# BEHAVIORAL AND ELECTROPHYSIOLOGICAL CORRELATES OF MORPHINE DEPENDENCE IN RATS

Sheryl Gene Beck

A DISSERTATION

Presented to the Department of Medical Psychology
and the Graduate Council of the
University of Oregon Health Sciences Center
in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy
December, 1980

APPROVED

[redacted] .....

(Professor in Charge of Thesis)

[redacted] .....

(Chairman, Graduate Council)

ACKNOWLEDGEMENTS

The completion of this dissertation was not possible without a combination of academic instruction and/or personal assistance from many individuals. The experiments reported in this dissertation were conducted in the laboratory of my advisor Dr. James H. O'Brien. I acquired a great deal of knowledge and technical skills while working under his guidance. I am particularly grateful for the many helpful suggestions supplied during the course of the development and execution of these experiments by my thesis advisory committee: Drs. Chris Cunningham, Hal Downes, Mary Meikle, and Robert Fitzgerald. In addition Chris Cunningham contributed essential programming skills and statistical advice. I also thank Dr. William Woodward for allowing me the use of his freezing microtome.

The telecommunications center and paging operators, especially Ms. Stoner, were very helpful and cooperative in setting up and maintaining the paging system which allowed me to spend time away from the lab and sleep at home at nights while running a 24-hr a day study.

Karen Gorton, Arlie Skurdal, Guy Mansfield and Jim Linakis provided friendship, strength and moral support at crucial times during the three long years it took to complete this dissertation. Without them these studies would never have been completed. Arlie Skurdal also contributed "invaluable assistance" as an editor during the writing of this manuscript. Keven Quinn and Barbara Hunter Quinn, my fellow cohorts in the laboratory, were always available for discussions, support, and assistance in trouble shooting equip-

ment problems. Guy Mansfield, Jim Linakis, Dan Hatton, Larry W lkin, and Dave Francisco also helped me in hooking up my animals and solving other technical problems.

This dissertation is dedicated to my family who have always supported me in my attempts to fulfill my career goals. I hope they realize by now that I do love them more than my rats.

This research was funded in part by a predoctoral fellowsh grant awarded to Sheryl Beck from NIDA (No. 05053).

Table of Contents

Table of Contents (continued)

List of Figures

List of Figures (continued)

List of Figures (continued)

List of Tables

# INTRODUCTION

Morphine, a potent narcotic, produces profound physiological effects, particularly on the central nervous system. With repeated administration a state of dependence to the drug results. Once dependence has been established, the drug must be present in order to maintain normal functioning. Extensive research has been conducted investigating the many physiological and neurological effects of morphine and morphine dependence. Behavioral studies have concentrated on establishing the effectiveness of morphine as a reinforcer using both instrumental and classical conditioning techniques. Pharmacological studies have investigated the physiological changes that occur with or may produce dependence. Biochemical, pharmacological, and electrophysiological techniques have been used to isolate the specific brain areas affected by morphine, the areas that mediate the many physiological effects of morphine, and the areas that may underlie development of dependence.

One area of morphine research that has attracted attention has been the high incidence of relapse to morphine use by humans after development of dependence and subsequent withdrawal from morphine. The exact mechanisms underlying morphine dependence and the relationship between withdrawal, dependence, and relapse are unknown, but both physiological and psychological processes have been implicated. It has been suggested that some changes occurring during the development of dependence may not be reversed by withdrawal, and may subsequently contribute to relapse. An effective animal model for the study of relapse phenomena would allow measurement and differentiation between the associative and physiological processes, in order to illuminate the

extent to which one or both processes may persist after withdrawal and contribute to relapse. Such a model has not yet been developed; available animal models have been designed to study only morphine dependence.

Self-administration Studies

Self-administration of morphine via lever pressing has become a useful and favored paradigm as an animal model for dependence. This procedure has been used successfully with monkeys and rats for intravenous, intragastric, or oral administration of morphine (Davis & Smith, 1974, 1976; Gotestam, 1973; Goldberg, 1976a; Kumar & Stolerman, 1973; Kumar, Steinberg, & Stolerman, 1969; Nichols & Davis, 1959; Nichols, Headlee, & Coppock, 1956; Weeks, 1962; Wikler, Pescor, Miller, & Norrell, 1971).

Weeks (1962) first described a method for inducing rats to press a lever to self-administer morphine. Rats were addicted to morphine by the administration of increasing doses of morphine hourly over 2 days. Then a lever was put into the cage and animals received 10 mg/kg of morphine for each lever press. Rats learned to respond regularly but the response rates were low, approximately 3-5 responses per hour. When the dose was decreased to 3.2 mg/kg, responding increased. The net morphine intake was less when the dose was low, even though the total number of responses was greater. Disconnecting the syringe for morphine delivery led to a seven-fold increase in response rate. Responding also increased when the rats were put on fixed ratio schedules, but net morphine intake decreased. The pattern of responding that developed consisted of periods of high rates of lever pressing alternating with periods of no lever pressing.

Since this study, many experiments have been conducted to determine factors that are important in the maintenance of self-administration. Animals will learn to lever press for morphine without prior addiction and will even maintain pressing for doses that do not lead to dependence (Weeks & Collins, 1964, 1979). The number of self-administered injections of morphine has been found to vary inversely with the size of the dose, while the net morphine intake varies directly with the dose size (Smith, Werner, & Davis, 1976; Weeks & Collins, 1964; Woods & Schuster, 1971). Intravenous administration maintains responding at lower rates than intragastric administration (Smith, Werner, & Davis, 1976). Response rates are also dependent upon the schedule of reinforcement. Weeks and Collins (1964) found that lever pressing by rats would increase as the fixed ratio schedule increased from 10 to 400 for 10 mg/kg of morphine delivered intravenously. Schuster and Woods (1968) and Schuster (1976) successfully conditioned monkeys to maintain addiction by lever pressing on variable interval schedules.

While these studies of self-administration have demonstrated that an animal model for development of dependence is available, and have elucidated some of the important variables in that development, they have not specifically addressed the question of changes during the development of dependence which may contribute to relapse. According to one theory that has been widely stated and accepted by many investigators, classical conditioning processes may mediate the relapse to self-administration behavior after withdrawal. A first step in support of such a theory is to demonstrate that morphine will support classical conditioning.

## Classical Conditioning

In recent years, many experiments have been performed using morphine as an unconditioned stimulus in order to examine the development of a conditioned response to morphine. The nature of the conditioned response has important implications for any theory relating conditioning factors to relapse. The exact nature of the conditioned response has been proposed to be of three forms: a conditioned drug response, a conditioned compensatory response, and/or a conditioned withdrawal response.

Conditioned drug response. One group of experiments conducted mainly in Lal's laboratory has indicated that the conditioned response is identical to that elicited by the drug. Drawbaugh and Lal (1974), Lal, Miksic, Drawbaugh, Numan, and Smith (1976), and Roffman, Reddy, and Lal (1973) found that a stimulus which had been associated with morphine injections would reverse withdrawal-induced hypothermia in the same manner as an injection of morphine in addicted rats. Lal et al. (1976) tested periodically for the development of a conditioned response by substituting saline injections for morphine during training. During early training trials, saline initially elicited a hypothermic response. After several days of pairing a 10 mg/kg morphine injection with a tone, the tone presented in conjunction with a saline injection elicited a morphine-like hyperthermic response. Kumar (1972) found that rats would choose an environment previously associated with daily intraperitoneal injections of morphine when tested during withdrawal. According to Kumar, this preference for the training environment was due to the association of the environmental stimuli with the effects of morphine and would, therefore, help to alleviate withdrawal

symptoms. In another study reported by Numan, Banerjee, Smith, and Lal (1976), a tone was paired with the injection of morphine to addicted rats after the animal entered the correct arm of a Y-maze. The extinction of the maze response was prolonged by the presentation of the tone during extinction because, according to the authors, the tone elicited a drug-like state. These authors suggested that if the morphine-like conditioned response elicited by the conditioned stimulus was not extinguished during withdrawal, after withdrawal the conditioned stimulus would elicit the conditioned response. The "high" of the conditioned response would be followed by withdrawal symptoms. The resultant withdrawal symptoms would elicit self-administration behavior, alleviating the aversive withdrawal state.

Conditioned compensatory response. A second hypothesis has maintained that a conditioned response that develops in response to injections of morphine will be in the form of a compensatory or adaptive response. Wikler (1948) first postulated that a conditioned response could be of an adaptive nature, maintaining homeostasis by counteracting the direct effects of the drug on the organism. For example, if morphine elicited a hyperthermic response, the conditioned response would be hypothermia. Rush, Pearson, and Lang (1970) found tachycardia in response to a conditioned stimulus that had been paired with morphine injections in dogs. The direct effect of the subcutaneous morphine injections was bradycardia. Lynch, Stein, and Fertziger (1976) have replicated the findings of Rush et al. using intravenous morphine administration and also found the conditioned heart rate response to be extremely resistant to extinction in both dogs and rats. The most extensive development of this compensatory response theory

has been accomplished by Siegel and his associates. Siegel's theory emphasizes the elicitation of a compensatory response to the environment in which the organism experienced the drug. Siegel (1975, 1976, 1977a; 1978a, 1978b) and Siegel, Hinson, and Krank (1978) have investigated the development of a conditioned response to the analgesic responsivity and hyperthermic effect of low doses of morphine (5 mg/kg) in nonaddicted rats. Generally, the training consisted of injecting rats subcutaneously with morphine in one environment, and every other day with saline in a different environment. Upon testing, the animals were given either saline or morphine injections, placed in the saline or morphine environment, and analgesic responsivity or rectal temperature was recorded. Whereas, the direct effect of morphine was to produce analgesia and hyperthermia, Siegel consistently found a conditioned hyperanalgesia and hypothermic response during testing. Both Wikler and Siegel proposed that the conditioned response was in the opposite direction of the direct drug effect because the organism was adapting, attempting to restore homeostasis after the disrupting effects of the drug. These authors also suggest that this conditioned compensatory response may be an important factor in relapse to drug use. "According to the analysis of addiction which emphasizes the role of drug associated environmental cues, the high relapse rate. . .results because patients evidence drug-compensatory responses when they return to the situation which previously signalled the effects of the narcotic" (Siegel, 1977b, page 21). The aversive compensatory response would be counteracted by self-administration behavior.

Conditioned withdrawal response. Reports from several laboratories have indicated that withdrawal signs can be elicited by a stimulus which

has been paired with the presentation of a narcotic antagonist (nalorphine or naloxone) in monkeys (Goldberg, 1970, 1976a, 1976b; Goldberg & Schuster, 1970; Goldberg, Woods, & Schuster, 1969; Irwin & Seevers, 1956), rats (Parker, Failor, & Weidman, 1973; Wikler, Pescor, Miller, & Nowell, 1971), and man (O'Brien, 1976; O'Brien, Testa, O'Brien, & Greenstein, 1976). These experiments have demonstrated that conditioned withdrawal responses can be elicited up to 4 months after complete abstinence from morphine. These authors suggest that the elicitation of conditioned withdrawal symptoms by a conditioned stimulus could be important in relapse if the conditioned withdrawal response had not been extinguished during withdrawal. The withdrawal symptoms would reappear upon reinstatement of the conditioned stimulus and self-administration behavior would be reinforced by alleviation of the conditioned withdrawal symptoms.

These classical conditioning studies that have used morphine as an unconditioned stimulus have demonstrated that morphine can support the development of a conditioned response. The next logical step is to demonstrate a relationship between the conditioned response and self-administration behavior.

Only one study has been reported in which the role of a stimulus paired with morphine delivery in response to lever pressing by rats has been examined. Davis and Smith (1976) used nondependent rats that were implanted with jugular cannulas. Doses of 60 µg/kg of morphine were delivered through the cannula contingent with a buzzer in response to lever pressing. Rats acquired the lever pressing response, and removing the buzzer and changing the drug infusion to saline resulted

in extinction of responding. In the testing situation, the buzzer was presented in response to a lever press. Lever pressing resumed even though saline, not morphine, was injected. If the buzzer was paired with the saline infusions during extinction, the buzzer was not able to support responding during testing and lever pressing rates were lower. The authors concluded that the buzzer had acquired secondary reinforcing properties. The buzzer maintained self-administration behavior even after extinction of the lever pressing response. However, resistance to extinction in the presence of a conditioned stimulus has been criticized as an unsatisfactory method for the measurement of conditioned reinforcement. The changes in response rates from acquisition to extinction may have been due to generalization decrement only, having nothing to do with the presence of the stimulus (Mackintosh, 1974). This experiment also was not an adequate test for the theory that conditioned stimuli may induce self-administration behavior.

No experiment has been conducted to follow the development of conditioning to a stimulus paired with morphine delivery in a self-administering animal as dependence is established. Even though the relapse theory has been accepted, no experimental evidence exists as to whether the presentation of a conditioned stimulus to a withdrawn animal previously trained to lever press for morphine will induce relapse to self-administration behavior. A straight-forward test of this theory would involve pairing a neutral stimulus with morphine delivery in response to a lever press, as in the Davis and Smith study. This conditioning process should continue through the development of dependence by the animal. Once dependence has been established, the animals would be withdrawn. As the animals go through withdrawal, the significance of

the conditioned stimulus would be maintained or extinguished. After withdrawal, the stimulus would be presented alone to determine if the non-extinguished conditioned stimulus would induce self-administration behavior.

The concern of the present study is not solely with behavioral processes which may underlie relapse. Morphine's most profound effects are exerted on the central nervous system. Physiological changes have been implicated as important to the development of dependence and may be responsible for relapse after withdrawal as well. A measurement technique that is sensitive to both conditioning and physiological changes and that would allow differentiation of these changes would be valuable for extracting the relevant factors in relapse. With regards to the physiological impact of morphine, previous studies have used electrophysiological techniques to monitor changes in brain activity. These electrophysiological recordings have also been used during conditioning studies, and changes in evoked potentials, multi-unit, and single-unit activity have been found due to conditioning processes. Single-unit and multi-unit activity is difficult to monitor in freely moving animals over extended periods of time. However, evoked potential activity can be recorded in freely moving animals for periods of days or weeks. This measurement technique has been reported to be sensitive to changes elicited by morphine administration and to conditioning procedures.

## Morphine Effects on Evoked Potentials

To present the information on morphine's effects on evoked potentials, it is necessary to review some of the literature concerned with

the physiological effects of morphine. Morphine exerts profound physiological effects, mainly on the central nervous system, to produce analgesia, drowsiness, changes in mood, depressed respiration, and nausea (Goodman & Gilman, 1975). Also, with the continuous use of morphine, tolerance and physical dependence on the drug results. The specific actions of morphine may be produced by the selective action of morphine on localized areas of the central nervous system. Evidence in support of this conclusion comes from many different areas of research.

Biochemical evidence has been reported on the existence of a specific receptor within the central nervous system that selectively binds morphine and other opiate agonists (Pert & Snyder, 1973a, 1973b; Simon, Hiller, & Edelman, 1973; Terenius, 1973). In the last two years, further evidence has been published on the existence of multiple opiate receptors (Martin, Eades, Thompson, Huppler, & Gilbert, 1976; Lord, Waterfield, Hughes, & Kosterlitz, 1977). Along with the definition of the opiate receptor subtypes, a theory was developed that these receptor subtypes could mediate the elicitation of the different effects of morphine within the central nervous system. Support for this theory has been provided by studies which have described a differential distribution in the brain of the opiate receptor subtypes. One receptor subtype, the mu receptor, has been found in high concentrations in the fourth layer of cortex, thalamic nuclei, and the periaqueductal-periventricular gray. The other receptor subtype, the delta receptor, has been localized to the striatum, amygdala and the second, third, fifth, and sixth layers of cortex (Goodman, Snyder, Kuhar & Young, 1980).

Other research techniques have also been used to investigate the role of localized areas of the central nervous system in the production of specific morphine effects. The most extensive research has been conducted to define the areas of the brain which mediate analgesia produced by morphine administration. Analgesia has been reported as one of the main effects of morphine at low doses (Goodman & Gilman, 1975). Using microinjections of morphine, three distinct regions of the brain have been demonstrated to have analgesic functions when tested behaviorally: periaqueductal and periventricular gray, lateral mesencephalic reticular formation and nucleus gigantocellularis of the reticular formation (reviewed by Yaksh & Rudy, 1978). All three of these brain areas contain a high concentration of the mu opiate receptor. Other evidence of site-specific analgesia has been provided by studies using electrical stimulation of the brain (reviewed by Mayer & Price, 1976). These sites included the periaqueductal-periventricular nuclei, lateral hypothalamus-medial forebrain bundle, and the septal nucleus.

Electrophysiological studies, using evoked potential recordings from subcortical areas implicated in morphine's analgesic effect, have reported both increases and decreases in evoked potential amplitude after morphine administration. Somatic-elicited evoked potentials recorded from periaqueductal gray and reticular formation were decreased in amplitude by low doses of morphine (1-2 mg/kg) administered intravenously (Chin & Domino, 1961; McKenzie & Beechey, 1962; Nakamura & Mitchell, 1972; Sinitsin, 1964; Straw & Mitchell, 1964). However, Chin and Domino (1961) also reported an increase in amplitude of evoked potentials elicited by tooth pulp stimulation as opposed to

sciatic nerve stimulation. This increase was found in recordings from the periaqueductal-periventricular gray and the reticular formation. Also, when visual or auditory stimuli were used to elicit the evoked potentials, an increase in evoked potential amplitude was recorded from the reticular formation (Burks & Dafny, 1977; Dafny & Burks, 1976; Salamy, Sands, & Dafny, 1979; Sinitsin, 1964). The latency of the evoked potential components most affected by morphine administration was approximately 80-140 msec.

The parafasciculariś-centromedian nucleus of the thalamus has been found to be an important brain area underlying the development of tolerance and dependence to morphine. This area of the brain contains a high concentration of the mu opiate receptor. Destruction of this nucleus has resulted in the loss of tolerance to morphine in dependent rats (Teitelbaum, Catravas, & McFarland, 1974) and naloxone crystals placed in this nucleus elicited severe withdrawal symptoms (Wei, Loh, & Way, 1972). Electrophysiological evidence on the effect of morphine administration on evoked potentials recorded from the parafascicularis nucleus comes from three studies. All of these studies reported an increase in somatic, visual or auditory elicited evoked potential amplitude with high morphine doses of approximately 10 mg/kg intravenously or intraperitoneally (Chin & Domino, 1961; Salamy, Sands, & Dafny, 1974; Sinitsin, 1964).

Evoked potentials have also been recorded from areas of the brain that were not analgesic in function or implicated in the development of tolerance or dependence to morphine. Some of these areas also have a substantial concentration of the mu or delta opiate receptors. Evoked potentials recorded from association nuclei in the thalamus were

decreased by low morphine doses (Chin & Domino, 1961; Sinitsin, 1964). Subcortical evoked potentials recorded from the caudate and substantia nigra (which have high concentrations of the delta opiate receptor) elicited by visual or auditory stimuli were reported to increase in amplitude with intraperitoneal injections of morphine (Dafny & Burks, 1976). Evoked potential changes due to morphine administration have also been recorded from other areas of the brain which have low or negligible morphine receptor concentrations and have not been implicated in mediating any of morphine's physiological effects. Intravenously administered morphine decreased the amplitude of the short latency components or had no effect on evoked potential amplitude in somatic elicited recordings from fiber tracts or nuclei involved in somatosensory input (McKenzie & Beechey, 1962; Sinitsin, 1964; Straw & Mitchell, 1964). Evoked potentials elicited by visual or auditory stimuli from the ventral medial hypothalamus were reported to increase in amplitude with intraperitoneal injections of morphine (Burks & Dafny, 1977; Dafny & Burks, 1976; Salamy, Sands, & Dafny, 1979).

Evidence for the effect of morphine on evoked potentials recorded from cortical areas comes from three studies. The cortex contains both mu and delta morphine receptors, depending on the layer of the cortex. The results of the evoked potential studies vary depending on the area recorded from, the stimulus used to elicit the evoked potential, and the dose of morphine. Low doses of morphine (1-2 mg/kg) were most effective in increasing the amplitude of the short-latency components of somatic elicited evoked potentials recorded from sensorimotor cortex (Jurna, Schlue, & Tamm, 1972; Sinitsin, 1964). However, somatic elicited evoked potentials recorded from visual or association cortex

were completely blocked by the same low doses of morphine (Sinitsin, 1964). Large doses of morphine (10 mg/kg) were required in order to affect the short or long latency evoked potential components recorded from auditory, visual, or association cortex in response to auditory or visual stimuli (Sinitsin, 1964).

The different changes in evoked potential amplitude induced by morphine across brain regions and within the same brain area cannot be easily explained. The differences probably can be attributed to the different types of somatic stimuli used to elicit the evoked potentials, different species of animals, doses of morphine, route of administration, and the placement of the recording electrodes.

Two studies have investigated the effects of morphine on evoked potentials recorded from addicted animals. Gildenberg, Murthy, Adler, and Frost (1976) recorded from the parafascicularis nucleus of the thalamus and the periaqueductal gray of naive, addicted, and withdrawn rats in response to sciatic nerve stimulation at various time periods after morphine administration. The rats received hourly administration of morphine intravenously, with doses of 2 mg/kg/hr the first day, 3 mg/kg/hr the second day, 5 mg/kg/hr the third day, and 8 mg/kg/hr from Day 4 on. Morphine suppressed evoked potential components, especially the late component recorded from the parafascicularis in naive animals. In order to obtain the same suppression of the evoked potential in addicted animals, a higher dose of morphine was necessary, and recovery was faster. When morphine was administered to a rat in withdrawal, the parafascicularis evoked potential was diminished, but then several minutes later an enhancement of both early and late

components of the evoked potential was apparent. The early component of the evoked potential recorded from the periaqueductal gray was not affected by morphine in either the addicted or naive rat. The late component was initially suppressed, but within an hour was enhanced. A higher dose was needed to elicit the initial suppression in recordings from the addicted rat. In the withdrawn animals, morphine suppressed the periaqueductal-evoked potential initially with recovery to baseline levels within an hour. The authors concluded that morphine had little effect on short latency components of the evoked potential, which are considered representative of the primary sensory system. The longer latency responses which were affected by the administration of morphine are representative of the multisensory system.

McClung, Burks, and Dafny (1977) also investigated changes in evoked potentials to auditory stimuli with the development of tolerance. Recordings were taken once a day after a challenge dose of morphine administered peritoneally. The effects of morphine were mainly seen in evoked potential components at latencies of 80-140 msec. Two types of effects were noted. In the periaqueductal gray and caudate, and increase in evoked potential amplitude developed over the first three days. The components then returned to control levels by Day 4. Within the parafascicularis and septal areas the increase in evoked potential amplitude was greatest on Day 1 and decreased over days. This study did not include saline control animals, so many of the evoked potential changes may have been due only to the repetitive presentation of the stimuli over days or other associative or non-associative factors.

Conditioning Effects on Evoked Potentials

Several review articles have been written on changes in evoked potentials during classical and instrumental conditioning (Galambos & Morgan, 1960; Galeano, 1963; John, 1961; Morrell, 1961). Since the majority of studies investigating the effects of conditioning on evoked potentials have not used morphine as the unconditioned stimulus or reinforcer, a review of this literature appears in Appendix A, with tables. Generally, the predominant effect of classical and instrumental conditioning procedures is an augmentation of evoked potential amplitude for aversive, appetitive, or sensory-sensory conditioning procedures. When reported, latency changes have primarily been found in the late components of the evoked potentials. The changes correlated with conditioning occurred in most areas of the brain including the classical sensory pathways activated by the conditioned and unconditioned stimuli.

Two studies have investigated changes in evoked potential amplitude during conditioning when morphine was used as the unconditioned stimulus. Stein (1976) and Stein, Lynch, and Ruchkin (1977) studied evoked potential changes which occurred during a classical conditioning paradigm with intravenous morphine as the unconditioned stimulus in nonaddicted rats. The animals were habituated for 10 trials that consisted of a click train paired with saline administration, in order to obtain baseline evoked potentials from the sensorimotor cortex. Conditioning for the four experimental animals consisted of the presentation of the click train for one minute prior to the administration of morphine, 30 sec during the presentation of morphine (0.5 mg/kg), and 30 sec after the morphine administration twice a day. At one

week intervals, the click train was presented with the infusion of saline in order to test for the acquisition of a conditioned response. Each weekly conditioning session plus saline session was considered a block, and the animals were trained for four blocks. As the animals exhibited tolerance to morphine, the dose was increased up to a maximum of 32 mg/kg. As a control, two animals received saline instead of morphine during conditioning. Comparison of the cortical average evoked potential recorded from the first click train presentations on Day 1 and the average evoked potential recorded from the last click train presentations after the first morphine administration was conducted to define any physiological effect of morphine on the cortical evoked potential. Morphine was reported to increase the amplitude and latency of the long latency cortical evoked potential peaks and changes were also found in the magnitude of the evoked potentials as training continued. One method of data analysis for the conditioning data compared the evoked potentials recorded during the saline test trials with the evoked potentials recorded during morphine conditioning trials. This analysis revealed a significant drug effect in the two early peaks (0-15 msec) of the cortical auditory evoked potential recorded during morphine conditioning trials. There was no evidence for the establishment of a conditioned response within the first three components of the evoked potential from the saline test trials. The fourth component (30-70 msec) did not demonstrate a significant drug effect but did show a significant increase in amplitude in the evoked potentials recorded during saline test sessions over the training sessions, which the authors stated was evidence for the development of a condi-

tioned response. A different method of data analysis for the conditioning data compared the evoked potential responses to the clicks 1 min prior to the morphine injection with the evoked potential responses elicited during the morphine injection (30 sec) and immediately after the injection (30 sec). This analysis revealed changes in the three short latency components of the evoked potentials recorded prior to morphine administration that were in the same direction as the changes in the evoked potentials that were recorded after the administration of morphine. No change was found in the fourth component using this second analysis. The authors concluded that the short latency changes reflected neural changes associated with conditioning. The first analysis technique revealed a conditioned response in the late latency component of the evoked potential, whereas the second analysis technique found the conditioned changes in the short latency components of the evoked potential. No attempt was made to reconcile the different results found by the two analysis techniques. Also, there was no control group available to differentiate conditioning effects from changes due to repeated morphine injections at increasing doses over days. This study was conducted with animals that were not dependent, and did not develop dependence during the study.

Wilder (1979) and Wilder and O'Brien (1980) recorded evoked potentials from four brain areas in response to forepaw stimulation during the establishment of morphine dependence in rats. The Sm, stimulation of one forepaw, was presented 100 times within an hour after the animals were given a bolus of morphine intravenously (20 mg/kg, increased to 30 mg/kg half-way through training). Thirty min after the end of the morphine session, the subjects were given an injection

of saline. The Ss, stimulation of the other forepaw, was also presented 100 times an hour, after the injection of saline. Thirty min after the completion of the saline session, the subjects were given an injection of morphine. Training sessions for each subject were at 8-hr intervals and the saline and morphine sessions were presented on a random schedule, with no session occurring more than twice in succession. After the end of training, half the animals were withdrawn for 4 days, and the other half were maintained on morphine. Testing consisted of two sessions in which both the Sm and Ss were presented 100 times. The groups had been further divided for testing so that four groups were tested: addicted and tested with morphine; addicted and tested with saline; withdrawn and tested with morphine; withdrawn and tested with saline.

A change in the cortical neural response over training trials was reported to develop to the Sm stimulus and not the Ss stimulus. The change was in a long latency component (120-175 msec) of the evoked potential. The change in the cortical evoked potential response persisted after withdrawal but was state dependent, only apparent when the animal was morphine intoxicated. Like the Stein et al. (1977) study, there was no control group available to differentiate conditioning effects from changes due to repeated morphine injections at increasing doses. Also, since the morphine or saline injections were administered 30 min prior to the start of the stimulus presentations, the conditioning paradigm used in the Wilder and O'Brien (1980) study was backward conditioning. The Sm was presented only when the animal was morphine intoxicated and never occurred preceeding the morphine administration.

Aim of the Study

A predominant and well-accepted hypothesis within the literature concerned with drugs of abuse has been that relapse to drug use after withdrawal may be elicited by non-extinguished conditioned stimuli. The objective of this experiment was to develop an animal model to test the validity of this theory, using behavioral and electrophysiological measures. Due to an unexpected result of lethal self-administration of morphine, the completion of the original experimental design was not accomplished.

According to the original experimental design, rats were to be trained to self-administer morphine intravenously by pressing a lever. In order to assess the effects of a conditioned stimulus, foreleg stimulation would be paired with morphine administration in response to a lever press. The electrical stimulation of the foreleg would occur after the lever press but prior to the morphine delivery in a trace conditioning paradigm. In an effort to separate conditioned from nonassociative changes, electrical stimulation of the other foreleg would be delivered so as to be explicitly unpaired with lever pressing behavior. Once dependence was established, the animals would be put through withdrawal. For one group of animals foreleg stimulation would continue during withdrawal, thus presumably extinguishing the conditioned response. The other group would not have the conditioned stimuli presented. During withdrawal the lever would not be available, so that lever pressing *per se* would be unaffected. Once the withdrawal procedures were completed, and during a final experi-

mental phase, the conditioned stimuli would be presented while lever pressing was possible.

Electrophysiological recordings were to be used to monitor physiological changes and conditioning changes during the establishment of dependence. Recordings would be made from four brain areas: cortex, parafascicularis nucleus of the thalamus, periaqueductal gray, and ventromedial hypothalamus. These areas were chosen because of the reported changes in evoked potential amplitude in all four brain sites due to morphine administration. Also, as discussed above, periaqueductal gray appears to be an important brain site mediating morphine's analgesic effect. Parafascicularis has been implicated as an important brain site for mediating tolerance and dependence to morphine. Both of these brain areas contain a high concentration of the mu opiate receptor subtype. The ventromedial hypothalamus was included as a control, since this area has not been implicated in mediating any of morphine's physiological effects, and has a low concentration of any opiate receptor. The cortical area was chosen because changes in cortical evoked potentials due to conditioning processes have been reported for this brain area by several laboratories, and evoked potential changes due to morphine administration have also been reported in this brain area.

The following results were expected:

1. The rats would learn to lever press for morphine reinforcement, thus replicating previous instrumental conditioning studies.

2. Evoked potential recordings would reflect:
   a. Changes due to conditioning, apparent in the difference between the evoked potentials elicited by the stimulus that had been paired with morphine injections, and the unpaired stimulus.
   b. Physiological effects of tolerance, dependence, and other non-associative factors that would be apparent in the difference between baseline evoked potentials and evoked potentials elicited during training by the unpaired stimulus.
3. The relapse theory would be supported if those animals for which the forelegs were not stimulated during withdrawal would lever press more than the extinguished group. Also, evoked potentials elicited by the stimulus which had been paired with morphine delivery would demonstrate the conditioned response, whereas the evoked potentials elicited by the unpaired stimulus would not.

# EXPERIMENT 1

## Methods

### Subjects

Twenty-seven female, Sprague-Dawley rats obtained from King Laboratories, Oregon, Wisconsin, weighing between 200-260 g prior to surgery started the experiment. All animals were housed individually in a colony room with a 12-h light-dark cycle and were maintained on an antibiotic regimen using Aureomycin (50 mg/liter) in the drinking water.

### Surgical Preparation

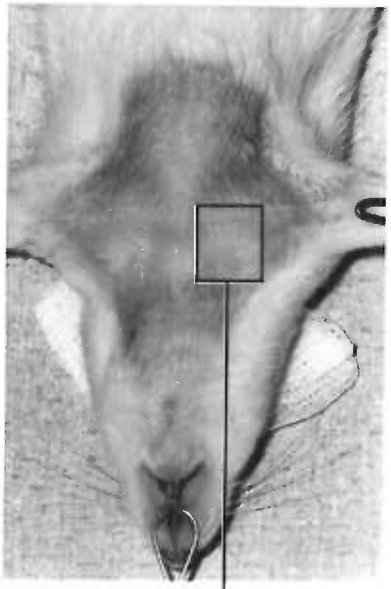
All surgical procedures were conducted under aspetic procedures. All animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and given atropine sulfate (0.3 mg/kg i.m.) to control mucous secretions.

An indwelling cannula, constructed from a 30-cm length of silastic tubing, 0.5 mm I.D. x 0.94 mm O.D. (Dow Corning), was inserted into the right jugular vein. An 8-cm incision was made on the top of the skull from between the eyes to the back of the head. For the cannula, a 2-cm incision was centered across the clavicle, 5 mm lateral of midline and the jugular was exposed using blunt dissection techniques (see Figure 1). Once exposed, excess tissue was removed carefully from around the vein so the tips of a pair of jeweler's forceps could be placed under it. A hemostat was pushed subcutaneously from the back of the head incision behind the front leg to the chest incision, and the cannula was grasped by the hemostat and pulled subcutaneously so that the cannula ran from the chest incision to the head incision.

