again in contrast to those of classical conditioning. In instrumental conditioning (Figure 5) the neuronal activity increased during the behavioral acquisition phase and remained high. This is the same pattern as that exhibited by the CN under the two differential training procedures. Moreover, during

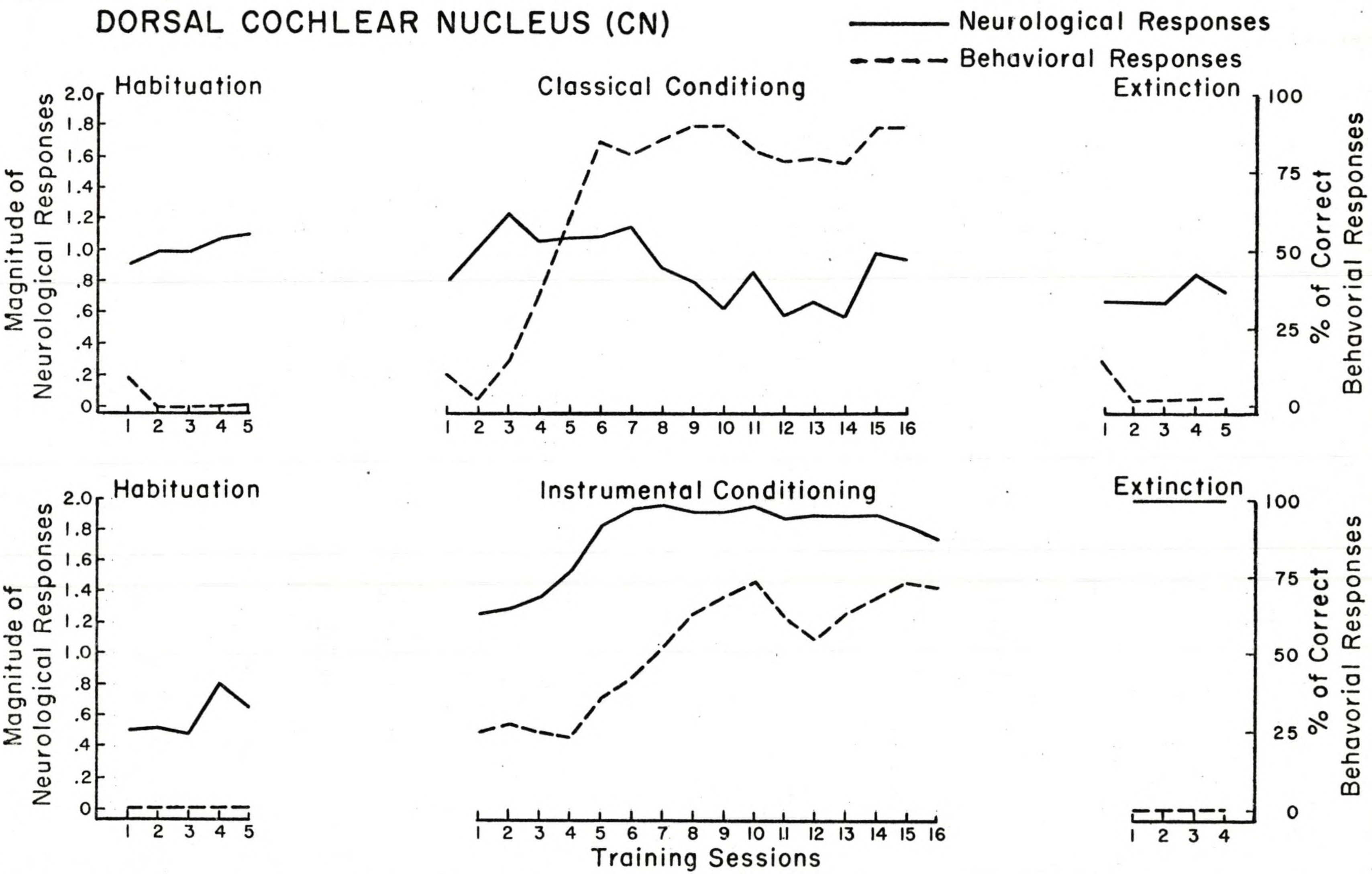

Figure 2. Three day running average of the neuronal activity and the behavioral responses for four of six cats associated with the right dorsal cochlear nucleus during classical aversive and instrumental avoidance conditioning.

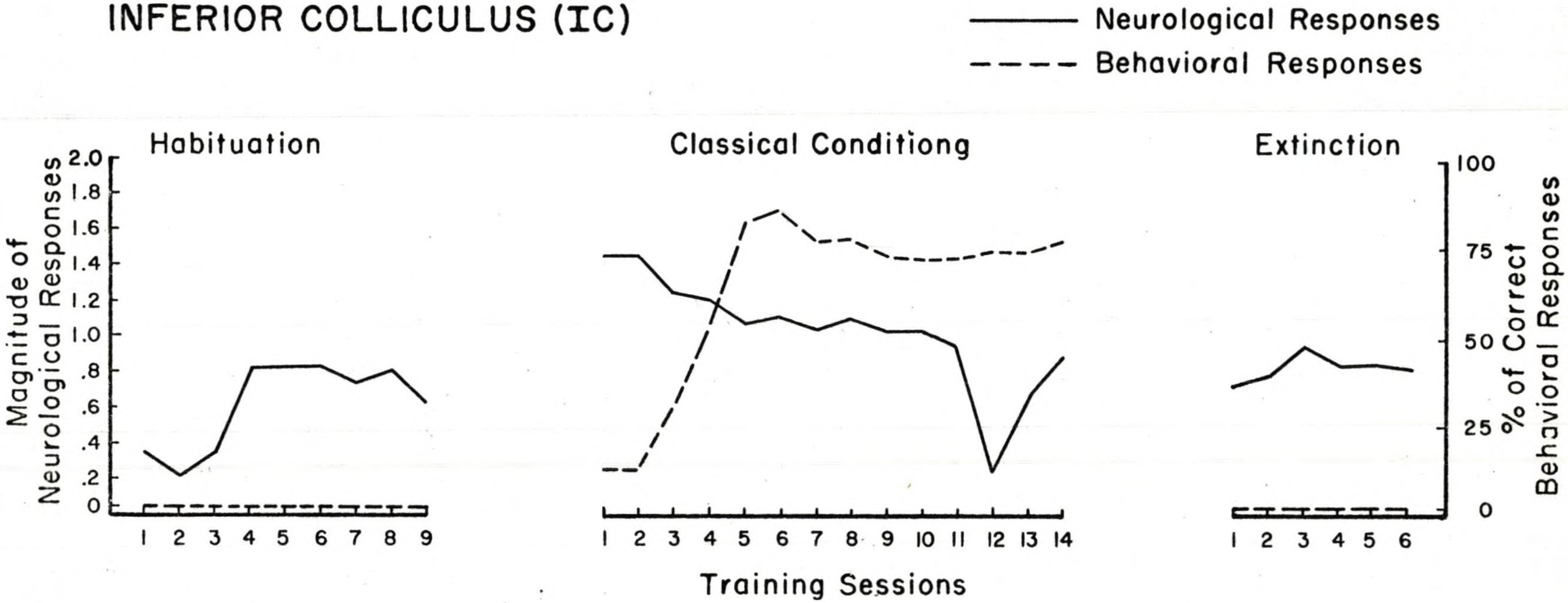

Figure 3. Three day running average of the neuronal activity and behavioral responses for four of six cats associated with the right inferior colliculus during classical conditioning.

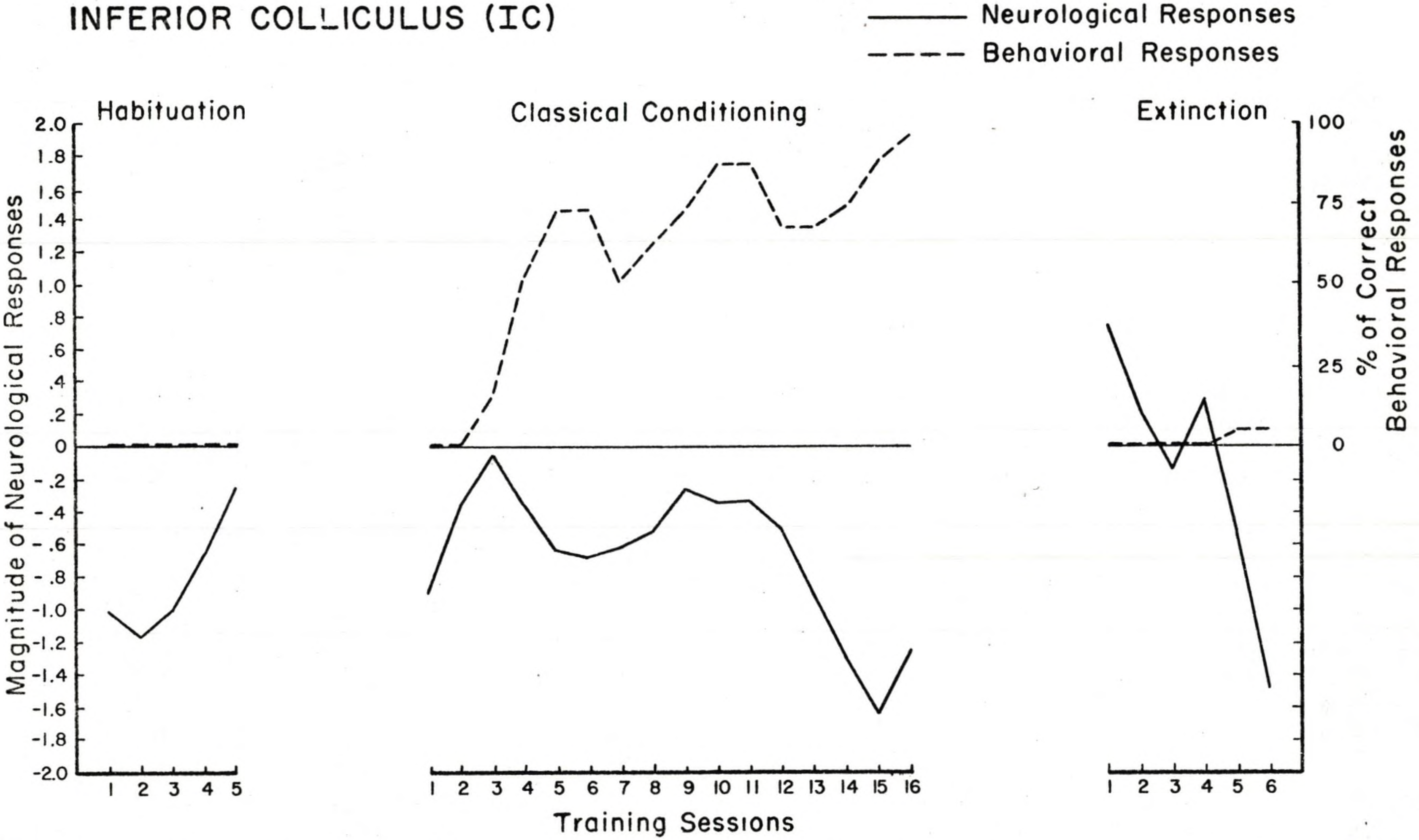

Figure 4. Three day running average of the neuronal activity and behavioral responses for two of six cats associated with the right inferior colliculus during classical aversive conditioning. These two cats emitted nearly all inhibitory neuronal responses.

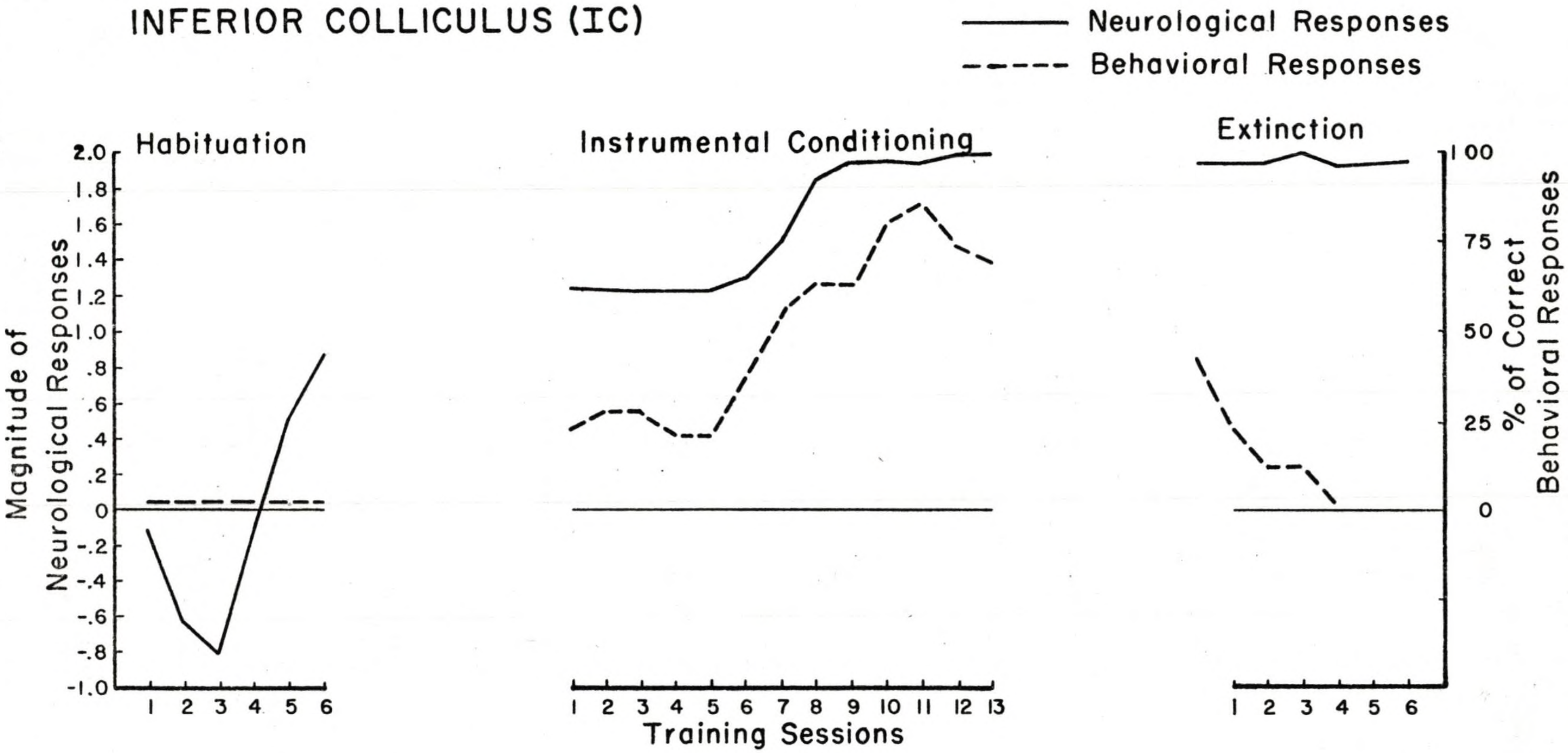

Figure 5. Three day running average of the neuronal activity and behavioral responses for three of six cats associated with the right inferior colliculus during instrumental avoidance conditioning.

extinction, the same high level of neuronal activity was found in instrumental conditioning and the same low level of activity in classical conditioning which was observed in the CN.

Another feature of instrumental conditioning which was common to the CN and IC was the rather high level of positive neuronal-behavioral relationship. In the CN the relationship was (.77) and in the IC the relationship was (.96).

Medial Geniculate (GM)

At the beginning of classical conditioning in the GM five of six cats showed an initial increase in neuronal activity, declined, and then continued to decline throughout the extinction period (Figure 6). This is a similar pattern to that found in the CN and IC. One cat was greatly atypical (Figure 7) in that its activity reached an immediately high level and remained rather high. Perhaps the explanation is that the electrode in this cat was somewhat less deeply implanted than were those in the other cats.