Figure 1: Implantation of jugular cannula

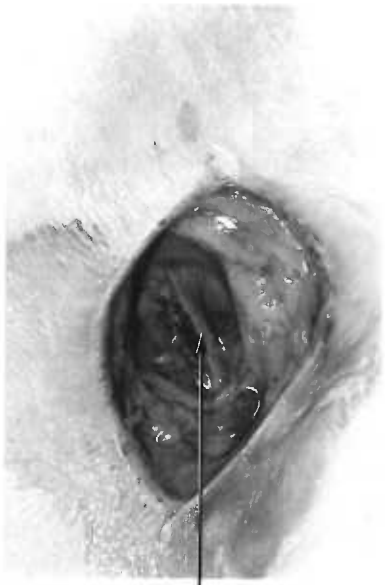
# CANNULA IMPLANTATION

Subject Prepared For Surgery



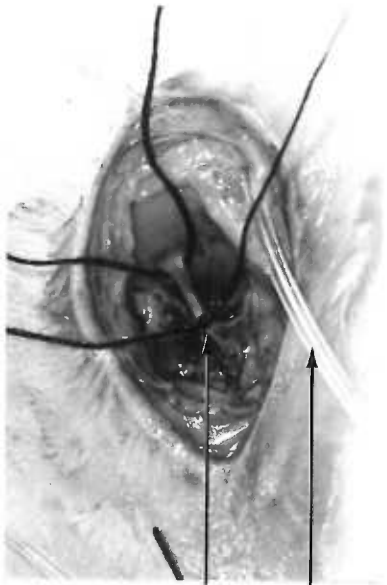
*Location of Incision*

Jugular Vein Exposed



*External Jugular Vein*

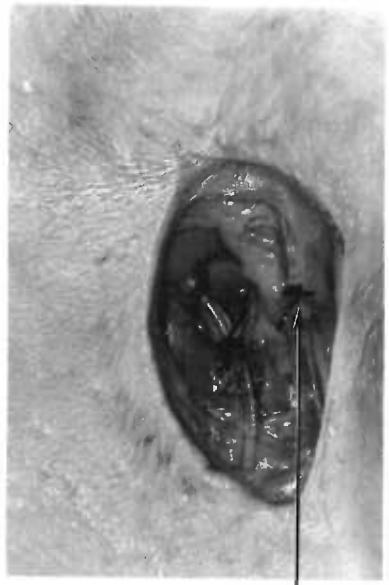
Vein Ligated Prior To Cannula Insertion



*Ligature*

*Cannula*

Cannula In Place



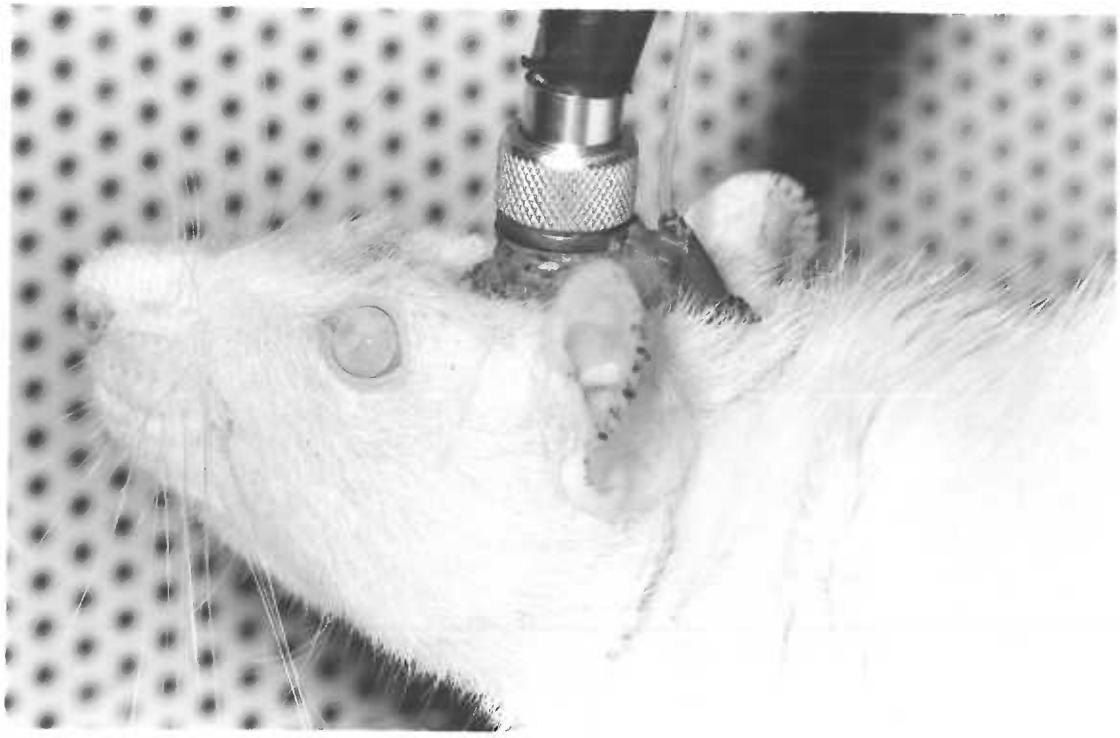
*Suture Securing Cannula To Neck Muscles*

The vein was tied off with 5-0 silk suture anteriorly. A small incision was made in the vein with iris scissors, and the cannula slipped into the jugular for approximately 2.5-3.0 cm so the tip of the cannula was in the vena cava. Another peice of suture was used to secure the cannula to the vein just caudal to the vein incision. The cannula was tacked to the pectoralis muscle by a suture and the muscle wall and skin were closed using 5-0 silk suture.

A 1-cm incision was made down each foreleg approxiamtely 1 cm above the paw and the skin was freed from the subcutaneous tissue and muscle using blunt dissection. A hemostat was pushed subcutaneously from each foreleg incision to the back of the head incision and the stimulating wires were grasped in the hemostat and pulled so that the wires ran subcutaneously from the head incision to the foreleg incision. The tips of the stimulating wires were sutured onto the flexor carpiulnaris and the extensor digiti foreleg muscles, and the incision was sutured.

The rat was than placed in a Kopf Model 1204 stereotaxic holder and the midline head incision was lengthened. The skin and muscle were retracted to the supraorbital ridge and the periosteum scraped from the bone. A small pin vise drill was used to drill holes for the implantation of three bipolar macroelectrodes and the cortical screw, with the location of the holes determined from stereotaxic coordinates (Pellegrino, Pellegrino, & Cushman, 1979). Bipolar electrodes were implanted in the left periventricular gray (PVG), the right parafascicularis nucleus of the thalamus (PFT), the left ventral medial nucleus of the hypothalamus (VMH), and a cortical screw was

Figure 2: Head post and cannula



placed over the right association cortex. The following stereotaxic coordinates (in mm) were used: for PVG, AP: 0.0, L: -0.8, V: -1.5; for VMH, AP: +6.0, L: -1.0, V: -3.5; for PFT, AP: +3.6, L: +1.3, V: -0.5. The cortical screw was placed +1.0 mm posterior to bregma, +1.0 mm lateral of midline and on the dura. The electrodes were cemented into the burr holes using dental cement. Four small screws were implanted to anchor the head post, lateral to each of the electrodes and screw and allowed to dry. The bipolar electrodes and cortical screw were soldered to the pins of a Microtech 123-4 12-pin connector and the head post secured to the top of the animal's head using dental acrylic. The cannula was guided through a 3-cm piece of 12-ga hypodermic stock or a 3-cm piece of polyethylene tubing (1.9 mm I.D. x 1.7 mm O.D.) and cemented close to the head plug. The skin was sutured around the head post and the animal allowed to recover for 1 week (see Figure 2).

Recording and Stimulating Electrodes

The foreleg stimulating wires were constructed from 9.0-cm and 8.0-cm Belden shielded phono pick-up arm cable (Allied Radio #8429). The tips of the wires were stripped of insulation for 1 cm, tinned with solder and sutured onto the leg musculature. The other ends of the wires were then stripped of insulation for 0.5 cm and soldered to the head plug prior to surgery. The shielding around the paired wires was used for animal ground.

The bipolar macroelectrodes were constructed from 250 μ stainless steel insulated wire. The tips of the wires were scraped of insulation for 0.5 mm and one wire was placed next to and parallel to the other

wire with one tip 1.0 mm below the other tip. The wires were coated with Epoxylite and baked for 30 min at 100° C, then recoated and baked for another 30 min at 170° C. The finished diameter of the electrodes was approximately 0.5 mm.

Apparatus

The experimental chamber was constructed from a Plexiglas cylinder 30 cm in height and 25 cm in diameter in which a rat lever (Gerbrands #66312) was mounted. The Plexiglas chamber was located in a sound-attenuating chamber (Industrial Acoustics) which was dimly lit with a 40-watt light from above. A one-way mirror allowed for observation of the animal. The leads from the stimulating and recording electrodes were passed through a custom-built mercury pool, 15-conductor swivel (Technical Concepts, Inc.).

A Digital Equipment Corporation PDP-12 computer controlled presentation of stimuli and on-line collection of data. The foreleg stimuli consisted of three 0.2 or 0.02-msec monophasic square wave pulses presented at a frequency of 300 pulses/sec. The stimulus intensity was adjusted for each animal and set at a level which reliably elicited a small foreleg twitch and a cortical evoked potential of approximately 200-300 μV. The voltages were approximately 3.0 to 6.0 V (for 0.2 msec/stimulus duration) or 5.0 to 13.0 V (for 0.02 msec duration). The frequency of the square wave pulses was controlled by a Digitimer D4030 while the intensity and duration were controlled by Digitimer Isolated Stimulators DS-2.

A Harvard lambda infusion pump (first 16 animals) or a pneumatic syringe (based on a design by Weeks, 1977) was used to control the

intravenous delivery of drug or saline. Coulbourn solid state modules were used to trigger the pneumatic syringe. Morphine sulfate or saline was infused in a total volume of 0.02 ml for each presentation. Morphine sulfate solutions were prepared in physiological saline.

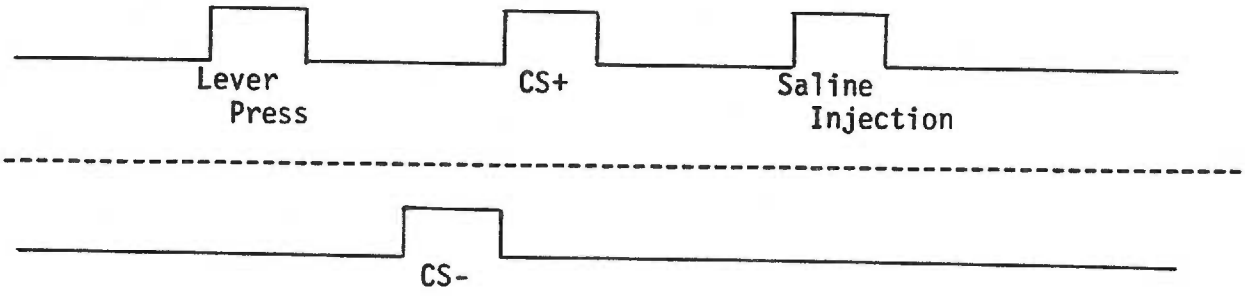
The bipolar electrodes and cortical screw were used to record gross evoked potentials from four brain sites. The signals from the electrodes were passed outside the sound-attenuating chamber and amplified by Tektronix AM502 differential amplifiers with adjustable gain. With the use of an oscilloscope, the gain of the amplifiers (1000-10,000x) was adjusted so the signal strength reaching the computer A/D converters was approximately 1.0 V peak to peak. The overall bandwidth on the amplifiers was set from 1.0 to 300 Hz. In order to avoid the stimulus artifact, the computer began sampling the evoked potential 10 msec after the onset of the foreleg stimuli, and the evoked potential amplitude from the four brain areas was sampled every 2 msec for 512 msec. For each area, an average evoked potential (AEP) was summed from 5 or 10 evoked potentials and stored on LINC tape in digital form. The area for each individual evoked potential was also stored on LINC tape. A cumulative record of the number of lever presses was stored in 6-min blocks by the computer and transformed to hard copy when the LINC tapes were changed.

## Experimental Design

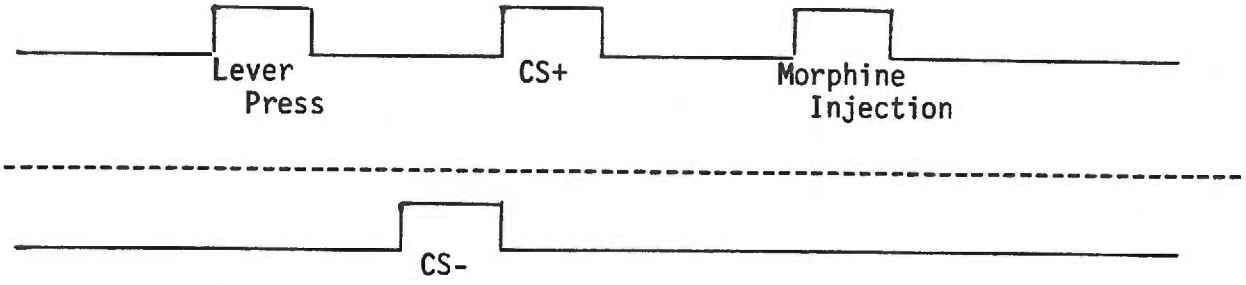
The experimental design is summarized in Table 1. Each rat was placed in the Plexiglas cage inside the sound-attenuating chamber (see Figure 3). Information about baseline behavior was collected

Table 1
Experimental Procedure

Baseline - All rats

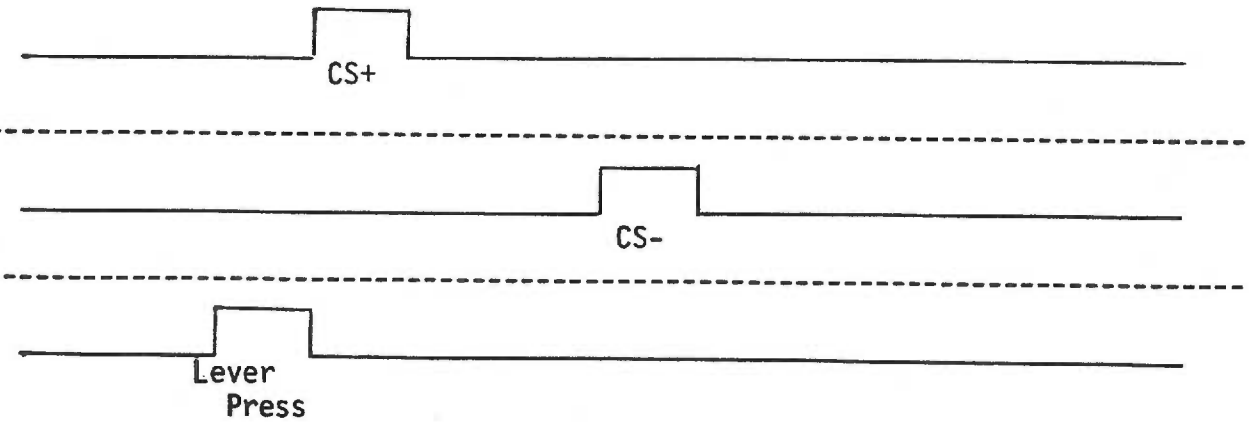



Training - All rats




Withdrawal - 6 days
Lever covered
No CS+ presentations
No CS- presentations

Testing

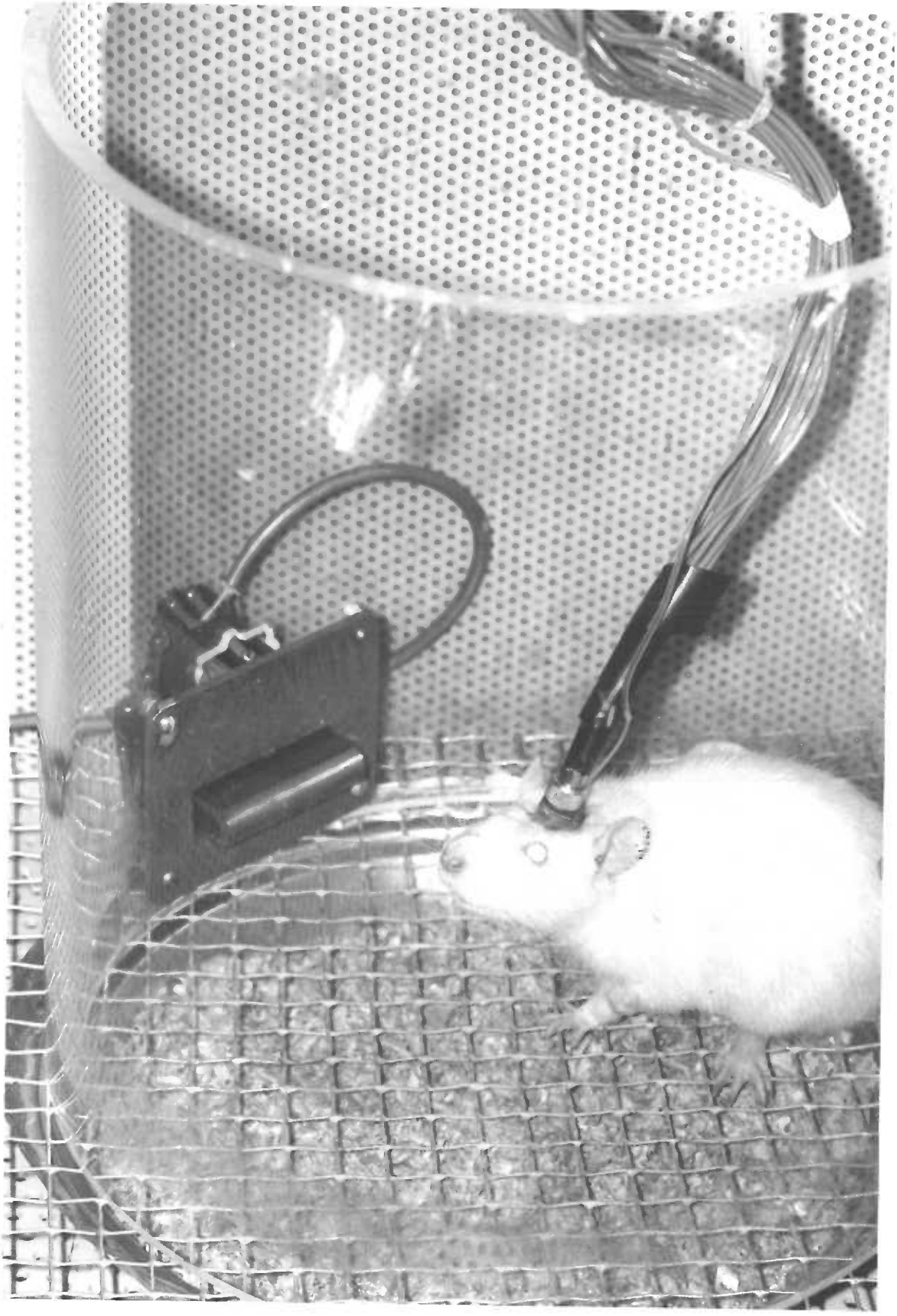


during a period of preliminary exposure to the experimental apparatus (8-44 hrs). During this time, the lever was baited with food, and AEPs were recorded in response to the CS+ presentation, which was elicited by a lever press. An equal number of responses was collected to the CS- stimulus, but the CS- presentation was random with respect to the lever press. The number of baseline trials varied from 80-200 for each animal. Saline was delivered after each lever press during this baseline period.

During the subsequent training phase, lever pressing resulted in the infusion of 1.0-2.0 mg/kg morphine sulfate. This dose was chosen based on a study by Weeks and Collins (1979), in which they found that most rats were dependent on morphine after 6 days, when self-administering doses of 1.0 mg/kg or more intravenously. For baseline and training periods, electrical stimulation of one foreleg (CS+) occurred 1.0 sec after the lever press. An equal number of stimuli (CS-) to the other foreleg were presented at random intervals, with a minimum delay of 5 min after a CS+ presentation. The designation of stimulation of the right or left foreleg as the CS+ was randomized across animals. The rat remained in the chamber with access to morphine 24 hr a day, with food and water available ad libitum. Once the rat achieved a response rate which resulted in the infusion of at least 100 mg/kg of morphine per day for 3 days, the training phase was terminated and the withdrawal phase began.

Only six animals survived to this stage of the experiment. These animals underwent withdrawal in the experimental apparatus with the lever covered. No saline or morphine injections or stimuli were presented during the withdrawal phase. The withdrawal phase lasted 6 days.

Figure 3: Animal connected to recording system and pump tubing in Plexiglas chamber



The testing procedure was the same for all animals: CS+ and CS- stimuli were presented in a randomized sequence with a mean intertrial interval of 5 min. No morphine or saline injections were given in response to a lever press or paired with the foreleg stimuli. Neither the CS+ nor CS- was presented following a lever press, and both stimuli were presented 100 times within a 24-hr time period.

Data Analysis

Several methods were used to analyze the AEP data. A correlation program was used to compare baseline AEPs to the training or testing AEPs. This analysis compared the shape of the AEPs based on 256 data points (2 msec each) and computed a Pearson's product moment correlation coefficient. This analysis was used to determine if any changes in the shape of the AEPs developed during training. Additional statistical analyses were conducted on three measurements: area under the AEP curve, latency of selected components, and peak-to-peak amplitude of selected AEP components.

The area analysis was conducted for the entire AEP curve or for a selected portion of the AEP. The area was quantified by integrating the AEP curve. The original form of the AEP was transformed so an equal positive and negative area existed around a zero baseline. The waveform was then rectified so all points of the AEP were positive and then integrated. This measure is sensitive to overall changes in the size of the AEP.

The latency and peak-to-peak measurements are sensitive to changes in individual components of an AEP waveform. These analyses were the most useful because the changes in the AEP waveform due to conditioning

and/or morphine were not uniform across all AEP components. Using an 80-trial AEP formed from baseline AEPs, peak components were determined, as well as the baseline latency of each peak. For each AEP collected during conditioning and testing, the latencies of the peaks were determined. Also, the amplitudes of the peaks were measured, and peak-to-peak amplitudes derived. When a peak was not apparent, the peak was defined as the largest deflection which occurred within ±10 msec of the latency for that peak as defined from the 80-trial baseline AEP.

# RESULTS

## Behavioral Results

Due to complications including broken stimulus wires, cannulas clogging during training, and pericardial infections, data from 7 out of 27 animals were discarded. Table 2 lists the various treatments and procedures for each of the 20 rats. The first five animals in the study were run on a continuous reinforcement schedule (1 mg/kg group). Unexpectedly, all five of the animals died due to unusually rapid lever-pressing that resulted in morphine overdose. The ratio and interval schedules of reinforcement were introduced in an attempt to limit the dose of morphine to a maximum of 10 mg/kg per hour; the objective was to prevent the rats from overdosing. However, it was difficult to adjust the schedules so that they would effectively control the rapid lever pressing behavior. If the ratio or time interval was set too high, the animals stopped responding entirely. It was, therefore, necessary to begin with low ratios and time intervals, and adjust these values upward as the rat increased its response rate. It was difficult to monitor the rats at all times (overnight, for example), and the rats managed to overdose, even with the ratio and interval schedules.

In the 1 mg/kg group, six rats were run under continuous reinforcement, two under variable interval, two under fixed ratio, and three under fixed interval. The fixed ratio schedules ranged from 3 to 18 lever presses and the variable interval schedules from 1 to 7 min. The fixed interval schedules ranged from 10 sec to 1 hr. Individual rats were run on only one paradigm, but the ratio or time interval was changed over time to control lever pressing behavior.

Table 2

| Dose/injection | Experimental Parameters: Number of Rats | Number Died | Shock Duration | Average Shock Intensity | Reinforcement Schedule: CRF | VI | FR | FI |
|---|---|---|---|---|---|---|---|---|
| 1 mg/kg | 13 | 11 | 0.2 msec | 5.4 V | 6 | 2 | 2 | 3 |
| 2 mg/kg | 3 | 3 | 0.2 msec | 5.3 V | 2 | 1 | 0 | 0 |
| 2 mg/kg | 4 | 0 | 0.02 msec | 8.5 V | 4 | 0 | 0 | 0 |

Morphine overdose produced respiratory depression resulting in the deaths of 14 out of 20 rats. Pathology reports on several of the animals confirmed that there were no other diseases or pathological causes of death. In the 1 mg/kg group, all animals overdosed except two that were run on a continuously adjusted fixed interval schedule. In the 2 mg/kg group, the only major difference was the pulse duration of the electrical stimuli to the forelegs. The three animals with a 0.2 msec pulse duration overdosed and died, whereas the four animals with a 0.02 msec duration did not overdose.

By looking at the pulse duration of the foreleg stimulus independent of the dose administered, it was possible to divide the rats into two groups: 16 animals in the 0.2 msec group and 4 in the 0.02 msec group. Those in the 0.2 msec group exhibited rapid lever pressing activity resulting in a larger average morphine intake than the rats in the 0.02 msec group.

The pattern of responding is illustrated in Figures 4, 5, and 6. Figure 4 contains cumulative bar pressing records (logarithmic) for four rats which demonstrated the rapid lever pressing behavior. Note that the two rats given 1 mg/kg per lever press had fairly constant low rates for the first 36-48 hr (F10 and F24). After this period, lever pressing rose dramatically and remained high until convulsions started. The other two animals given 2 mg/kg/press acquired rapid rates within the first 24 hr (F47 and F50). Figure 5 contains cumulative records from the four rats that did not demonstrate the rapid lever pressing behavior. These were the four rats which had 0.02 msec pulse durations for the foreleg stimuli. The rats demonstrated consistent lever pressing behavior which continued from 5 to 10 days.

Figure 4. Cumulative lever pressing records in 12-hr periods for rats that overdosed. Dose for F10 and F24 1 mg/kg; for F47 and F50 2 mg/kg. Duration of stimulus pulse, 0.2 msec. Ordinate is logarithmic scale.

# LETHAL ADMINISTRATORS

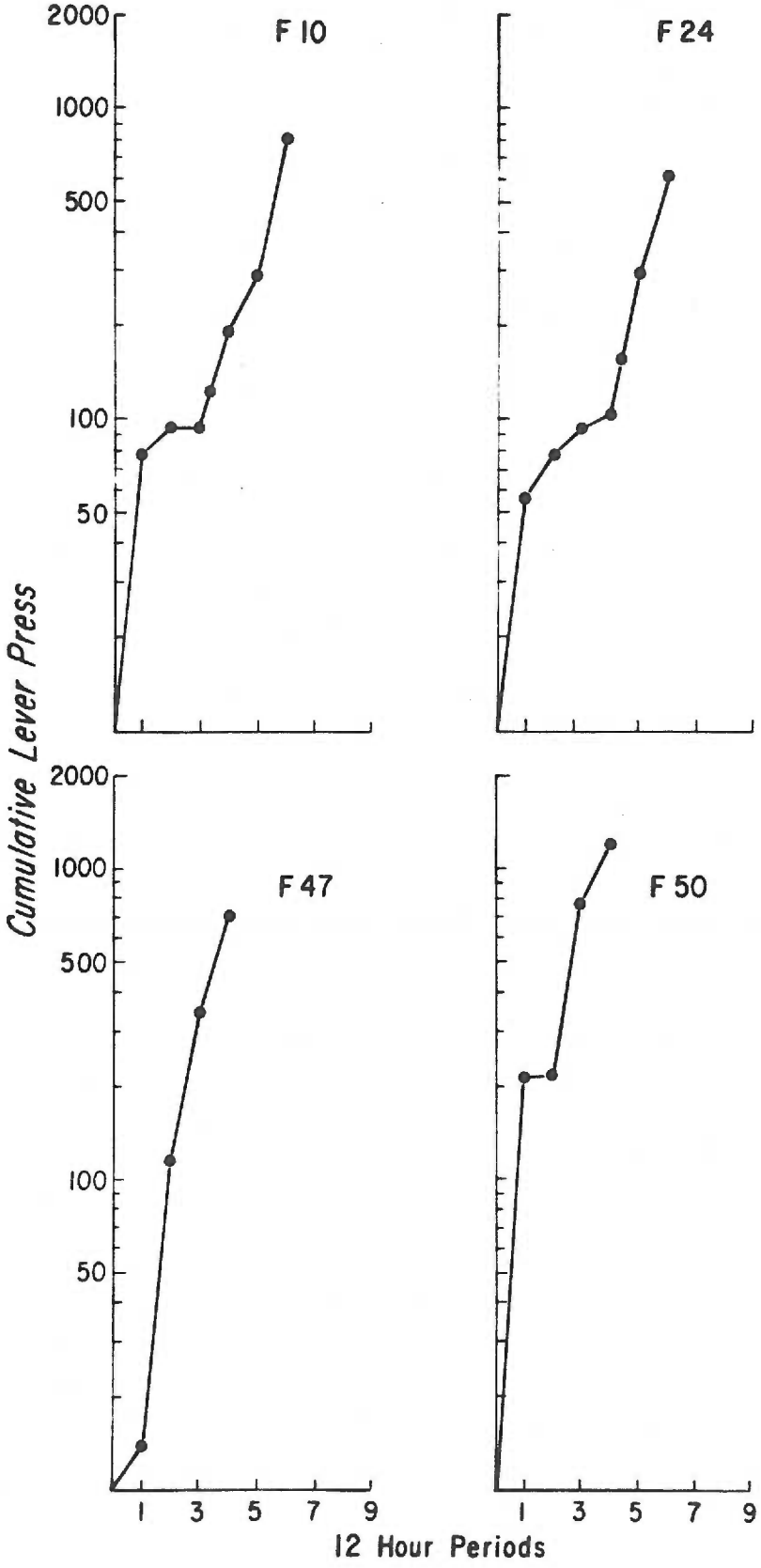
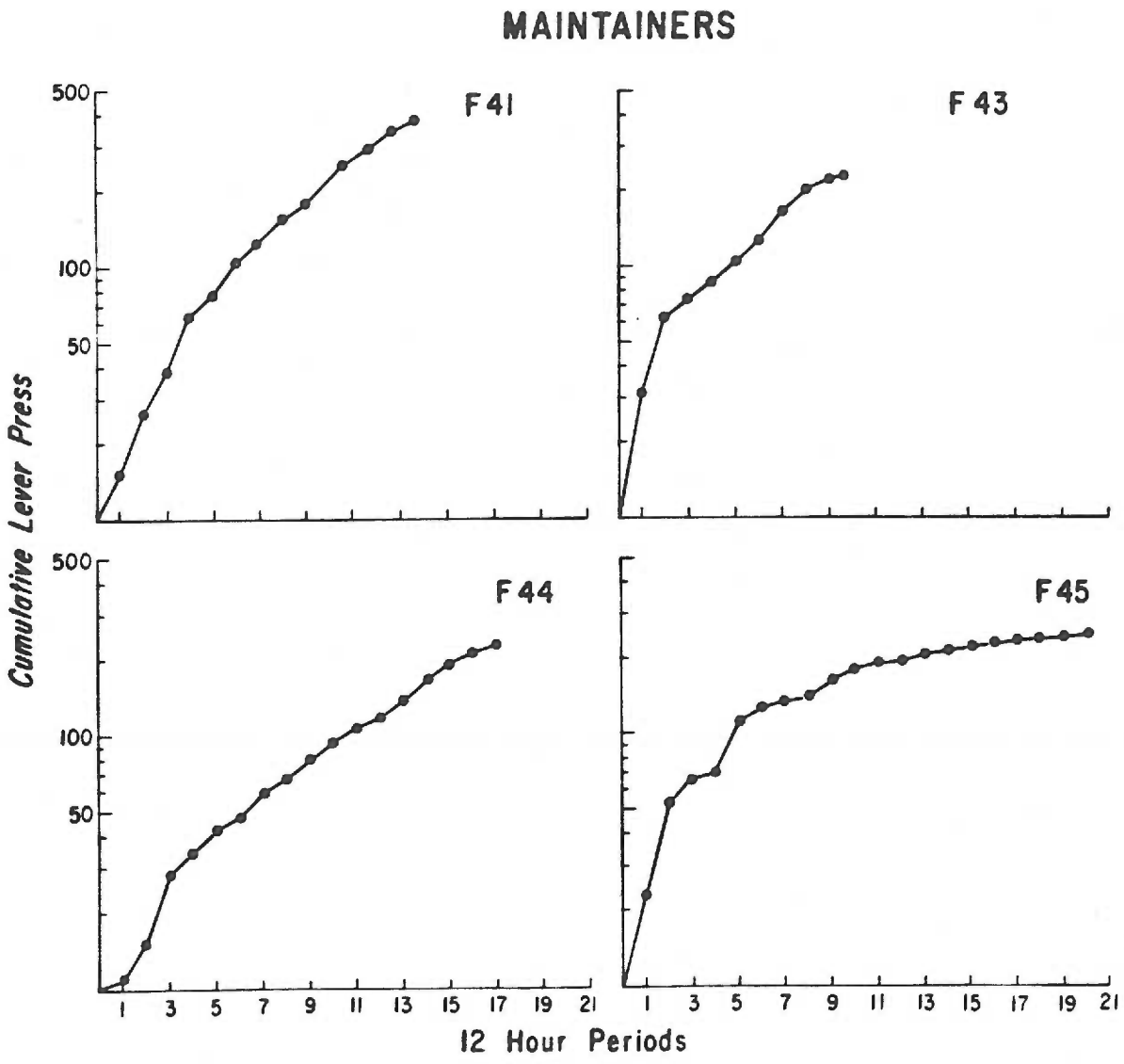


Figure 5. Cumulative lever pressing records in 12-hr periods for maintaining rats. Dose for all rats, 2 mg/kg/injection. Duration of stimulus pulse, 0.02 msec. Ordinate is logarithmic scale.


MAINTAINERS
F 41
F 43
F 44
F 45
500
100
50
Cumulative Lever Press
1 3 5 7 9 11 13 15 17 19 21
12 Hour Periods

A comparison of the temporal distribution of lever pressing for the last 24 hr of pressing activity is presented for a representative rat from each group in Figure 6. For both animals, clusters of lever pressing activity were typically followed by periods of inactivity. The animal that overdosed pressed more frequently with less time between clusters and more presses within each cluster.

Table 3 provides information concerning the morphine intake for the eight animals on the CRF schedule which demonstrated rapid lever pressing behavior compared to the four animals with low maintained lever pressing. The average amount administered was significantly different between the two groups (Mann Whitney $U(8, 4) = 0.0$, $p < .002$). There was also a significant difference between the two groups of animals in the largest dose administered in any 6-hr period, $U(8, 4) = 0.0$, $p < .002$). The average largest 6-hr dose for the rapid pressers was 4.5 times that of the maintainers.

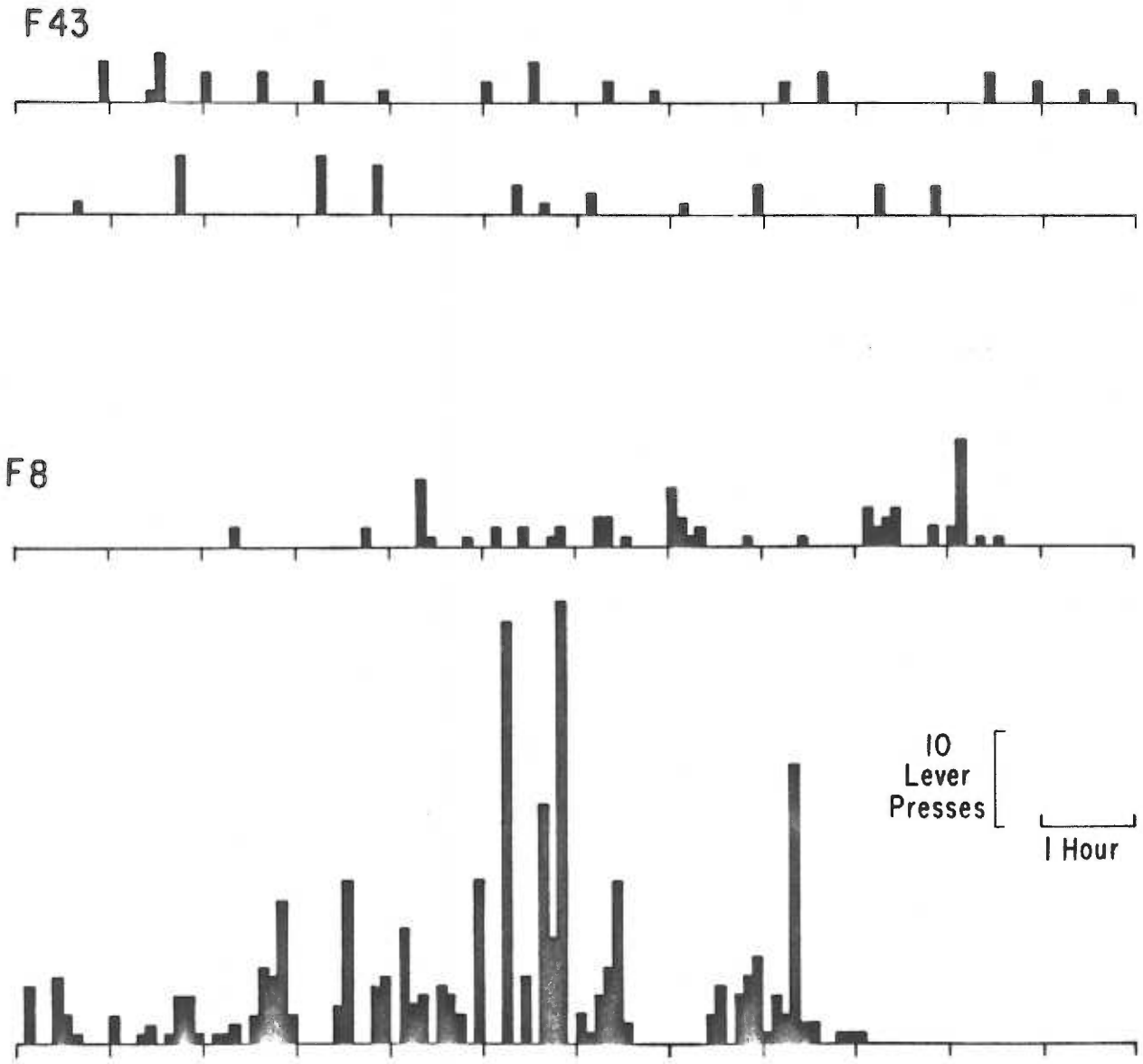
Testing. The four animals which maintained their morphine intake were allowed to withdraw from morphine for 6 days with the lever covered. During these 6 days, periodic behavioral observations were made for the appearance of withdrawal symptoms. All of the animals exhibited wet dog shakes which began on the first day, were most frequent on the second and third days, and grew less frequent during the last 3 days. Food pellets were available in the chamber throughout the withdrawal phase. For the first 2 days of the withdrawal period the rats did not eat and appeared to lose weight. All of the rats had loose stools or diarrhea, primarily during the first 3 days of withdrawal.

Table 3

Comparison of Morphine Intake for Rats on CRF Schedule

| | | Foreleg Pulse Duration | |
|---|---|---|---|
| | | 0.2 msec (8 rats) | 0.02 msec (4 rats) |
| Average Drug Administered/Hr | Range | 6.8-26 mg/kg | 1.0-4.5 mg/kg |
| | $\bar{X}$ ± SEM | 14.0 ± 7.0 mg/kg | 2.2 ± 1.4 mg/kg |
| Largest Dose/ 6-hr period | Range | 229-482 mg/kg | 30-66 mg/kg |
| | $\bar{X}$ ± SEM | 330.9 ± 88 mg/kg | 44.5 ± 14.7 mg/kg |

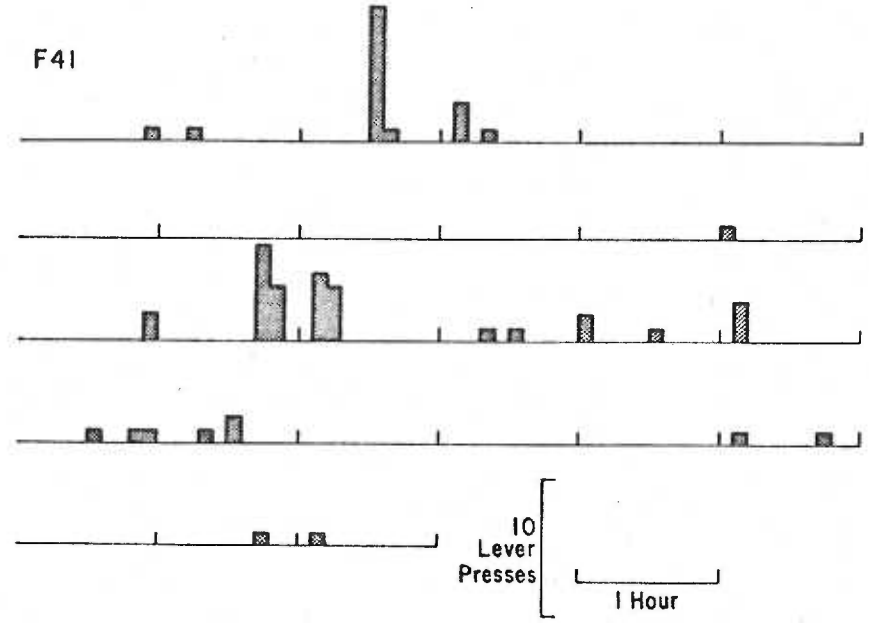
Figure 6. Temporal distribution of pressing (in 6 min blocks) in the last 24 hr for two rats: F8 overdosed and F43 did not overdose. Dose for F8 was 1 mg/kg and for F43 was 2 mg/kg.

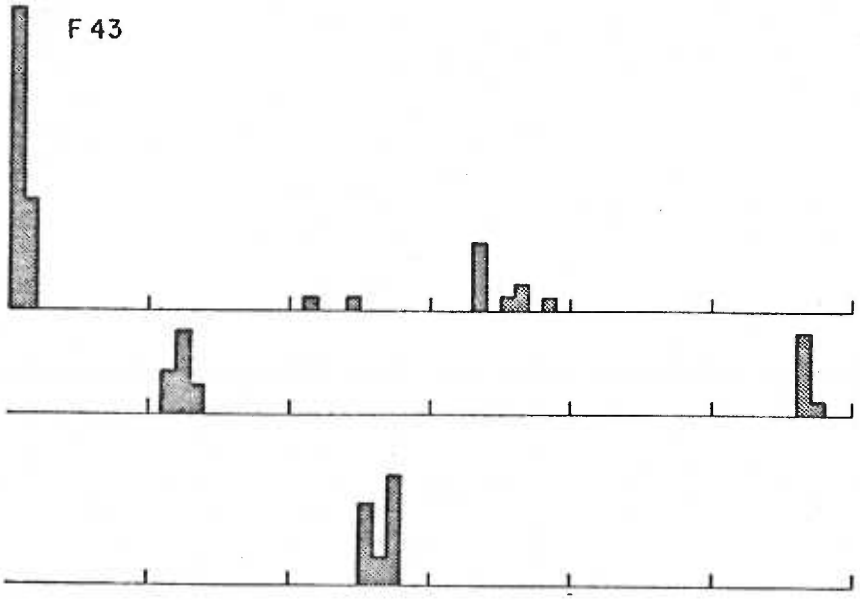

F43
F8
10
Lever
Presses
1 Hour

Three of the four animals that underwent withdrawal were used in the testing phase. One of the rats was not tested becuase of a broken stimulating wire. The three tested animals were given a randomized sequence of CS+ and CS- presentations. The lever was uncovered but stimulus presentations were not paired with lever presses. Neither saline nor morphine injections were delivered at any time during the testing phase. All three animals pressed the lever during testing: Rat F41 pressed a total of 56 times, F43 pressed 76 times and F44 pressed 100 times. Figures 7 and 8 present the data from these three animals. Figure 7 presents the temporal distribution of lever pressing behavior. For all three rats, lever pressing appeared to be distributed throughout the testing period and responding was clustered. Rat F43 exhibited a burst of responding at the start of testing prior to the presentation of any stimuli. Figure 8 shows the latency to lever press after the presentation of the CS+, the CS-, or a preceding lever press. The lever pressing activity did not appear to be correlated with the presentation of the CS+ for any subject. Most of the lever presses occurred within 20 sec after another lever press. Only five to six lever presses ever occurred within 30 sec after the presentation of either the CS+ or the CS-. The purpose of this test procedure, and the analysis presented in Figure 8, was to determine if the CS+ stimulus had any control over the lever pressing behavior of the rat. If there had been conditioning to the CS+ and this conditioning survived withdrawal, then presentation of the CS+ might elicit lever pressing by the rat. The data from the three rats that were tested did not support this idea.

Figure 7. Temporal distribution of lever pressing activity during the testing period for Rats F41, F43 and F44.

TEMPORAL DISTRIBUTION OF PRESSING DURING TESTING



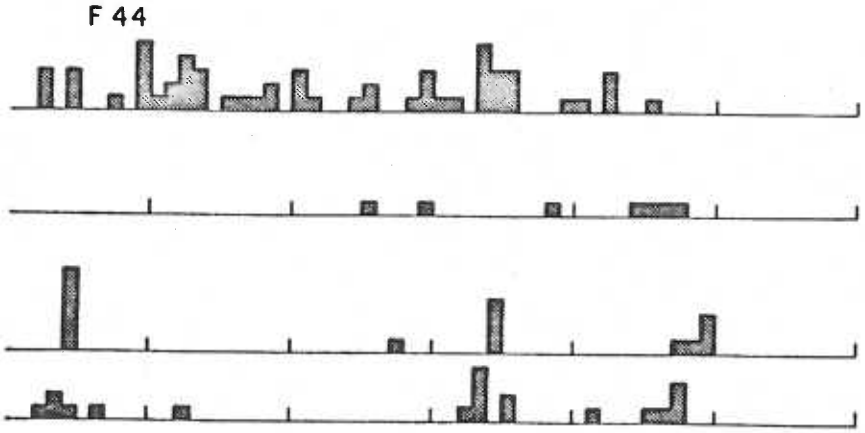
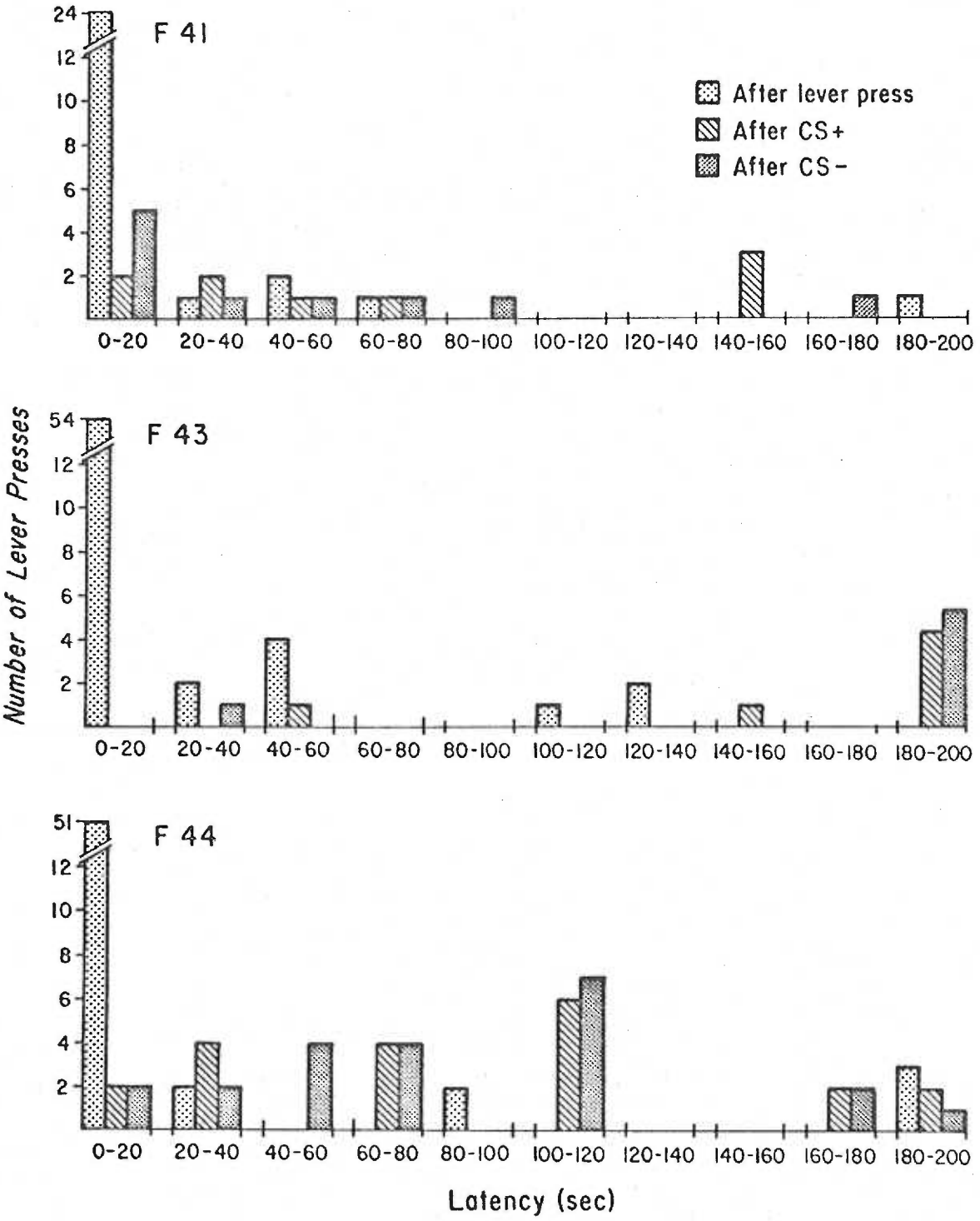



Figure 8: Latency to lever press after the presentation of the CS+, the CS-, or following a lever press for Rats F41, F43 and F44. Latency plotted in 20-sec blocks.

# LATENCY TO LEVER PRESS DURING TESTING



Electrophysiological Results

Due to the unexpected results of overdosing behavior, a majority of the rats' brains were not saved and histological verification of electrode placements was not possible. Since a bone screw was used as an epidural electrode to record cortical activity, histological analysis was not necessary and the AEPs recorded from the cortex were analyzed. Usable cortical AEPs were obtained from seven of the lethal administrators and from all four of the rats which maintained their morphine intake. The loss of cortical records from the other nine animals was due in part to the manner in which the AEPs were stored and the rapid pressing behavior of these animals. The computer LINC tapes used for storage of AEP data have a finite storage capacity. Once the tape storage blocks are filled, the computer returns to the beginning of the tape to store subsequent AEPs, writing over previously stored data, which are then lost. Once the animal started pressing rapidly, if the program was not monitored continuously, AEP data were lost.

The total number of trials for each animal was different, since the rats were controlling their morphine intake. In order to facilitate comparison of the data across animals and in order to conduct statistical analyses, the Vincent method of organizing the data was used (cf. Stevens, 1951). This method divides the total number of trials from each animal into an equal number of fractions. The number of trials within each block varies between animals, with slow lever pressers having a smaller number of trials than fast lever pressers. The lever pressing records for the lethal administrators

(see Figure 4) illustrate that morphine intake was maintained for a period of time prior to the start of the rapid lever pressing phase. During this maintenance period, the number of CS+ and CS- presentations were approximately equal. Lever pressing occurred in bursts, with an average of 5-10 lever presses per burst. The CS- presentations occurred during the periods of inactivity between bursts of pressing. However, once the rapid pressing began, there were no periods of inactivity during which the CS- could be presented. Therefore, the number of CS- presentations was not equal to, and was much less than, the CS+ presentations. In an effort to compare the CS+ and CS- AEPs for learning changes, the cumulative records for each animal were examined. The end point for analysis was arbitrarily defined as the point where 50 CS+ presentations occurred without any CS- presentations interspersed. This point usually coincided with the start of the rapid pressing phase. Also, the lever pressing activity prior to this point had been maintained with bursts of pressing so that the CS+ and CS- presentations were equal. The number of trials from the start of conditioning to the selected end point was divided into 10 blocks. Also, since the data were stored in 10-trial AEPs, the trials were further divided by 10 to determine how many 10-trial AEPs would be averaged together to form a trial block for baseline, conditioning and test data (see Appendix C).

Since the amplifier settings were different for each animal, a difference score measure was used to represent the peak-to-peak amplitude of selected AEP components. For each rat, the peak-to-peak amplitude for each data block was compared to the mean of the peak-to-peak amplitude of the baseline blocks: $\overline{X}_n - \overline{X}_B$.

An unweighted means three-way analysis of variance for unequal number of subjects per group was used to analyze the conditioning data, with one between-groups factor and two within measures. The between-groups factor was the duration of the electrical stimulus. One within measure was the CS+ versus CS-; the other within factor was the 10 conditioning blocks. Eight out of the eleven animals received stimulation of the left foreleg as the CS+. The test data were analyzed separately because only the three rats which maintained their morphine intake made it through both the withdrawal and the testing phases of the experiment. The first five data blocks from each animal were used in the analysis of variance.

A representative cortical AEP is shown in Figure 9, with the peaks labelled. The latency to the AEP components were: $N_1$ - 18 msec; $P_1$ - 27 msec; $N_2$ - 41 msec; $P_2$ - 96 msec; $N_3$ - 197 msec; and $P_3$ - 260 msec. The changes in the CS+ and CS- AEPs during conditioning for one subject, F16, are presented in Figure 10. The AEPs from the first baseline block and Blocks 1, 3, 5, 7, and 9 of conditioning are presented in the figure. The most dramatic change appeared in the long latency $P_2$-$N_3$-$P_3$ component of the cortical AEP. The development of this new AEP component is most apparent in the CS- AEPs. The formation of the new AEP component and the other changes which developed during conditioning are discussed for each AEP component below.

Latency

Only one of the selected AEP components demonstrated a shift in latency during conditioning. The long latency positive $P_3$ peak increased in latency over blocks of conditioning, $F(9, 81) = 3.32$, $p < .001$. This shift is illustrated in Figure 11. The difference in

Figure 9. Cortical AEP with peaks labelled.

# Cortical AEP

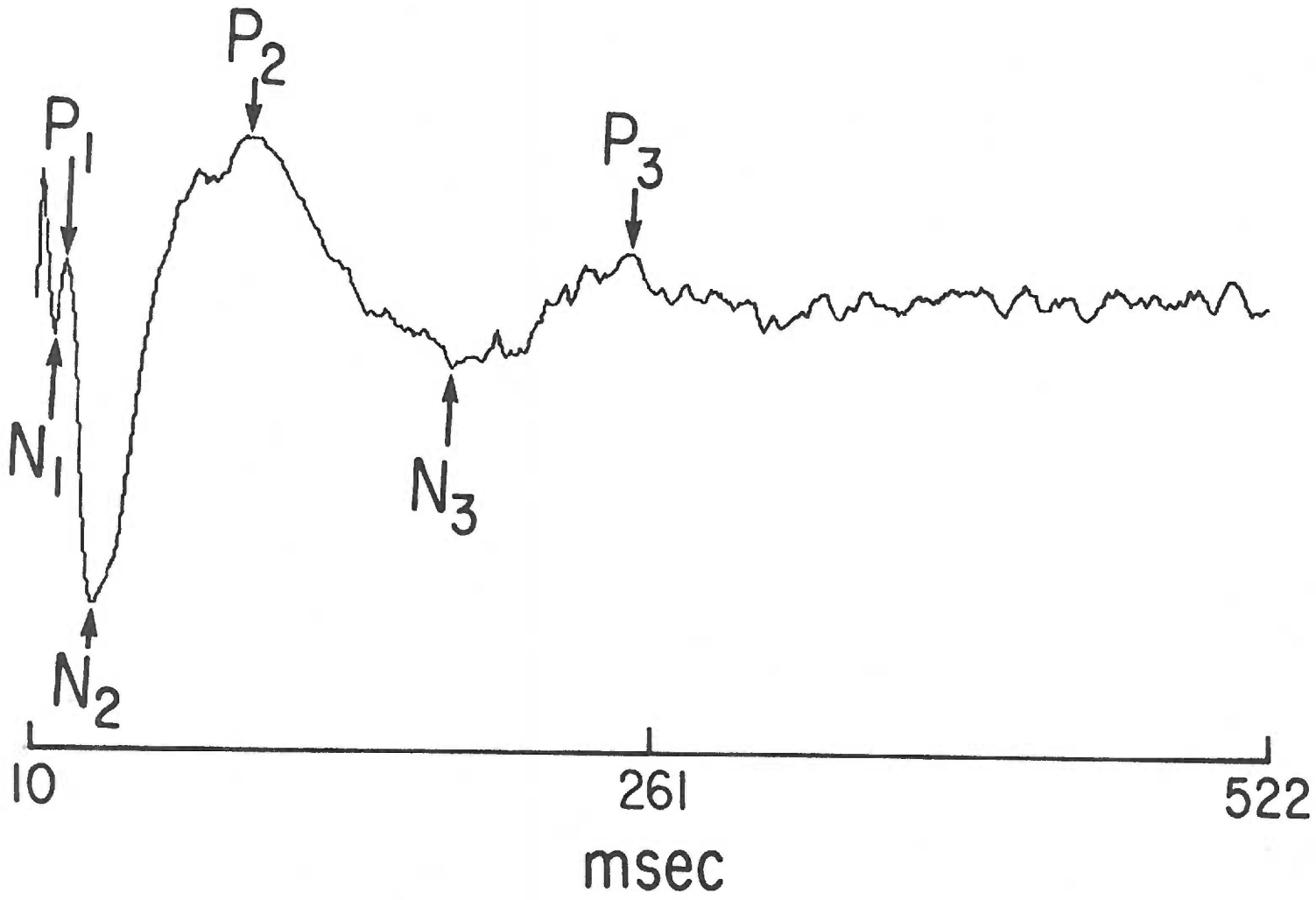


Figure 10. Individual CS+ and CS- cortical AEPs recorded during baseline and conditioning for Rat F16. This animal received 0.2 msec pulse duration electrical stimulation of the left foreleg as the CS+. The AEPs were taken from the first baseline block and Blocks, 1, 3, 5, 7, and 9 of conditioning. One block was defined as one-tenth the total number of conditioning trials calculated separately for each animal.

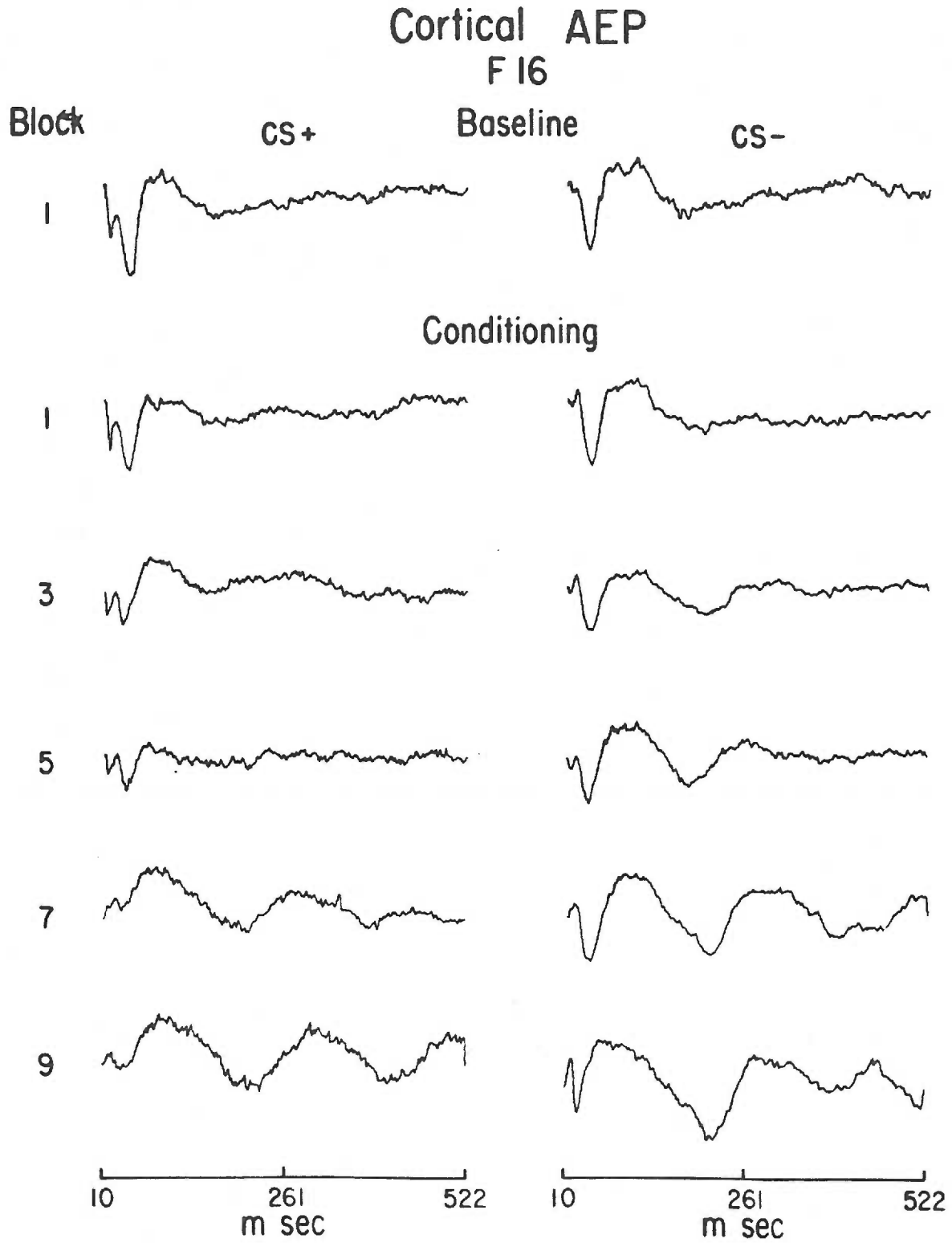
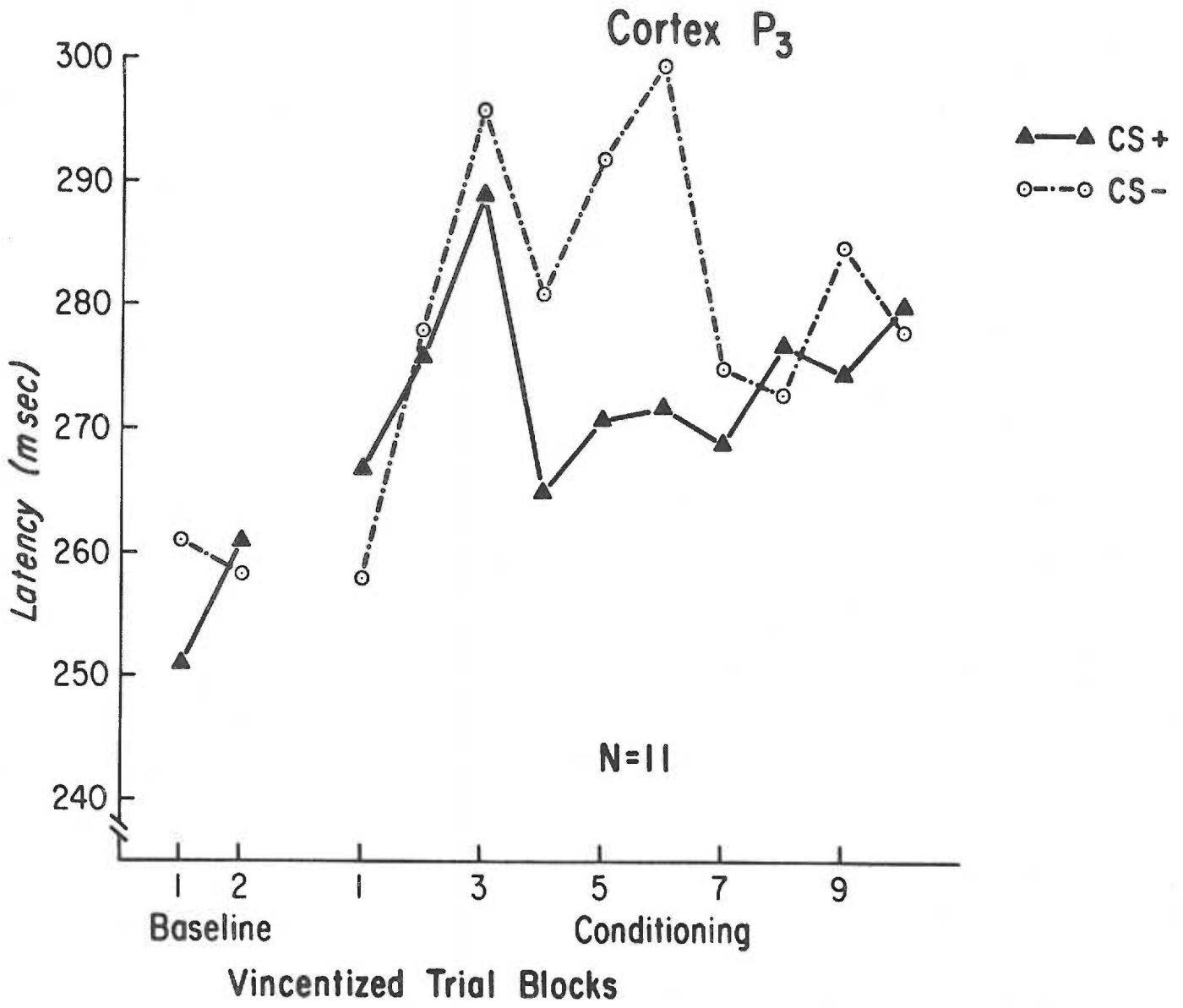

Cortical AEP
F 16
Block
CS+
Baseline
CS-
1
Conditioning
1
3
5
7
9
10
261
522
m sec
10
261
522
m sec

Figure 11. Shift in latency of cortical AEP peak $P_3$ during conditioning. The axis has been expanded starting at 240 msec. Latency was analyzed for blocks of conditioning. A Vincentized trial block was equal to the total number of trials of conditioning for each animal divided into 10 equal portions. One block for each animal equalled one-tenth the total number of conditioning trials.


Cortex $P_3$
300
290
280
270
260
250
240
Latency (m sec)
CS+
CS−
N=11
1
2
1
3
5
7
9
Baseline
Conditioning
Vincentized Trial Blocks

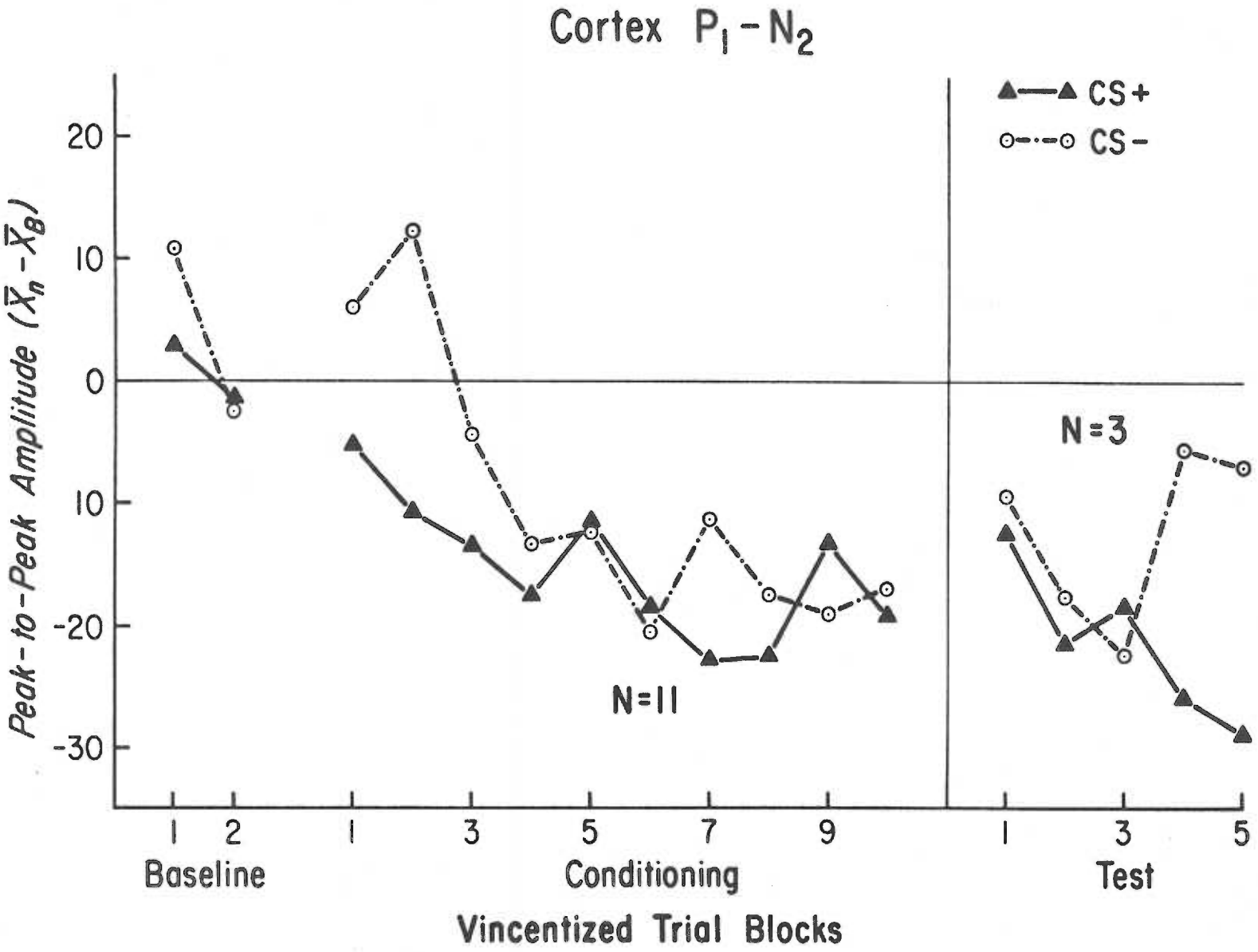
latency between the CS+ and CS- for the $P_3$ component was also significant, $F(1, 10) = 5.22$, $p < .05$. The average latency during conditioning for the CS+ peak $P_3$ was 271 msec, whereas the CS- AEP latency for Peak $P_3$ was 278 msec. As can be seen in the figure, the latency to the peak was longer in the CS- AEPs during the middle blocks of conditioning. This shift in latency can be seen in the CS- AEPs recorded during conditioning from Rat F16 (Figure 10). In the right-hand side of Figure 10, the latency of the $P_3$ component in the CS- AEP for Block 3 was 266 msec, whereas for Block 9, it was 324 msec.

Peak-to-Peak Amplitude

There were no significant differences between the AEP changes for the animals that received 0.2 msec as opposed to 0.02 msec stimulation for any of the peak-to-peak amplitude measures ($P_1$-$N_2$, $F(1, 9) = 1.43$, $p > .05$; $N_2$-$P_2$, $F(1, 9) = 1.92$, $p > .05$; $P_2$-$N_3$, $F(1, 9) = 1.52$, $p > .05$; and $N_3$-$P_3$, $F(1, 9) = 0.84$, $p > .05$). Therefore, in the results presented below, the data from the two groups have been combined.

$P_1$-$N_2$. A progressive decrease in peak-to-peak amplitude for both the CS+ and CS- AEPs during conditioning for this short latency component can be seen in Figure 12. The only significant outcome of the analysis of the $P_1$-$N_2$ component was a main effect of blocks, $F(9, 81) = 2.51$, $p < .01$. In the first two blocks of conditioning, there appeared to be a differentiation between CS+ and CS- AEPs for this component. After an initial increase in amplitude in the CS+ component, both the CS+ and CS- components decreased in amplitude as conditioning trials continued. The change in the CS- component did

Figure 12. Change in peak-to-peak amplitude of Component $P_1$-$N_2$ of the CS+ and CS- elicited cortical AEPs during baseline, conditioning, and testing, for all 11 subjects. One-tenth the total number of conditioning trials for each animal determined the number of trials in each Vincentized trial block for each animal. Peak-to-peak amplitude for each data block was compared to the mean of the peak-to-peak amplitude of the baseline blocks for each rat: $\overline{X}_n - \overline{X}_B$. Baseline data were taken from the first and last blocks of the baseline session for each animal. Testing data were obtained from three animals that maintained their morphine intake during conditioning. The first five blocks of testing data were used from each animal.