Although the pattern followed during classical conditioning was very similar to that found in the CN and IC, the pattern during instrumental conditioning was slightly different from that of the others. No great increase in activity occurred as in the other, but nevertheless a slight increase did occur. Thus, a degree of similarily may be found in the overall trend of the data.

By comparing the three sites (CN, IC, GM) with respect to the training sessions in which the decline in classical conditioning and the increase in instrumental conditioning took place, one may see another pattern emerge that is related to the level of the nucleus in

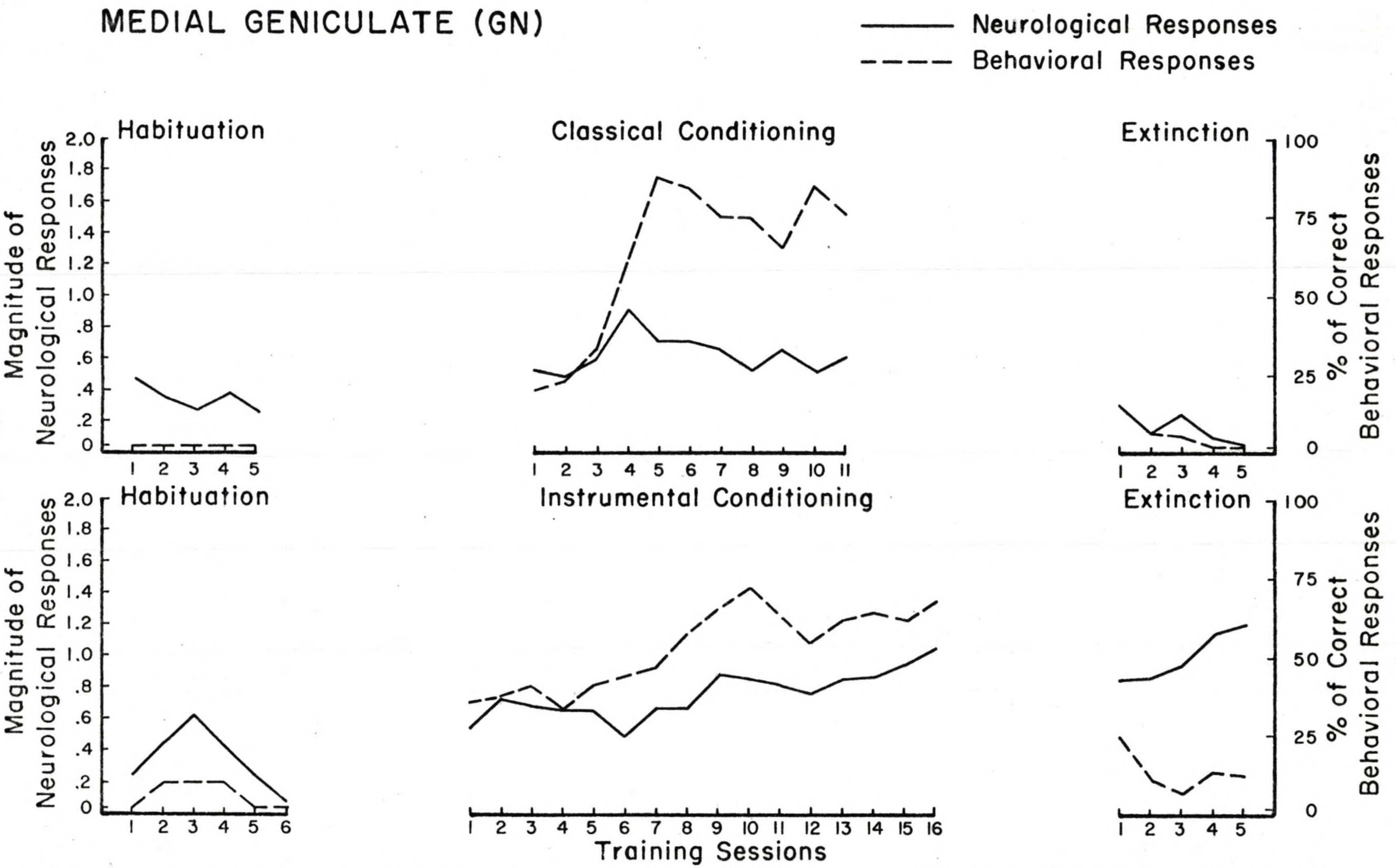

Figure 6. Three day running average of the neuronal activity and behavioral responses for five of six cats associated with the left medial geniculate during classical aversive and instrumental avoidance conditioning.

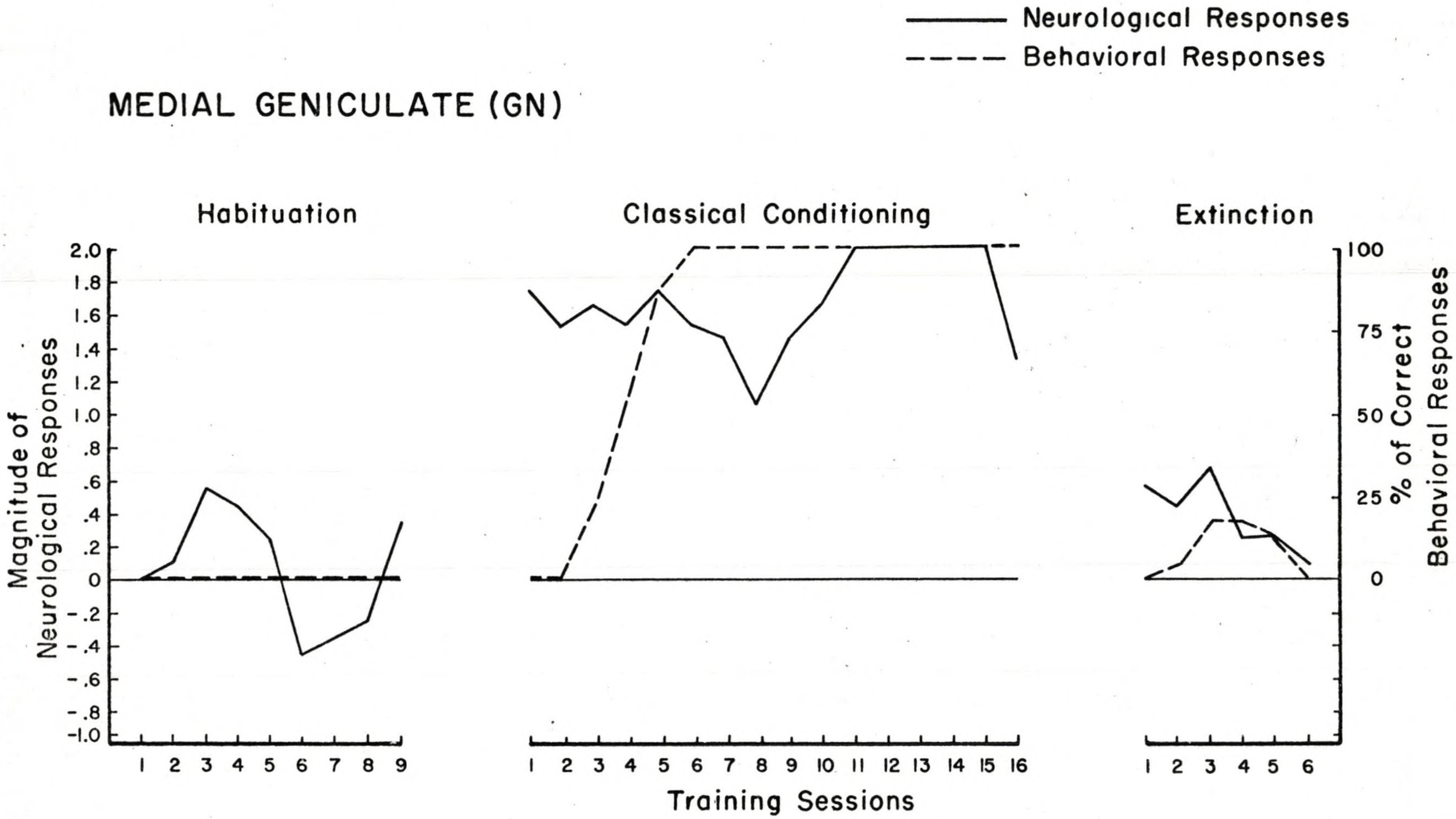

Figure 7. Three day running average of the neuronal activity and behavioral responses for one of six cats associated with the left medial geniculate body during classical aversive conditioning.

the brain stem. Except for one reversal, the changes in neuronal activity level (decline in classical; increase in instrumental) occurred first in the lowest centers. Table 6 summarizes the data.

TABLE 6

EARLIEST TRAINING SESSION AT WHICH DECELERATION AND ACCELERATION IN NEURONAL ACTIVITY TOOK PLACE DURING CLASSICAL AND INSTRUMENTAL CONDITIONING IN THE CN, IC, AND GM

Number of Cats During Classical Conditioning	Training Session Number at Which Downward Change Occurred	Number of Cats During Instrumental Cond.	Training Session Number at Which Upward Change Occurred
4 of 6 cats CN	4	4 of 6 cats	3
4 of 5 cats IC	3	3 of 6 cats	6
6 of 6 cats GM	5	6 of 6 cats	9

Such a pattern suggests that as the learning task is integrated within the neuronal system, the first step occurred in the lower centers and then moved successively upward to higher centers. Also, one may infer that integration in these centers came earlier for classical conditioning than for instrumental conditioning. Another inference may be made from the direction of the integration of neuronal responses reported in this data. Since the direction of integration is from lower center to higher center, some expression of an associative process may be involved. The behavioral data indicate that these cats learned the classical task more quickly than the instrumental task (using the highest peak of learned behavior as the criterion). This is consistent with the neuronal data.

Invariance across cats in discrimination I and II was not discernable and therefore no data is presented.

Brachium Colliculi Inferioris (BCI)

Although only the data for one cat is available for the BCI (Figure 8) it is interesting to note its activity level was quite different from the other auditory centers. In this site instrumental activity declined throughout the training period whereas in the others it increased throughout. In other words, instrumental conditioning in the BCI acted like the activity of classical conditioning in the other auditory sites. However, similar to the other sites in instrumental conditioning, extinction began at a maximumly high level. But unlike the others, it declined to a "no response" level rapidly.

Mesencephalic Reticular Formation (RF)

Sites from the right and left mesencephalic reticular formation have been combined because of the high concordance found between the activity levels of the two sites. An example of the remarkable similarity in response is illustrated by cat F in Figure 9.

While all seven of the cats demonstrated behavioral learning, only four animals exhibited neuronal changes that correlated closely with the conditioned behavioral responses. The other three cats showed conditioned behavioral responses, but there were no corresponding neuronal changes in their brain sites.

Figure 10 illustrates the results of the neuronal and behavioral responses of the four cats for classical conditioning. There was an overall increase in neuronal response during classical conditioning as compared to the habituation period. There was also an overall increase in behavioral response during conditioning as compared to habituation.

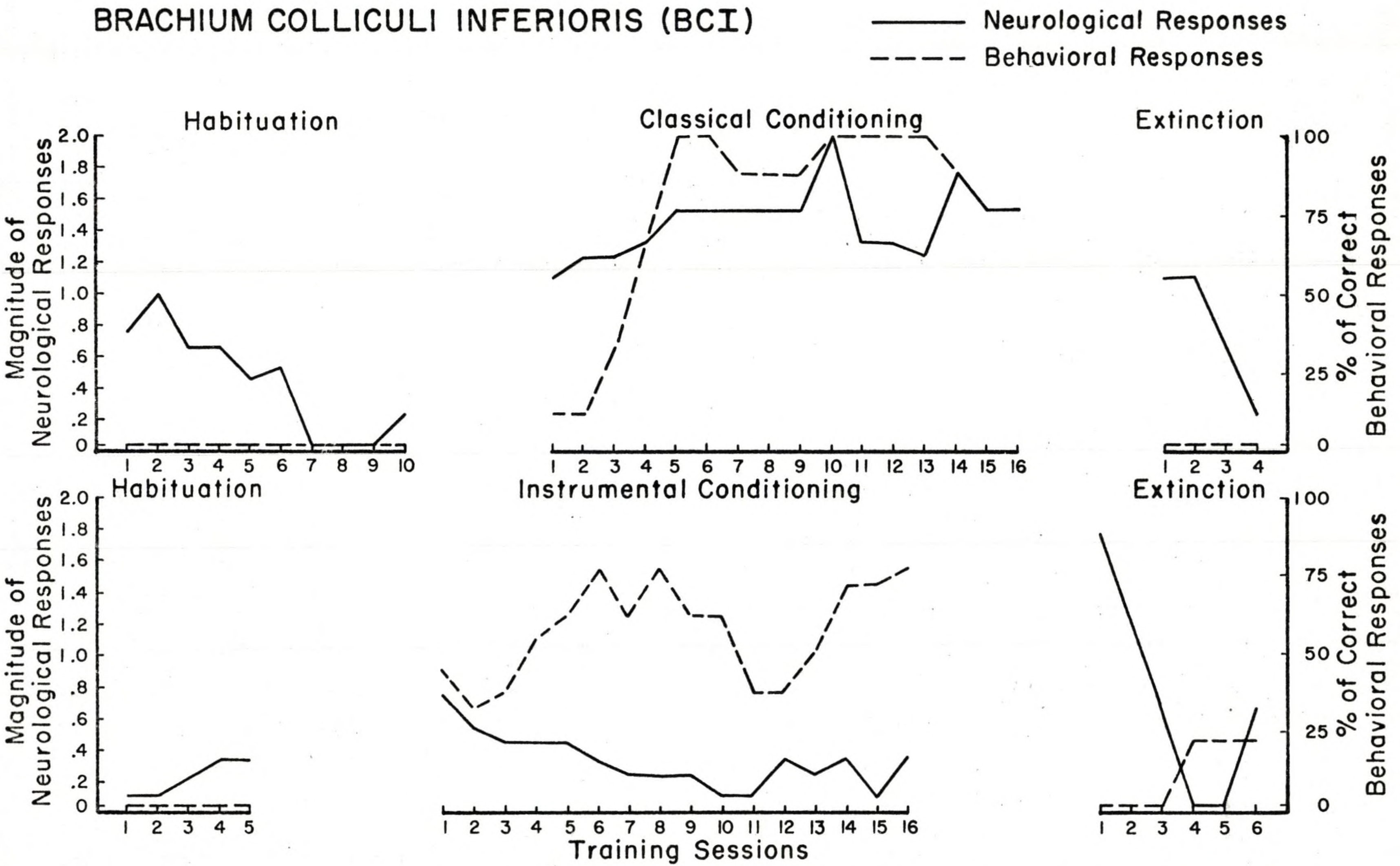

Figure 8. Three day running average of the neuronal activity and behavioral responses for one of two cats associated with the right brachium of the inferior colliculus during classical aversive and instrumental avoidance conditioning.

These results are not unexpected. However, although there is an increase in both neuronal and behavioral responses during conditioning, the two are not related (r = .03).

Figure 10 shows the results of the neurological and conditioned behavioral responses of the same four cats for instrumental conditioning. It is obvious that both the neuronal and behavioral responses increased during the instrumental conditioning period as compared to the habituation period. This was not predicted by Hall and Mark in their discussion cited in Chapter II. Furthermore, the correlation between neuronal and behavioral responses was greater during instrumental conditioning (r = .84) than it was for classical conditioning (r = .03). Whereas Hall and Mark predicted a decrease in neuronal activity after an initial increase, the neuronal responses actually increase in tandem with the behavioral responses. In general, the correlation between behavioral and neuronal responses was much higher in instrumental conditioning than it was for classical conditioning.

Since the activity during instrumental conditioning increased throughout the training period the pattern is similar to that which was found in the CN, IC, and GM. The gradual nature of the increase is reminiscent of that in the GM as the increases in the CN and IC were rather steep.

However, the responses during classical conditioning were not at all like those in the CN, IC, and GM since the auditory activity declined as training progressed and in this site the activity remained constant or even increased slightly.