Cortex $P_1 - N_2$
CS+
CS-
20
10
0
10
-20
-30
Peak-to-Peak Amplitude ($\bar{X}_n - \bar{X}_B$)
N=11
N=3
1
2
1
3
5
7
9
1
3
5
Baseline
Conditioning
Test
Vincentized Trial Blocks

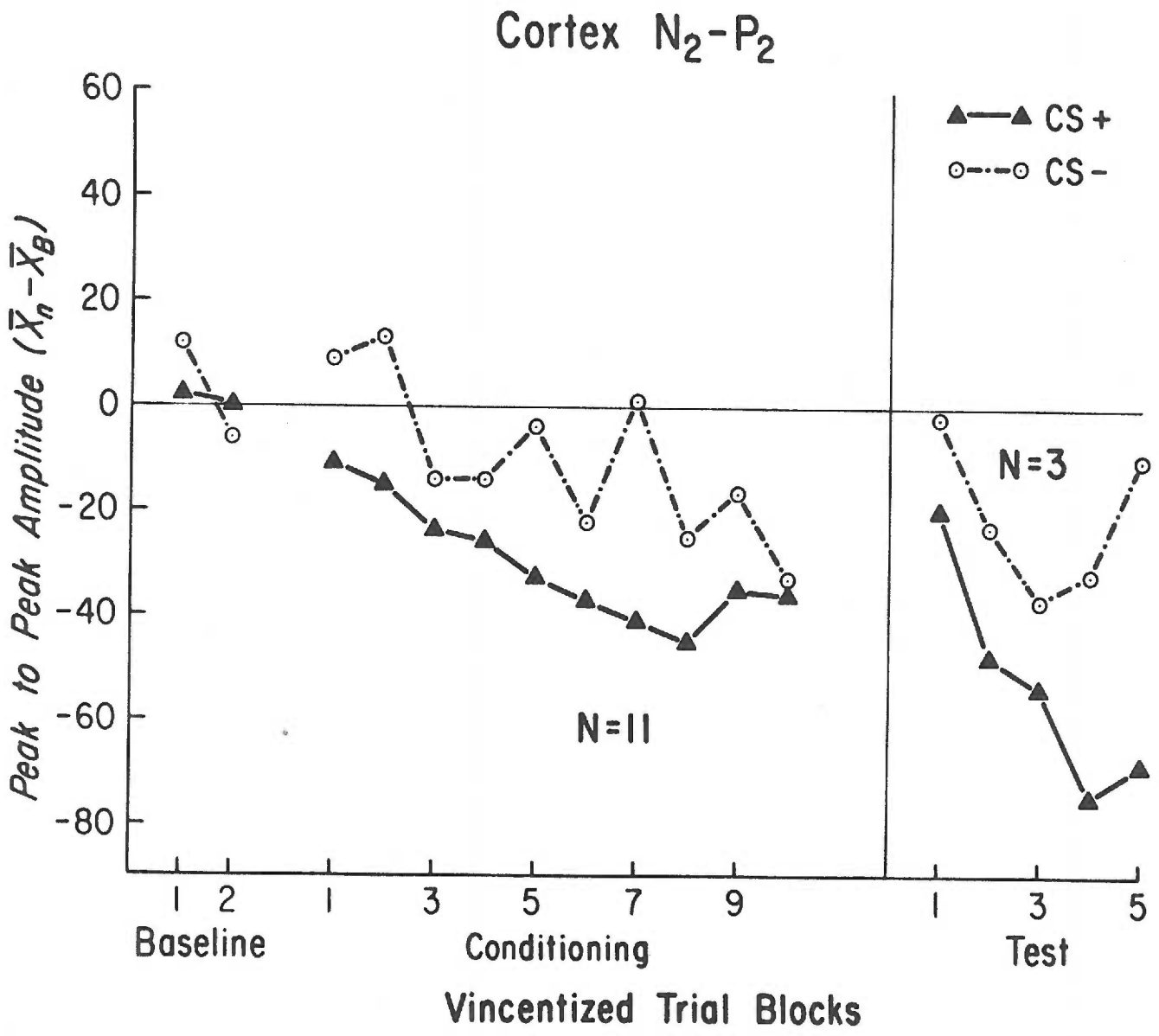
not differ from the progressive decrease in amplitude of the CS+ component. Therefore, the progressive decrease in amplitude of the $P_1$-$N_2$ component can be concluded to be a direct physiological effect of morphine and no conditioned response developed in this AEP component.

For the three animals that were successfully tested, the peak-to-peak amplitude of $P_1$-$N_2$ from the first block of testing was not significantly different from the baseline $P_1$-$N_2$ amplitude. The amplitude of the CS+ and CS- AEPs had returned to baseline levels. Statistical analysis of the data from the five blocks of testing revealed no significant effects, possibly due to the small number of subjects which were tested.

$N_2$-$P_2$. The general decrease in peak-to-peak amplitude for this component during conditioning is illustrated in Figure 13. The main effect of blocks was significant in the analysis of the $N_2$-$P_2$ component data, $F(9, 81) = 2.90$, $p < .005$. Although there appears to be an overall difference between the CS+ and CS- AEP amplitude for this component during conditioning, no significant difference was found. The course of the decrease to the CS- was much more erratic than the change to the CS+ AEP component. Since no differentiation occurred between the CS+ and CS- component amplitude, this decrease in amplitude of the $N_2$-$P_2$ component can be considered to be a direct physiological effect produced by morphine administration.

The test data from the three rats which maintained their morphine intake is shown in the right hand side of Figure 13. A dependent $t$ test revealed that the peak-to-peak amplitude of the first test block AEP had returned to baseline levels. The analysis of the data

Figure 13. Change in peak-to-peak amplitude of Component $N_2$-$P_2$ of the CS+ and CS- elicited cortical AEPs during baseline, conditioning, and testing, for all 11 subjects. One-tenth the total number of conditioning trials for each animal determined the number of trials in each Vincentized trial block for each animal. Peak-to-peak amplitude for each data block was compared to the mean of the peak-to-peak amplitude of the baseline blocks for each rat: $\overline{X}_n - \overline{X}_B$. Baseline data were taken from the first and last blocks of the baseline session for each animal. Testing data were obtained from three animals that maintained their morphine intake during conditioning. The first five blocks of testing data were used from each animal.

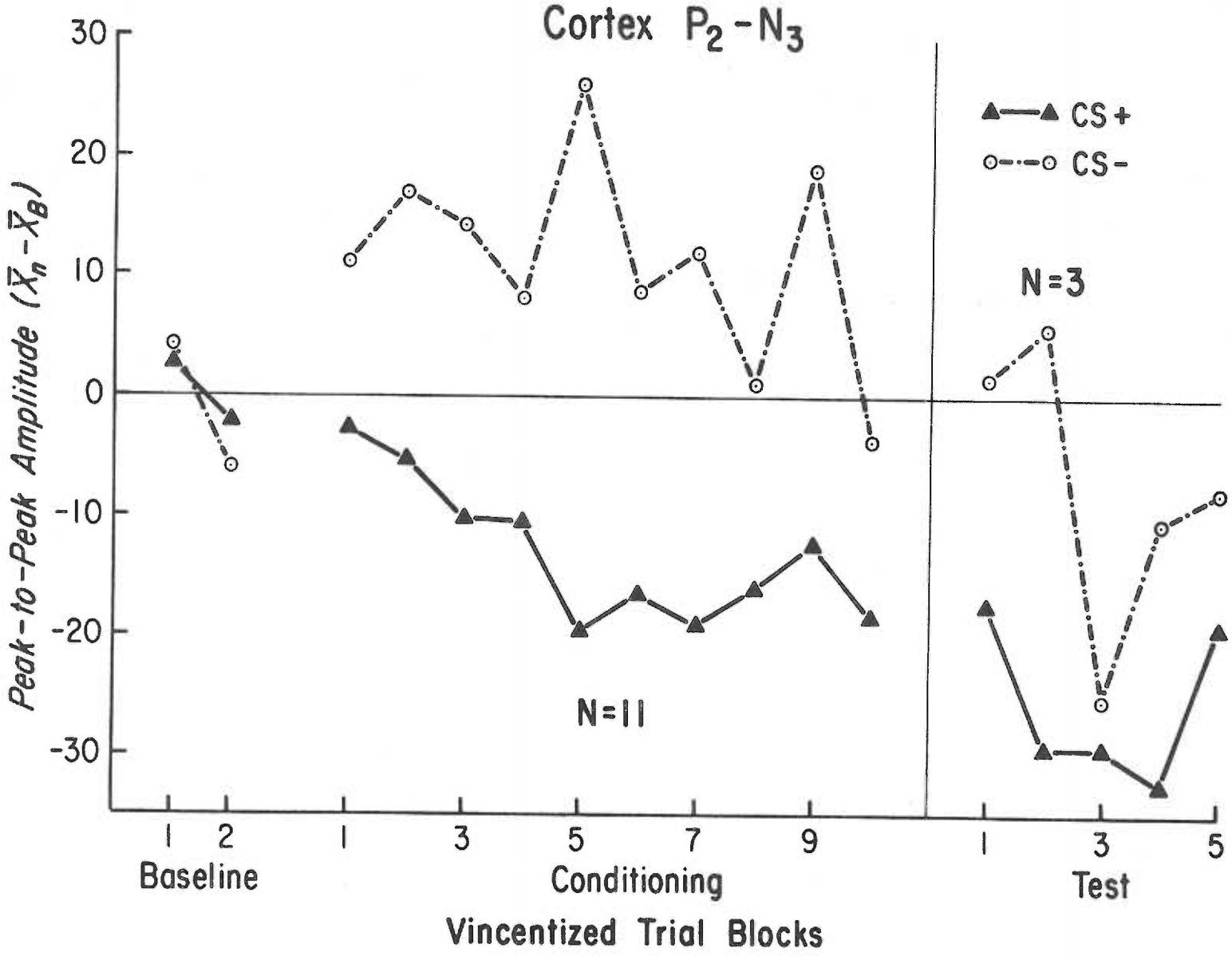

Cortex $N_2$-$P_2$
60
40
20
0
-20
-40
-60
-80
Peak to Peak Amplitude ($\bar{X}_n - \bar{X}_B$)
CS+
CS-
N=11
N=3
1 2
1 3 5 7 9
1 3 5
Baseline
Conditioning
Test
Vincentized Trial Blocks

from the five blocks of testing produced a significant change in amplitude over the testing blocks, $F(4, 8) = 7.80$, $p < .01$. Both the CS+ and CS- AEPs decreased in amplitude over the first four testing blocks, with a tendency to return to baseline levels in the last test block.

The progressive decrease in amplitude of the two short-latency components can be seen in the individual records of F16 for both the CS+ and CS- AEPs in Figure 10. This decrease in AEP amplitude due to morphine administration is most striking in the CS+ AEPs on the left-hand side of the figure.

$P_2$-$N_3$. The only AEP component which demonstrated a differentiation in peak-to-peak amplitude measures between CS+ and CS- was the $P_2$-$N_3$ component which occurred with a latency of 98-197 msec. Figure 14 depicts the changes in amplitude of this component over conditioning blocks. The amplitude for CS+ and CS- were the same during the two blocks of baseline, with a decrease in amplitude from the first to last block. During conditioning, the amplitude of the $P_2$-$N_3$ component for the CS+ AEP decreased over the first five blocks. The change in amplitude of this component for the CS- AEP was an increase in amplitude initially, then a decrease to baseline levels in the final conditioning blocks. The greatest differentiation between CS+ and CS- appeared to occur in the middle blocks. These observations were confirmed statistically. The main effect, reflecting a level difference between CS+ and CS-, was significant, $F(1, 9) = 10.59$, $p < .01$. Also, the change in the CS+ AEP amplitude was different than the change in the CS- AEP amplitude over blocks, as indicated by the significant

Figure 14. Change in peak-to-peak amplitude of Component $P_2$-$N_3$ of the CS+ and CS- elicited cortical AEPs during baseline, conditioning, and testing for all 11 subjects. One-tenth the total number of conditioning trials for each animal determined the number of trials in each Vincentized trial block for each animal. Peak-to-peak amplitude for each data block was compared to the mean of the peak-to-peak amplitude of the baseline blocks for each rat: $\overline{X}_n - \overline{X}_B$. Baseline data were taken from the first and last blocks of the baseline session for each animal. Testing data were obtained from three animals that maintained their morphine intake during conditioning. The first five blocks of testing data were used from each animal.


Cortex $P_2 - N_3$
CS+
CS-
N=3
N=11
Peak-to-Peak Amplitude ($\bar{X}_n - \bar{X}_B$)
30
20
10
0
-10
-20
-30
1
2
1
3
5
7
9
1
3
5
Baseline
Conditioning
Test
Vincentized Trial Blocks

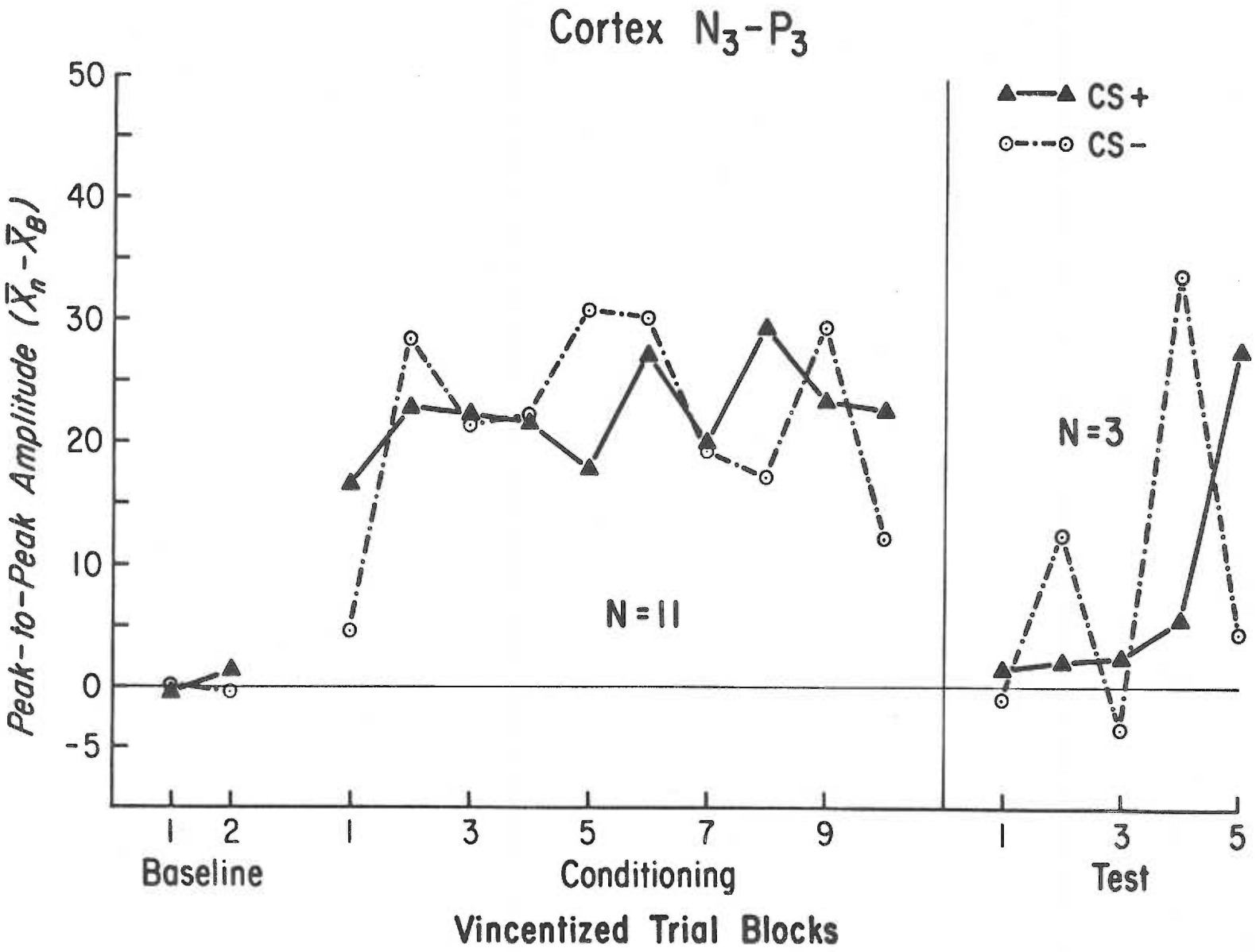
interaction of CS+ versus CS- over blocks, $F(9, 81) = 2.02$, $p < .05$. This differentiation between CS+ and CS- cannot be attributed to a physiological effect of morphine alone. The increase in the CS- $P_2$-$N_3$ amplitude from baseline can be considered to be the result of the physiological effect of morphine. The change which developed to the CS+ AEP component was in the opposite direction. The difference in the changes which developed to the CS+ AEP and CS- AEP components can be concluded to be a conditioned effect. This differential change can be seen in the individual cortical AEPs of F16 in Figure 10. The difference between the CS+ and CS- AEPs is especially noticeable in the fifth block of conditioning.

The conditioned difference between the CS+ AEPs and CS- AEPs which developed during conditioning appeared to persist after withdrawal as can be seen in Figure 14. However, the difference between the CS+ component amplitude and CS- component amplitude recorded from the first block of testing were not significantly different from the amplitude of the respective baseline components, $t(2) = 1.69$, and $t(2) = -0.07$, $p > .05$. Also, none of the effects from the statistical analyses of the five blocks of testing data were significant. The lack of statistical support for this observation can be attributed to the small number of subjects tested and the large variability in the data. Inspection of the data from the individual animals determined that the conditioned difference in the amplitude of the $P_2$-$N_3$ component of the CS+ and CS- AEPs persisted after withdrawal in two out of the three animals.

$N_3$-$P_3$. There was no significant change in the AEP amplitude of the $N_3$-$P_3$ component over blocks, $F(9, 81) = 1.62$, $p > .05$. As can be seen in Figure 15, the largest increase in AEP amplitude appeared to occur between baseline and the first two blocks of conditioning for both the CS+ and CS-. Dependent $t$ tests were conducted to determine if this change was significant. There was a significant increase in amplitude of the $N_3$-$P_3$ component for the CS+ between baseline and the first conditioning blocks, $t(10) = -2.64$, $p < .05$. There was not a significant difference between the baseline amplitude of the CS- component and the first block of conditioning, $t(10) = -1.06$, $p > .05$; however, the difference was significant for the second block of conditioning, $t(10) = -4.96$, $p < .002$. Generally, the amplitude for both the CS+ and CS- AEPs remained at a constant increased amplitude as compared to baseline from the second block of conditioning to the final block of conditioning. The increased amplitude of this component can also be seen in the individual AEP records of Rat F16 in Figure 10. This increased amplitude of the $N_3$-$P_3$ component was produced by the physiological effects of morphine, since the change was the same for both the CS+ and CS- AEPs.

During testing, the amplitude of $N_3$-$P_3$ returned to baseline levels. One effect was significant from the analysis of the five blocks of testing data: The interaction of CS+ versus CS- over testing blocks, $F(4, 8) = 5.05$, $p < .025$. The change in the CS+ and CS- AEPs occurred differently over testing blocks. The CS+ demonstrated a progressive increase in AEP amplitude in the last block of testing. However, the changes in the amplitude of the CS- AEP component fluctuated over the testing blocks.

Figure 15. Change in peak-to-peak amplitude of Component $N_3$-$P_3$ of the CS+ and CS- elicited cortical AEPs during baseline, conditioning, and testing for all 11 subjects. One-tenth the total number of conditioning trials for each animal determined the number of trials in each Vincentized trial block for each animal. Peak-to-peak amplitude for each data block was compared to the mean of the peak-to-peak amplitude of the baseline blocks for each rat: $\overline{X}_n - \overline{X}_B$. Baseline data were taken from the first and last blocks of the baseline session for each animal. Testing data were obtained from three animals that maintained their morphine intake during conditioning. The first five blocks of testing data were used from each animal.


Cortex $N_3-P_3$
CS+
CS−
N=11
N=3
Peak-to-Peak Amplitude ($\bar{X}_n - \bar{X}_B$)
50
40
30
20
10
0
-5
1
2
1
3
5
7
9
1
3
5
Baseline
Conditioning
Test
Vincentized Trial Blocks

In summary, the major finding of this instrumental conditioning experiment was that 16 out of 20 rats self-adminstered lethal doses of morphine. The explanation for this phenomenon was not definitely known; however, from the information available from this experiment, the duration of the electrical stimulation of the forelegs appeared to be a possible factor.

Only three animals completed the testing phase of the experiment. Based on the test results from these three animals, it appeared that the stimulus which had previously been paired with the injection of morphine did not elicit lever pressing behavior when presented to a withdrawn animal.

The cortical AEPs from 11 of the subjects were analyzed. Changes in peak-to-peak amplitude for both CS+ and CS- elicited AEPs due to morphine administration were found in three of the AEP components. In all cases, the amplitude of the peak-to-peak components returned to baseline levels following withdrawal.

In one of the cortical AEP components, $P_2$-$N_3$, the change in amplitude during conditioning was different for the CS+ elicited AEPs than the CS- elicited AEPs. The difference in the change in amplitude in the CS- and CS+ AEP components was defined as a conditioned response. This conditioned response appeared to persist after withdrawal; however, there was no statistical support for this observation.

## EXPERIMENT 2

Due to the unexpected self-administration of lethal doses of morphine by the rats in the first experiment, the original objective of the experiment was not adequately tested. In an attempt to test the relapse theory but control the morphine intake of the rats, a classical conditioning experiment was designed. The purpose of the experiment was to determine if a conditioned response would develop to a stimulus paired with the injections of morphine during the establishment of dependence. Then, after a period of withdrawal, a testing session would determine if the conditioned response persisted after withdrawal.

Rats were trained in a classical conditioning paradigm for 5 days after 1 baseline day. Electrical stimulation of one foreleg (CS+) was paired with morphine injections. In order to differentiate associative from physiological changes due to the drug or to dependence, electrical stimulation of the other foreleg (CS-) occurred randomly. Once dependent, the rats were taken from the experimental apparatus and allowed to go through withdrawal for 6 days. During a final experimental phase, the rats were divided into two groups. Both groups of rats were presented with electrical stimulation to both forelegs, the CS+ and CS-. One group of rats had a morphine injection paired with the CS+ in order to test whether any effects which developed during conditioning persisted after withdrawal, but were present only when the animal was morphine intoxicated. The second test group received saline injections paired with the CS+ in order to test whether the evoked potential changes which developed during conditioning persisted after withdrawal and were present in a drug-free animal.

Another group of animals was also run in the conditioning paradigm but received saline injections paired with the CS+ instead of morphine injections throughout conditioning. All other experimental parameters were the same as the group that received morphine. Due to computer malfunctions, the saline group was not tested. This group of animals was included to differentiate changes due to the effects of morphine or conditioning from other factors such as sensitization or habituation to the repeated electrical stimulation of the forelegs during training.

The following results were expected:

1. Evoked potential recordings would reflect:
   a. Changes due to conditioning, apparent in the difference between the evoked potentials elicited by the stimulus that had been paired with morphine injections and the unpaired stimulus, and
   b. Physiological effects of tolerance, dependence, and other non-associative factors, which would be apparent in the difference between baseline evoked potentials and evoked potentials elicited during training by the unpaired stimulus.
2. The relapse theory would be supported if the conditioned neural response was present in the evoked potentials elicited by CS+ during the testing phase. This conditioned neural response would be present in the CS+ elicited evoked potentials in both groups of animals during the testing phase. The CS- elicited evoked potentials should not demonstrate the conditioned neural response.

# METHODS

## Subjects

Fifteen female, Sprague-Dawley rats obtained from King Laboratories, Oregon, Wisconsin, weighing between 200-260 g at the beginning of the experiment were used. All animals were housed individually in a colony room with a 12-h light-dark cycle and were maintained on an antibiotic regimen of Aureomycin (50 mg/liter) in the drinking water.

## Surgical Preparation

All surgical procedures were conducted under aseptic conditions. Two animals were anesthetized with pentobarbitol (50 mg/kg i.p.). Atropine sulfate (0.30 mg/kg i.p.) was administered to control mucous secretions. Due to the loss of rats during surgery to respiratory complications, Ketamine hydrochloride (60 mg/kg i.m.) and Acepromazine (2 mg/kg i.m.) were used as the anesthetic agents on the remaining thirteen animals. The surgical procedures were the same as those described for the first experiment.

## Recording and Stimulating Electrodes

The foreleg stimulating wires were constructed from two 8.5-cm and two 7.5-cm lengths of 28-ga multistranded stainless steel suture (Davis & Geck) with polyvinyl tubing (0.40 mm I.D. x 0.80 mm O.D.) as insulation. One end of each wire was soldered to separate pins of the head plug. One 8.5-cm and 7.5-cm electrode was implanted in each foreleg. Animal ground was two 6-cm lengths of stainless steel suture wrapped around each pair of stimulating leads and soldered together to one pin of the head plug.

The bipolar macroelectrodes used for recording were the same as those described for the first experiment, constructed from 0.25 mm stainless steel insulated wire.

## Apparatus

The apparatus was the same as that used in the first experiment, with a few exceptions. The intensity of the stimulating pulses was less with the stainless steel stimulating electrodes. The voltage was adjusted between 2.0-5.0 V for the three 0.2 msec monophasic square wave pulses which were presented at a frequency of 300 pulses per second. This voltage was selected for each rat so as to elicit a slight foreleg twitch and a cortical evoked potential of approximately 300 μV.

The pneumatic syringe was used to control the intravenous delivery of morphine or saline. Also, the mercury commutator was bypassed in order to obtain a signal which was not contaminated by movement artifact.

## Experimental Design

The experimental procedure is summarized in Table 4. All animals received the same training procedure. The only difference was whether the animals received morphine or saline as the US during conditioning. Each rat was placed in the Plexiglas cage inside the sound-attenuating chamber (see Figure 3). For the first 24 hours, the animals was presented with 80 foreleg stimuli to one paw (CS+) which were paired with the injection of 0.025 ml of saline into the jugular cannula 1 sec after the onset of the CS+. The same number of foreleg stimuli were presented

Table 4

| | Experimental Group | Control Group |
|---|---|---|
| | **Baseline** | |
| Day 1: | 80 CS+ - US (saline 0.025 ml)<br>80 CS-* | 80 CS+ - US (saline 0.025 ml)<br>80 CS-* |
| | **Conditioning** | |
| Day 2: | 80 CS+ - US (morphine 0.2 mg/kg in 0.01 ml)<br>80 CS- | 80 CS+ - US (saline 0.01 ml)<br>80 CS- |
| Day 3: | 80 CS+ - US (morphine 0.4 mg/kg in 0.02 ml)<br>80 CS- | 80 CS+ - US (saline 0.02 ml)<br>80 CS- |
| Day 4: | 80 CS+ - US (morphine 0.6 mg/kg in 0.03 ml)<br>80 CS- | 80 CS+ - US (saline 0.03 ml)<br>80 CS- |
| Day 5: | 80 CS+ - US (morphine 0.8 mg/kg in 0.04 ml)<br>80 CS- | 80 CS+ - US (saline 0.04 ml)<br>80 CS- |
| Day 6: | 80 CS+ - US (morphine 0.8 mg/kg in 0.04 ml)<br>80 CS- | 80 CS+ - US (saline 0.04 ml)<br>80 CS- |
| | **Withdrawal - Six Days** | |
| | **Testing** | |
| | Group 1 | |
| | 80 CS+ - US (saline 0.025 ml<br>80 CS- | No Test |
| | Group 2 | |
| | 80 CS+ - US (morphine 0.5 mg/kg in 0.025 ml)<br>80 CS- | |

*CS+ and CS- were stimulation of opposite forelegs, counterbalanced within groups for left vs right

to the other foreleg (CS-) explicitly unpaired with the CS+-saline presentations. The designation of stimulation of the right or left foreleg as the CS+ was counterbalanced across animals. The evoked potentials collected during this time period constituted baseline data.

The training phase began immediately following the collection of the baseline data. For the next 5 days, the rats were presented with 80 CS+ and 80 CS- presentations per day. The CS+ was paired with an injection of morphine 1 sec after the onset of the CS+. In order to offset tolerance and to establish dependence, the dose of morphine was 0.2 mg/kg per presentation on the first day, 0.4 mg/kg the second day, 0.6 mg/kg the third day, and 0.8 mg/kg the fourth and fifth days. The total morphine dose per day was 16 mg/kg the first day, 32 mg/kg the second day, 48 mg/kg the third day and 64 mg/kg the fourth and fifth days. The volume per injection was 0.01 ml the first day, 0.02 ml the second day, 0.03 ml the third day, and 0.04 ml the fourth and fifth days.

The CS+-US and CS- presentations occurred every 9 min, on the average, varying between 5 min and 13 min. The sequence of stimulus presentation was determined by the Fellows progression (Fellows, 1967). Three separate progressions were made. The progression used for each animal for each day was determined randomly, with the restriction that a progression was not repeated for more than 2 days in succession. The CS- presentations were explicitly unpaired with the CS+-US presentations. The average ITI from a US presentation to either a CS+ or CS- presentation was also equated. Therefore, the average drug level was the same during the CS+ and CS- presentations (see Appendix B). Food and water were available at all times in the chamber.

After 5 days of conditioning, withdrawal began. The animal was taken out of the chamber and returned to its home cage for 6 days and was weighed once a day. Once the animals had gone through withdrawal, testing began. The morphine animals were divided into two groups. For the next 24 h, one group of morphine animals received 80 presentations of the CS+ paired with 0.025 ml saline injections, and 80 presentations of the CS-. The second group of animals received a preliminary injection of 2.5 mg/kg morphine just prior to the start of testing. These animals also received 80 presentations of the CS+ and CS-, but the CS+ was paired with the injection of 0.5 mg/kg morphine in 0.025 ml of solution. Four control animals were run after all the morphine animals had been through the paradigm. For the control animals, saline injections were administered instead of morphine as the US, with all other experimental parameters the same. Due to computer problems, the saline control animals were not tested.

Once the testing procedure was completed, the animals were anesthetized with sodium pentobarbital (50 mg/kg) and perfused with 10% buffered formalin. The brain was blocked at -2.0 - +9.0 mm and the brain allowed to sit in 10% buffered formalin for at least a week. The brains were sliced in 50 μ sections using a freezing microtome. The serial sections were mounted and later stained with a Nissl stain (Thionin).

## Data Analysis

The data analysis techniques were the same as those described for Experiment 1.

# RESULTS

## HISTOLOGY

The epidural bone screw used to record cortical AEPs was always placed according to bone landmarks on the skull. Therefore, the only histological analysis for the cortical electrode was an examination of the cortex after the animal was perfused. There was no evidence of cortical damage due to infection,hemorrhage or necrosis on any of the experimental or control animals' brains.

Histological verification was conducted for the subcortical electrode placements for all experimental and control animals. The actual location of the electrodes was determined by comparing light projected sections with the atlas of Pellegrino, Cushman, and Cushman (1979). The anterior and lateral coordinates were determined from the center of the electrode, whereas the vertical coordinate was taken from the tip of the electrode at its deepest penetration point.

Fourteen out of fifteen animals in the study produced good recordings from the electrodes directed at PFT. Twelve of the fourteen electrode placements were found to be in the nucleus, nine from experimental animals and three from the control animals. The actual location of the electrode for each animal is reported in Table 5. Figure 16 contains coronal brain sections from the atlas of Pellegrino et al., with the electrode locations marked with a cross. The anterior-posterior coordinates ranged from +3.2 mm to +3.8 mm with a mean of +3.55 mm. The lateral placements ranged from +1.0 mm to +2.0 mm, with a mean of +1.41 mm. The vertical placement ranged from -1.0 to +1.0, with a mean of -0.7. One electrode, which was not in PFT, was found

Figure 16: Actual location of electrodes directed at PFT. The tip of each electrode for each animal is marked with a cross. The anterior-posterior coordinates are shown under each coronal section. Abbreviations:

PVG--Periventricular gray

PV--Paraventricular nucleus of the thalamus

PF--Parafascicularis nucleus of the thalamus

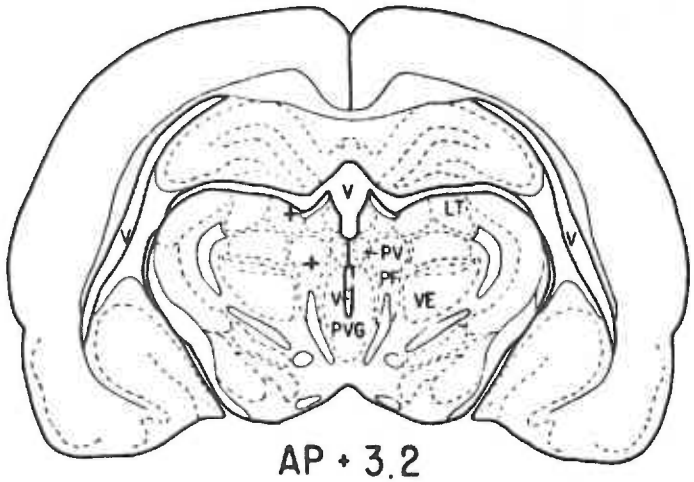
VE--Ventral nucleus of the thalamus

V--Ventricle

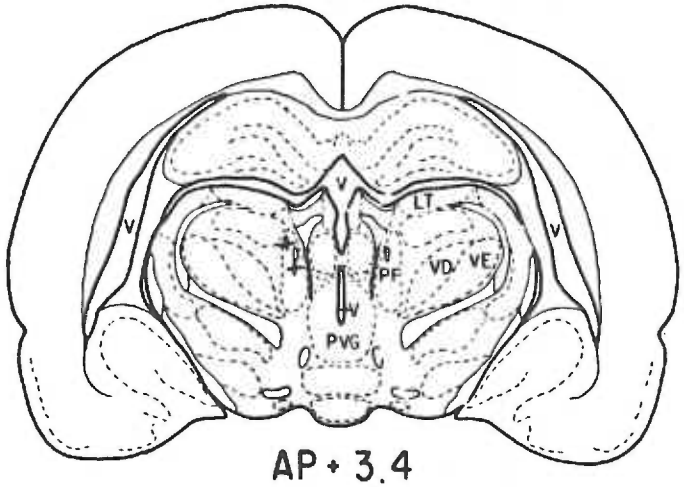
LT--Lateral nucleus of the thalamus

MD--Medial dorsal nucleus of the thalamus

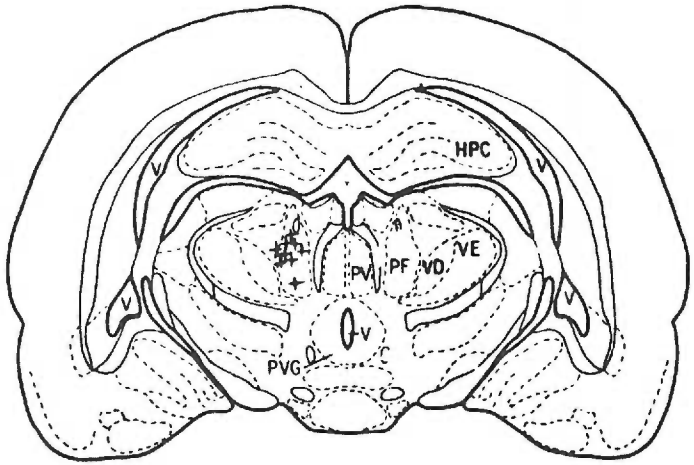
# PFT Electrode Placements



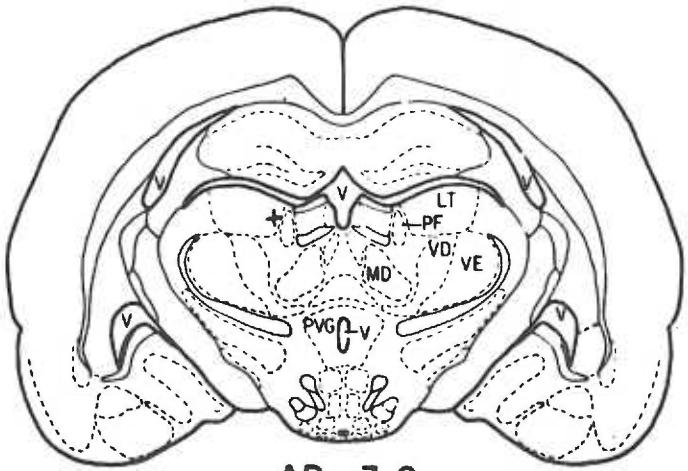

AP + 3.2




AP + 3.4




AP + 3.6




AP + 3.8

Table 5

PFT Electrode Locations

| Subject | AP | L | V |
|---|---|---|---|
| F61 | +3.5 | +2.0 | -0.5 |
| F63 | +3.6 | +1.5 | +0.0 |
| F67 | +3.6 | +1.2 | -0.5 |
| F70 | +3.8 | +2.0 | +0.5 |
| F75 | +3.6 | +1.2 | -0.5 |
| F76 | +3.7 | +1.5 | +0.0 |
| F79 | +3.6 | +1.2 | +0.0 |
| F81 | +3.6 | +1.2 | +0.0 |
| F86 | +3.5 | +1.5 | +0.0 |
| F89 | +3.6 | +1.0 | -1.0 |
| F91 | +3.2 | +1.0 | -0.5 |
| F97 | +3.2 | +1.5 | +1.0 |
| F95 | +3.7 | +1.3 | +0.0 |
| F100 | +3.5 | +1.2 | +0.5 |

in the pretectal area. The other electrode was in the lateral nucleus of the thalamus.