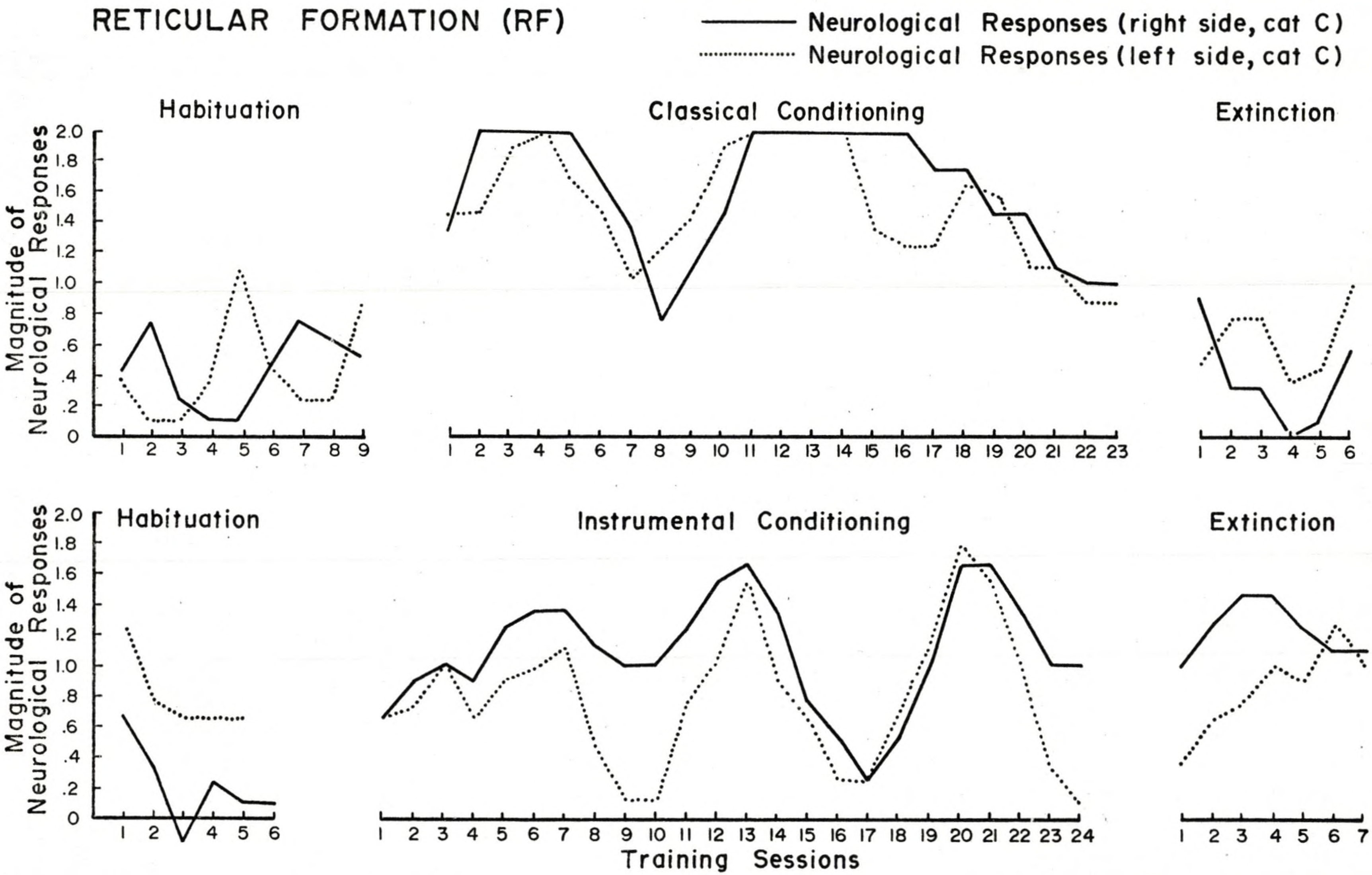

Figure 9. Three day running average of the neuronal activity only of the right and left mesencephalic reticular formation for Cat C. The data illustrate the close concordance of the neuronal activity between the left and right sides in this site.

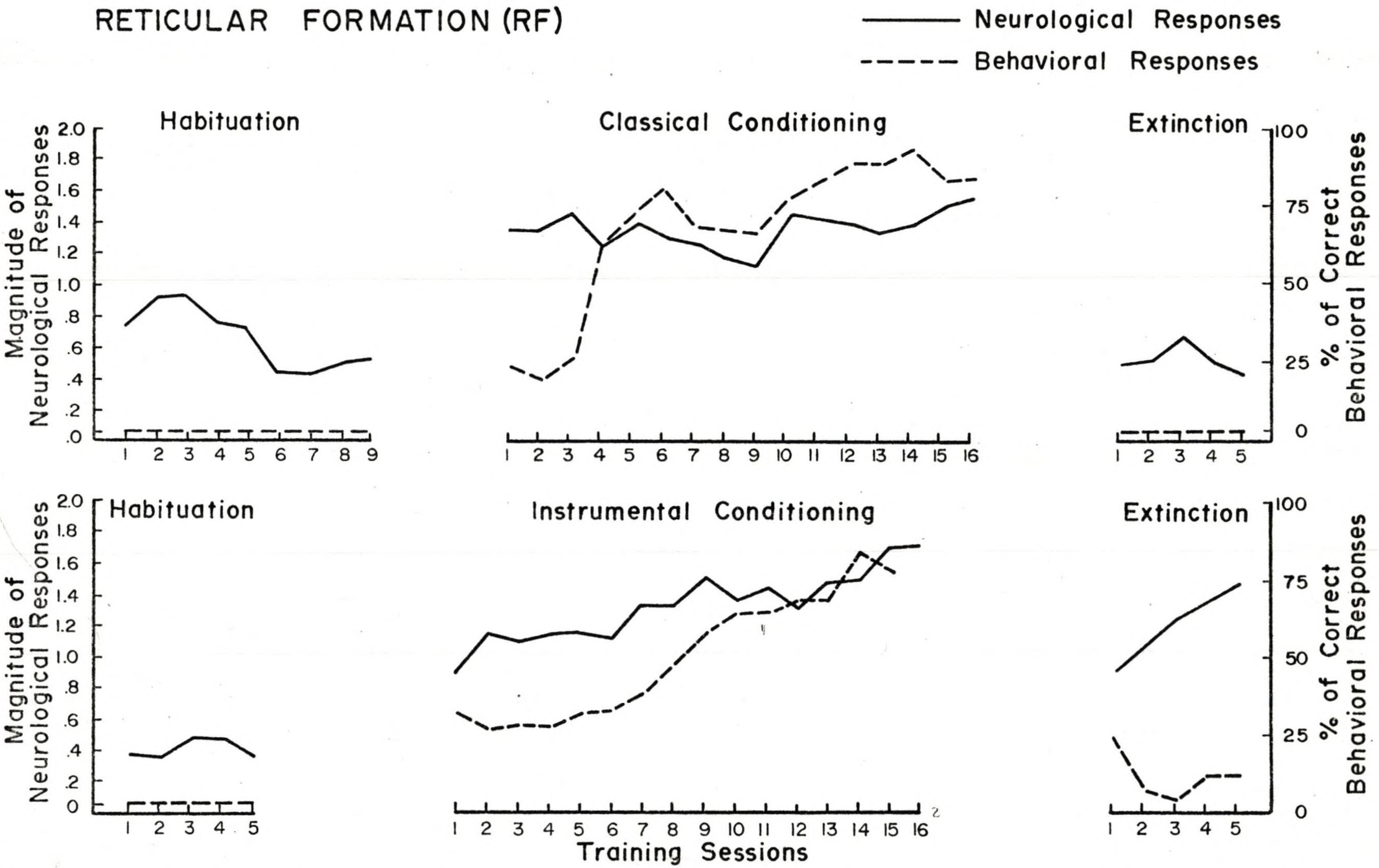

Figure 10. Three day running average of the neuronal activity and behavioral responses for four of seven cats associated with the mesencephalic reticular formation during classical aversive and instrumental avoidance conditioning.

Medullary Reticular Nucleus (MRF)

Only one cat received classical conditioning in this site (Figure 11). The same cat plus another one received instrumental conditioning. However, even with so few animals represented, the same general pattern as that found in the mesencephalic reticular formation is discernable. That is, the activity during classical conditioning remained relatively flat at a rather low level whereas the instrumental activity tended to rise throughout.

The high level of activity achieved during the extinction period of instrumental conditioning is reminiscent of the high levels attained in the auditory sites (CN, IC, GM).

Nucleus Centrum Medianum (CM)

The responses in the CM (Figure 12) during classical conditioning most closely resembled those in the auditory nuclei where a decline in activity took place as training progressed. However, in the CM the decline was quite regular throughout with no marked change as behavioral acquisition occurred. The nucleus that the CM resembled most closely in classical conditioning was the IC.

Again, the rise in activity that has become characteristic of instrumental conditioning occurred as well. The shape of the rise resembles that found in the GM, CL, and RF. Those in the IC and CN rose rather sharply by comparison. In fact, it appeared characteristic in this site during instrumental conditioning, and in the other sites presented so far, that as one moves up the brainstem the rate of increase in activity level slows down. When extinction was taken into account, the nucleus that the CM resembled most closely in instrumental conditioning was the GM.

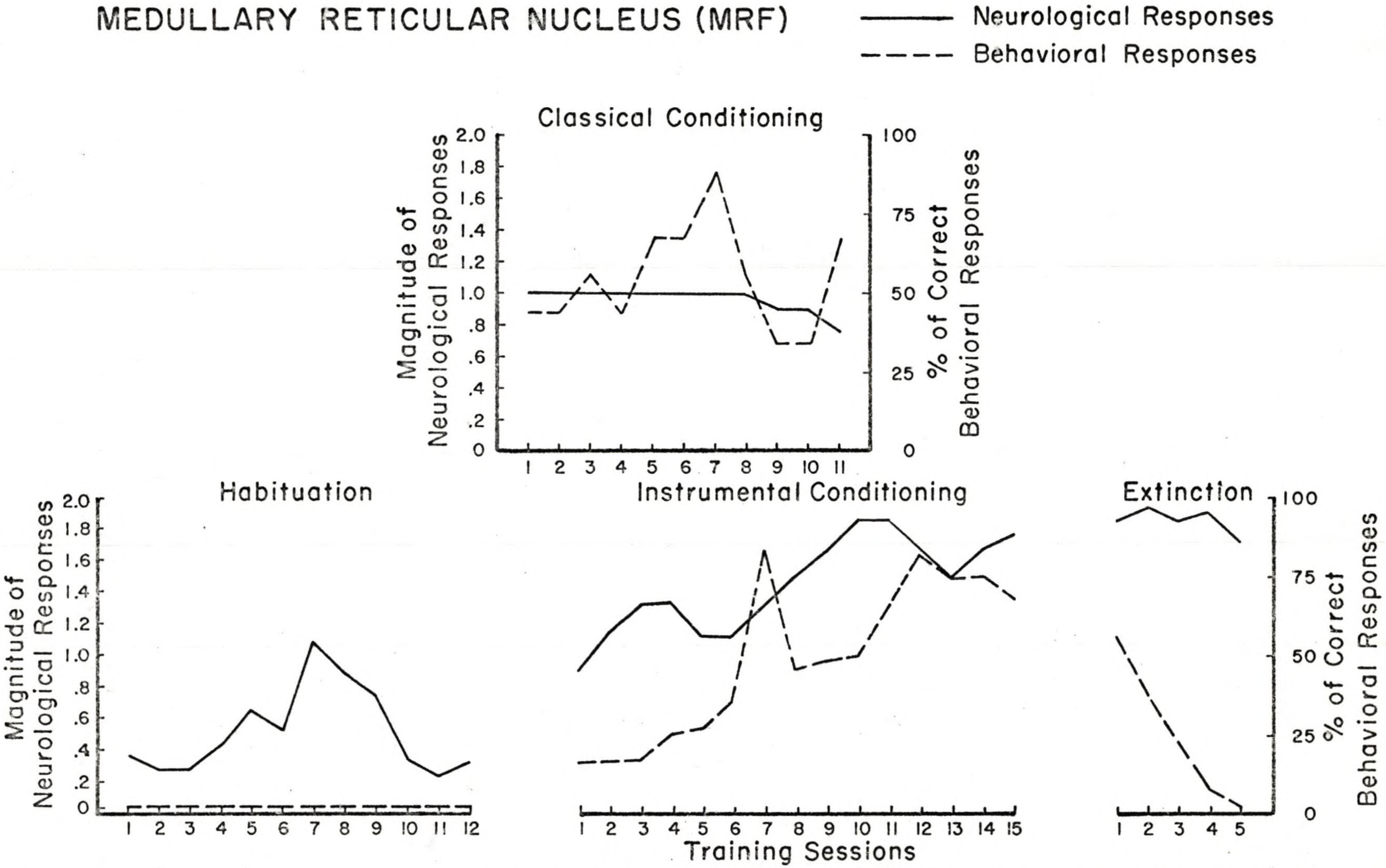

Figure 11. Three day running average of the neuronal activity and behavioral responses for two of two cats associated with the medullary reticular nucleus during classical aversive and instrumental avoidance conditioning. Habituation and extinction responses are missing for classical conditioning.

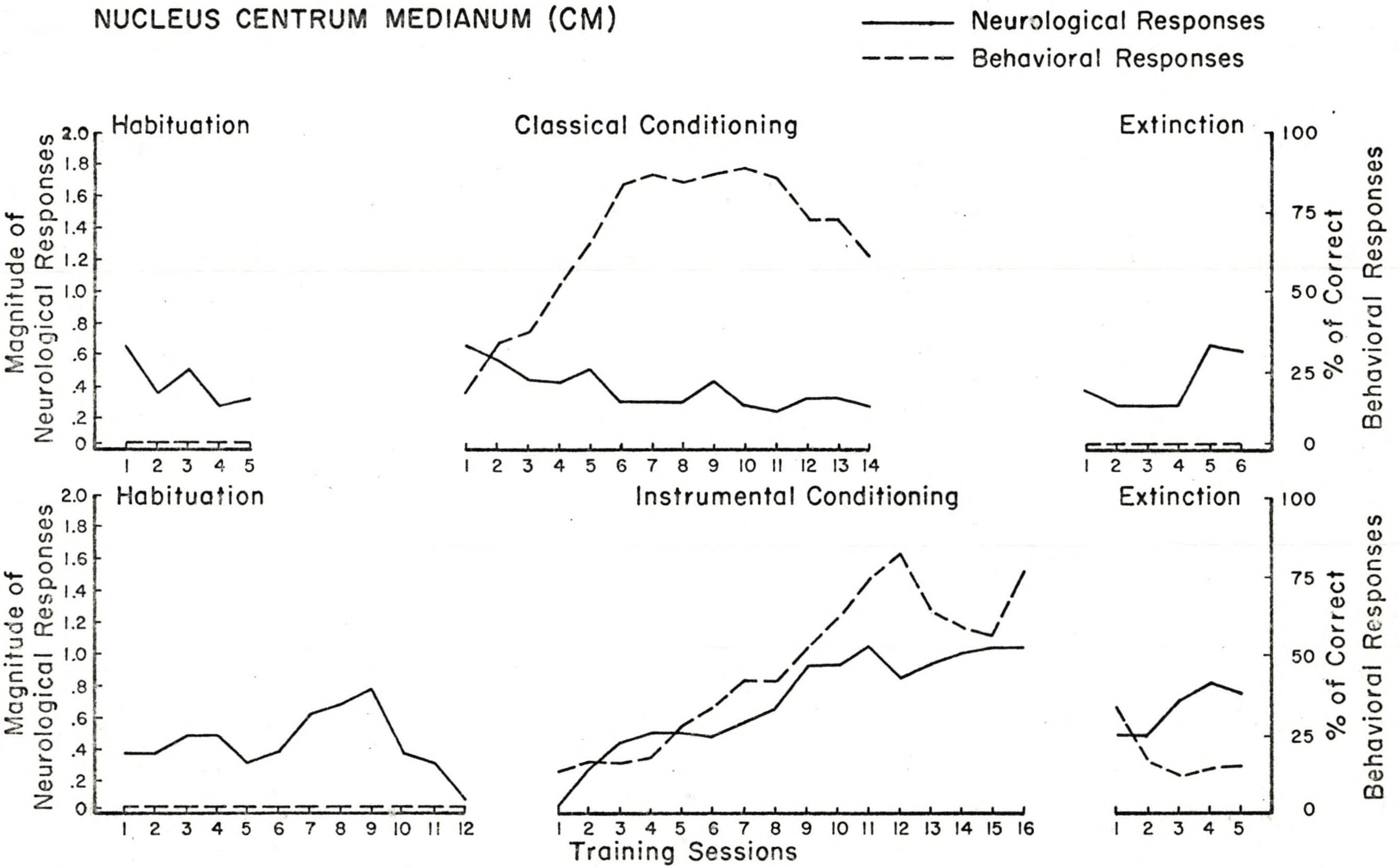

Figure 12. Three day running average of the neuronal activity and behavioral responses for three of four cats associated with the left centrum medianum during classical aversive and instrumental avoidance conditioning.