Thirteen out of fifteen animals produced usable recordings from the electrodes directed at PVG. Ten out of the thirteen electrode placements were found to be in PVG, as shown in Figure 17. Table 6 contains the coordinates for the electrode placements of each animal. Six of the experimental animals had accurate PVG placements and all four of the control animals had accurate placements. The anterior-posterior coordinates for the electrodes ranged from 0.0 mm to +0.2, with a mean of +0.09 mm. The lateral placements ranged from -0.5 mm to -1.25 mm, with a mean of -0.95 mm. The vertical placements ranged from 0.0 mm to -2.0 mm, with a mean of -1.34. Those electrodes which were not found in PVG were located in the commissure of the superior colliculus, lateral to PVG in the reticular formation, and in the superior colliculus.

Usable recordings from electrodes directed at VMH were available from 13 out of 15 animals. None of the electrodes were found to be in VMH, as can be seen by examination of the electrode locations plotted on coronal sections in Figure 18. Instead, 5 of the 14 electrodes were in LH, 1 was in the median forebrain bundle, and 6 were below and lateral to VMH, and 1 was directly above VMH. Table 7 gives the electrode location for each animal.

## BODY WEIGHT

Loss of body weight after discontinuing the administration of morphine has previously been used as an index of the withdrawal syndrome in rats (cf. Weeks & Collins, 1979). The weights of 13 of the

Figure 17: Actual location of electrodes directed at PVG. The tip of each electrode for each animal is marked with a cross. The anterior-posterior coordinates are shown under each coronal section. Abbreviations:

CS--Superior colliculus

CIF--Inferior colliculus

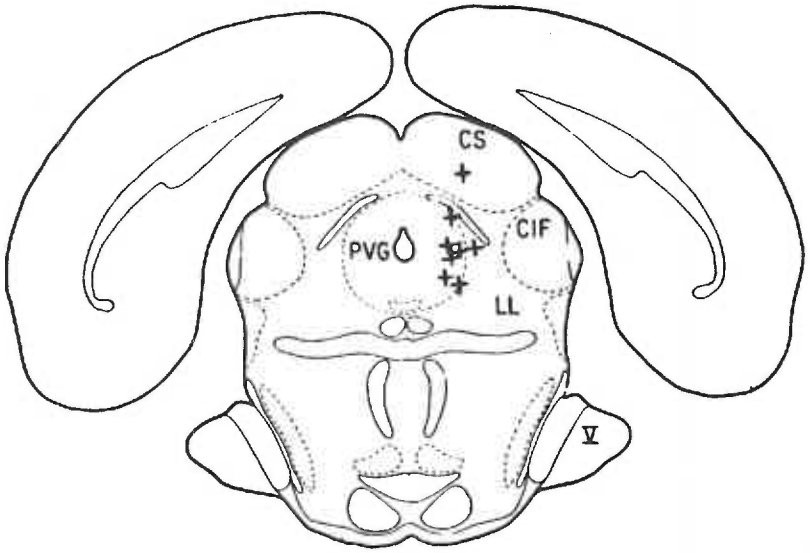
LL--Lateral lemniscus

V--Fifth cranial nerve

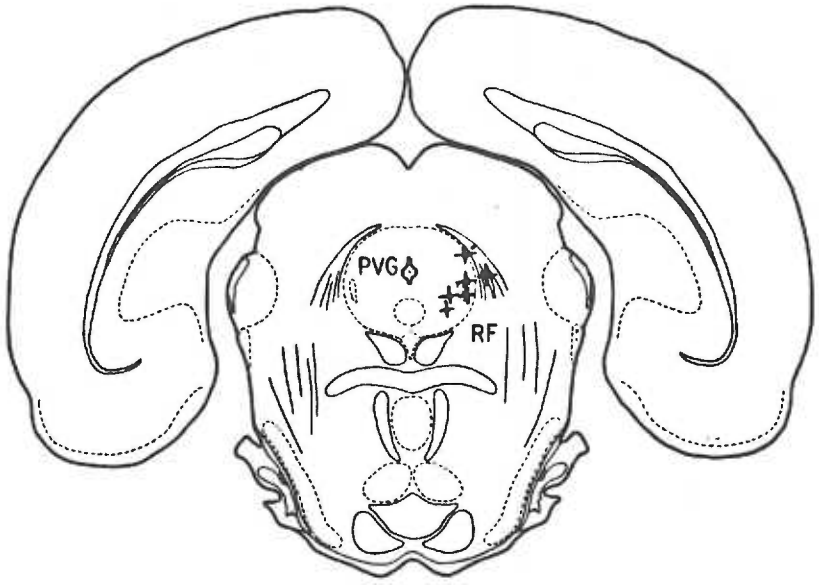
PVG--Periventricular gray

RF--Reticular formation

# PVG Electrode Placements




AP 0.0




AP + 0.2

Table 6

PVG Electrode Locations

| Subject | AP | L | V |
|---|---|---|---|
| F61 | +0.2 | +0.8 | -2.0 |
| F63 | +0.2 | +0.5 | -1.5 |
| F67 | +0.2 | +1.0 | -1.5 |
| F70 | +0.2 | +1.25 | -1.0 |
| F75 | +0.0 | +1.25 | -1.5 |
| F76 | +0.2 | +1.25 | -1.5 |
| F79 | +0.0 | +1.0 | +0.0 |
| F81 | +0.0 | +0.75 | -1.5 |
| F86 | +0.0 | +0.75 | -2.0 |
| F89 | +0.0 | +1.0 | -2.0 |
| F91 | +0.2 | +1.0 | -1.25 |
| F97 | +0.0 | +1.0 | -0.5 |
| F100 | +0.0 | +0.8 | -1.0 |

Figure 18: Actual location of electrodes directed at VMH. The tip of each electrode for each animal is marked with a cross. The anterior-posterior coordinates are shown under each coronal section. Abbreviations:

AHA--Anterior hypothalamic area

CPU--Caudate putamen

IC--Internal capsule

FX--Fornix

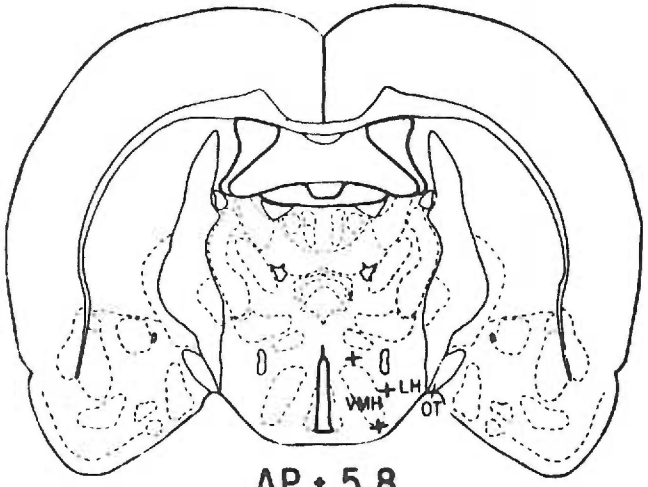
LHA--Lateral hypothalamus

OT--Optic tract

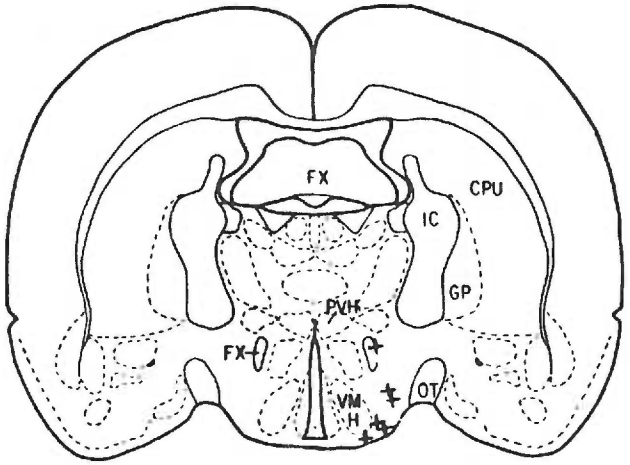
GP--Globus pallidus

PVH--Periventricular hypothalamus

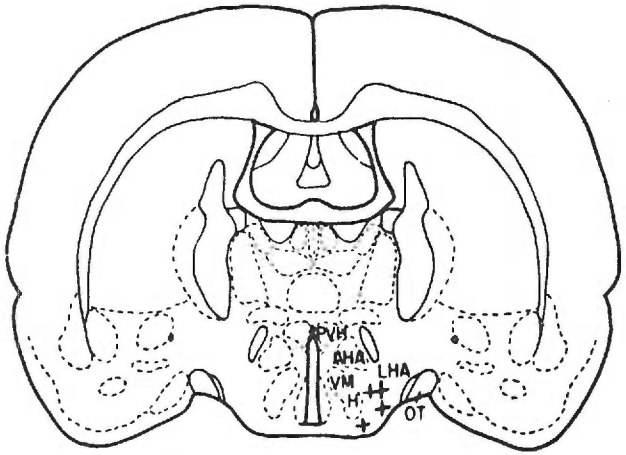
# VMH Electrode Placements



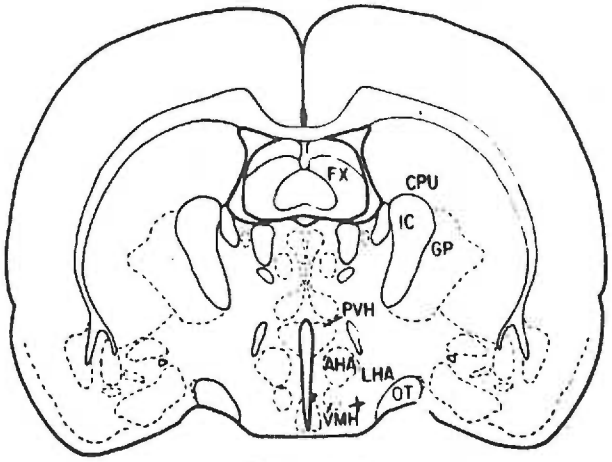

AP • 5.8




AP • 6.0




AP • 6.2




AP • 6.4

Table 7

VMH Electrode Locations

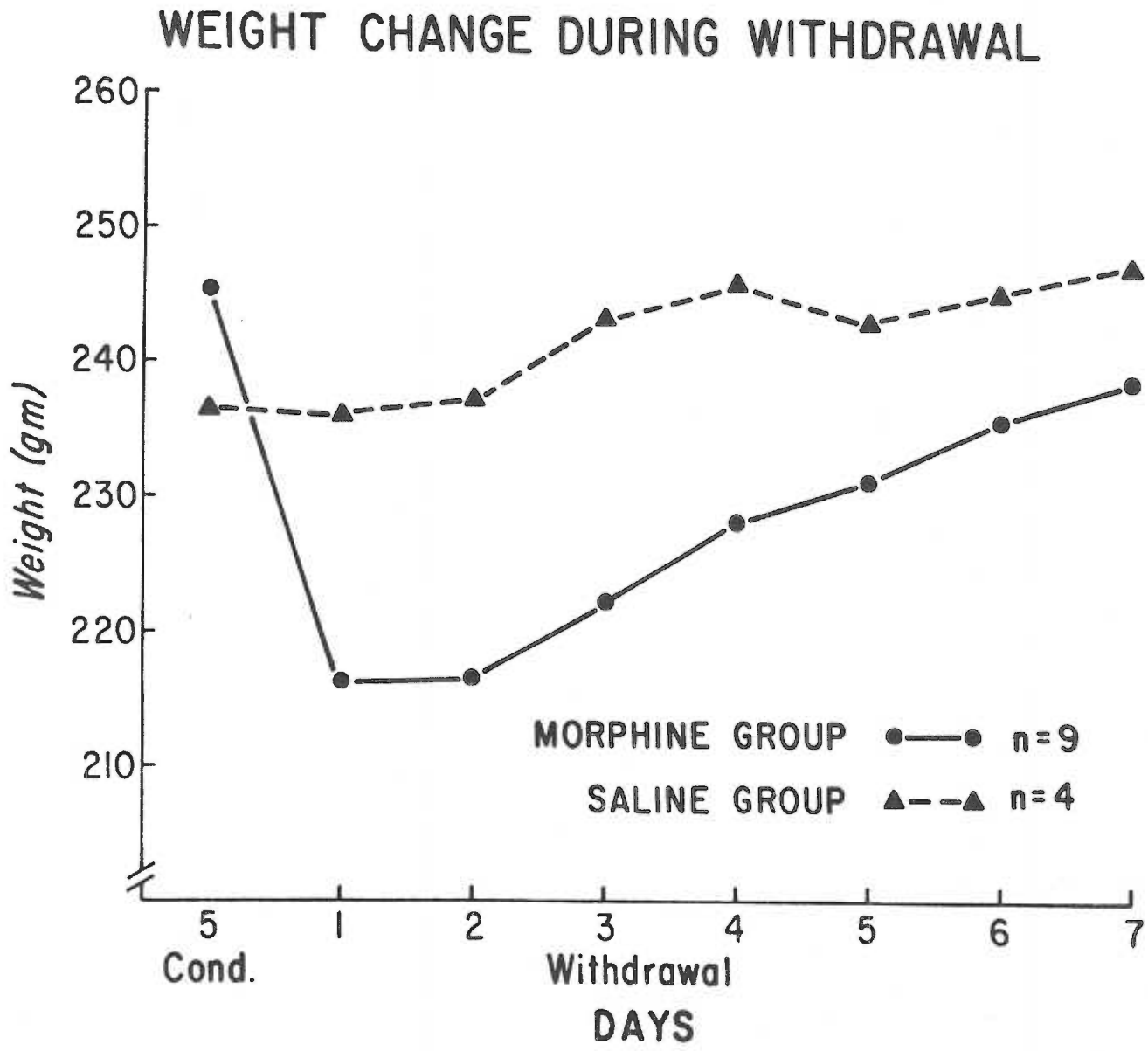
| Subjects | AP | L | V |
|---|---|---|---|
| F61 | +6.0 | +1.5 | -4.0 |
| F63 | +5.8 | +1.25 | -4.0 |
| F67 | +6.2 | +1.5 | -3.5 |
| F70 | +6.0 | +1.5 | -2.0 |
| F75 | +6.0 | +1.0 | -4.0 |
| F76 | +6.2 | +1.25 | -4.0 |
| F79 | +5.8 | +2.0 | -3.0 |
| F81 | +6.4 | +1.25 | -3.5 |
| F86 | +6.0 | +2.0 | -3.25 |
| F89 | +6.0 | +1.75 | -3.5 |
| F91 | +6.2 | +1.5 | +3.0 |
| F95 | +6.0 | +1.5 | -4.0 |
| F97 | +5.8 | +1.0 | -2.0 |
| F100 | +6.2 | +1.25 | -3.5 |

15 animals were monitored during withdrawal. As shown in Figure 19, there was a significant difference between the weight change in the post-conditioning period for the saline control animals and the animals which received morphine during conditioning, $F(7, 77) = 13.61$, $p < .001$. The morphine animals exhibited a 30-gm loss of weight between the last day of conditioning and the first day of withdrawal. The weight loss was maintained throughout the second day; then the rats steadily gained weight during Days 3-7 of withdrawal. In contrast, the saline animals exhibited a gradual weight gain throughout the post-conditioning period. The significant weight loss by the morphine animals, as compared to the control animals, is evidence that the morphine animals had become dependent on morphine during conditioning.

ELECTROPHYSIOLOGY

Each animal received 80 CS+ and 80 CS- presentations per day. The evoked potentials from each brain area were stored in 10-trial AEPs. For statistical analysis, measurements were taken from 20-trial AEPs as a trial block. The AEPs from the four brain areas were analyzed separately for changes in latency and peak-to-peak amplitude for the conditioning and testing data. Analysis of the latencies of the AEP peaks recorded from all four brain areas revealed a significant shift in latency of only two peaks from only the cortical AEP during conditioning. Accordingly, a discussion of latency results will be found only for the cortical brain area. A description of the statistical analyses used to examine the latency data is also presented there.

Figure 19: Change in body weight for morphine and saline animals during the post-conditioning period and prior to testing.


WEIGHT CHANGE DURING WITHDRAWAL
260
250
240
230
220
210
Weight (gm)
MORPHINE GROUP n=9
SALINE GROUP n=4
5
Cond.
1
2
3
4
5
6
7
Withdrawal
DAYS

Conditioning

The peak-to-peak amplitude measure was more sensitive to the effects of the experimental manipulations. Preliminary analysis of the peak-to-peak amplitude data determined that there was no effect of trial blocks (20 trials) within days (80 trials). Therefore, statistical analyses were conducted on the mean peak-to-peak amplitudes of the four 20-trial AEPs (80 trial block) for the baseline day and each day of conditioning. Thus, for each AEP component from each brain area of each rat, there were six dependent measures used in each analysis (i.e., the baseline day and the 5 conditioning days).

Because it seemed possible that stimulation of one foreleg as opposed to the other might be more effective in producing evoked potentials in a given brain area, initial analysis of the data included paw assignment as a factor. Recall that designation of stimulation of the right or left foreleg as the CS+ was randomized across animals, whereas the bipolar electrodes were implanted in the same hemisphere of the brain in all animals.

An unweighted means, four-way analysis of variance for unequal subjects was conducted, with two between and two within measures. One between factor was the drug condition (i.e., morphine vs saline) as the US. The other between factor was paw assignment (contralateral versus ipsilateral paw stimulation in reference to the cortical screw placement as the CS+). Since all animals received both the CS+ and CS-, one within measure was the stimulus type. The other within measure was trial blocks. The paw assigment factor was only significant in two cases. In one peak-to-peak component, $P_1$-$N_2$, of the cortical AEP, this factor was significant in an interaction with CS+

and CS-, $F(1, 11) = 22.49$, $p < .001$. Examination of the data showed that the interaction arose because the $P_1$-$N_2$ peak-to-peak amplitude was greater for CS+ than CS- when the CS+ was presented to the ipsilateral paw, whereas the reverse was true when CS+ was presented to contralateral paw. This effect is probably due to the neuroanatomical organization of the contralateral versus ipsilateral fiber pathways to the cortex.

The paw assignment was also a significant factor in an analysis conducted on the data from the cortical $P_2$-$N_3$ component, and will be discussed in detail in that section. The counterbalancing procedure produced no other significant outcomes and did not interact significantly in any other case with independent measures, so in all further analyses the data were collapsed across the paw assignment factor.

Examination of the baseline and conditioning data continued with an unweighted-means, three-way analysis of variance for an unequal number of subjects per group. The between-groups factor was drug treatment as the US. The within measures were the stimulus type (CS+ or CS-) and trial blocks. Appropriate follow-up analyses were used as necessary.

Testing

The test data were analyzed separately from the conditioning data. In accordance with the goals of the experiment (to detect physiological changes induced by conditioning and/or morphine which withstand withdrawal), only significant effects discovered in conditioning data were pursued in the examination of test data. The peak-to-peak

amplitudes of each of the 20-trial AEPs were obtained. Dependent *t* tests were used to compare the amplitudes of components of the first 20-trial AEP of the testing session to the amplitudes of the baseline AEP components, in order to determine if the changes which developed during conditioning were maintained after withdrawal. If a conditioning effect had been noted, one *t* test compared the amplitude of the testing CS+ AEP component to the amplitude of the respective component from the baseline CS+ AEP and a second *t* test compared the amplitude of the testing CS- component to the amplitude of the respective component from the baseline CS- AEP. However, if no conditioning effect had been found in the data from the conditioning phase analysis, one test was conducted on the combined amplitude data from the testing CS+ and CS- AEPs compared to the respective baseline AEPs. By comparing the test AEP components to the baseline AEP components, it was possible to determine if a difference existed and also whether any obtained difference was in the same direction as the change which developed during conditioning. This procedure would, at the same time, avoid the error of attempting to prove the null hypothesis which would occur if these comparisons referred to the final data of the conditioning phase.

As a next step in the analysis of test data, two test-period measures were derived for each animal by calculating a mean amplitude for the appropriate components from the first two 20-trial AEPs and the mean amplitude of the same components from the second two 20-trial AEPs. A three-way analysis of variance was conducted on these amplitude measurements. The between-groups factor was drug treatment during the testing session. The within measures were stimulus type

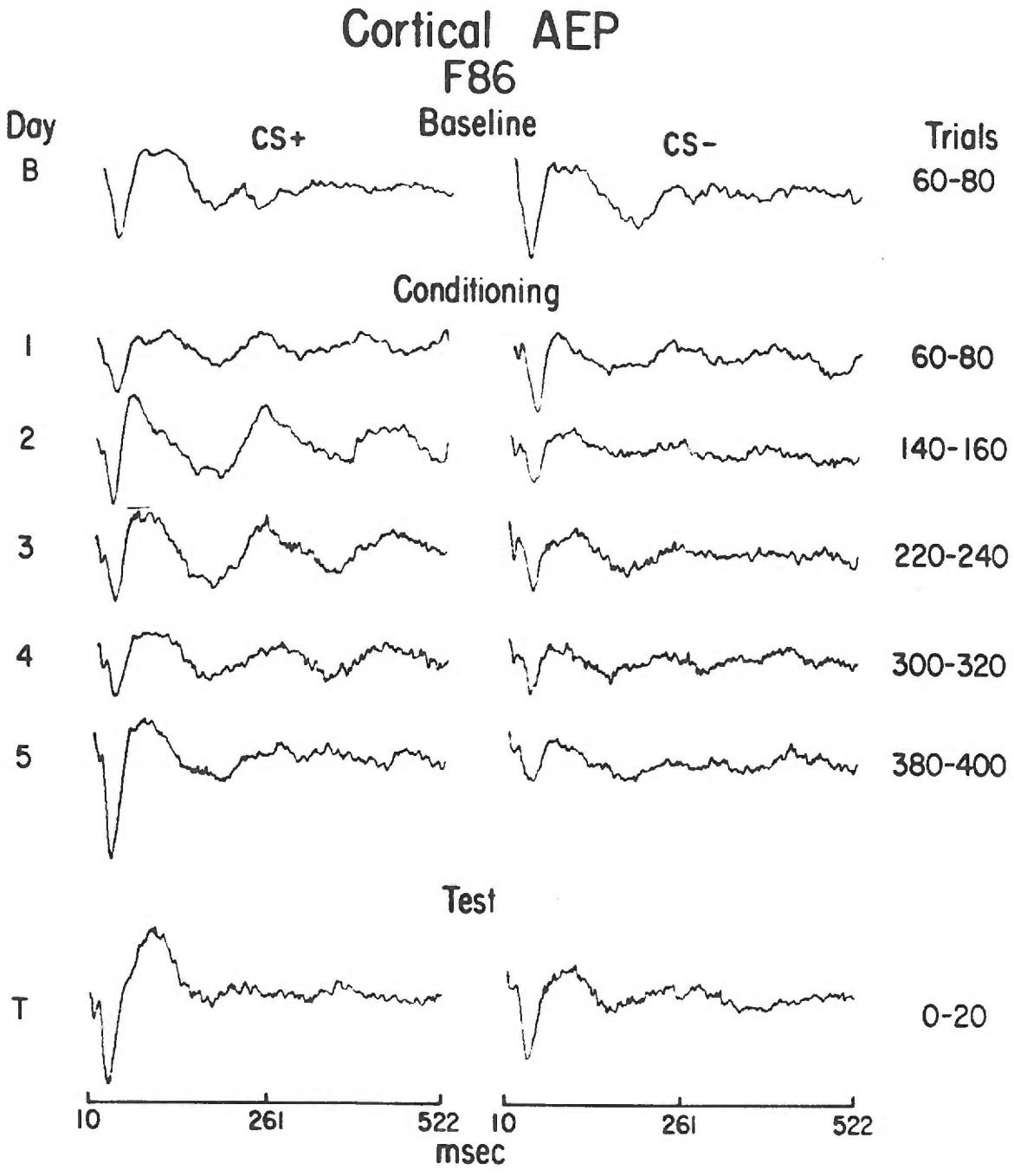
and trial blocks. Nine of the eleven morphine-conditioned animals were tested because two animals pulled their head posts off prior to the testing session and could not be tested. None of the saline-control animals were tested due to computer malfunctions.

## Cortex

The cortical AEP data from all 14 animals were used; 11 of the animals were from the morphine group and 4 from the saline group. A cortical AEP with the peaks labelled is presented in Figure 9, Page 49. The average latencies of the peaks for the saline and morphine animals were: $P_1$ - 26 msec, $N_2$ - 40 msec, $P_2$ - 96 msec, $N_3$ - 195 msec, and $P_3$ - 264 msec. To illustrate the kinds of changes observed in the cortical AEPs, the AEPs from a subject that demonstrated obvious changes during conditioning are presented in Figure 20. The AEPs for CS+ and CS- from the last 20 trials of the baseline period and the 5 days of conditioning are shown, as well as the first 20-trial AEP for both CS+ and CS- from the testing period. There were two particularly noticeable changes. As can be seen in the left of Figure 20, a late component, at approximately 200 msec, developed in the CS+ AEPs during conditioning. Also, the amplitude of components of the CS- AEPs were reduced during conditioning, as compared to the baseline CS- AEPs. These and other changes which developed to any AEP component during conditioning are discussed below.

Latency. A latency measure for each of the five peaks was taken from each 20-trial AEP from the baseline and conditioning sessions. Three-way analyses of variance were conducted on these data. The

Figure 20: Cortical CS+ and CS- AEPs recorded during baseline, conditioning and testing for Rat F86. The AEPs were taken from the last 20 trials of the baseline day and each of the five days of conditioning. The test AEP was taken from the first 20 trials of the test session. Rat F86 received contralateral foreleg stimulation as the CS+, ipsilateral foreleg stimulation as the CS-. Saline injections were paired with the CS+ for this subject during testing.


Cortical AEP
F86
Day
CS+
Baseline
CS-
Trials
B
60-80
Conditioning
1
60-80
2
140-160
3
220-240
4
300-320
5
380-400
Test
T
0-20
10
261
522
10
261
522
msec

between-subjects factor was drug treatment and the two within measures were stimulus type and the trial blocks (1-24).

As stated earlier, a significant shift in latency occurred in two peaks of the cortical AEP. The statistical analysis of the data from Peak $P_2$ revealed a significant two-way interaction of drug treatment by trial blocks, $F(23, 299) = 2.37$, $p < .001$. This interaction is presented graphically in Figure 21. As can be seen in this figure, there was a progressive decrease in the $P_2$ peak latency for the morphine animals, whereas the latency of the $P_2$ peak showed no consistent change for the saline animals. The greatest difference in the latency of this peak between the two groups appeared to be in the baseline day. The mean latency of Peak $P_2$ was 15 msec longer during the baseline session for the morphine animals than for the saline animals, but during the last day of conditioning the mean latency was 5 msec shorter. Since the decrease in latency occurred in both the CS+ and CS- AEPs for Peak $P_2$, and was different from the saline animals, this effect may be attributed to a physiological effect of morphine. This decreased latency of Peak $P_2$ is apparent in the individual 20-trial CS+ AEPs shown in Figure 20 for Rat F86.

There was also a significant shift in latency for the $P_3$ peak in the AEP recordings from morphine animals. The three-way analysis of variance disclosed two significant main effects: a change in latency over trial blocks, $F(23, 299) = 1.80$, $p < .025$, and a significant difference in the latency of $P_3$ between the morphine and saline groups, $F(1, 13) = 5.48$, $p < .05$. Both effects are shown in Figure 22. The average latency of $P_3$ for the morphine animals was 263 msec, whereas

Figure 21: Shift in latency of cortical AEP Peak $P_2$. An 80-trial block was composed of the mean of the latency of the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning.

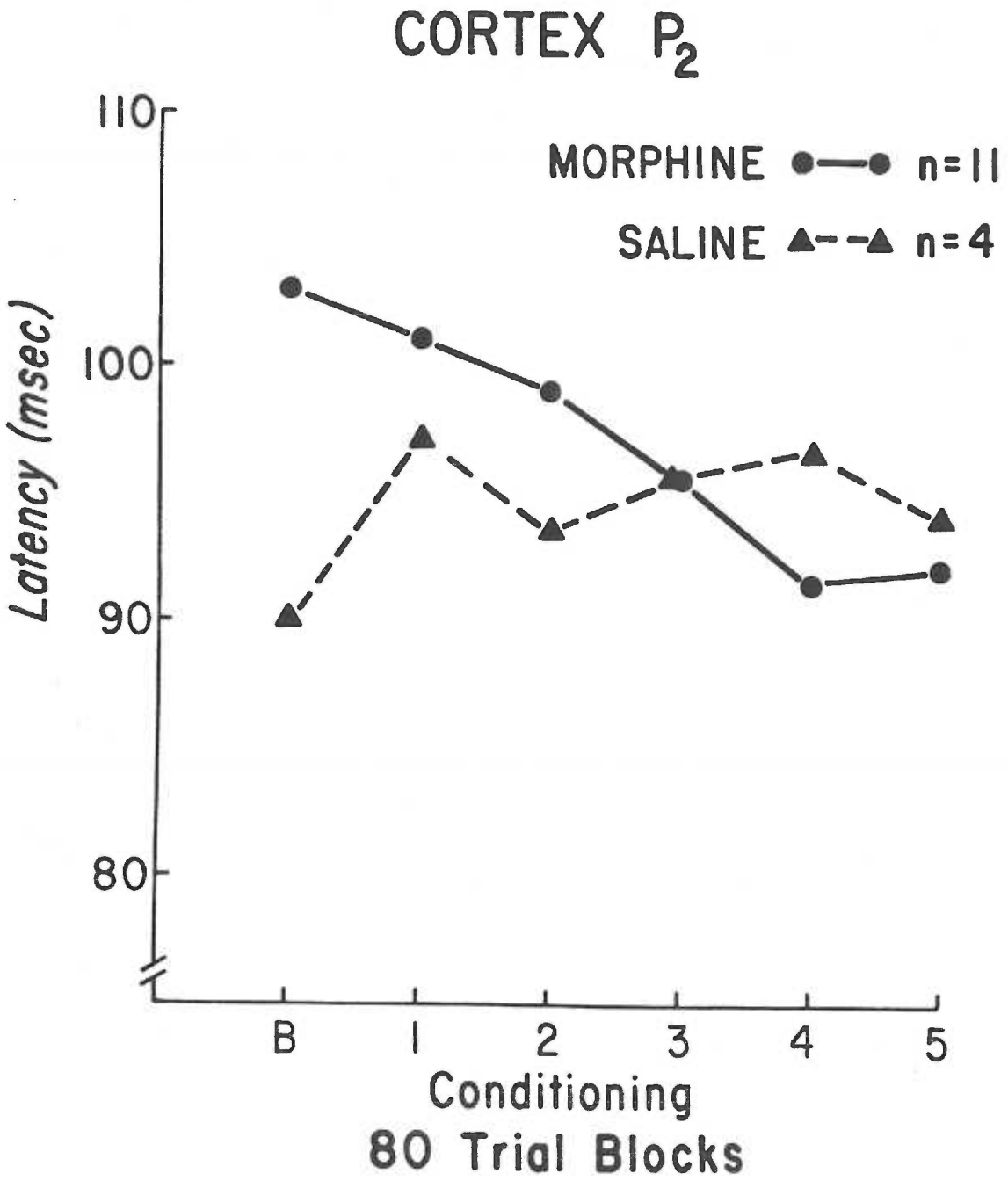
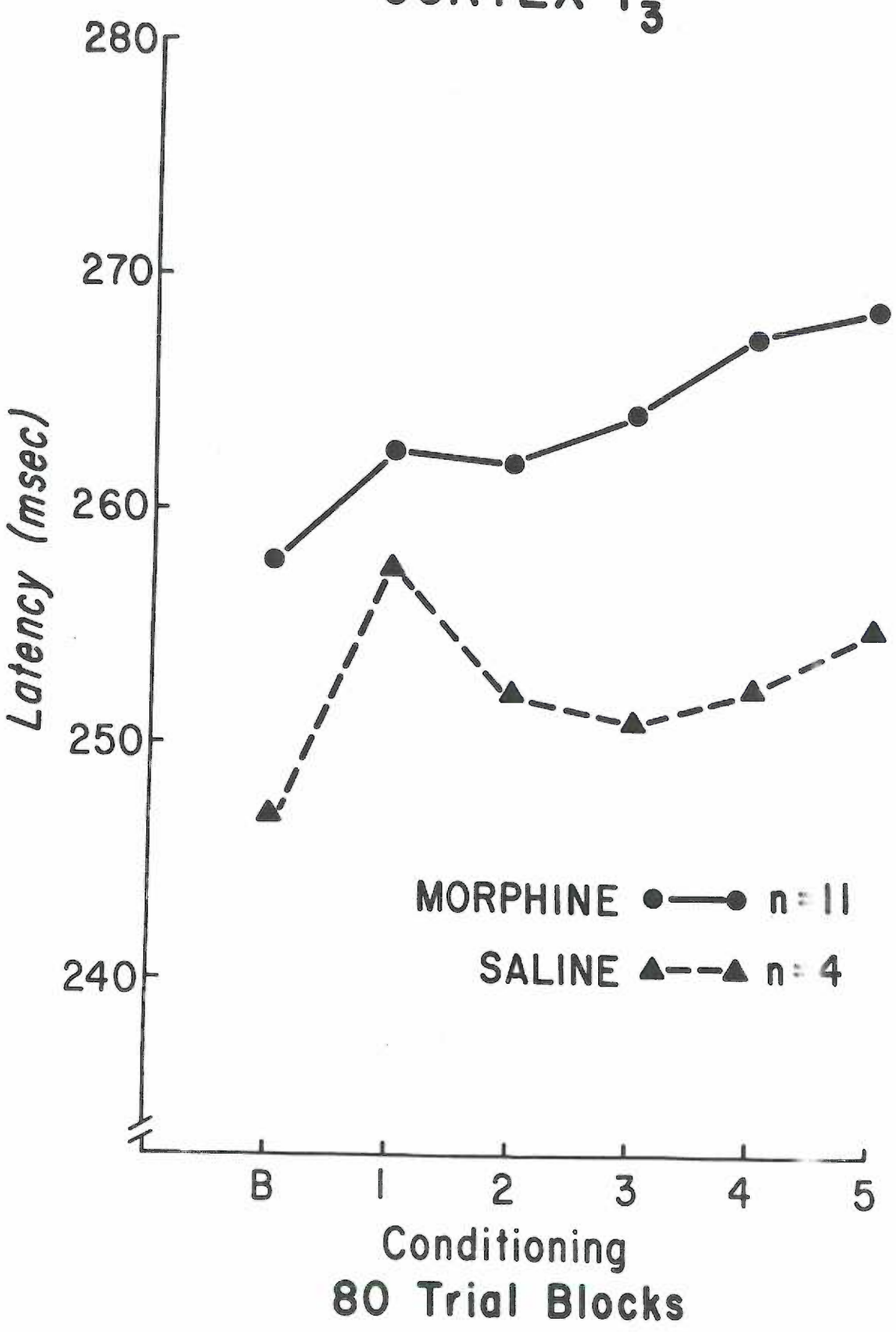

CORTEX P2
MORPHINE n=11
SALINE n=4
Latency (msec)
110
100
90
80
B
1
2
3
4
5
Conditioning
80 Trial Blocks

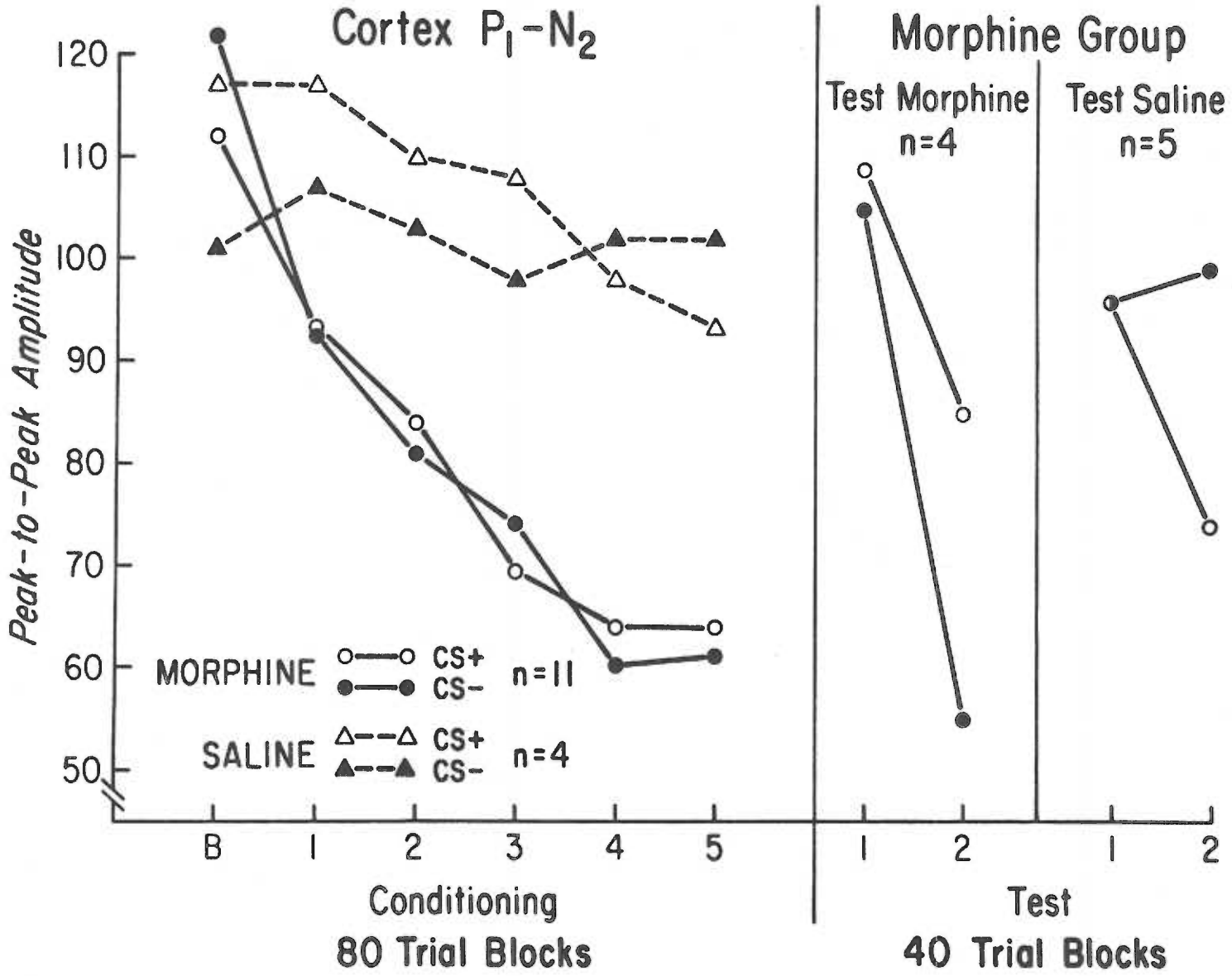
Figure 22: Shift in latency of cortical AEP Peak $P_3$. An 80-trial block was composed of the mean of the latency of the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning.


CORTEX P3
280
270
260
250
240
Latency (msec)
MORPHINE n: 11
SALINE n: 4
B
1
2
3
4
5
Conditioning
80 Trial Blocks

the latency was 253 msec for the saline animals. The latencies gradually increased over conditioning for both the morphine and saline animals. Since the difference in latency of the $P_3$ peak between the morphine and saline animals was constant throughout training, the shift in latency cannot be attributed to any progressive physiological effect produced by the administration of morphine.

$P_1$-$N_2$ amplitude. As can be seen in Figure 23, both the saline and morphine animals exhibited a decrease in amplitude for Component $P_1$-$N_2$ over trial blocks. However, the decrease in amplitude appeared to be much greater in both the CS+ and CS- AEPs from the morphine animals. Statistical analysis of the peak-to-peak amplitude change for Component $P_1$-$N_2$ during conditioning disclosed one significant main effect and one significant two-way interaction. The significant main effect was a change in amplitude over trial blocks, $F(5, 65) = 7.32$, $p < .001$. The interaction between drug condition and trial blocks was also significant, $F(5, 65) = 3.33$, $p < .01$. Therefore, the change in amplitude over trials was greater for the morphine animals than the saline animals, and in fact, a two-way followup for each group indicated the decrease in amplitude to be nonsignificant for the saline animals, $F(5, 15) = 1.52$, $p > .05$, whereas the decrease was significant for morphine animals, $F(5, 50) = 16.52$, $p < .001$. The decrease in amplitude which developed in both the CS+ and CS- AEPs recorded from the morphine animals can be attributed to the increasing dose of morphine over days. No evidence for a conditioned response was found in this component.

Figure 23: Change in peak-to-peak amplitude of component $P_1$- $N_2$ of CS+ and CS- elicited cortical AEPs during baseline, conditioning and testing for the morphine and saline animals. An 80-trial block was composed of the mean of the amplitude measures from the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning. During testing, a 40-trial block was composed of the mean amplitude measure of two 20-trial AEPs. Peak-to-peak amplitude in arbitrary units.


Cortex $P_1-N_2$
Peak-to-Peak Amplitude
120
110
100
90
80
70
60
50
MORPHINE CS+ CS- n=11
SALINE CS+ CS- n=4
B
1
2
3
4
5
Conditioning
80 Trial Blocks
Morphine Group
Test Morphine
n=4
Test Saline
n=5
1
2
1
2
Test
40 Trial Blocks

During testing, the animals conditioned with morphine were divided into two groups. It should be recalled that no test data were obtained from the saline-conditioned animals because of computer malfunctions. Of the morphine-conditioned rats, one group received morphine injections paired with the CS+ during testing; the other received saline. A dependent *t* test was used to compare the amplitude of Component $P_1$-$N_2$ from the baseline session to the same component in the first 20-trial AEP from the testing session for each of the test groups separately. The *t* test conducted on the data from the morphine-tested animals determined that the amplitude of the $P_1$-$N_2$ component from the CS+ and CS- AEPs was not different from the amplitude of the baseline component. The second group of morphine animals received saline injections paired with the CS+ during the testing session. The dependent *t* test comparing the CS+ and CS- component amplitude from the testing session to the baseline amplitude was also non-significant. For these animals, then the amplitude of this component of the CS+ and CS- AEPs returned to baseline levels after withdrawal. This return in amplitude can be seen in Figure 20, where the individual records of Rat F86 are presented.

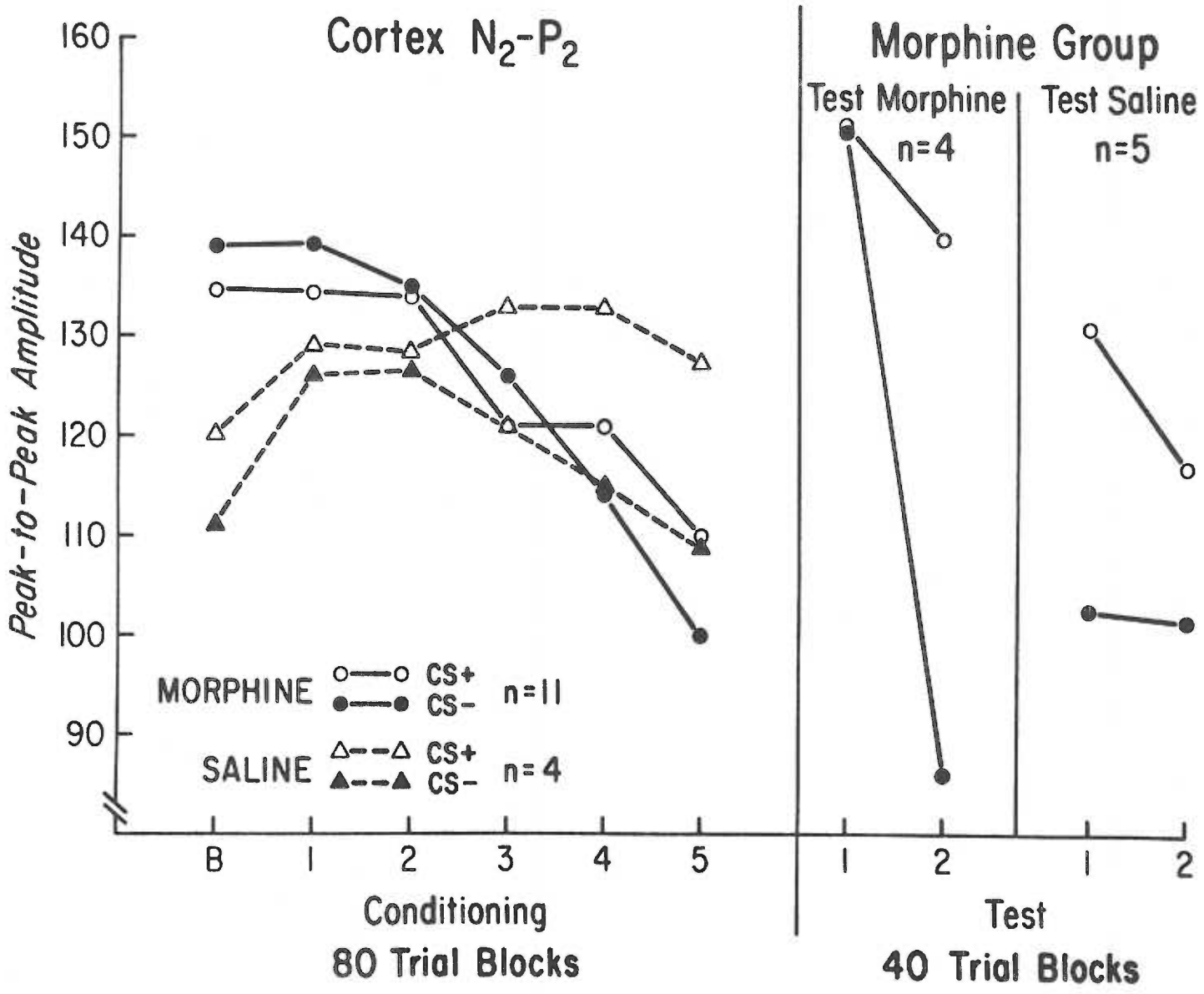
The three-way analysis of variance conducted on the test data produced a single significant outcome: the change in amplitude of Component $P_1$-$N_2$ over trials, $F(1, 7) = 11.85$, $p < .025$. As can be seen in the right hand side of Figure 23, this main effect was mainly due to the decrease in amplitude of Component $P_1$-$N_2$ over testing trials for the animals tested with morphine. This decrease in amplitude, as seen during conditioning, was due to a direct physiological effect of morphine. There appeared to be a difference between the

amplitude of this component in the CS+ and CS- AEPs for the saline-tested animals. The CS+ amplitude decreased over trials, whereas the CS- remained at baseline levels. This effect though was not statistically significant.

In summary, a decrease in amplitude of Component $P_1$-$N_2$ in the CS+ and CS- AEPs recorded from the morphine animals developed over conditioning trials. Since a decrease in amplitude of this component did not develop in the AEPs recorded from the saline animals, this effect was attributed to a direct physiological effect of morphine. There was no evidence for the development of a conditioned response in this cortical AEP component. After withdrawal, the amplitude of this component returned to baseline levels for both the animals tested with morphine and saline.

$N_2$-$P_2$ amplitude. As can be seen on the left hand side of Figure 24, a progressive decrease in amplitude of the $N_2$-$P_2$ component recorded from both the CS+ and CS- AEPs of the morphine animals appeared to develop over trials. The amplitude changes recorded from the saline animals were smaller and less consistent. These observations were confirmed statistically. The significant factors were a main effect of trial blocks, $F(5, 65) = 4.61$, $p < .001$, and the interaction of drug treatment and trial blocks, $F(5, 65) = 3.12$, $p < .025$. The decrease in amplitude of the $N_2$-$P_2$ component recorded from the morphine animals was significantly greater than the amplitude change over trial blocks for the saline animals. In fact, follow-up analyses determined the decrease in amplitude over trial blocks was significant for the morphine animals, $F(5, 50) = 11.20$, $p < .001$, whereas the amplitude

Figure 24: Change in peak-to-peak amplitude of component $N_2$-$P_2$ of CS+ and CS- elicited cortical AEPs during baseline, conditioning and testing for the morphine and saline animals. An 80-trial block was composed of the mean of the amplitude measures from the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning. During testing, a 40-trial block was composed of the mean amplitude measure of two 20-trial AEPs. Peak-to-peak amplitude in arbitrary units.


Cortex $N_2$-$P_2$
Morphine Group
Test Morphine
n=4
Test Saline
n=5
Peak-to-Peak Amplitude
160
150
140
130
120
110
100
90
MORPHINE CS+ CS- n=11
SALINE CS+ CS- n=4
B
1
2
3
4
5
1
2
1
2
Conditioning
80 Trial Blocks
Test
40 Trial Blocks

change was non-significant for the saline animals, $F(5, 15) = 1.18$, $p > .05$. Because of the difference in the change in amplitude in the recordings from the morphine and saline animals, the decreased amplitude can be considered to be a physiological effect of morphine. This decrease in amplitude of the $N_2$-$P_2$ component can also be seen in the individual record of Rat F86 in Figure 20. The effect is most dramatic in the CS- AEPs in the right hand side of the figure.

The test results for both groups of morphine-conditioned animals are shown in the right hand side of Figure 24. For both the animals that received morphine injections or saline injections paired with the CS+, there was no significant difference in the amplitude of Component $N_2$-$P_2$ for the first 20-trial AEP from the testing session as compared to the baseline component amplitude. As can be seen from the figure, the amplitude of the $N_2$-$P_2$ component returned to baseline levels after withdrawal for both of the test groups.

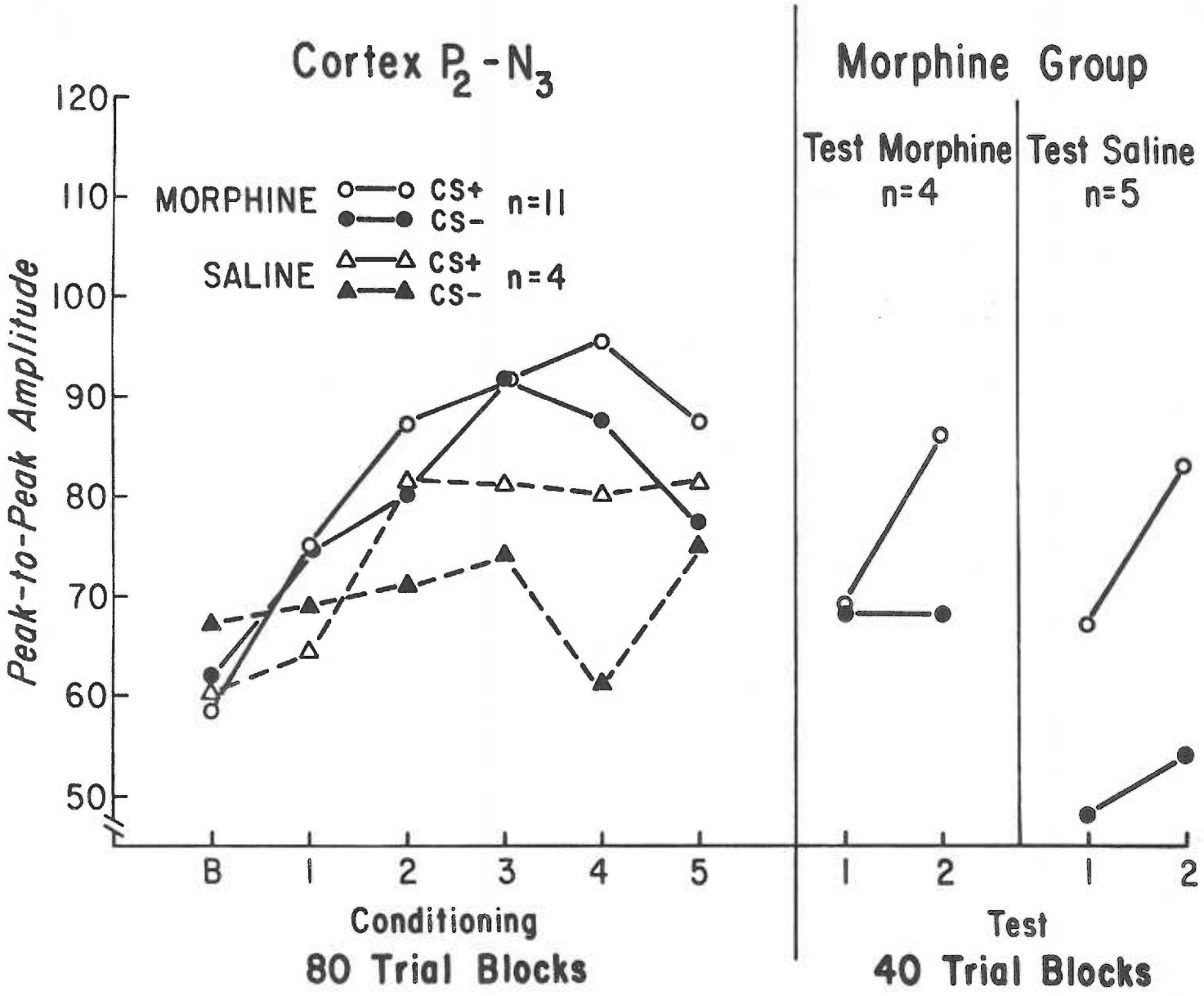
A three-way analysis of variance conducted on the test data from the morphine-conditioned animals revealed a significant main effect of trials, $F(1, 7) = 7.71$, $p < .05$. As can be seen in the right hand side of Figure 24, a decrease in AEP amplitude for Component $N_2$-$P_2$ was present in both sets of animals tested with morphine or saline injections. There appeared to be a difference in the amplitude of the CS+ and CS- AEPs for both groups, but was most apparent for the saline-tested animals. There was no statistical difference between the amplitude changes of the two stimuli.

To summarize the results from the analysis of Component $N_2$-$P_2$, a progressive decrease in amplitude in both the CS+ and CS- AEPs

developed over trial blocks for the morphine animals. This same effect was not seen in the AEPs recorded from the saline animals. Therefore, this effect can be attributed to a direct physiological effect of morphine. There was no evidence for the development of a conditioned response in this component. The amplitude of the $N_2$-$P_2$ component returned to baseline levels after withdrawal.

$P_2$-$N_3$ amplitude. The data for the $P_2$-$N_3$ component are presented graphically in Figure 25 for both the morphine and saline animals. A general increase in amplitude was noted in the AEPs elicited by both the CS+ and CS- AEPs from both groups of animals. There also appears to be some differentiation between the CS+ and CS- component amplitudes for both the saline and morphine animals. One main effect and a two-way interaction were the significant outcomes of the statistical analysis of the conditioning phase data. The main effect of trial blocks confirms the observation that an increase in amplitude occurred during conditioning, $F(5, 65) = 6.96$, $p < .001$. The two-way interaction between CS+ and CS- over trial blocks, $F(5, 65) = 2.64$, $p < .025$, means that the amplitude of this component changed differently over trial blocks for the CS+ and CS- AEPs. Follow-up $t$ tests were conducted according to the method described by Winer (1971, page 544). These $t$ tests determined that there was a significant difference between the CS+ and CS- AEPs for Component $P_2$-$N_3$ on Days 2, 4, and 5 of conditioning, $ts \geq 1.72$, $p < .05$. This difference in the change of Component $P_2$-$N_3$ between the CS+ and CS- AEPs can be attributed to conditioning, but it should be emphasized that this conditioned response developed in the component for both the saline and morphine animals.

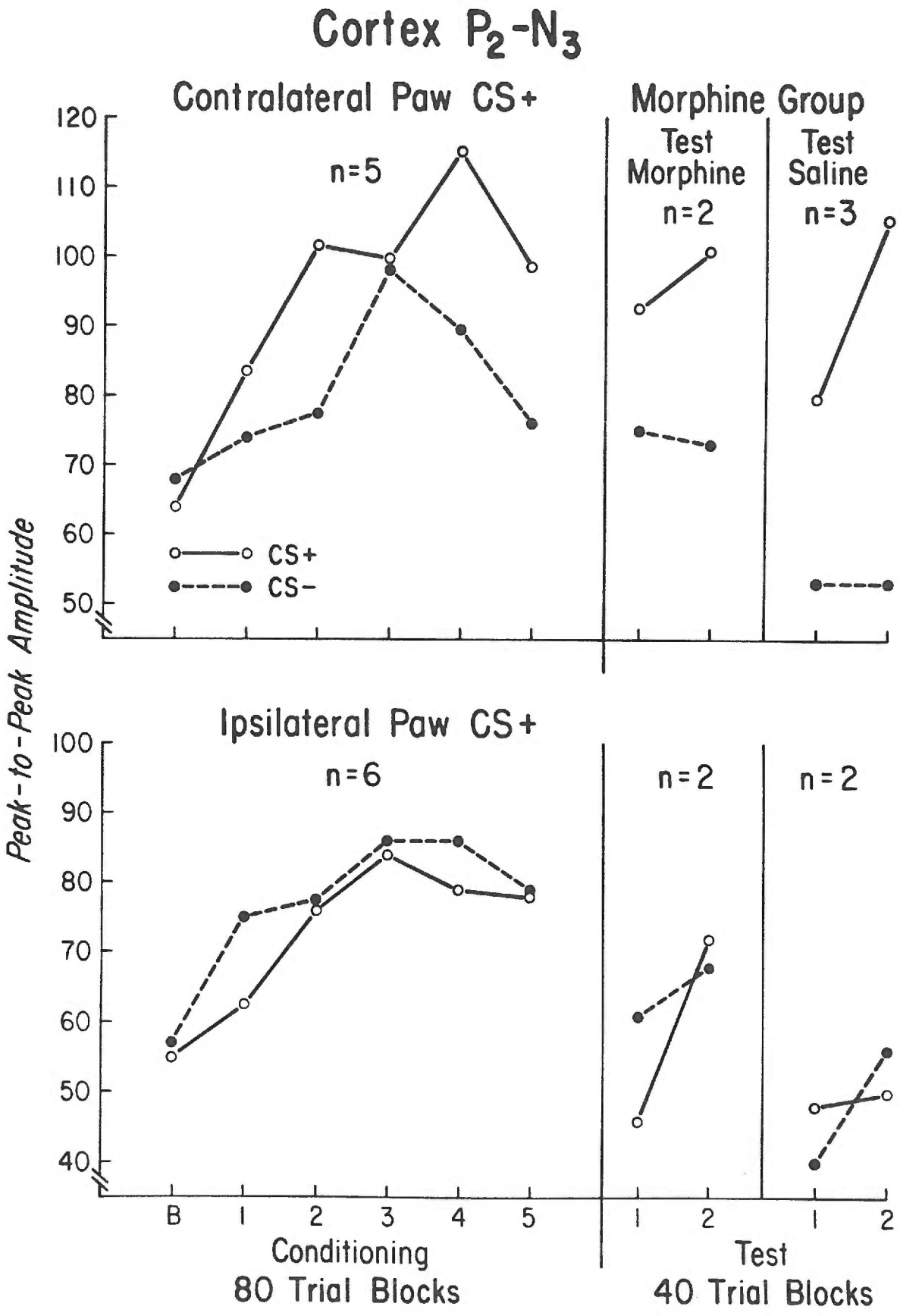
Figure 25: Change in peak-to-peak amplitude of component $P_2$- $N_3$ of CS+ and CS- elicited cortical AEPs during baseline, conditioning and testing for the morphine and saline animals. An 80-trial block was composed of the mean of the amplitude measures from the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning. During testing, a 40-trial block was composed of the mean amplitude measure of two 20-trial AEPs. Peak-to-peak amplitude in arbitrary units.


Cortex $P_2$ - $N_3$
Morphine Group
Test Morphine
n=4
Test Saline
n=5
MORPHINE CS+ n=11
CS-
SALINE CS+ n=4
CS-
Peak-to-Peak Amplitude
120
110
100
90
80
70
60
50
B
1
2
3
4
5
1
2
1
2
Conditioning
80 Trial Blocks
Test
40 Trial Blocks

Due to the anomolous nature of these results, and since the two drug groups had been run as two separate groups, analysis of the data from the morphine and saline treatment groups was conducted separately. A three-way analysis of variance was conducted on the data from the morphine animals alone. The between-groups factor was paw assignment (contralateral versus ipsilateral foreleg stimulation as the CS+). One within measure was the stimulus type (CS+ or CS-) and the other within measure was trial blocks. This analysis revealed a significant main effect, a significant two-way interaction and a reliable three-way interaction. The main effect was trial blocks $F(5, 45) = 11.92$, $p < .001$, confirming the observation that there was an increase in amplitude of the $P_2$- $N_3$ component over trials. However, the counter-balanced factor of paw assignment interacted significantly with CS+ versus CS-, $F(1, 9) = 6.68$, $p < .025$. Also, the triple interaction of paw assignment by CS+ versus CS- by trial blocks was significant $F(5, 45) = 2.87$, $p < .025$. These results mean that the change of amplitude to the CS+ and CS- AEPs during conditioning was different, depending on whether the contralateral paw was stimulated as the CS+, or stimulation of the ipsilateral paw was the CS+. In order to evaluate this interaction, the data from the paw assignment groups were analyzed separately.

Five of the morphine animals received stimulation of the contralateral paw as the CS+ and stimulation of the ipsilateral paw as the CS-. The data from these five animals are presented graphically in the top of Figure 26. As can be seen in the figure, there was a significant increase in amplitude over trial blocks, $F(5, 20) = 6.26$, $P < .01$. Also, the interaction of CS+ versus CS- over trial blocks

Figure 26: Change in peak-to-peak amplitude of component $P_2$- $N_3$ of CS+ and CS- elicited cortical AEPs during baseline, conditioning and testing for the two groups of morphine-conditioned animals. The top graph illustrates the change in amplitude for CS+ and CS- AEPs for the group that received contralateral foreleg stimulation as the CS+ and ipsilateral foreleg stimulation as the CS-. The bottom graph illustrates the change in amplitude for CS+ and CS- AEPs for the group that received ipsilateral foreleg stimulation as the CS+ and contralateral foreleg stimulation as the CS-. An 80-trial block was composed of the mean of the amplitude measures from the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning. During testing, a 40-trail block was composed of the mean amplitude measure of two 20-trial AEPs. Peak-to-peak amplitude in arbitrary units.


Cortex $P_2$-$N_3$
Contralateral Paw CS+
n=5
Morphine Group
Test Morphine
n=2
Test Saline
n=3
CS+
CS-
Ipsilateral Paw CS+
n=6
n=2
n=2
Peak-to-Peak Amplitude
120
110
100
90
80
70
60
50
40
B
1
2
3
4
5
1
2
1
2
Conditioning
80 Trial Blocks
Test
40 Trial Blocks

was significant, $F(5, 20) = 4.51$, $p < .01$. This means that the change in amplitude of the $P_2$-$N_3$ component for the CS+ AEPs was different from the change in amplitude for the CS- AEPs over trial blocks. As can be seen in the figure, the CS- AEP amplitude for $P_2$-$N_3$ increased gradually over days, with a large increase on Day 3. The increase in amplitude to the CS+ AEPs for Component $P_2$-$N_3$ was much larger than the increase in the amplitude seen in the CS- AEPs, with the largest amplitude increase occurring on Day 4. Followup $t$ tests revealed a significant difference between the CS+ and CS- on Days 2, 4, and 5 of conditioning ($ts \geq 1.94$, $p < .05$). Also, a test for linear and quadratic components was conducted on the change in AEP amplitude for the CS+ AEPs over days and CS- AEPs over days. The trend test revealed a significant linear component, $F(1, 20) = 59.4$, $p < .001$, and quadratic component, $F(1, 20) = 105$, $p < .001$ for the CS+ amplitude curve. The CS- AEPs curve exhibited a quadratic component, $F(1, 20) = 5.71$, $p < .05$, but no linear component, $F(1, 20) = 1.7$, $p > .05$. This difference in the existence of the linear component between the CS+ and CS- AEP amplitude curves for the $P_2$-$N_3$ component is further evidence for the differentiation between the CS+ and CS- AEP amplitudes over trial blocks.

In contrast, as can be seen in the bottom graph of Figure 26, the six morphine animals that received ipsilateral stimulation as the CS+ and stimulation of the contralateral paw as the CS- did not demonstrate any differentiation between the increase in amplitude of component $P_2$- $N_3$ between the CS+ and CS- AEPs. Statistically, the only significant effect was a main effect of trial blocks, $F(5, 25) = 5.72$, $p < .001$.

None of the statistical outcomes of the three-way analysis of variance conducted on the data from the four saline animals were significant. As was noted earlier and can be seen in Figure 25, there appeared to be some differentiation between the CS+ and CS- AEP amplitudes, but because of the small number of subjects within the saline group, the statistical results only approached significance, $F(5, 15) = 2.60$, $p < .07$.