The neuronal-behavioral relationship was similar to that in all the other nuclei presented so far since the correlation coefficient for instrumental conditioning was (.90) and for classical conditioning, (-.88). The opposite relationship exhibited by the two training conditions suggests again that a general arousal factor such as that hypothesized by Mark and Hall cannot account for the data. Some differential effect involving motivational or associative processes was implied. Since this site, which is so similar to the main auditory nuclei, did not exhibit the acceleration - deceleration pattern midway in the first third of conditioning, some sort of differential motivational process may have occurred.

Central Lateral Nucleus (CL)

The responses in the CL (Figure 13) closely resemble those in the mesencephalic reticular formation (Figure 9). In both nuclei the classical conditioning responses did not decline but remained at moderate and constant levels with a characteristic drop occurring during the extinction period.

In both nuclei during instrumental conditioning the responses began a slow rise that paralleled the behavioral increase. Such an increase was also reminiscent of the slow rise in the CM during instrumental conditioning (Figure 12). The neuronal-behavioral relationship was quite high for instrumental conditioning (.90) and was low for classical conditioning (-.06).

Perhaps the trend of the activity in this site during classical conditioning should be viewed with caution since the standard deviation was quite high (Table 4) and was minimumly reduced by the transformation

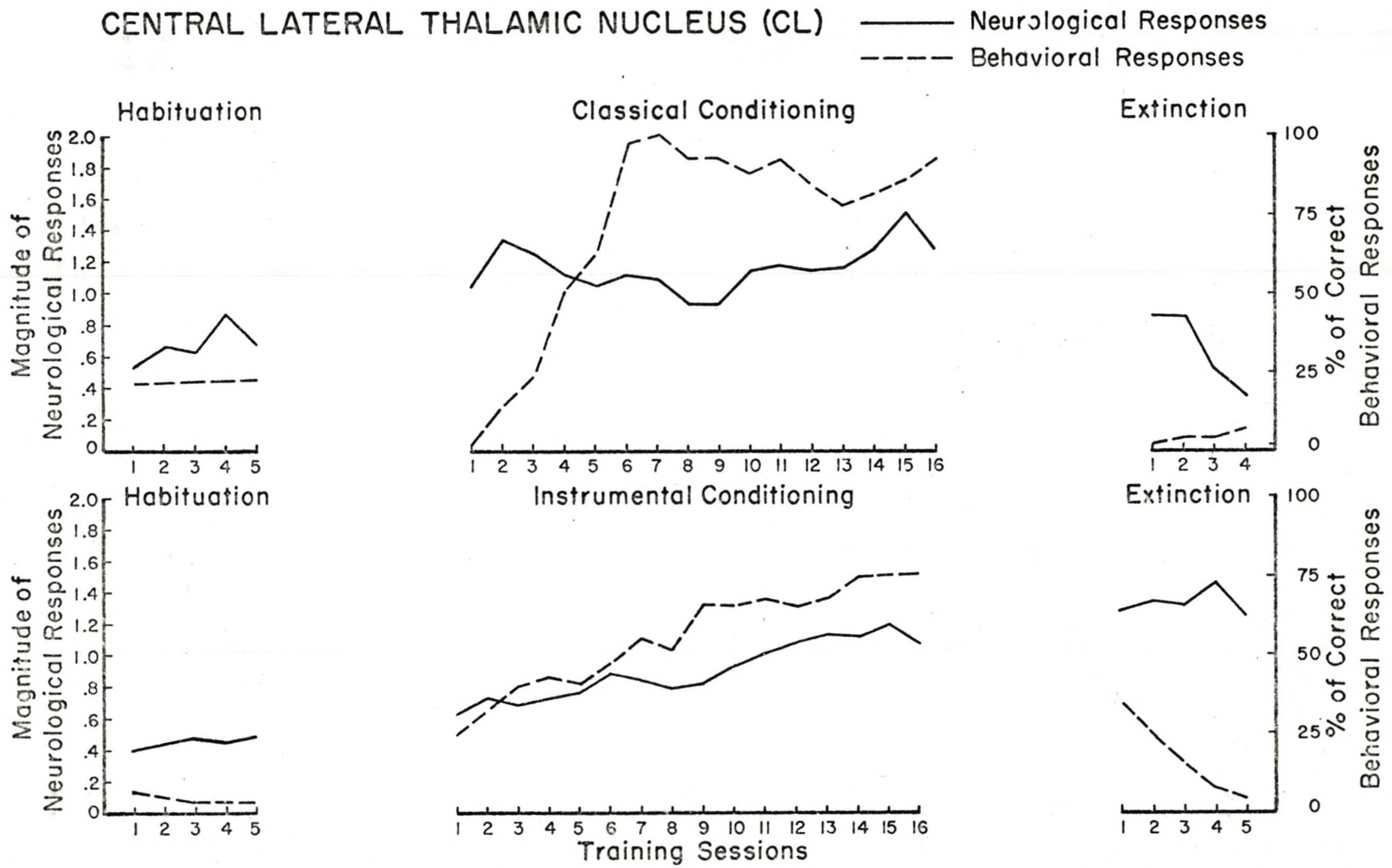

Figure 13. Three day running average of the neuronal activity and behavioral responses for four of five cats associated with the right central lateral thalamic nucleus during classical aversive and instrumental avoidance conditioning.

described in Appendix B. The standard deviations exhibited in instrumental conditioning were moderate to low.

Nucleus Centralis Medialis (NCM)

Although only two cats were implanted in this site (Figure 14) the results are interesting because of the differential responses which occurred between the sides of implantation. The neuronal activity on the side contralateral to the shock during classical conditioning was highly elevated, whereas the activity on the ipsilateral side declined. The latter declined in a fashion similar to that of the auditory nuclei and some of the diffuse thalamic nuclei (CM, RN).

On the other hand during instrumental conditioning the activity declined to zero on the ipsilateral side. This pattern in instrumental conditioning was not found in most other reticular sites. One location in the RN and the BCI responded similarly to the NCM.

Nucleus Reticularis of the Thalamus (RN)

Two sets of graphs are presented for each of the two available implanted cats (Figure 15 and Figure 16). Each is presented separately because of the contrary trends exhibited during conditioning. The remarkable difference in activity levels may be due to the quite different electrode placements. Cat B's electrode (Figure 15) was three mm anterior to Cat D's. The sustained high levels of neuronal activity in the posterior site (Figure 16, Cat D) may have reflected general emotional or motivational factors underlying both kinds of conditioning.

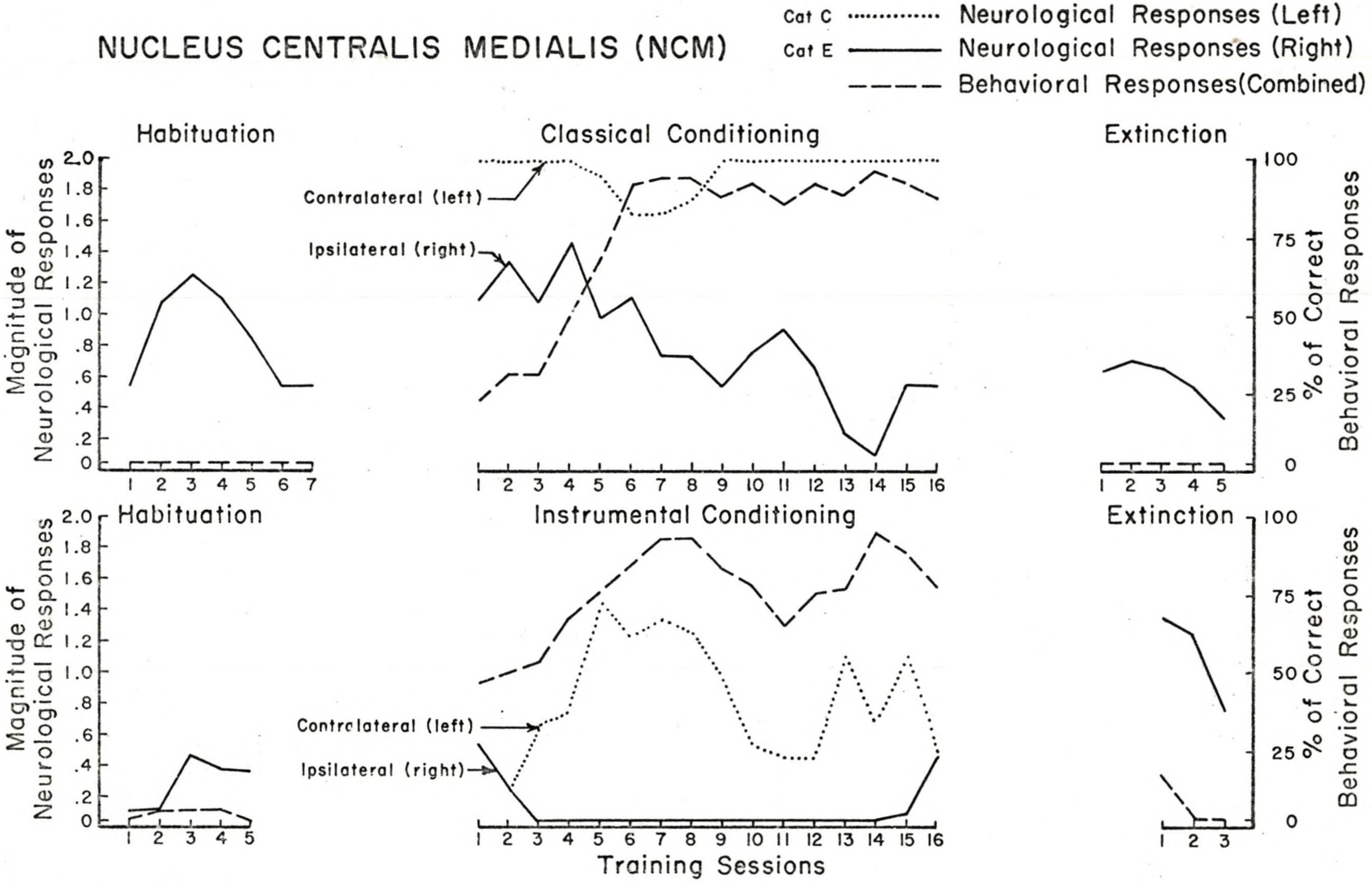

Figure 14. Three day running average of the neuronal activity and behavioral responses for two of two cats associated with the right and left nucleus centralis medialis of the thalamus. Cat C was implanted in the left side and Cat E was implanted in the right side.

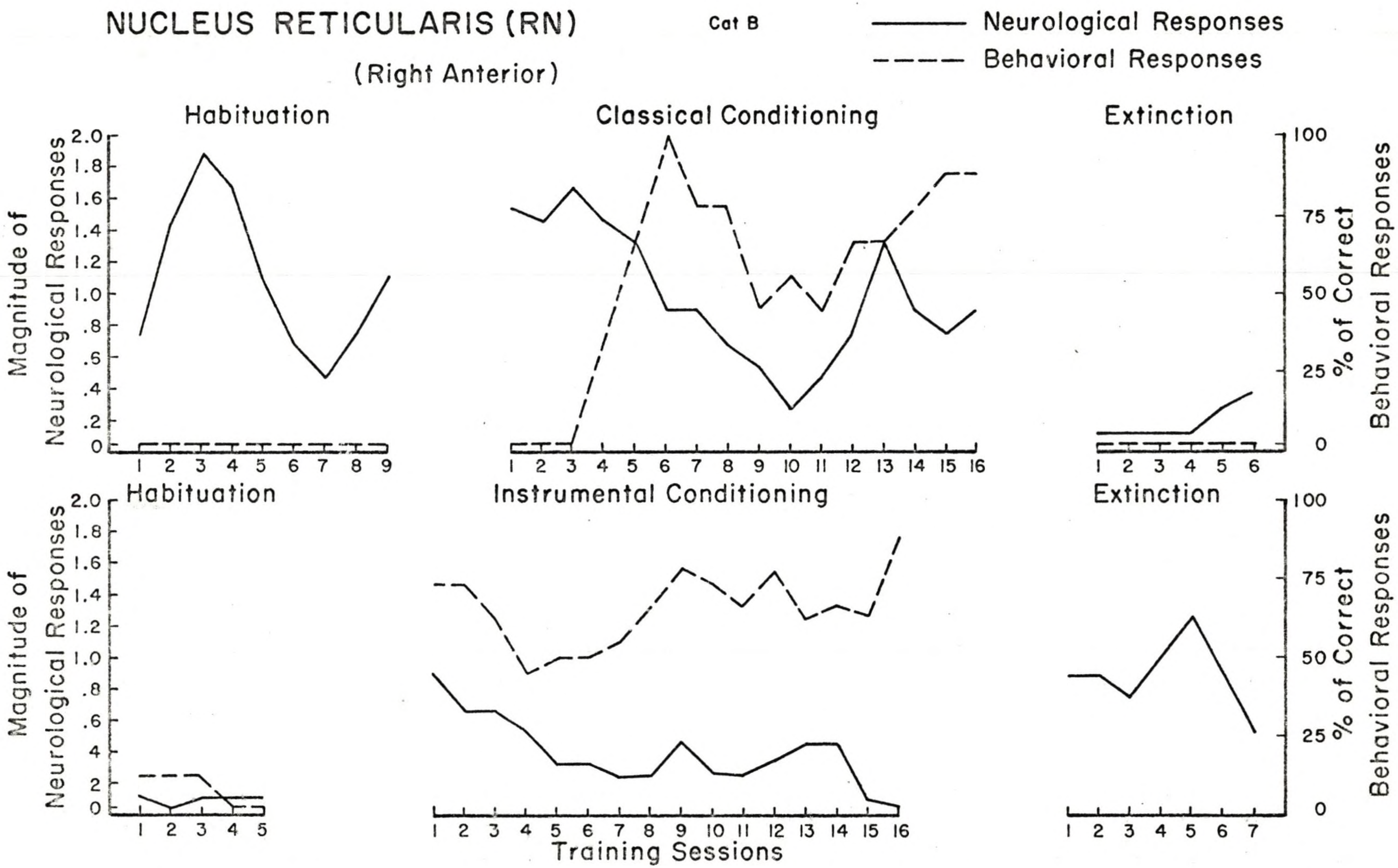

Figure 15. Three day running average of the neuronal activity and behavioral responses for Cat B (one of two cats) in the anterior thalamic reticular nucleus during classical aversive and instrumental avoidance conditioning.

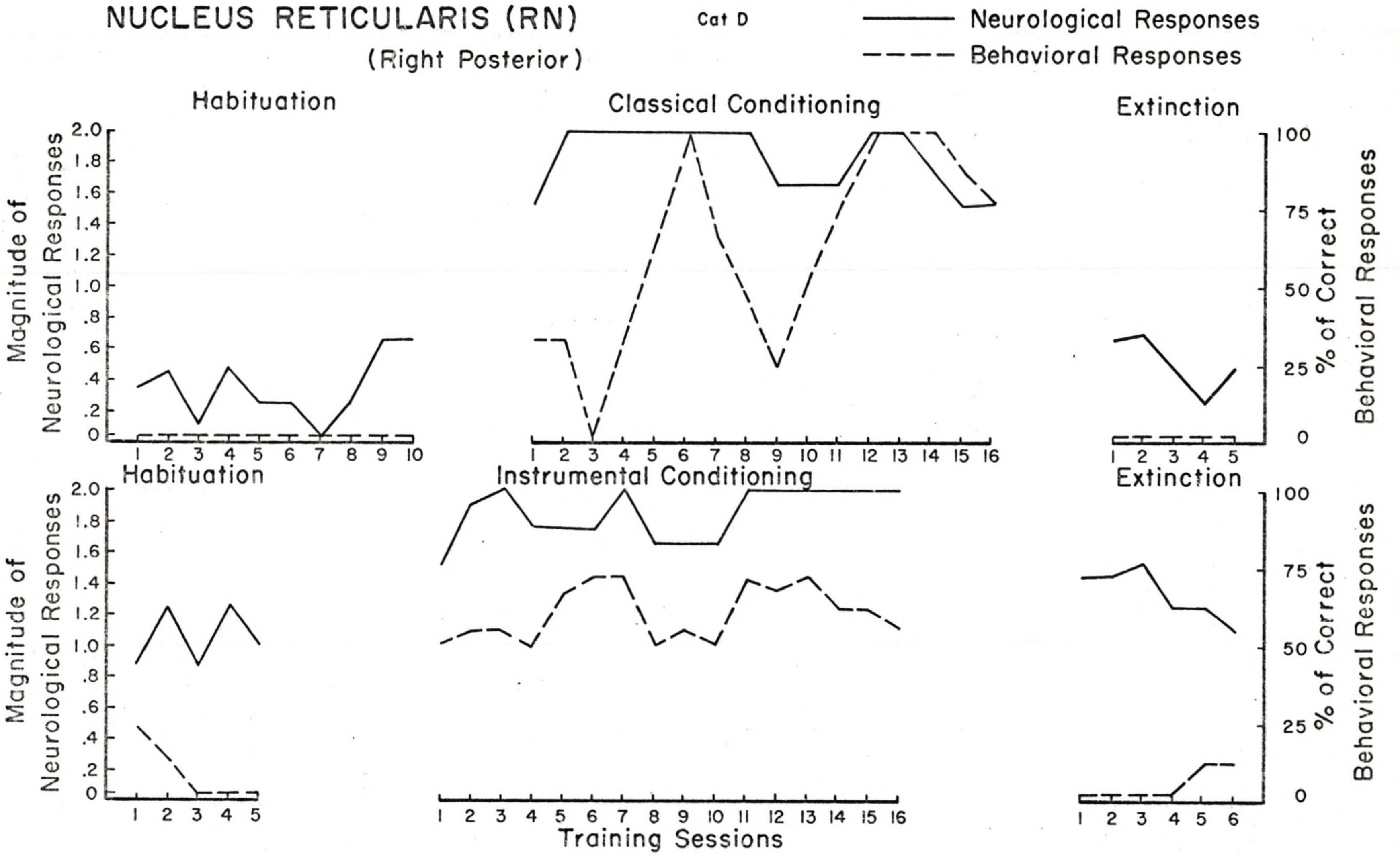

Figure 16. Three day running average of the neuronal activity and behavioral responses for Cat D (one of two cats implanted in the RN) in the posterior thalamic reticular nucleus during classical aversive and instrumental avoidance conditioning.

Ectosylvian Cortex (Right and Left Anterior; Right and Left Posterior)

In three of the four cortex sites (Figures 17 - 20) the multiple unit activity during instrumental conditioning tended to increase, whereas in three of three sites during classical conditioning the trend was downward. The one exception was in the right posterior ectosylvian (Figure 18) where the neuronal activity tended to be low and decreasing during instrumental conditioning.

In a pattern often seen before, especially in the subcortial auditory sites, activity in all four cortex sites after instrumental conditioning (extinction) increased; the activity after classical decreased. The interpretation of such a pervasive widespread event is difficult to make. One would be tempted to presume that systematic equipment artifacts were the cause if there had not been contrary trends displayed in other sites. In addition, not all cats were run at the same time. Some cats started months earlier than others.

Nucleus Ventralis Postero-lateralis (VPL)

No definite trend appeared in this site (Figure 21). Worthy of note, perhaps, is the drop in activity which appeared in the classical conditioning extinction period. This pattern has been observed in many other sites.

One of the problems encountered in this site was the high degree of variability which appeared during both classical and instrumental conditioning (Table 4).

Nucleus Amygdaloideus Lateralis (Al)

The most interesting feature of this site (Figure 22) is the action of the level of multiple unit activity during the initial phases

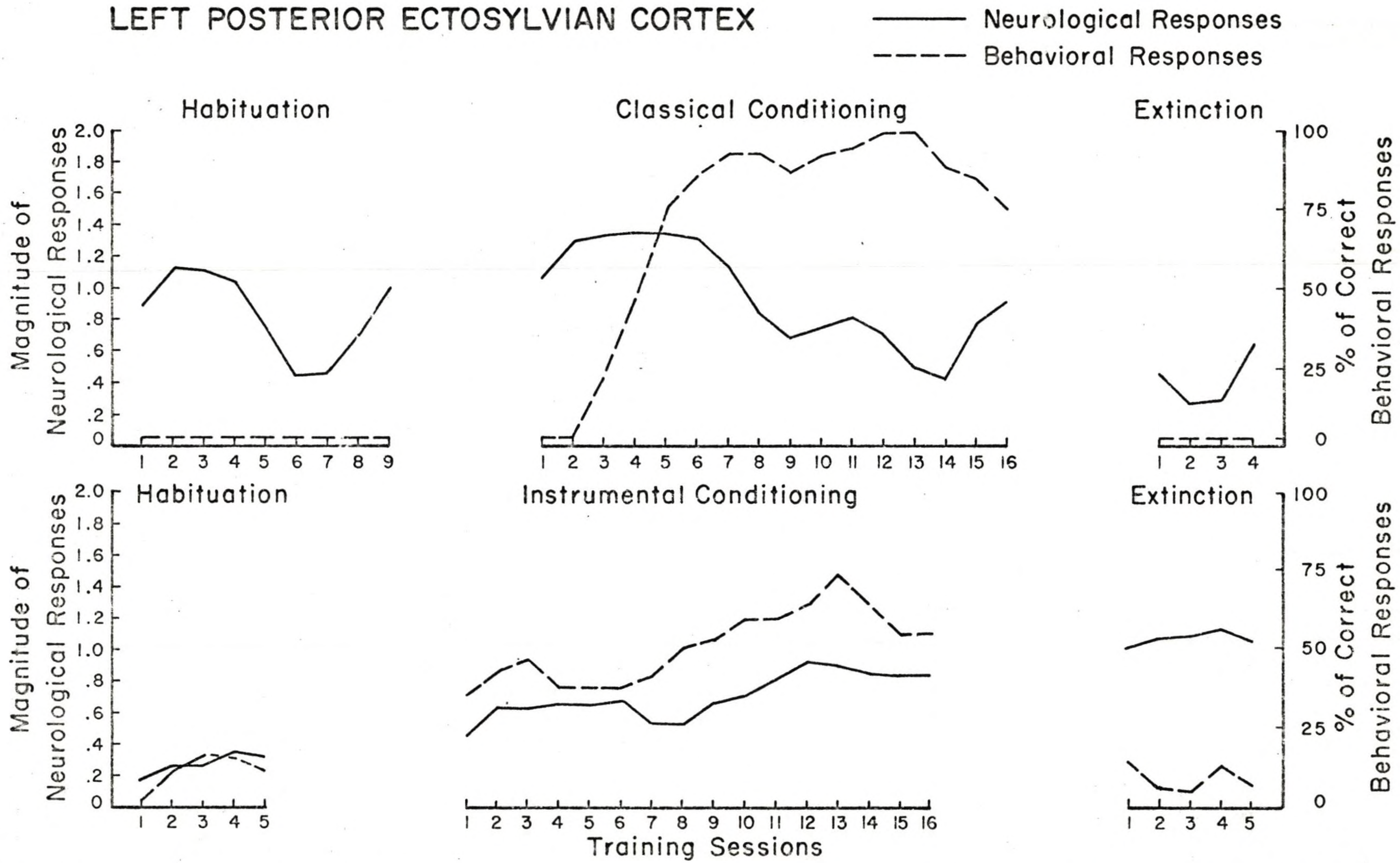

Figure 17. Three day running average of the neuronal activity and behavioral responses for four of five cats associated with the left posterior ectosylvian cortex during classical aversive and instrumental avoidance conditioning.

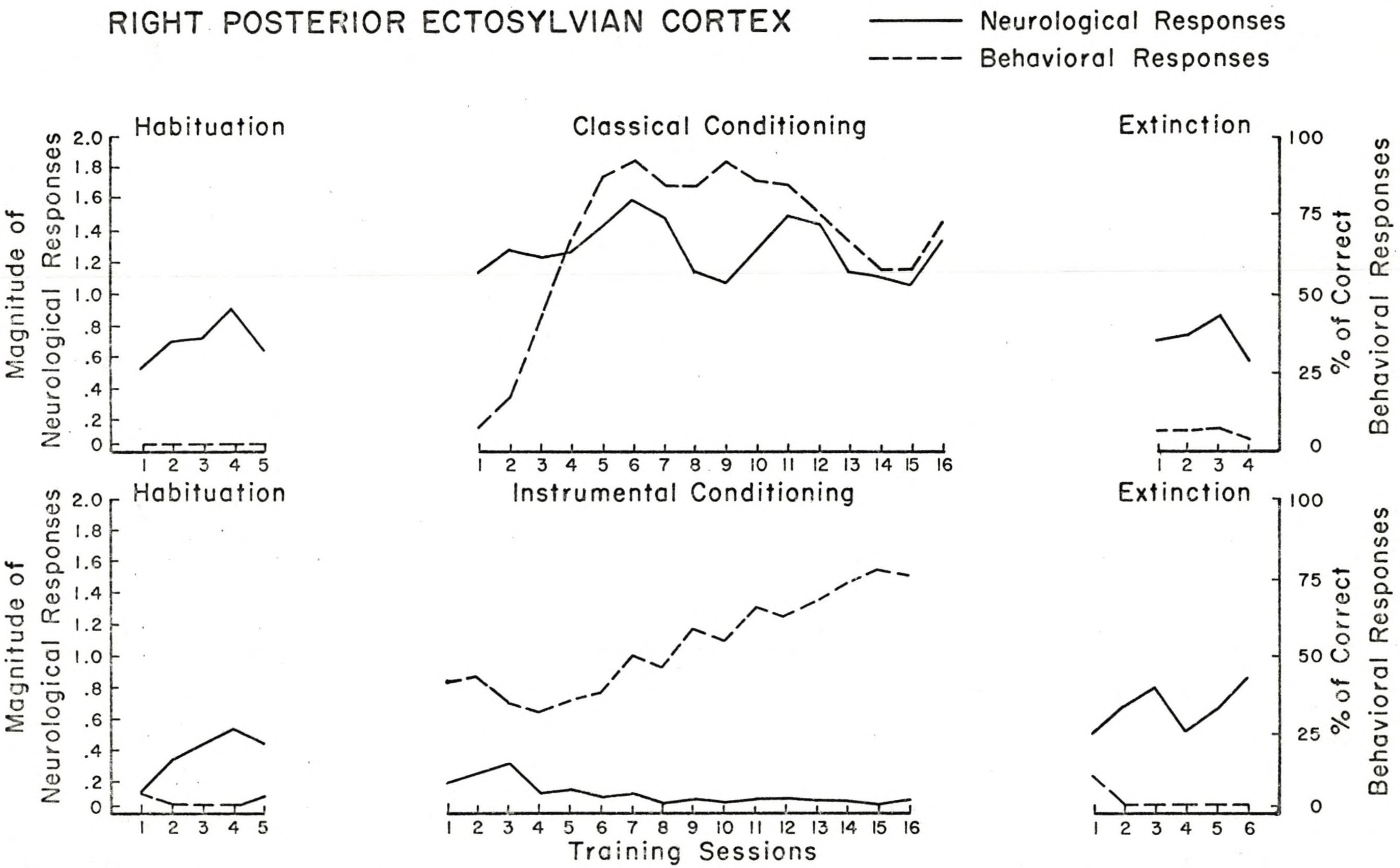

Figure 18. Three day running average of the neuronal activity and behavioral responses for five of five cats associated with the right posterior ectosylvian cortex during classical aversive and instrumental avoidance conditioning.

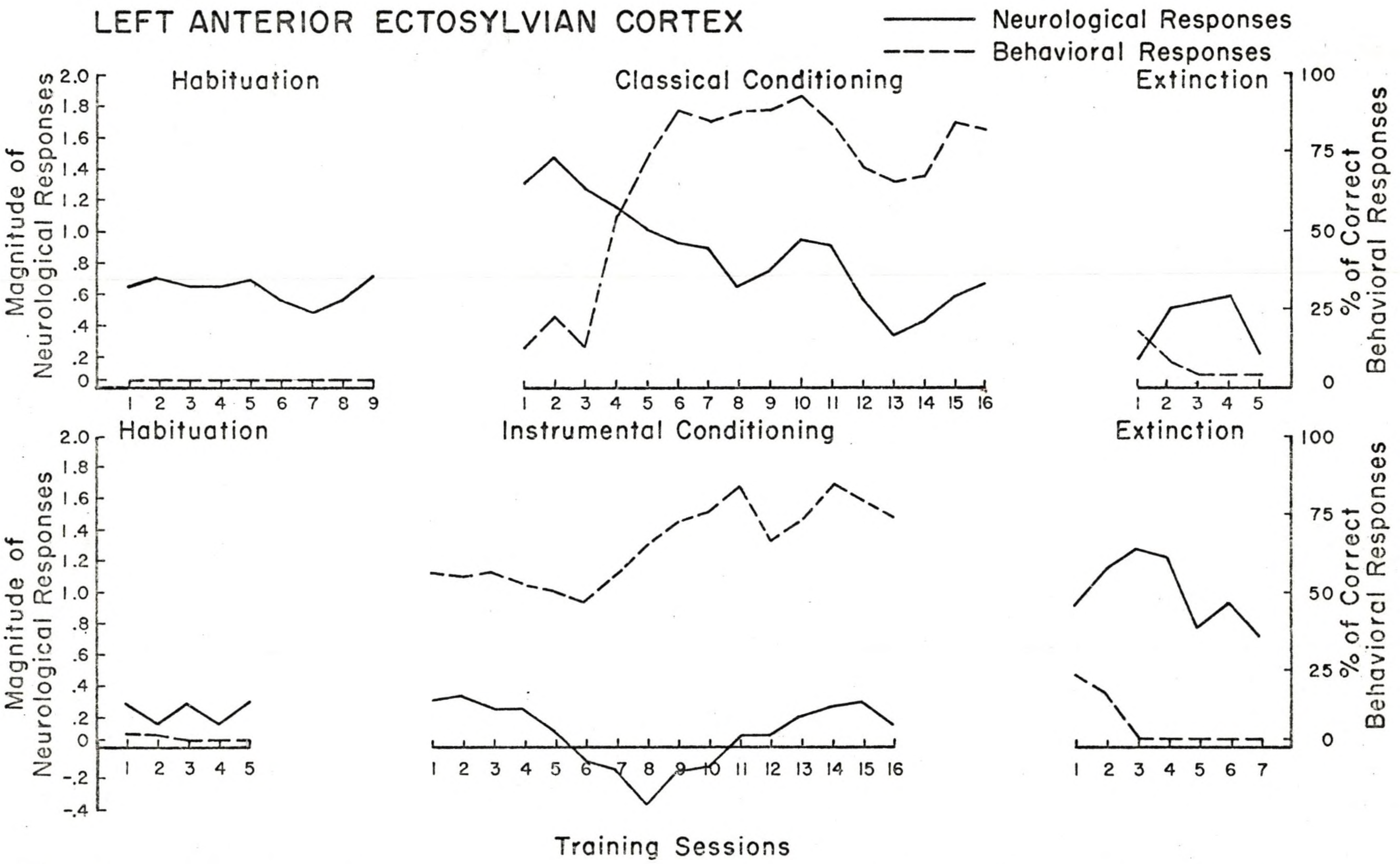

Figure 19. Three day running average of the neuronal activity and behavioral responses for four of four cats associated with the left anterior ectosylvian cortex during classical aversive and instrumental avoidance conditioning.