As can be seen on the right hand side of the graphs in Figure 26, it appeared that the conditioned difference between the CS+ and CS- was still present at the start of testing in the morphine-conditioned animals that received contralateral paw stimulation as the CS+. The amplitude of the CS- AEPs were back to baseline levels for both the animals tested with morphine and saline, whereas the amplitude of the $P_2$- $N_3$ component of the CS+ AEPs appeared to be at the same increased amplitude level as the last day of conditioning. The dependent $t$ tests did not confirm this observation statistically, probably due to the small number of subjects per group (n = 2 or 3). However, since the CS+ amplitude appeared to be larger than the CS- amplitude for both the saline-tested and morphine-tested animals, a dependent $t$ test was conducted between CS+ and CS- for the two groups combined for the data from the first 20-trial AEP from the testing session. This difference between the CS+ and CS- component amplitude was significantly different, $t(4) = 2.20$, $p < .05$. It appears, then, that the conditioned difference was still present for the contralateral-paw group after withdrawal when tested with either saline or morphine. In contrast, this difference between the CS+ and CS- amplitudes was not apparent

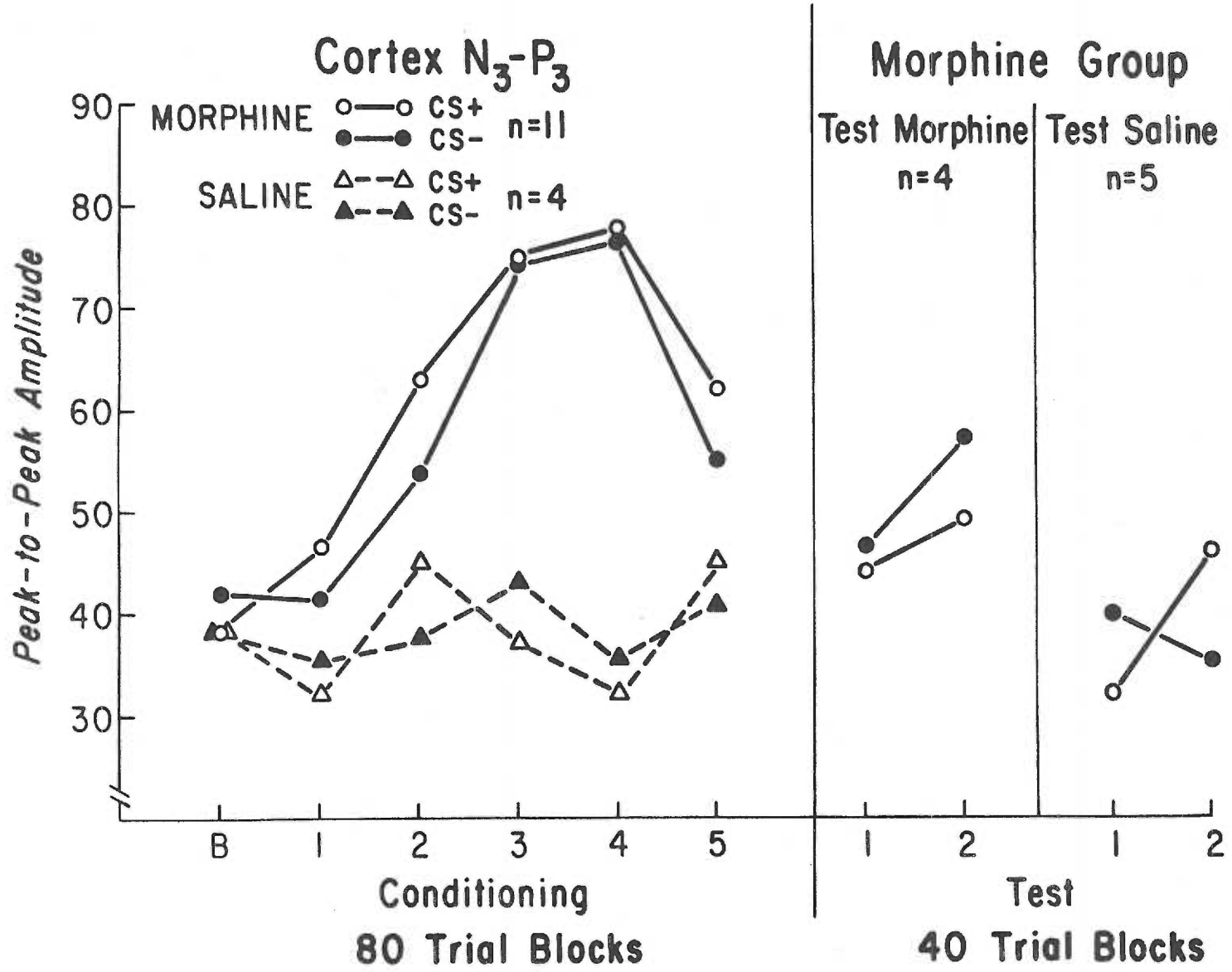
in the data for the ipsilateral paw stimulation group that received either morphine or saline during testing. The dependent *t* tests revealed no significant differences for the ipsilateral paw group. There were no significant results from the three-way analysis of variance on the test data, probably due to the small number of subjects in each test group.

In summary, the conditioned response which developed in the $P_2$- $N_3$ component over trials was present in the AEPs from both the saline and morphine animals. Separate analysis of the data from the saline and morphine groups revealed that the conditioned response developed predominantly in those animals that received contralateral paw stimulation as the CS+ paired with morphine injections. This conditioned response persisted after withdrawal for the animals tested with either morphine or saline injections paired with the contralateral paw CS+.

The differential change in the amplitude of this component can be seen in the individual AEP record of Rat F86 presented in Figure 20. This animal received contralateral paw stimulation as the CS+. Notice the increase in amplitude of Component $P_2$- $N_3$ in the CS+ AEPs throughout conditioning, and that this conditioned response appears in the first 20-trial AEP recorded during testing.

$N_3$- $P_3$ amplitude. As can be seen in the left of Figure 27, an increase in amplitude of Component $N_3$- $P_3$ appeared to develop only in the CS+ and CS- elicited AEPs recorded from the morphine animals. This observation was confirmed statistically. There was a significant main effect during the conditioning phase of drug treatment, $F(1, 13) = 5.50$, $p < .05$. In addition, the main effect of trial blocks

Figure 27: Change in peak-to-peak amplitude of component $N_3$- $P_3$ of CS+ and CS- elicited cortical AEPs during baseline, conditioning and testing for the morphine and saline animals. An 80-trial block was composed of the mean of the amplitude measures from the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning. During testing, a 40-trial block was composed of the mean amplitude measure of two 20-trial AEPs. Peak-to-peak amplitude in arbitrary units.


Cortex $N_3$-$P_3$
MORPHINE CS+ CS- n=11
SALINE CS+ CS- n=4
Peak-to-Peak Amplitude
90
80
70
60
50
40
30
B
1
2
3
4
5
Conditioning
80 Trial Blocks
Morphine Group
Test Morphine
n=4
Test Saline
n=5
1
2
1
2
Test
40 Trial Blocks

was significant, confirming the observation that the amplitude of the AEPs increased over trials, $F(5, 65) = 8.01$, $p < .001$. Also, the two-way interaction of drug treatment by trial blocks was significant, $F(5, 65) = 7.76$, $p < .001$. This interaction means that there was a larger increase in amplitude of the $N_3$-$P_3$ component recorded from the morphine animals than from the saline animals. Since the increase in amplitude of this component was present only for the morphine group, $F(5, 50) = 25.37$, $p < .001$, and since there was no difference between CS+ and CS- for these data, this effect can be attributed to a physiological effect of morphine.

The test results are presented graphically in the right-hand side of Figure 27. The dependent $t$ tests comparing the first 20-trial AEP from testing with the baseline amplitude were non-significant for both the animals tested with morphine and those tested with saline injections. None of the results from the analysis of variance conducted on the test data were significant. Thus, as Figure 27 illustrates, the amplitude of Component $N_3$-$P_3$ returned to baseline levels after withdrawal.

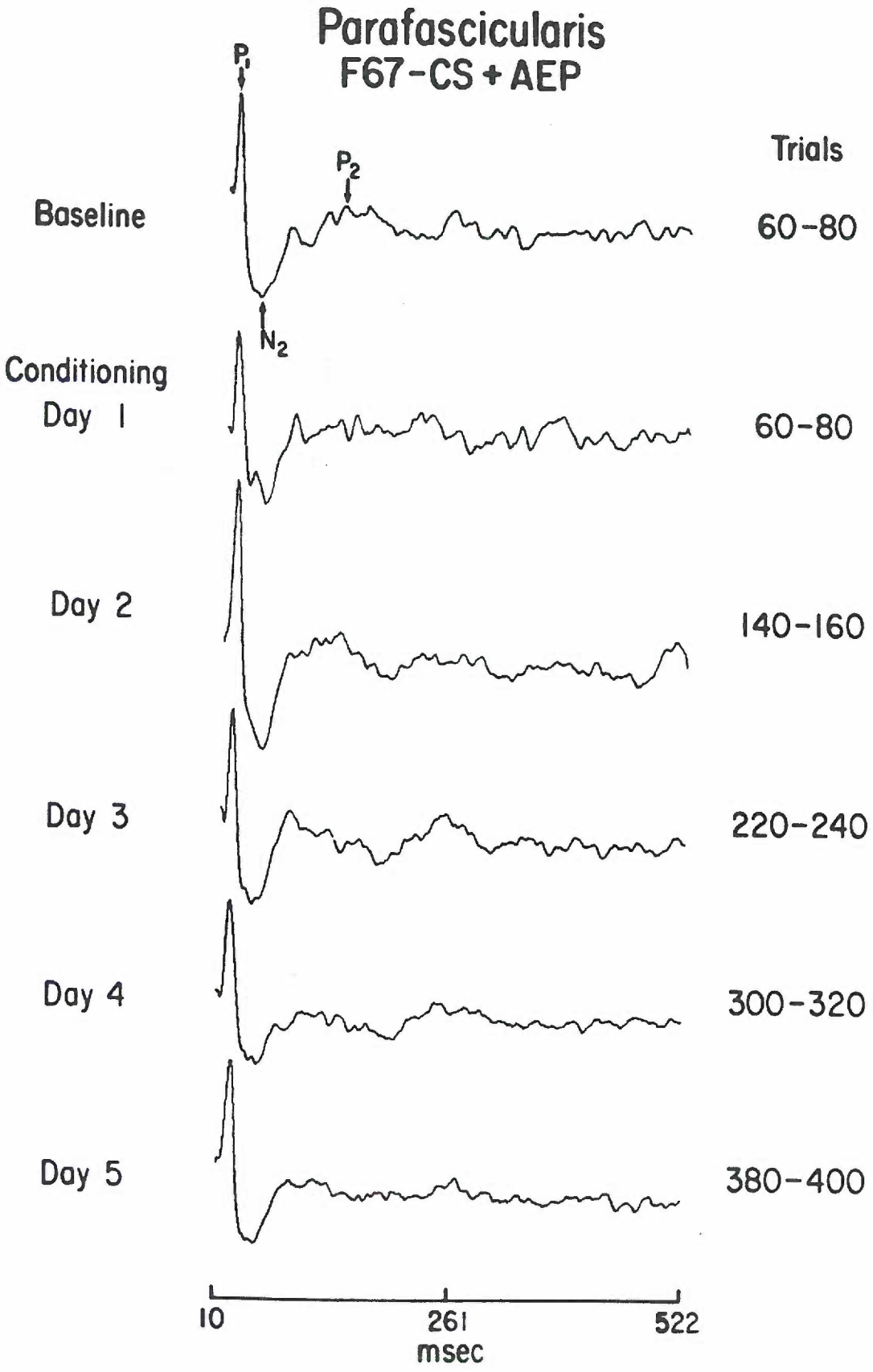
In summary, a progressive increase in amplitude of Component $N_3$-$P_3$ was recorded in the AEPs elicited by both the CS+ and CS- over trials. Since the saline animals did not exhibit the same progressive increase in amplitude of this component, and there was no difference between the CS+ and CS-, this effect can be attributed to the administration of increasing doses of morphine. This physiological effect did not persist following withdrawal in either of the test groups.

Cortex: Summary. A change in AEP component amplitude due only to an effect of morphine was recorded in three components. The two short-latency components decreased in amplitude, whereas the $N_3$-$P_3$ component increased in amplitude. These responses to morphine did not persist following withdrawal. Only one AEP component developed a difference in the change in amplitude recorded in response to CS+ versus CS- stimulation. This conditioned response was present in the $P_3$-$N_3$ component when the data were collapsed across drug treatment groups. However, follow-up analyses determined that the conditioned response appeared the strongest in those animals trained with contralateral paw stimulation as the CS+ paired with morphine administration. The conditioned differentiation between the CS+ and CS- persisted after withdrawal for the animals trained with contralateral paw stimulation as the CS+.

## Parafascicular Nucleus of the Thalamus

Based on the histological results, AEP records from 12 of the 15 subjects were used for statistical analyses. Nine of the subjects were morphine-conditioned animals, and three received saline injections paired with CS+ presentation during conditioning. Representative 20-trial CS+ AEPs recorded from PFT for Subject F67 for the baseline period and the 5 days of conditioning are presented in Figure 28. The peaks are labelled on the 20-trial AEP taken from the baseline session. No obvious changes in the amplitude of these components are apparent in the individual 20-trial AEPs recorded from Subject F67, and no new AEP components developed during conditioning.

Figure 28: Inidividual 20-trial CS+ AEPs recorded from parafascicularis nucleus of the thalamus of Rat F67 during the last 20-trials of the baseline day and each of the five days of conditioning.

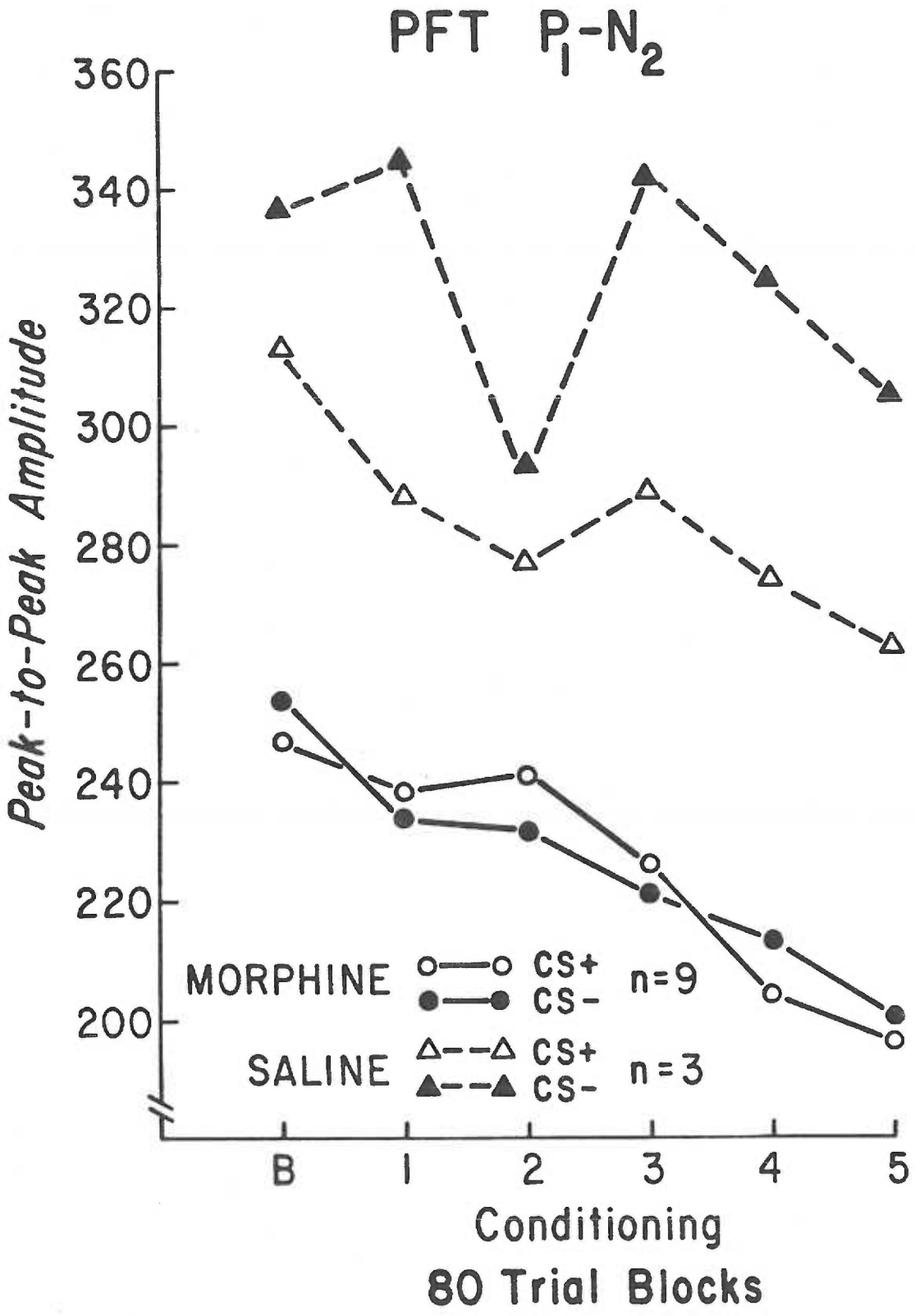

Parafascicularis
F67-CS + AEP
P1
P2
N2
Trials
Baseline
60-80
Conditioning
Day 1
60-80
Day 2
140-160
Day 3
220-240
Day 4
300-320
Day 5
380-400
10
261
522
msec

The average latencies of the AEPs peaks for all the animals were: $P_1$ - 26 msec, $N_2$ - 57 msec, and $P_2$ -109 msec.

$P_1$-$N_2$ amplitude. As can be seen from the data for this PFT component presented graphically in Figure 29, there appeared to be a level difference in amplitude between the saline and morphine animals on the baseline day which remained essentially constant over trial blocks. Also, a decrease in amplitude of the $P_1$-$N_2$ component for both the CS+ and CS- AEPs recorded from both the saline and morphine animals over trial blocks was evident. There did not appear to be any difference in the change in amplitude of this component between the two drug treatment groups. These observations were confirmed statistically. The statistical analysis of the $P_1$-$N_2$ component data from the conditioning phase resulted in two significant main effects. There was a reliable difference between the drug treatment groups, $F(1, 10) = 4.98$, $p < .05$, and a significant main effect of trial blocks, $F(5, 50) = 5.87$, $p < .001$. There was not a significant interaction of drug treatment groups and trial blocks. Since the decrease in amplitude for Component $P_1$-$N_2$ was the same for both saline and morphine animals, it cannot be due to a physiological effect of morphine. Finally, since the CS+/CS- factor was nonsignificant both as a main effect and in any interactions with the other factors, this outcome cannot be attributed to conditioning. Because the effect was attributed to other factors than conditioning or a morphine effect, the test data were not analyzed.

$N_2$-$P_2$ amplitude. Figure 30 contains a graphic representation of the data from PFT component $N_2$-$P_2$. The amplitude of $N_2$-$P_2$ was

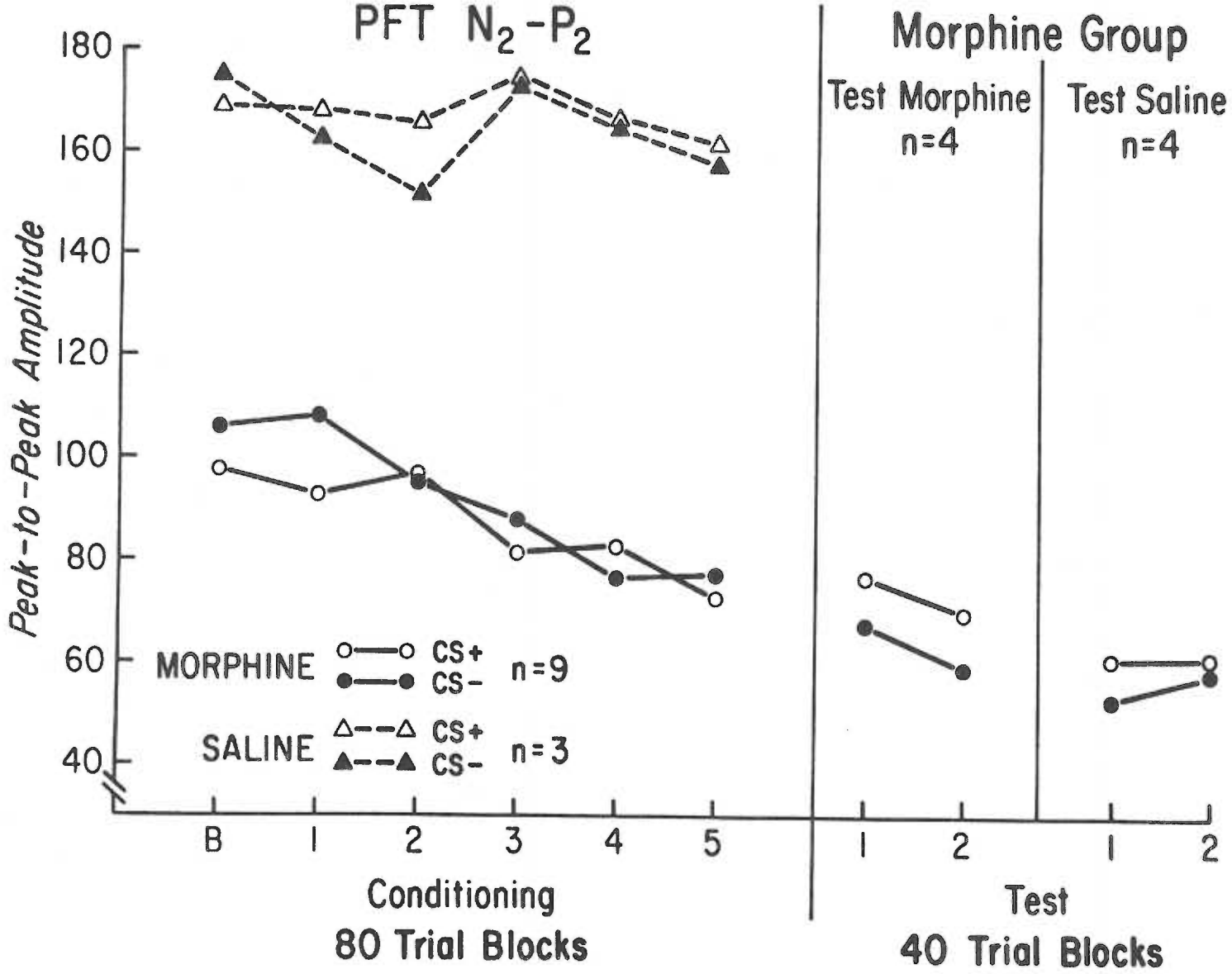
Figure 29: Change in peak-to-peak amplitude of component $P_1$- $N_2$ of CS+ and CS- elicited PFT AEPs during baseline and conditioning for the morphine and saline animals. An 80-trial block was composed of the mean of the amplitude measures from the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning. Peak-to-peak amplitude in arbitrary units.


PFT P1-N2
360
340
320
300
280
260
240
220
200
Peak-to-Peak Amplitude
MORPHINE
CS+
CS-
n=9
SALINE
CS+
CS-
n=3
B
1
2
3
4
5
Conditioning
80 Trial Blocks

greater in the AEP recordings taken from the saline animals than the morphine animals. This level effect was apparent during baseline as well as conditioning. Since the difference was apparent during baseline, this level effect may be attributed to non-associative factors such as the amplifier settings and resistance of the recording and stimulating electrodes. There also appears to be a progressive decrease in amplitude of this component in the AEPs from both the morphine and saline animals. The decrease in amplitude for the morphine animals appears to be greater than the decrease recorded from the saline animals. These observations were confirmed statistically. Two main effects and a two-way interaction were significant from the statistical analysis of the $N_2$-$P_2$ amplitude data from the conditioning phase. The main effects were a difference between the drug treatment groups, $F(1, 10) = 5.73$, $p < .05$, and trial blocks, $F(5, 50) = 5.09$, $p < .01$. The significant interaction was of drug treatment and trial blocks, $F(5, 50) = 3.77$, $p < .01$. The interaction reflects the change during conditioning for the morphine animals, $F(5, 40) = 12.82$, $p < .001$, while the amplitude of this component recorded from the saline animals fluctuated around baseline values, $F(5, 10) = 1.88$, $p > .05$. Even though a level difference existed between the drug treatment groups, the change in the $N_2$-$P_2$ component for morphine rats reflects a progressive impact of the drug which was not found in the AEPs of the saline rats.

The testing results from the morphine animals are shown in the right hand side of Figure 30. Even though it appears that the decreased amplitude of Component $N_2$-$P_2$ was still present after withdrawal

Figure 30: Change in peak-to-peak amplitude of component $N_2$- $P_2$ of CS+ and CS- elicited PFT AEPs during baseline, conditioning and testing for the morphine and saline animals. An 80-trial block was composed of the mean of the amplitude measures from the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning. During testing, a 40-trial block was composed of the mean amplitude measure of two 20-trial AEPs. Peak-to-peak amplitude in arbitrary units.


PFT $N_2-P_2$
Morphine Group
Test Morphine
n=4
Test Saline
n=4
Peak-to-Peak Amplitude
180
160
140
120
100
80
60
40
MORPHINE CS+ CS- n=9
SALINE CS+ CS- n=3
B
1
2
3
4
5
1
2
1
2
Conditioning
80 Trial Blocks
Test
40 Trial Blocks

for both test groups, the results of the dependent t tests comparing the amplitude of the test AEP with the baseline AEP were non-significant. Excessive variability in the test data presumably underlies these outcomes. There were no significant results from the three-way analysis of variance on the test data.

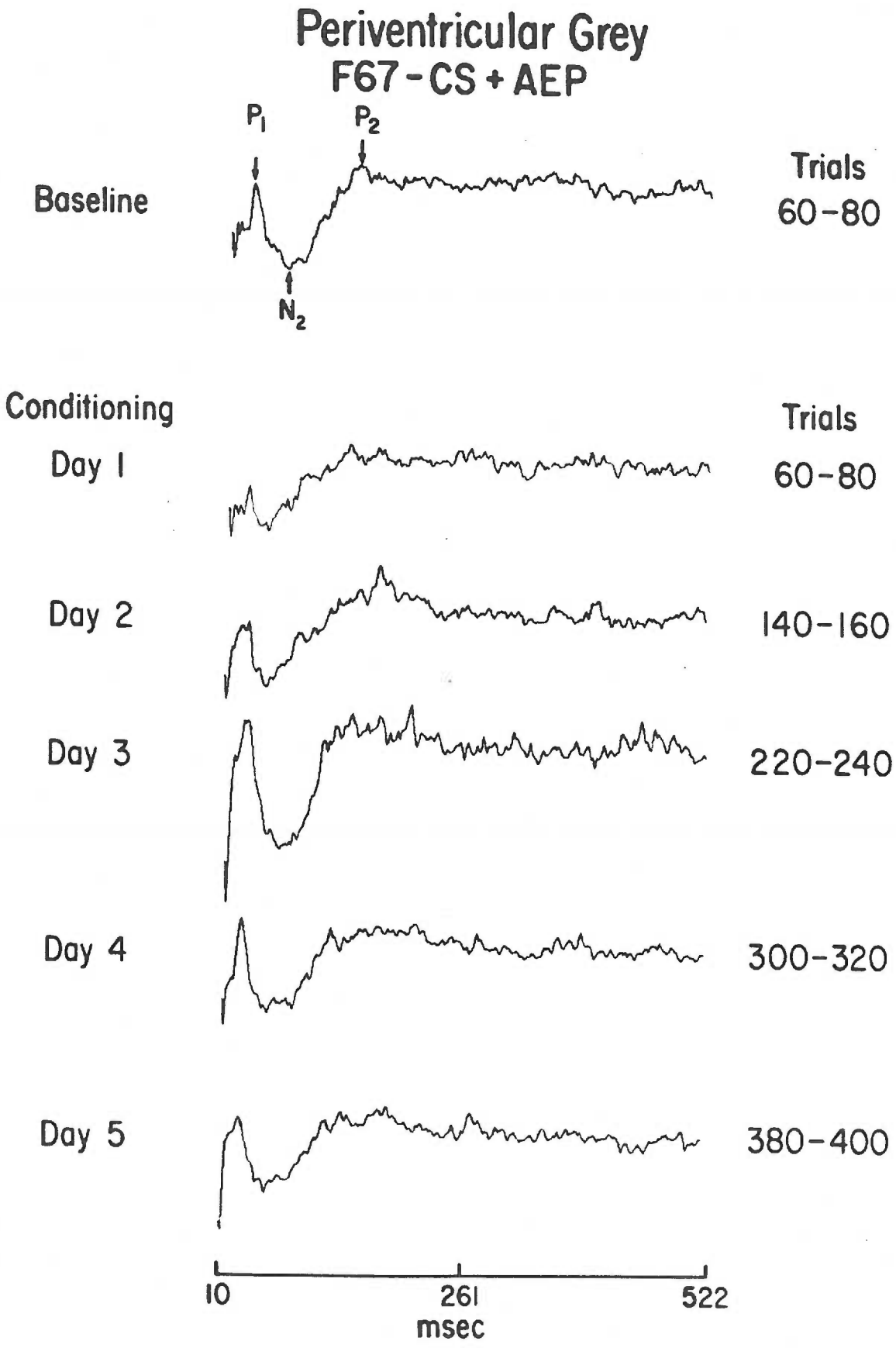
In summary, a significant change in amplitude due to conditioning or morphine administration occurred in only one component of the AEPs recorded from PFT. Component $N_2$-$P_2$ of both the CS+ and CS- AEPs demonstrated a progressive decrease in amplitude which was greater than the change in amplitude recorded from the saline animals. There was no evidence for the development of a conditioned response in this component. After withdrawal, the amplitude returned to baseline levels.

## Periventricular Gray

Data from the nine animals that had electrodes in the PVG were used for statistical analysis. Six of the animals were from the morphine group and three were from the saline group. Figure 31 presents a representative record of AEPs from the last 20 trials of the baseline day and from each day of conditioning for Subject F67. The prominent peaks are labelled for the baseline AEP. The average latency of the $P_1$ peak for all animals was 30 msec, the average latency of the $N_1$ peak was 51 msec, and the average latency of the $P_2$ peak was 134 msec.

$N_2$-$P_2$ amplitude. From the PVG data, only the $N_2$-$P_2$ component demonstrated a reliable amplitude change over trials. These data are presented graphically in Figure 32. There appears to be a large difference in the $N_2$-$P_2$ amplitude in the AEPs recorded from the morphine

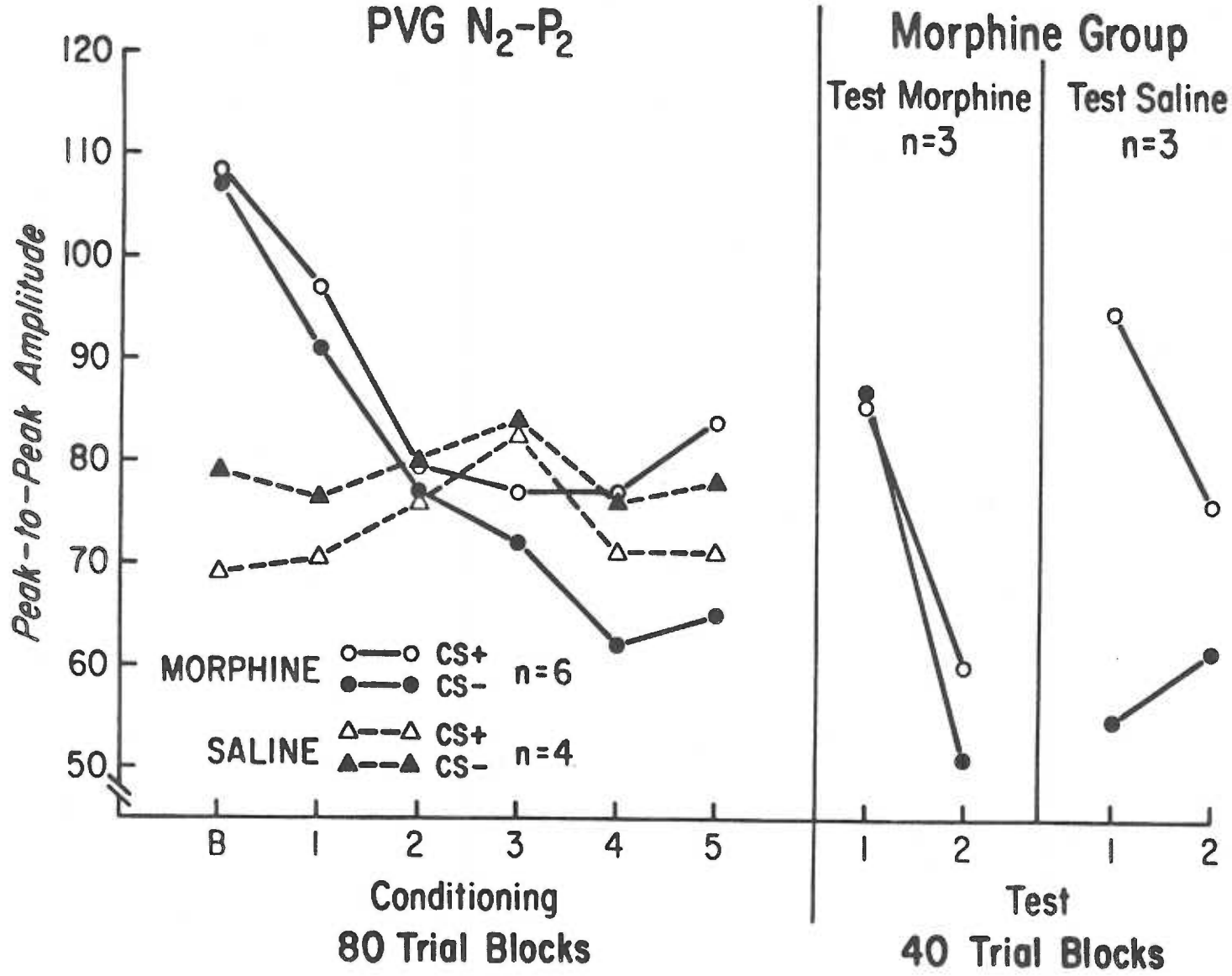
Figure 31: Individual 20-trial CS+ AEPs recorded from the peri-ventricular gray of Rat F67, during the last 20 trials of baseline, and each of the five days of conditioning.


Periventricular Grey
F67 - CS + AEP
P1
P2
N2
Baseline
Trials
60-80
Conditioning
Trials
Day 1
60-80
Day 2
140-160
Day 3
220-240
Day 4
300-320
Day 5
380-400
10
261
522
msec

and saline animals. Since this difference was apparent at the onset of conditioning, it is mainly due to nonassociative factors such as amplifier settings and electrode resistance. The most apparent feature of this data was the larger decrease in amplitude in the CS+ and CS- AEPs of the morphine animals as compared to the saline animals. In fact, statistical analysis of this data revealed no main effect of drug treatment groups. The only significant effect in these data was an interaction between drug treatments and trial blocks, $\underline{F}(5, 40) = 2.88$ $\underline{p} < .025$. As can be seen in Figure 32, this component from the morphine animals demonstrated a consistent decrease in amplitude over trial blocks, $\underline{F}(5, 25) = 3.79$, $\underline{p} < .025$, whereas for the saline animals the values fluctuated near baseline, $\underline{F}(5, 15) = 1.41$, $\underline{p} > .05$. Since the decrease in amplitude for this component occurred only in the AEPs recorded from morphine animals, this effect must have been produced by the drug.

As seen in Figure 32 for the saline-tested animals, it appeared as if the amplitude of the $N_2$-$P_2$ component of the CS- elicited AEPs from the saline animals had remained at the decreased amplitude that had developed during conditioning. However, comparison of the test data with baseline data for this component disclosed that the amplitudes did not differ from baseline for the animals tested with saline injections or morphine injections. Also, even though it appeared as if there was a difference between the CS+ and CS- component amplitudes recorded from the saline-tested animals, the three-way analysis of variance conducted on the test data produced no significant results.

Figure 32: Change in peak-to-peak amplitude of component $N_2$- $P_2$ of CS+ and CS- elicited PVG AEPs during baseline, conditioning and testing for the morphine and saline animals. An 80-trial block was composed of the mean of the amplitude measures from the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning. During testing, a 40-trial block was composed of the mean amplitude measure of two 20-trial AEPs. Peak-to-peak amplitude in arbitrary units.