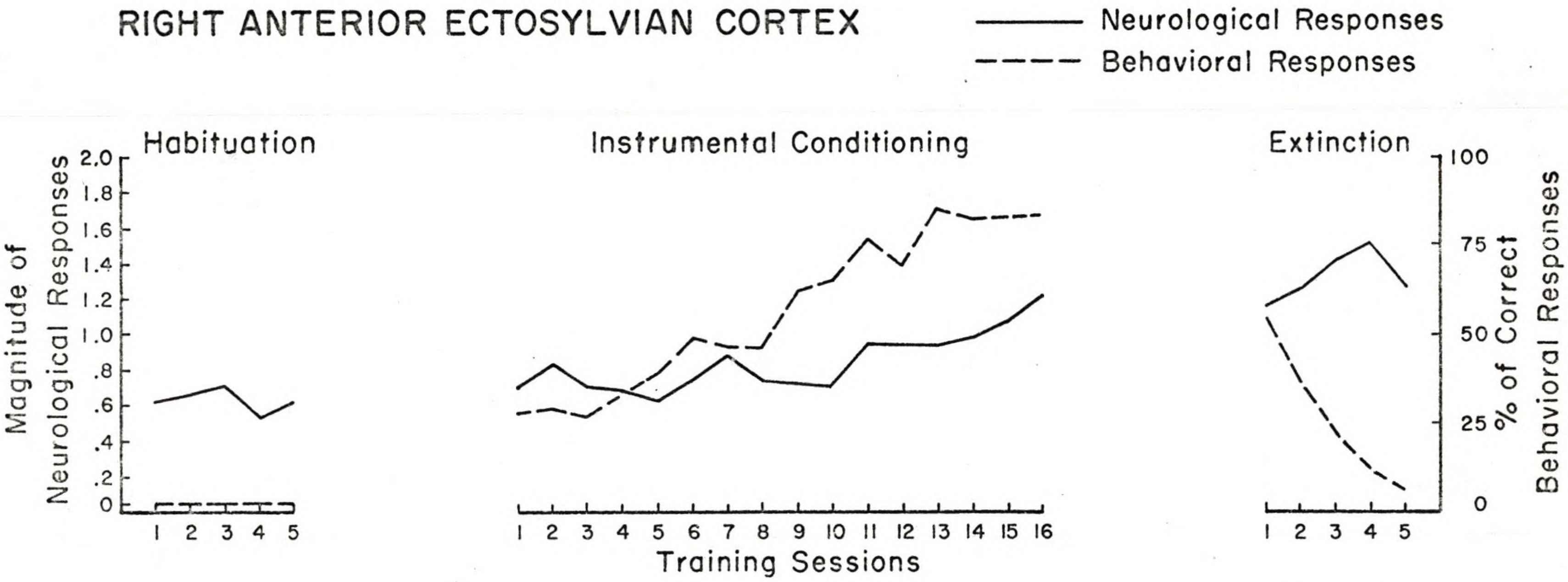

Figure 20. Three day running average of the neuronal activity and behavioral responses for four of four cats associated with the right anterior ectosylvian cortex during instrumental avoidance conditioning.

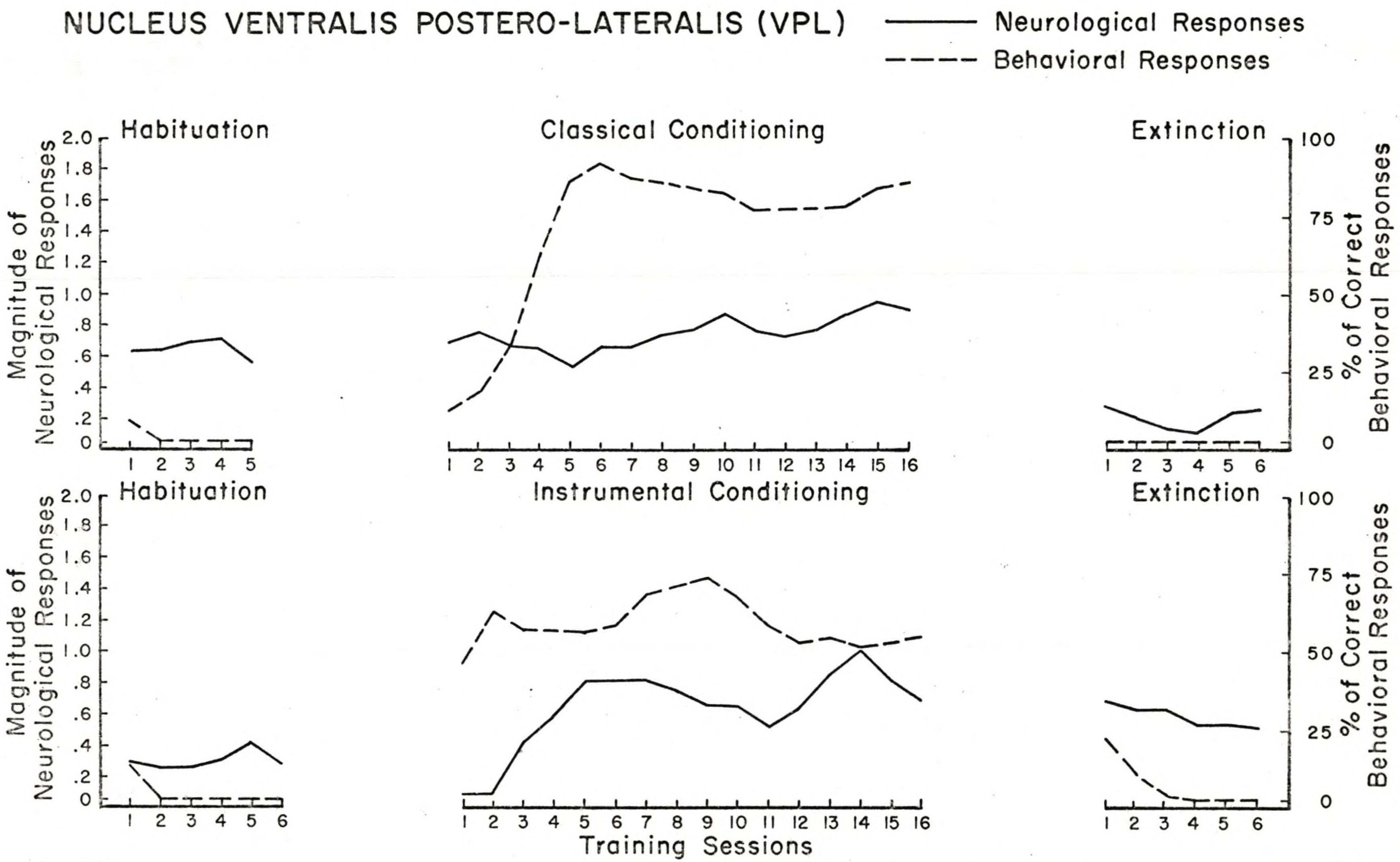

Figure 21. Three day running average of the neuronal activity and behavioral responses for four of five cats associated with the nucleus ventralis postero-medialis (left) during classical aversive and instrumental avoidance conditioning.

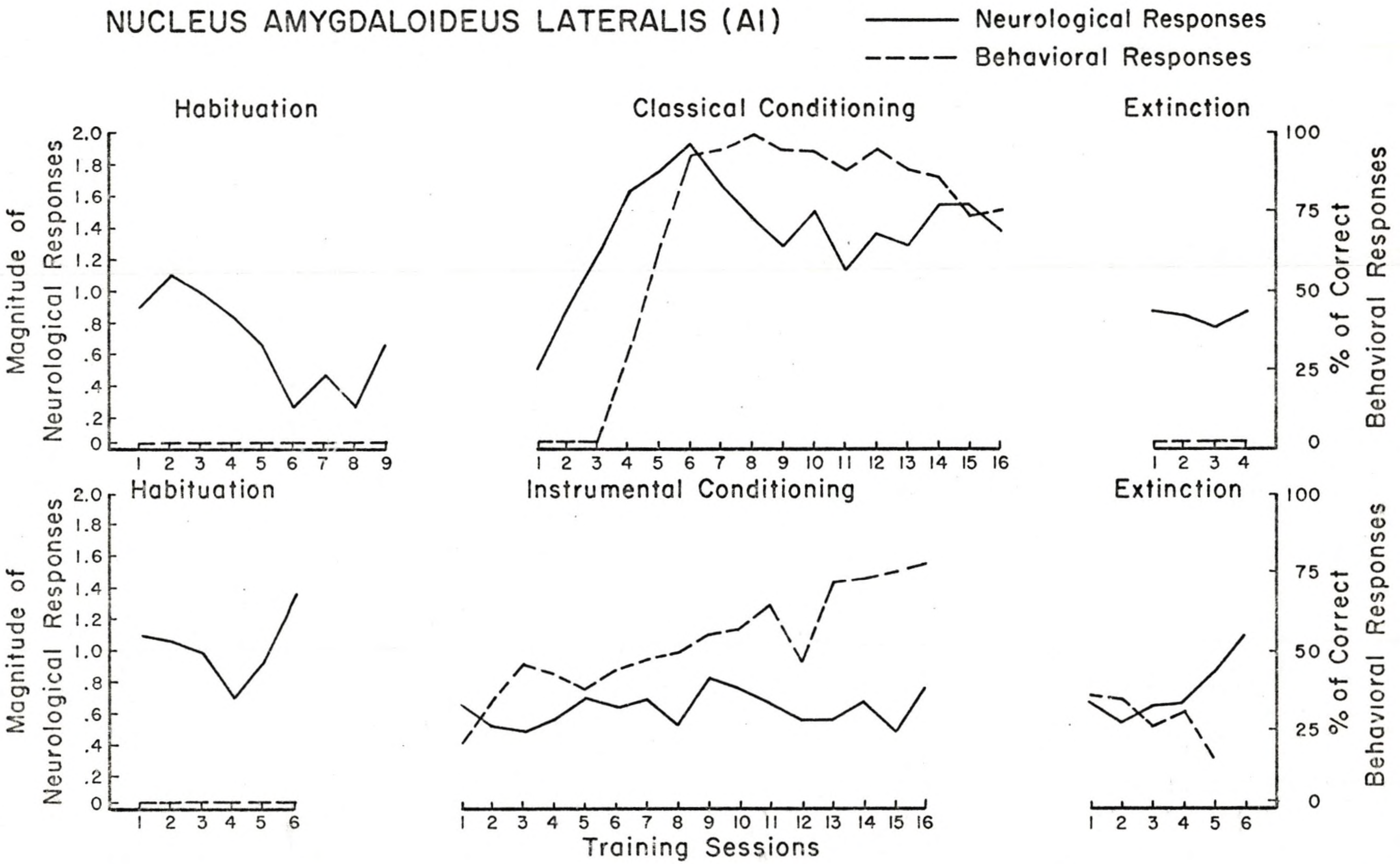

Figure 22. Three day running average of the neuronal activity and behavioral responses for two of four cats associated with the left nucleus aymgdaloideus lateralis during classical aversive and instrumental avoidance conditioning.

of classical conditioning--the rise in activity is very sharp. Such a rise in classical conditioning is very atypical as compared to the other sites sampled in this experiment. The drop in activity level at the point of completed behavioral acquisition suggested that the A1 might be involved in an energizing process which faded as soon as acquisition was complete. However, the general low level of activity exhibited in instrumental conditioning makes such an interpretation difficult.

Hippocampus (Hipp)

The data for this site was partially lost. Little is revealed by the meager data in Figure 23. No consistent trend is observable. One of the major problems encountered in this site was the judges' evaluation of the high degree of bursting multiple unit activity. The neuronal bursting often occurred between trials as well as during the training periods. Thus, it seemed to appear as random activity with no relation to observable stimulus conditions. This site was the most unreliably judged.

Caudate Nucleus (CdN)

The neuronal activity in classical conditioning (Figure 24) declined during the first one-half of the learning period; the trend remained stable thereafter. The overall neuronal activity level was higher than that in instrumental conditioning. Instrumental activity began low and remained at a constant low level throughout. As a result, negative neuronal-behavioral correlation coefficients occurred.

Again, the characteristic drop in classical extinction and the

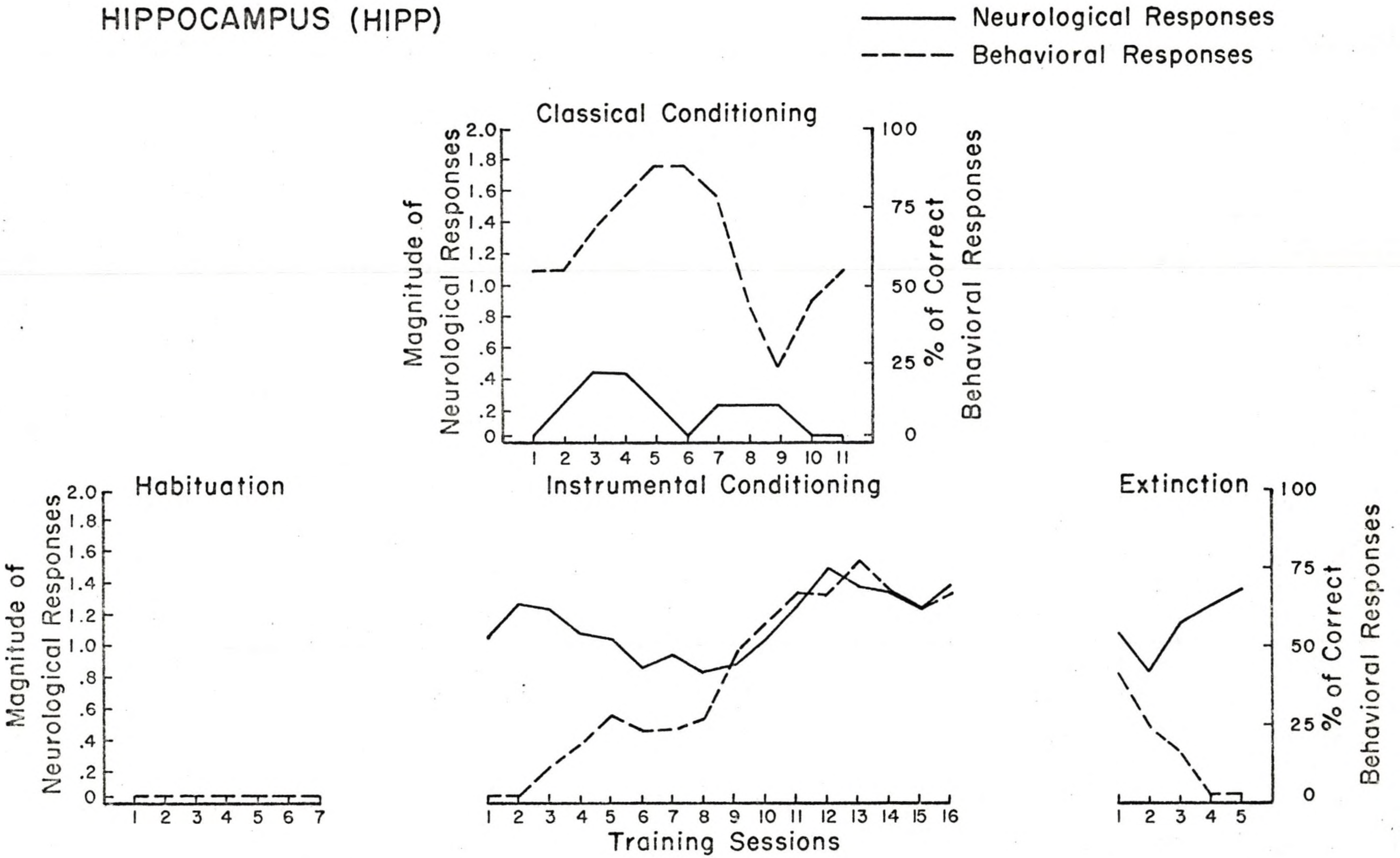

Figure 23. Three day running average of the neuronal activity and behavioral responses for two of two cats associated with the hippocampus during classical aversive and instrumental avoidance conditioning.

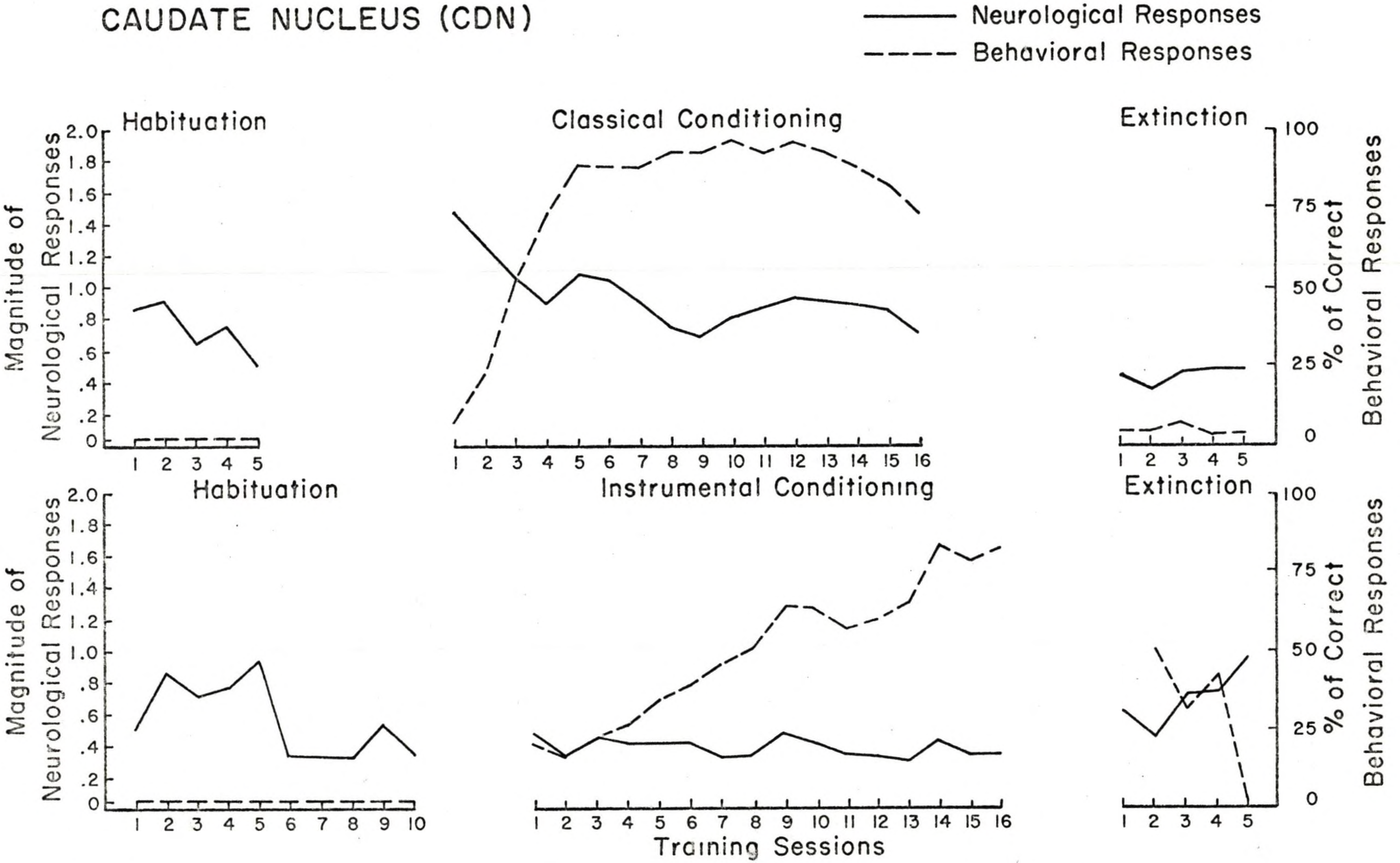

Figure 24. Three day running average of the neuronal activity and behavioral responses for three of four cats associated with the left and right caudate nucleus during classical aversive and instrumental avoidance conditioning.

increase in instrumental extinction that has been noted in most other sites has occurred here.

CHAPTER V

DISCUSSION

An attempt was made to meet two major overall objectives in this study. The first objective was to study the method of multiple unit recording by correlating it with three fairly well-known learning procedures: classical conditioning; instrumental conditioning; and discrimination conditioning, using a noxious stimulus as the unconditioned stimulus. To meet the first objective, certain questions concerning the method were posed. Could multiple units be quantified so that the resulting neuronal responses would reflect stable relationships among neurological sites, as well as between neural responses and behavior? Would the resulting quantified dependent variable be sensitive enough to assist the neurophysiologist in deciding for or against a viable hypothesis found in current literature? Is the multiple unit method sensitive enough to register a difference neurologically among different behavioral procedures? To test the latter possibility the behavioral parameters were kept as similar as possible. The results indicate that the method was quite successful in answering the questions in the affirmative.

A second major overall objective was to use the method as a tool to uncover phenomena about learning. The larger portion of this study was concerned with the second objective.

One of the problems in the employment of the method was that of evaluating a huge amount of data. At this point the decision was made to use a measure that simply reflected a gross overall change in the neuronal population during the application of conditioning procedures. The hope was that the basic existence of some general and meaningful neuronal-behavioral phenomenon could be demonstrated. However, in the process of this general evaluation, potentially important detailed data had to be ignored. For instance, no distinction was made among short onset neuronal responses, sustained neuronal responses, and delayed neuronal responses during the CS period. We are certain that the equal treatment of these responses masked important relationships. In the future, the assistance of a computer to digest such data, and in such volume, would be required. Concerted efforts are presently being made in our laboratory to secure this assistance. Therefore, the data gathered in this study accentuate the necessity of computer assisted data collection and analysis in future studies.

Another major problem of data evaluation was the reduction of excessive variability in responses among cats. Often in a common electrode site among several cats an obvious neuronal invariance over training sessions occurred. However, one or two cats sometimes emitted quite different patterns. At this point a purely arbitrary, unscientific, decision was made to omit the two deviant cats from consideration. Reliable criteria for future studies definitely must be developed. Another problem of variability emerged as a function of the small number of cats, i.e., the cats learned at different rates. Consequently, the pattern of one cat would lag somewhat behind another. Hoping that the differences would average out, the lag effect was simply ignored and the responses averaged across cats. However, we feel that the procedure adapted here is an improvement over most past studies (multiple unit and EEG studies) where only the records of individual subjects were presented.

Little is claimed for precisely what was measured beyond the assertion that some measure of overall neuronal spike activity was tapped. On the basis of other studies (Buchwald, Halas, and Schramm, 1966_b; Halas and Beardsley, 1968) the spike activity of a population of neurons firing within .5 mm of the electrode tip was probably recorded. At the present time little is known conclusively about the exact relationship among the spikes. Preliminary results from another study presently being conducted in the Halas laboratory indicate that the spike record consists of a combination of coincidentally firing neurons, individually spiking neurons, and the chain-like firing of a sequence of neurons.

Results from microelectrode studies indicate that in most sites many spontaneously firing neurons cease firing or reduce their rate of firing when a stimulus is introduced. In certain sites under various conditions (e.g., IC, cortex) the data in the present study illustrated a similar effect in the inhibition of multiple unit activity. However, inhibition of multiple unit activity did not occur as often as that reported in microelectrode studies. Two possibilities exist: either multiple unit recordings masks the overall reduction in rate of firing of some neurons by the increase in rate of others, or overall increases in neuronal firing are the more general occurrence. The latter is more consistent with evoked potential records. After many stimulus presentations these potentials persist over widespread areas of the brain.

One of the important results of the application of the multiple unit method was its ability to discriminate neurologically between two quite similar behavioral procedures. As the conditioned stimulus (1500 hz tone) and the unconditioned stimulus (shock to the right hind paw) for the two types of training procedures were kept as congruent as possible, the pervasive, remarkably different neuronal patterns which resulted not only attest to the sensitivity of the method but imply that classical and instrumental conditioning procedures share very little in common in the CNS. The trend for classical conditioning was downward throughout the period and the trend for instrumental was upward. Both trends for all the sites, averaged together, were statistically significant ($p \leq .01$). However, some sites exhibited opposite trends which indicate that the overall pattern was pervasive but not complete. Notable exceptions were the nucleus amygdaloideus lateralis of the limbic system, the brachium of the inferior colliculus, the central lateral thalamic nucleus, and the nucleus ventralis posteromedialis. Some sites exhibited no upward or downward trend.

Since behavioral learning occurred and the neuronal trend was upward in instrumental multiple unit activity, positive neuronal-behavioral correlation coefficients tended to occur--and some of them quite high--for instrumental conditioning. Since the classical neuronal trend was downward, negative correlation coefficients for classical conditioning tended to occur.

Among psychologists there has been a long standing controversy on whether "one process" or "two processes" underlie the two conditioning procedures (Kimble, 1961). On the basis of this study, substantial evidence is presented for the two (or more) process approach. The pervasive difference in neuronal responses to the two procedures even extended to their respective extinction periods. Obviously, two quite different reactions are occurring in rather widespread areas in the brain.

Using classical aversive conditioning Hall and Mark (1967) found that evoked potential responses were positively correlated with behavior in reticular and auditory projection sites. However, they quickly pointed out that it was their opinion that the neuronal increases (evoked potentials) were related to unconditioned changes in behavior rather than conditioned changes. That is, a conditioned emotional response (CER) rather than a conditioned behavioral response to the CS had occurred and as such explained the data. They thought Mowrer's concept of "fear" fitted the data. Hall and Mark then hypothesized that during instrumental avoidance conditioning the neuronal CER ought to undergo extinction as appropriate avoidance behavior became established. Thus, they predicted a decline in neural activity once the acquisition of behavioral instrumental avoidance had begun.

Our data do not confirm this prediction since a general increase in neuronal activity occurred throughout behavioral acquisition. Moreover, if emotion had been reflected in the neuronal activity then there should have been a sharp drop in activity during extinction. Instead, there was a sustained, high level of neuronal activity long after the overt behavior had extinguished. Furthermore, if emotion had been responsible for the neuronal responses, the decline in classical conditioning should not have occurred. Clearly, a CER cannot account for the data in the study. Nor is the explanation for these contrasting results, particularly during extinction after classical and instrumental conditioning, readily available through any other theory of learning or by the writer of this paper. What is perhaps happening is that brain processes which are related to learned behavior can now be monitored with reasonable accuracy. As more substantive information is gathered about these brain processes, then new theories will evolve.

Some sort of a neuronal alerting or vigilance reaction may have occurred in the rather widespread initial increase in activity in the first training session of both classical and instrumental conditioning. This reaction is more visible in classical than in instrumental conditioning. During classical conditioning a decline in activity often began in the second session whereas during instrumental conditioning the neuronal activity continued to increase. In both cases, the very first conditioning training session revealed a heightened level of neuronal activity which then served as a starting point for their respective downward and upward trends. It is difficult to think that such an initial increase is related to emotional arousal because behavioral emotionality, such as that found during classical conditioning, lasts considerably longer.

A very suggestive pattern occurred in the three main subcortical auditory nuclei where inflections in the trend of activity for both kinds of conditioning may have reflected some sort of an associative (learning) process. That is, during classical conditioning a sharp decline in neuronal activity often occurred during the first one-third of the training sessions whereas during instrumental conditioning an accelerated increase often occurred during the first one-third of training sessions. Interestingly, the inflection points (Table 6) appear to occur first in the lowest center (CN) and latest in the highest center (GM) for both types of conditioning (one exception occurs in the IC during classical conditioning). These data suggest that the subcortical auditory nuclei function as a unit during learning. There is already considerable data showing that these same nuclei function as a unit during the transmission of sensory information which therefore indicate that these nuclei are capable of mediating several quite different functions. This is not surprising in view of the size and neural complexities of these structures.

These data are not inconsistent with the single unit data of Mountcastle, et al. (1963) while studying proprioception. These researchers found that individual first order neurons in the medulla (cuneatus and gracilis nuclei) responded in all or none fashion to a specific change in limb movement whereas an individual neuron in higher centers (ventrobasal nuclear complex of the thalamus) appeared to integrate the data from the many lower neurons by simply firing at different rates. Others have found similar integrative phenomenon in the auditory system relative to sound localization (Masterton, et al., 1967). Thus one may speculate with some justification that we may see here the reflection of a similar integrative, but associative, process.

Some reinforcement of this view may be obtained tangentially by noting that the overall neuronal pattern for instrumental conditioning occurred later than that for classical. This is consistent with the speed with which the animals learned behaviorally. Thus, the neuronal difference may be related to a difference in learning.

Electrodes in three levels of reticular nuclei were implanted: one site in the medulla, one in the mesencephalon, and four in the thalamic diffuse system. In three of the sites (RN, MRF, NCM) only two cats were implanted, and in one of them (MRF) part of the data was lost.

Only the RF, CL and the CM involved enough cats so that reliable conclusions are possible. The neuronal activity during classical conditioning acted quite ambiguously as a slight increase (CL), a slight decrease (CM), and no change (RF) occurred. The instrumental activity in all three sites increased in a fashion similar to the instrumental activity of the auditory nuclei. These data support the data from the auditory nuclei which suggest that certain neural structures will display different neuronal activity for different types of learning. The significance of this is that various neural structures are able to integrate their functions in different combinations for different types of learning. Thus, one neural structure does not mediate just one type of learning. Rather it is capable of collaborating with other structures in the mediation of two or more different types of learning. The brain has generally been thought of as being very plastic and this data clearly demonstrate some of this plasticity.

Certain sites exhibited trends contrary to the overall neural downward (classical) and upward (instrumental) trends found generally. The most noteworthy were the neuronal responses found in two of four cats in the Al, Cat D in the posterior RN, and Cat C in the NCM. In all three sites, very high levels of neuronal activity occurred during classical conditioning. The high levels obtained suggest that they may have reflected sustained emotional arousal or motivation. Only the single cat implanted in the posterior RN emitted correspondingly high neuronal activity levels for both conditioning procedures. Interestingly, two of the four cats during classical conditioning in the Al exhibited a sharp increase in activity during the behavioral acquisition period, but declined thereafter. Such a pattern suggests that the A1 may have participated in motivational processes such that as acquisition was complete its operation was no longer in effect. It must be recognized that with so few cats implanted in these sites that conclusions drawn are highly speculative.

In conclusion, the writer feels that the two main overall objectives of this study were met. It was found that minimal quantification could reveal stable relationships among the responses of different anatomical sites and between neuronal response and behavior. Furthermore, the method proved sensitive enough to register consistent neuronal differences between highly similar behavioral conditioning procedures. Thus, as it was used in this study, multiple unit recording method proved to be sensitive enough to detect neuronal differences as a function of behavioral procedures; and very importantly, demonstrated that these neuronal differences would remain stable across cats and across time.

Besides being adequately sensitive and stable, the multiple unit method revealed interesting phenomena about learning. Apparently, neurons in the same implanted sites are capable of mediating remarkably different neuronal response patterns to different, but superficially similar, behavioral training procedures. Moreover, the patterns exhibited revealed that a reliable decreasing neuronal trend occurred for classical conditioning and an increasing neuronal trend occurred for instrumental conditioning. Such consistent trends made rather high correlation coefficients possible between neuronal and behavioral responses. Moreover, patterns of curve inflections for the auditory nuclei under the two types of behavioral training procedures suggested that progressive integration of associative processes may have occurred.

These and other substantive observations lead us to conclude that multiple unit recording is a fruitful method for correlating brain-behavior relationships.

CHAPTER VI

SUMMARY

Multiple unit recording began as a special way of observing gross nerve action potentials. At that time a technique was needed to record the asychronous potentials that resulted from a long train of stimuli such as the irregular neuronal firing from gustatory or olfactory stimuli. Beidler (1953) probably developed the first satisfactory technique for summarizing the train of unit activity by way of an electronic integrator. His integrator and the ones subsequently developed in other laboratories reflected the combined effect of the frequency and amplitude of the massed spike activity.

Other laboratories (Starr and Livingston, 1963; Schlag and Balvin, 1963; Buchwald et al., 1965_a) extended the method by recording from grey matter. A diversity of stimuli were used ranging from white noise to levels of sleep state. One laboratory (Albe-Fessard et al., 1962) used the method in a clinical setting to study characteristic bursting activity which was seen to accompany Parkinsonian tremor. Other laboratories, whose work is of particular interest here, studied multiple unit responses in relation to conditioned and unconditioned stimuli in learning (Buchwald et al., 1965_b, 1966_a; Galin, 1964; Halas and Sandlie, 1966).

Encouraging results emerged from these studies. The method was found sensitive to changes in behavior while EEG recordings were dissociated from behavior (Buchwald et al., 1965_a; Podvoll and Goodman, 1967). The method was found to be specific to sense modalities (Halas and Beardsley, 1968). That is, visual stimuli evoked responses in visual anatomical locations and not in auditory locations, and vice versa. Moreover, the method was found sensitive enough to detect changes in overall firing activity as an electrode was moved from nucleus to nucleus.

In the use of multiple unit recording with behavioral conditioning procedures, regular and systematic conditioned neuronal changes occurred. Others have observed similar neuronal conditioned changes using evoked potentials and single units. However, in a review of the literature by Mark and Hall (1967) the only consistent changes, when all training and recording methods were taken into account, occurred under classical aversive conditioning procedures (Unfortunately, no one had used instrumental training procedures with multiple units.) These authors concluded that emotional arousal (specifically, Mowrer's "fear") could account for the data better than the concept of a neural associative process and that the neural correlations with behavior were highest when the neural component was considered as a conditioned emotional reaction (CER).

Such a hypothesis is directly testable. Hall and Mark predicted that if fear would account for the neuronal data then during classical aversive conditioning high sustained neuronal responses should occur throughout behavioral training. Furthermore, as acquisition is completed under instrumental training the neuronal activity level should decrease. The latter reflects a decline in the emotionality of the animal which is ordinarily observable behaviorally.

The present study was designed to test the Hall and Mark hypothesis, to explore the sensitivity and stability of the multiple unit method, and to see, generally, if the multiple unit method could reveal unique phenomena related to classical, instrumental and discrimination learning. Unfortunately, equipment problems eventually precluded the usefulness of the discrimination data.

Seven cats were each permanently implanted with thirteen deep monopolar gross electrodes in twenty anatomical locations. The sites implanted were in the main auditory nuclei, the reticular formation, the limbic system, and the cortex. The cats were trained behaviorally in a counterbalanced fashion in classical aversive and instrumental avoidance conditioning. The conditions were kept as similar as possible and in both cases the conditioned stimulus (CS) was a 1.5 sec 1500 hz tone and the unconditioned stimulus (US) an external shock applied to the right hindpaw during the last .5 sec of the tone. The only difference between the two conditions behaviorally was the fact that the cats could avoid the shock in the instrumental situation by lifting their paws during the 1 sec interval prior to the shock period.

The animals were trained in daily sessions of thirty nine trials each where three trials for each site were recorded on film. Up to twenty habituation sessions, or enough sessions so that most sites yielded no response to the CS alone were given. Extinction sessions were not as long, lasting up to ten sessions with the same criterion. The conditioning sessions varied from twenty sessions (the cats learned behaviorally at least by the fifteenth session) to fifty sessions.

Three judges independently judged the magnitude of the neuronal responses. Five categories were used: no response (i.e., no deflection of the integrator line), slight positive, great positive, slight negative, and great negative. A three day running average (total of nine responses) was computed from the combined judges' scores and displayed as lines on a line graph. The resulting scores for most cats in each site were combined and means and standard deviations for each training condition calculated. The data for some cats in each site were ignored when their pattern of scores differed greatly from the others. Ordinarily, most of the cats displayed very similar neuronal patterns (see Table 4).

The results of the experiment were very rewarding. The most important results were these: Positive neuronal responses occurred almost always. Occasionally, at certain times in the inferior colliculus, medial geniculate, and the cortex inhibition of responses occurred. Similarly, studies of single units indicate a mixture of inhibition, no response, and acceleration of responses to external stimuli. However, this study indicates that the usual overall firing rate, reflected in multiple unit recording, is one of increased activity. This is consistent with evoked potential responses except that evoked responses rarely, if ever, show any consistent reversal of potentials which would be similar to multiple unit inhibition of activity. Moreover, evoked potentials rarely disappear even after prolonged stimuli presentation without shock, whereas no response often occurred for multiple units under the same conditions.

A very general, pervasive difference in neuronal response between classical and instrumental conditioning occurred. Marked differences emerged between the two in the acquisition periods and in the extinction periods. During classical conditioning the neuronal activity generally decreased throughout whereas during instrumental conditioning the neuronal activity increased. Furthermore, the neuronal activity generally declined even more during classical extinction, but increased during instrumental extinction. The different patterns were so general and pervasive that the combined neuronal trends of all the sites taken together (with some reversals of trend included) proved to be linearly negative for classical conditioning and linearly positive for instrumental conditioning. The extinction periods were obviously different. Thus we concluded that these behaviorally similar training procedures showed very little in common in the CNS. We feel that these data strongly support the hypothesis that classical and instrumental conditioning represent two quite different processes.

The data did not support the Hall and Mark hypotheses that conditioning procedures only reflect various levels of the neuronal CER. Nearly opposite results were obtained. Where the CER should have augmented a high rate of neuronal activity a declining trend was observed (classical conditioning); on the other hand, where a CER should have been reflected in a lower rate, an increasing neuronal trend was observed. Moreover, where the activity should have decreased sharply during both kinds of extinction, the activity for instrumental conditioning increased further. We are at a loss to explain these results using existing theory. Perhaps, as more substantive information is gathered about these differential brain processes, then new theories will evolve.

A very suggestive pattern emerged from the three main subcortical auditory nuclei (IC, GM, and dorsal CN) in that inflections in the trends of neuronal activity indicated that some process of associative neural integration may have occurred during both types of behavioral conditioning. These data also suggest that the subcortical auditory nuclei function as a unit during learning.

Certain sites exhibited trends contrary to the overall downward (classical) and upward (instrumental) trends found generally. The nucleus amygdaloideus lateralis (Al) was such a site. During classical conditioning a sharp rise in neuronal activity occurred in acquisition but then declined once acquisition was completed. Such a pattern suggests that the Al may have participated in a motivational process such that as acquisition was completed its operation was no longer in effect. For instrumental conditioning however, a very low level of neuronal activity was emitted throughout. Perhaps other limbic structures would show an opposite pattern. Such patterns are said to be typical in limbic structures.

Only three reticular sites (nucleus centralis lateralis of the thalamus, mesencephalic reticular formation, and the centrum medianum) were implanted in enough cats to allow reliable conclusions. All three sites consistently increased their activity levels throughout instrumental conditioning. This is a similar pattern to the neural responses found in the auditory sites. However, the classical neuronal responses were quite inconsistent. Only the activity of the CM resembled the activity of the auditory nuclei throughout.

Finally, since an increasing neuronal trend was generally evident in all sites during instrumental conditioning and a decreasing one generally evident in all sites during classical conditioning, rather high correlation coefficients resulted from coefficients calculated between the neuronal and behavioral variables. Correlations as high as .96 (IC) resulted for instrumental conditioning and ones as high as -.88 (CM) were obtained for classical conditioning. Very rarely in the literature are such high correlations found, especially when the responses of several animals are combined into an average.

In conclusion, the multiple unit method has proven in this study to be sufficiently sensitive and stable to provide fruitful clues for unraveling the enigma of brain-behavior relationships. Further development of the method by further quantification and greater computer assistance ought to prove even more fruitful.

APPENDIX A

TABLE 7

HORSLEY-CLARKE COORDINATES FOR EACH SITE IMPLANTED

Site	Anterior	Lateral	Height
AUDITORY SYSTEM			
Dorsal Cochlear N. (Right)	(-5.0)-(-8.0)	(5.5)-(8.0)	(1.5)-(6.0)
Inferior Colliculus (Right)	(-2.7)-(-2.0)	(4.0)	(10.0)-(11.0)
Medial Geniculate (Left)	(4.0)-(4.5)	(8.0)-(9.0)	(-1.0)-(0.5)
Brachium of the Inferior Colliculus (Right)	(3.0)	(7.0)	(-1.0)
RETICULAR SYSTEM			
Mesencephalic Reticular (Right)	(2.0)	(2.0)-(4.5)	(-5.0)-(-1.0)
Mesencephalic Reticular (Left)	(2.0)	(2.0)-(4.0)	(-4.0)-(-1.0)
Medullary Reticular Nucleus (Left)	(-5.5)	(2.0)	(3.0)
Centrum Medianum (Left)	(7.0)-(7.5)	(2.5)-(3.0)	(0.0)-(1.0)
Central Lateral Thalamic N. (Right)	(9.5)	(3.5)	(2.0)-(2.7)
Nucleus Centralis Medialis (Right)	(9.0)	(1.5)	(0.0)
Nucleus Centralis Medialis (Left)	(9.0)	(1.0)	(0.0)
Nucleus Reticularis (Right)	(9.5)-(12.0)	(6.0)-(8.5)	(2.5)-(3.5)
CORTEX			
Right Anterior Ectosylvian	(8.0)-(9.0)	(15.0)	
Right Posterior Ectosylvian	(3.0)	(15.0)	
Left Anterior Ectosylvian	(8.0)-(9.0)	(15.0)-(16.0)	
Left Posterior Ectosylvian	(3.0)	(15.0)	
MISCELLANEOUS SITES			
Nucleus Ventralis Postero-medialis (Left)	(9.0)-(9.5)	(6.5)-(7.0)	(0.5)-(1.0)
Area Hypothalamica Dorsalis (Left)	(10.5)	(2.0)	(-3.0)
Nucleus Amygdaloideus Lateralis (Al)	(12.0)	(8.5)-(11.0)	(-6.0)-(-5.7)
Hippocampus (Right)	(6.0)-(7.8)	(8.0)-(12.5)	(-5.5)-(-2.5)
Caudate Nucleus (Left)	(15.0)-(15.3)	(4.0)-(4.5)	(4.0)-(4.5)
Caudate Nucleus (Right)	(14.5)-(16.0)	(3.5)-(5.0)	(2.4)-(5.0)

APPENDIX B

TABLE 8

STANDARD DEVIATIONS COMPUTED ON THE UNTRANSFORMED NEURONAL OBSERVATIONS FOR THE THREE DAY RUNNING AVERAGE FOR EACH SITE DURING HABITUATION, CLASSICAL CONDITIONING, INSTRUMENTAL CONDITIONING AND EXTINCTION

			Conditioning			Total		Number
Site		Habituation	1/3	1/3	1/3	Average	Extinction	of Cats
AUDITORY SYSTEM								
Dorsal Cochlear Nucleus (Right)	Class.	.72	.21	.45	.52	.40	.31	4 of 5
	Instr.	.52	.42	.06	.23	.24	.00	4 of 6
Inferior Colliculus (Right)	Class.	.46	.28	.48	.35	.37	.21	4 of 6
	Instr.	.39	.28	.24	.03	.17	.93	3 of 6
Medial Geniculate (Left)	Class.	.50	.45	.30	.37	.38	.20	5 of 7
	Instr.	.20	.30	.38	.37	.35	.41	6 of 6
Brachium of the Inferior Colliculus (Right)	Class.	-	-	-	-	-	-	1 of 2
	Instr.	-	-	-	-	-	-	1 of 1
RETICULAR SYSTEM								
Mesencephalic Reticular N. (Right and Left)	Class.	.45	.24	.24	.23	.24	.25	4 of 7
	Instr.	.15	.36	.31	.49	.39	.48	4 of 7
Medullary Reticular Nucleus (Left)	Class.	-	-	-	-	-	-	1 of 2
	Instr.	.20	.56	.28	.35	.39	.16	2 of 2
Centram Medianum (Left)	Class.	.16	.20	.24	.26	.23	.14	3 of 4
	Instr.	.20	.20	.26	.38	.29	.39	3 of 4
Central Lateral Thalamic Nucleus (Right)	Class.	.30	.36	.36	.44	.39	.45	4 of 4
	Instr.	.47	.47	.53	.70	.57	.52	4 of 4

TABLE 8--Continued

Site		Habituation	Conditioning 1/3	1/3	1/3	Total Average	Extinction	Number of Cats
Nucleus Centralis Medialis (Right and Left)	Class.	.20	.37	.58	.79	.60	.36	2 of 2
	Instr.	.07	.42	.33	.37	.37	.04	2 of 2
Nucleus Reticularis Anterior (Right)	Class.	-	-	-	-	-	-	1 of 1
	Instr.	-	-	-	-	-	-	1 of 1
Nucleus Reticularis Posterior (Right)	Class.	-	-	-	-	-	-	1 of 1
	Instr.	-	-	-	-	-	-	1 of 1
CORTEX								
Right Anterior Ectosylvian	Class.	1.01	.68	.56	.49	.58	.60	4 of 4
	Instr.	.37	.46	.66	.54	.55	.51	4 of 4
Right Posterior Ectosylvian	Class.	.51	.44	.43	.41	.42	.40	5 of 6
	Instr.	.40	.31	.10	.07	.16	.56	4 of 6
Left Anterior Ectosylvian	Class.	.38	.26	.23	.19	.23	.34	3 of 4
	Instr.	.22	.32	.32	.39	.34	.69	4 of 4
Left Posterior Ectosylvian	Class.	.53	.13	.25	.29	.23	.33	3 of 4
	Instr.	.25	.35	.47	.63	.49	.49	4 of 5
MISCELLANEOUS SITES								
Nucleus Ventralis Postero-medialis (Left)	Class.	.29	.27	.39	.46	.37	.06	4 of 4
	Instr.	.16	.28	.30	.46	.35	.54	3 of 5
Area Hypothalamica Dorsalis (Left)	Class.	-	-	-	-	-	-	1 of 1
	Instr.	-	-	-	-	-	-	1 of 1

TABLE 8--Continued

Site		Habituation	Conditioning 1/3	1/3	1/3	Total Average	Extinction	Number of Cats
Nucleus Amygdaloideus Lateralis (Left)	Class.	.49	.21	.10	.11	.14	.15	2 of 4
	Instr.	.53	.34	.34	.47	.38	-	3 of 4
Hippocampus (Right)	Class.	-	-	-	-	-	-	1 of 1
	Instr.	-	.32	.17	.08	.18	.20	2 of 2
Caudate Nucleus (Left and Right)	Class.	.59	.46	.33	.46	.42	.25	3 of 3
	Instr.	.42	.44	.32	.41	.40	.14	3 of 3
Total Standard Deviation	Class.	.47	.33	.35	.38	.36	.29	
	Instr.	.30	.36	.32	.37	.35	.40	

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