PVG $N_2$-$P_2$
Morphine Group
Test Morphine
n=3
Test Saline
n=3
120
110
100
90
80
70
60
50
Peak-to-Peak Amplitude
MORPHINE CS+ CS- n=6
SALINE CS+ CS- n=4
B
1
2
3
4
5
1
2
1
2
Conditioning
80 Trial Blocks
Test
40 Trial Blocks

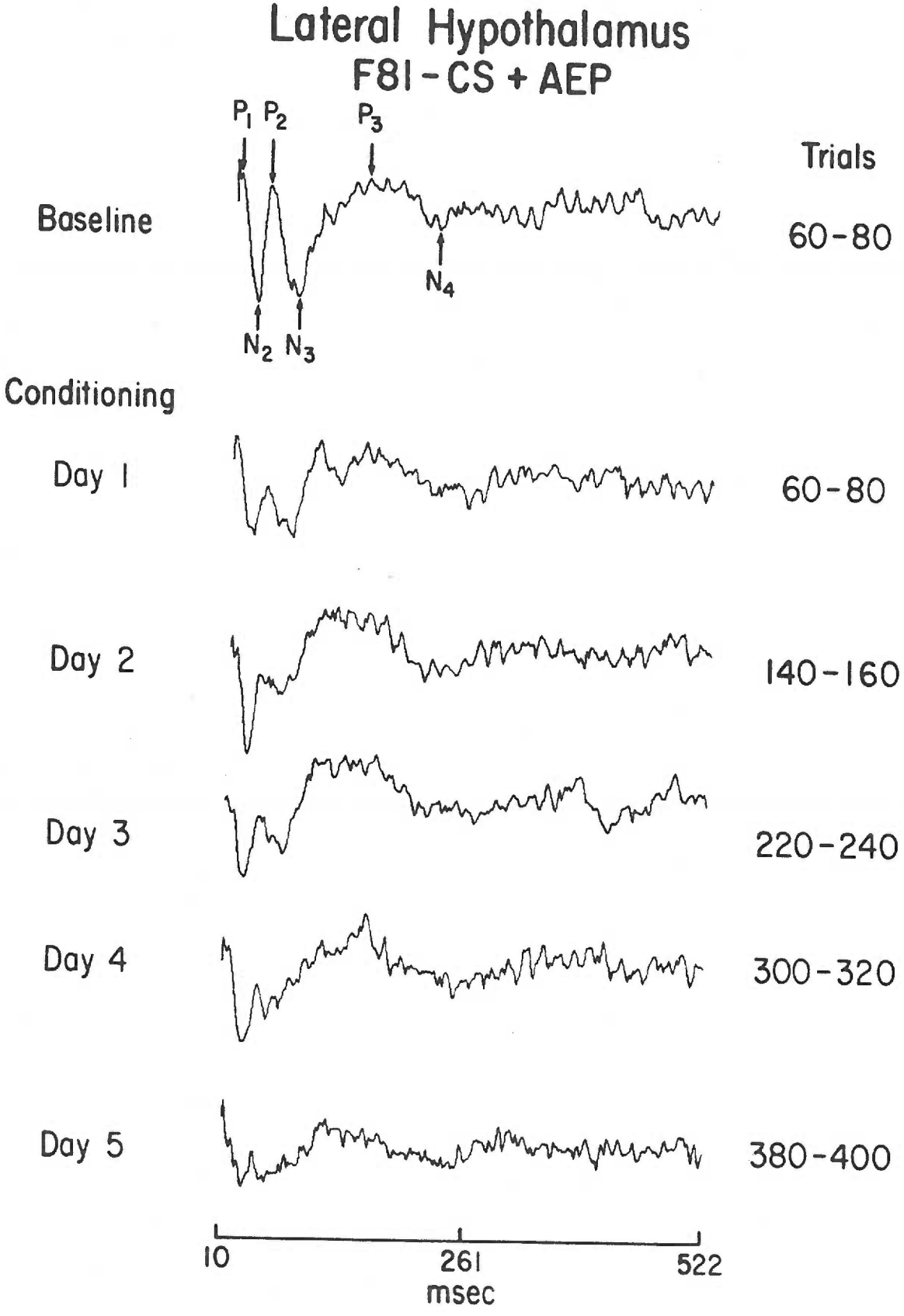
In summary, only one component of the AEPs recorded for PVG demonstrated any reliable change in amplitude over conditioning trials. The $N_2$-$P_2$ component recorded from the morphine animals decreased in amplitude in both CS+ and CS- AEPs, whereas the saline animals demonstrated no change in amplitude of this component. There was no evidence for the development of a conditioned response. The amplitude of this component returned to baseline levels after withdrawal.

## Lateral Hypothalamus

The AEP data from the five animals that had electrodes in LH were analyzed. The hypothalamic AEPs had a characteristic shape as shown in the individual record for Rat F81 in Figure 33. Six identifiable peaks were present with the following average latencies for all animals: $P_1$ - 18 msec, $N_2$ - 34 msec, $P_2$ - 45 msec, $N_3$ - 77 msec, $P_3$ - 165 msec, and $N_4$ - 238 msec. Only two peak-to-peak components demonstrated any significant changes in amplitude over conditioning trials. It should be noted, however, that there were only three animals in the morphine group and two animals in the saline group.

$P_2$-$N_3$ amplitude. The results for Component $P_2$-$N_3$ were limited to a significant change in amplitude over trial blocks, $F(5, 15) = 28.44$, $p < .001$. These results are illustrated in Figure 34. The change in amplitude recorded from the morphine and saline animals did not differ. Since the amplitude decrease occurred in the AEPs from both the saline and morphine animals, this decrease over conditioning trial blocks must not have been specific to morphine. Since the change in amplitude for this component was not due to morphine or conditioning effects, the test results were not analyzed.

Figure 33: Individual 20-trial CS+ AEPs recorded from the lateral hypothalamus of Rat F86 during baseline and each of the five days of conditioning.


Lateral Hypothalamus
F81-CS + AEP
P1 P2 P3
N2 N3 N4
Trials
Baseline
60-80
Conditioning
Day 1
60-80
Day 2
140-160
Day 3
220-240
Day 4
300-320
Day 5
380-400
10
261
522
msec

$N_3$-$P_3$ amplitude. The data for this LH component are presented graphically in Figure 35. The only significant outcome from the analysis of the $N_3$-$P_3$ component was a main effect of trial blocks, $F(5, 15) = 4.71$, $p < .01$. From inspection of Figure 35, it can be seen that primary responsibility for the trial blocks effect may lie with the change in AEP amplitude recorded from the morphine animals. However, the variability of the data and small number of subjects limits the certainty with which such statements can be made. Since the results indicated that the change in amplitude recorded in this LH component was not due to morphine effects or conditioning, the test data were not presented.

In summary, the amplitude changes that were recorded in the AEPs from LH of the morphine animals, were also recorded in the AEPs from the saline animals. Therefore, these changes were concluded to be a result of other factors than a physiological effect of morphine or conditioning.

Figure 34: Change in peak-to-peak amplitude of component $P_2$- $N_3$ of CS+ and CS- elicited LH AEPs during baseline and conditioning for the morphine and saline animals. An 80-trial block was composed of the mean of the amplitude measures from the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning. Peak-to-peak amplitude in arbitrary units.

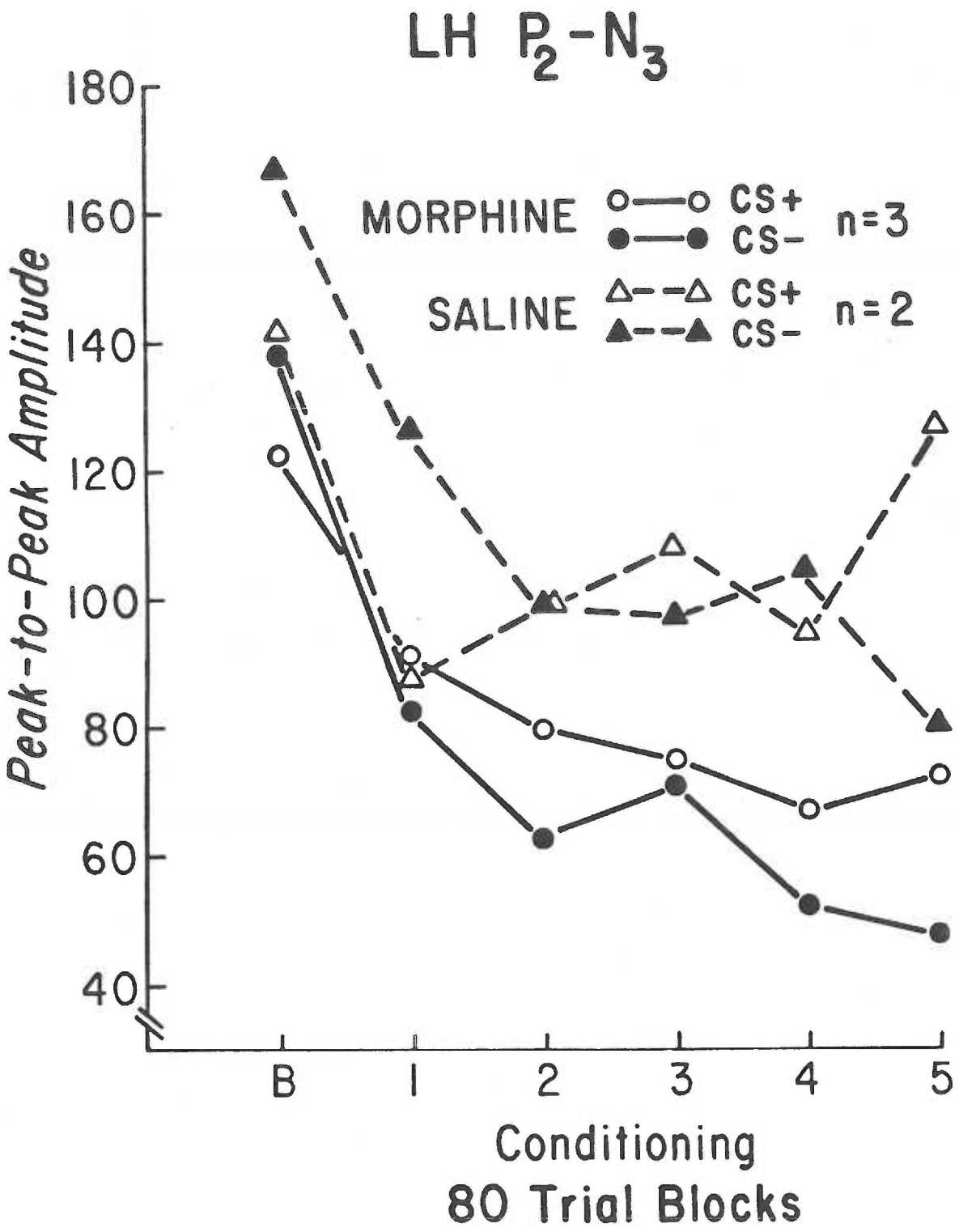
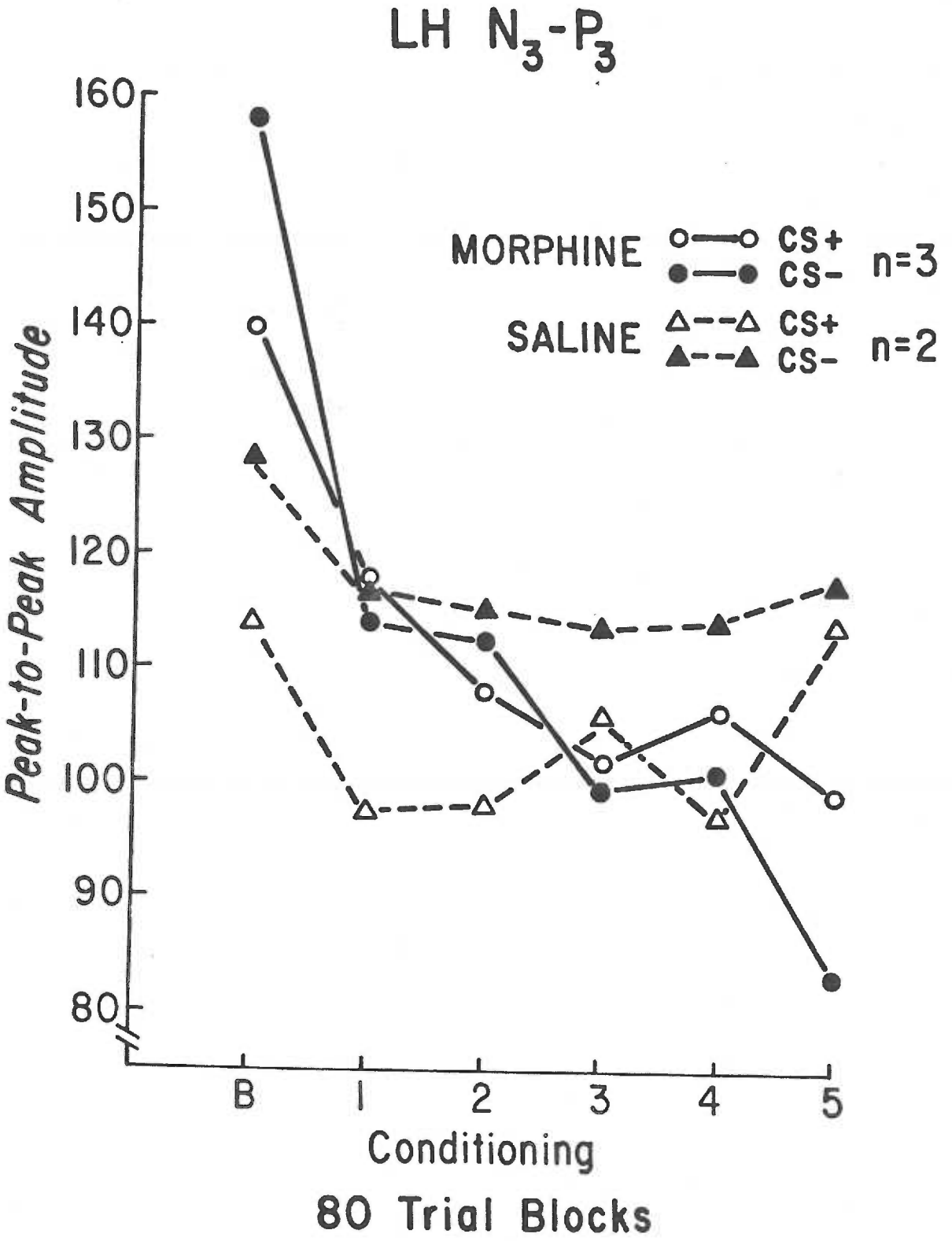

LH $P_2$-$N_3$
MORPHINE CS+ CS- n=3
SALINE CS+ CS- n=2
Peak-to-Peak Amplitude
180
160
140
120
100
80
60
40
B
1
2
3
4
5
Conditioning
80 Trial Blocks

Figure 35: Change in peak-to-peak amplitude of component $N_3$- $P_3$ of CS+ and CS- elicited LH AEPs during baseline and conditioning for the morphine and saline animals. An 80-trial block was composed of the mean of the amplitude measures from the four 20-trial AEPs from the baseline day (B) and each of the five days of conditioning. Peak-to-peak amplitude in arbitrary units.


LH $N_3$-$P_3$
160
150
140
130
120
110
100
90
80
Peak-to-Peak Amplitude
MORPHINE CS+
CS- n=3
SALINE CS+
CS- n=2
B
1
2
3
4
5
Conditioning
80 Trial Blocks

# DISCUSSION

## Lever-pressing Behavior

One major result of the first experiment was that rats self-administered lethal doses of morphine by lever pressing for intravenous injections. Previous reports have demonstrated that the pattern of lever pressing can be controlled by schedules of reinforcement (Schuster & Woods, 1968; Weeks, 1962; Woods & Schuster, 1971). Also, rates of lever pressing have been found to vary inversely with the size of the dose (Smith, Werner, & Davis, 1976; Weeks, 1962; Weeks & Collins, 1964, 1979; Woods & Schuster, 1971). The animals in this study engaged in very rapid lever pressing which was not easily controlled by schedules of reinforcement. The animals either pressed rapidly, or if reinforcement was too infrequent, stopped pressing entirely. Also, the lethally administering rats with the 2.0 mg/kg dose pressed faster than most of the rats with the 1.0 mg/kg dose.

In an effort to pinpoint possible causes for this lethal self-administration, a number of variables were examined. One possibility was that the morphine solution was contaminated. To check this, samples from the morphine reservoirs of the pumps were incubated for bacterial growth, but there was no evidence of the presence of gram-positive or gram-negative bacteria.

Another possible cause was some kind of blockage within the pump system which, when relieved, delivered a large quantity of morphine into the animal. This possibility was contraindicated by two kinds of evidence. First, normal functioning of the pump system was apparent

in the stereotyped behavior of rats immediately after each injection, (e.g., freezing, bulging eyes, and increased respiration). Second, if a block existed, the back pressure within the system produced leaks at the tubing junctions. The behavior of the rats and the tubing junctions were checked several times daily throughout the experiment.

Another possible cause of lethal self-administration could be lever activation during respiratory convulsions due to morphine overdose. If a rat was close to the lever while convulsing, it might press the lever as a result of the convulsion. Observation of the animals led to the opposite conclusion, since lever pressing rarely occurred after the onset of convulsions.

Because of the small size of the headpost, there was also a possibility of voltage leaking across the pins to the recording electrodes, enabling the animal to press for direct stimulation of the brain instead of morphine injections. Ventral medial hypothalamus, where one of the recording electrodes was placed, is known as a site supporting self-stimulation behavior (Margules & Olds, 1962). However, the recording system was tested for any voltage leak from the stimulating leads when the shock stimuli were presented, and there was no measurable voltage. Also, several animals have been run since the conclusion of this study with no recording electrodes implanted in the animals' brains. These animals also demonstrated the rapid lever pressing behavior leading to the administration of lethal doses of morphine.

The only parameter that appeared to differentiate the overdosing from the maintaining rats was the pulse duration of the foreleg stimuli. In the present study, the animals which controlled their morphine input received foreleg stimuli with 0.02 msec duration pulses. Even though the behavioral and electrophysiological responses to the 0.02 and 0.2 msec pulse durations were similar, there probably was a difference in the fiber pathways excited by the stimuli. Both durations probably produced excitation of the large myelinated, fast conducting fibers which are afferent fibers for touch and proprioception. In contrast, there probably would be a difference in the excitation of the small, unmyelinated, slowly conducting fibers which are the afferent fibers for pain. It is known that as pulse duration is decreased, it is much more difficult to excite the slowly conducting small fibers (Erlanger & Glasser, 1937). Consistent with this notion, since morphine acts as an analgesic, the animals that received the 0.2 msec stimulation would lever press faster to alleviate the painful stimulation. However, the animals did not exhibit any behavior, such as squeaking or jumping, during the initial establishment of the stimulus levels which might indicate that the stimuli were painful. The usual behavioral response was a slight twitch of the stimulated paw. Also, during the collection of baseline responses, usually 80-100 trials, the animals pressed the lever while licking off the bait and received the paired foreleg stimuli and saline injections. If the stimuli were painful, the animals would presumably have learned to avoid the lever during baseline trials, but no avoidance behavior was noted. How this possible elicitation

of the pain fibers interacts with morphine delivery to produce the self-administration of lethal doses of morphine is unknown and awaits further experimentation.

Physiological Effect of Morphine

Since morphine acts primarily on the central nervous system, changes in evoked potential components due only to the physiological effects of morphine were expected. Previous studies have reported that morphine affects the amplitude and latency of evoked potential components, but there appears to be little consistency to the data (Burks & Dafny, 1976; Chin & Domino, 1960; Dafny & Burks, 1977; Jurna, Schlue, & Tamm, 1972; McKenzie & Beechey, 1962; Sinitsin, 1964; Straw & Mitchell, 1964). There is very little data available concerning changes in evoked potentials during the development of dependence. The overwhelming majority of the studies investigating morphine effects of evoked potentials used naive animals and recorded changes following delivery of a single dose of morphine (e.g., Burks & Dafny, 1976; Chin & Domino, 1960; Dafny & Burks, 1977; Jurna, Schlue, & Tamm, 1972; McKenzie & Beechey, 1962; Sinitsin, 1964; Straw & Mitchell, 1964). Only one study has investigated changes in evoked potentials to somatic stimulation in naive or tolerant/dependent rats (Gildenberg, Murthy, Adler, & Frost, 1976). Recordings were made from the animals on first exposure to the drug or after dependence had been established by 5 days of morphine injections. In contrast, the present experiments allowed the monitoring of progressive physiological changes as dependence developed.

In the present studies, an effect due only to the physiological effects of morphine was defined as a change which occurred to both the CS+ and CS- evoked potential components during the conditioning phase. These changes occurred both as shifts in the latency of evoked potential peaks and as changes in the peak-to-peak amplitude of evoked potential components. In Experiment 2, a saline control group was also included to differentiate evoked potential changes due to morphine from changes due to habituation, sensitization, or other non-associative factors.

In both experiments, a shift in latency of cortical evoked potential peaks occurred: Peak $P_2$ decreased in latency, whereas Peak $P_3$ increased in latency. Previous studies have also reported shifts in cortical evoked potential latencies due to morphine administration (Stein, 1976; Wilder, 1978). Another aspect of the effect of morphine appears to involve a change in the shape of the long latency peaks of the cortical evoked potentials, whereby they become sharper (see Figures 10 and 20). The shift in latency of the two long latency positive peaks occurs in conjunction with the change in the shape of these peaks. As can be seen in the individual evoked potential records from Rats F16 and F86, the two long latency positive peaks become more compact with the increasing doses of morphine during conditioning and the latency of the peaks shifted. Thus, morphine may synchronize the underlying neural activity of the cortex, leading to a sharpening of the evoked potential peaks.

Morphine-induced changes in peak-to-peak amplitudes were recorded in cortical evoked potentials in both Experiments 1 and 2,

as well as in evoked potentials recorded from PFT and PVG during Experiment 2. No effects were found on the short latency components of the evoked potentials recorded from any of the brain areas. Since the short latency components of the evoked potentials are mediated by the neural input from the primary sensory systems, it appears as if morphine does not alter the direct sensory information. These results are in agreement with previous experiments that have reported that morphine administration does not affect the short latency components of evoked potentials recorded from somatosensory pathways (McKenzie & Beechey, 1962; Straw & Mitchell, 1964).

In the present study, morphine's main effects were exerted on the evoked potential components that occurred with latencies greater than 30 msec. These evoked potential components are believed to be mediated by input from multi-synaptic pathways or the activity of reverberating circuits.

Morphine's main effect at low doses appears to be analgesia, produced via inhibition of the spinothalamic pathway which carries afferent pain information. This fiber pathway, arising in the dorsal horn of the spinal cord is a multi-synaptic pathway. The afferent fibers carrying pain information ascend through the spinal cord and brain stem through interneurons in the reticular formation, including PVG, to end in several thalamic nuclei, including the parafascicularis-centromedian. However, the brain areas PVG and PFT have also been implicated in other effects of morphine such as the development of tolerance and dependence, and contain a high concentration of morphine receptors. Since these effects are presumably mediated through poly-

synaptic pathways, it cannot be determined whether the changes in evoked potential amplitudes recorded from PVG, PFT, or association cortex were due to effects of morphine at the spinal cord, brain stem, thalamus, and/or cortex.

Acquisition of Conditioned Response

The development of a conditioned response was found in the same cortical evoked potential component during conditioning in both Experiment 1 and Experiment 2. The $P_2$-$N_3$ component decreased in amplitude over conditioning trials in the cortical evoked potentials elicited by the CS+ during the first experiment, as compared to the progressive increase in amplitude in the cortical evoked potentials elicited by the CS-. For the second experiment, the conditioned response also developed in the $P_2$-$N_3$ component of the cortical evoked potential. However, the conditioned response was in the opposite direction: an increase in amplitude that was greater than the increase in amplitude recorded in the CS- evoked potentials.

Experiment 1. In the first experiment, the CS+ was presented following a lever press but prior to the administration of morphine. The CS- occurred explicitly unpaired with the CS+-US presentation. As stated above, the conditioned response which developed in the $P_2$-$N_3$ component was a decrease in amplitude in the evoked potential elicited by the CS+. In addition, there was an increase in amplitude in the CS- evoked potentials during conditioning, due to a direct physiological effect of morphine. No previous experiments have used this same paradigm in analyzing changes in evoked potentials during instrumental conditioning experiments. Most studies analyzed changes

in evoked potentials elicited by a discriminative stimulus (S+) which was presented prior to the elicitation of the instrumental response by the animal. Using this paradigm, several experimenters have reported a decrease in evoked potential amplitude elicited by the S+ (Beer, Sheatz, & Galambos, 1960; Cherubini, Bilancia, & Ricci, 1976; Majkowski & Sobieszek, 1975).

Since the design of the first experiment involved the presentation of the CS+ after the lever press but prior to the administration of morphine, the relationship of the CS+ to the morphine administration can be considered analogous to a trace conditioning paradigm for classical conditioning. The stimulus preceded the presentation of the US, in this case morphine administration. Previous studies, using classical conditioning procedures with food, tones, or airpuffs as the US have also reported decreases in evoked potential amplitudes during conditioning (Feeney, 1971; Kitai, Cohen, & Morin, 1965; Sugawara, Kitajima, & Konoh, 1977). One study that used morphine as the US in a classical conditioning paradigm with non-dependent animals also reported a decrease in evoked potential amplitude (Stein, 1976).

Experiment 1 did demonstrate a conditioned neural response based on morphine as a US. The evoked potential changes which developed in Component $P_2$-$N_3$ over conditioning blocks in the first experiment are in agreement with previous reports. The conditioned changes take the form of a decrease in amplitude and the changes occur in the long latency components (100-200 msec) of the cortical evoked potential.

Experiment 2. In the second experiment, the CS+ was paired with the injection of morphine in a classical conditioning paradigm. The nature of the conditioned response was an increase in amplitude of the $P_2$-$N_3$ component of the cortical evoked potential elicited by the CS+, which was greater than the increase in evoked potential amplitude elicited by the CS-. Many experiments have reported an increase in association cortex evoked potential amplitude during either appetitive or aversive classical conditioning procedures (Begleiter & Platz, 1969; Boyd, Boyd, & Brown, 1977b; Galambos & Sheatz, 1962; Khachaturian & Gluck, 1969). The increase in amplitude was reported to occur at latencies around 100-200 msec of the evoked potential. One study by Wilder and O'Brien (1980) also reported an increase in association cortex evoked potentials in association with morphine in a conditioning paradigm with electrical stimulation of the forepaws presented as CS's following a single large dose of morphine. The conditioned response developed at a latency of 120-175 msec, which is the same approximate latency as the $P_2$-$N_3$ component of the cortical evoked potential reported in this study.

The conditioned response which developed in the $P_2$-$N_3$ component was found in the evoked potential data that had been collapsed across both the morphine and saline animals, indicating that the conditioned response was not specific to morphine as the US. The implication of this result is that conditioning may occur not only to morphine as the US, but also to some concomitant aspect of the injection procedure.

The question also arises as to why the conditioned response was a decrease in amplitude in the first experiment, and an increase in amplitude in the second experiment, even though the relationship between the CS+ and morphine injections was essentially the same in both experiments. The answer may lie in the fact that a lever press preceded the stimulus presentation in the first experiment. During training, the animal was also learning an association between the lever press response and the administration of morphine. The change that developed to the CS+ might be a product of the association of the lever press and morphine delivery or of the association of the CS+ and morphine administration, or both. No other instrumental conditioning experiments have investigated changes in evoked activity to a stimulus presented after the lever press, but prior to reinforcement.

Unilateral development of a conditioned response. Analysis of the data from the morphine animals in Experiment 2 revealed that the animals that received contralateral stimulation of the foreleg as the CS+ demonstrated the greatest differentiation between the CS+ and CS-. That is, the conditioned response was most apparent in the evoked potentials recorded from the right association cortex when electrical stimulation of the left foreleg was the CS+ and electrical stimulation of the right foreleg was the CS-. It was possible to identify this unilateral conditioning phenomena because of the counterbalancing procedures that were used in specifying the foreleg stimulation for each rat.

Other examples of unilateral conditioning effects are available. For example, studies on split-brain cats and monkeys have found that ". . .the primary learning process takes place within the parietal lobe of the hemisphere opposite to the hand which undergoes the manipulative experience" (Myers & Ebner, 1976; page 461). This conclusion is based on results from studies which have investigated the transfer of both classically conditioned and instrumentally conditioned somesthetic responses. Sectioning of the corpus callosum following a training period has been found to interfere with the transfer of learned somesthetic discriminations in cats and monkeys (Ebner & Meyrs, 1962; Glassman, 1970; Meikle, Sechzer, & Stellar, 1962). For example, Meikle, Sechzer, and Stellar (1962) trained cats to avoid a shock to the forelimb by paw flexion in response to a tactile CS. After training, two of the cats had the corpus callosum sectioned. The untrained forelimb was tested in the tactile discrimination task 4 weeks later. There was a complete failure to transfer the limb flexion response to the presentation of the tactile CS. These results, and the results from other split-brain studies indicate that the locus of the initial learning process resides in the association cortex of the hemisphere contralateral to the forelimb which receives the somatic discrimination training.

In the present studies, the association cortex evoked potentials were always recorded from the right hemisphere by an epidural bone screw in both Experiments 1 and 2. Based on the neuroanatomy of the fiber pathways to the association cortex, evoked potentials would be recorded in the right hemisphere in response to either right

(secondary pathways) or left (primary pathway) foreleg stimulation. However, any changes which occur due to conditioning would reside in the hemisphere contralateral to the foreleg that was stimulated as the CS+. Therefore, the conditioned response would develop in the right hemisphere for stimulation of the left foreleg as the CS+. Those learning changes which occur to right paw stimulation would be most prevalent in the opposite left hemisphere. It seems reasonable to expect, then, that recordings will most reliably reflect conditioning changes if they are recorded from the appropriate hemisphere. To test this notion, cortical recordings would be obtained from both the right and left hemispheres.

Testing

The original objective of this experiment was to develop an animal model for relapse to morphine use after the development of dependence and subsequent withdrawal from morphine. Both physiological and psychological processes have been implicated as mechanisms underlying the development of dependence, tolerance, and subsequent relapse to morphine use after withdrawal. It has been suggested that some changes occurring during the development of dependence may not be reversed by withdrawal, and may subsequently contribute to relapse. This experiment was originally designed to allow for measurement of both associative and physiological changes during the development of dependence and, further, to determine whether either or both processes may persist after withdrawal and contribute to relapse.

Physiological effects. For the three animals from the first experiment, and the animals that were tested with saline in the

second experiment, components that had demonstrated morphine-induced changes were expected to return to baseline levels by the start of testing. During withdrawal, morphine was eliminated from the system, and so, therefore, morphine-produced changes should no longer be present. The results in most cases confirmed this expectation.

For the animals that were tested with morphine in the second experiment, components that demonstrated a change due to morphine administration were expected to be close to baseline levels at the start of testing. As morphine administration continued throughout the testing sessions, these components would presumably change in the same direction as during the conditioning phase. In most of the brain areas, the results of the testing session for the morphine-tested animals confirmed this expectation. This effect was most dramatic in the short latency components of the cortical evoked potentials. Components that had demonstrated changes due only to the administration of morphine had returned to baseline levels at the start of testing. As testing continued, the morphine-induced evoked potential changes which had developed during conditioning were manifested again (see Figures 23 and 24).

Persistent associative effects in Experiment 1. A conditioned response was found to develop in the $P_2$-$N_3$ component of the cortical evoked potential during conditioning. Testing was conducted on the three animals which survived to determine whether the changes in this component persisted after a period of withdrawal. The expectation was that the conditioned response would still be apparent in the evoked ptoentials elicited by the CS+ during the first testing blocks,

even though the animals were not morphine intoxicated. Probably because of the small number of subjects, the statistical analysis of the data failed to reach significance for the differences between the CS+ and CS- evoked potentials. Nonetheless, two out of the three animals did demonstrate a difference in the amplitude of the $P_2$-$N_3$ component of the CS+ and CS- evoked potentials. Further, this difference was in the same direction as the difference which had developed during conditioning. The third animal also demonstrated a difference in amplitude between the CS+ and CS- evoked potential components during conditioning. However, during testing, the difference between the responses to the CS+ and CS- had reversed in direction. These results indicate that the conditioned neural response may have persisted after withdrawal.

Persistent associative effects in Experiment 2. A conditioned response also developed in the $P_2$-$N_3$ component of the cortical evoked potentials during conditioning in the second experiment. This conditioned response was most apparent in those animals conditioned with contralateral stimulation of the foreleg as the CS+. In this second experiment, one group of animals was tested with saline injections and another group was tested with morphine injections. These procedures allowed determination of whether the conditioned response was present after withdrawal in either a drugged or nondrugged animal.

The test results from the second experiment provided statistical support for the maintenance of the conditioned response after withdrawal, but only for the animals that received contralateral stimulation as the CS+ during conditioning. The difference between the CS+

and CS- was still present for both the group tested with morphine and the group tested with saline (see Figure 26). There was no evidence for the maintenance of the conditioned response for the animals conditioned with ipsilateral foreleg stimulation as the CS+.

Lever-press behavior during testing. The original objective of these experiments was to develop an animal model to test a particular relapse theory. This theory proposes that relapse to drug use after withdrawal may be elicited by non-extinguished conditioned stimuli. In the first experiment, electrical stimulation of the foreleg was paired with the injection of morphine after a lever press by the animal. Theoretically, the previously neutral stimulus of electrical stimulation of the foreleg would acquire conditioned properties throughout the establishment of dependence. If the conditioned response which had developed to the CS+ persisted through withdrawal, the expectation, according to the relapse theory, was that lever pressing behavior would be resumed in conjunction with the conditioned response elicited by the presentation of the CS+ after withdrawal.

Three of the animals which had maintained their morphine intake successfully completed the testing phase of the first experiment. Based on the lever-pressing results from these three animals, it appeared that the conditioned stimulus presentation after withdrawal had no influence on the lever pressing behavior of the animals. All of the animals exhibited lever pressing activity which occurred in clusters throughout the testing period (see Figures 7 and 8). Most lever pressing behavior occurred following another lever press, and

and did not follow the presentation of either the CS+ or the CS-. Based on the evoked potential results from the testing period, it appears as if the conditioned neural response persisted after withdrawal and was apparent in both a drug-free and drug-intoxicated animal. However, presentation of the conditioned stimuli after withdrawal did not elicit the resumption of lever pressing behavior.

# SUMMARY AND CONCLUSIONS

The main objective of this experiment was to develop an animal model to study relapse to morphine use after the development of dependence and subsequent withdrawal from morphine. One prominent theory which has been well accepted in the literature is that relapse to drug use after withdrawal may be elicited by non-extinguished conditioned stimuli. In order to test this theory, rats were trained to lever press for intravenous doses of morphine. Electrical stimulation of a foreleg (CS+) was paired with the administration of morphine following a lever press. Stimulation of the other foreleg (CS-) ocurred explicitly unpaired with the morphine administration. Once dependent, the animals were allowed to withdraw from morphine with the lever covered for 6 days. A final testing session involved the random presentation of both foreleg stimuli with the lever uncovered. Lever pressing behavior and changes in cortical evoked potentials were monitored throughout baseline, conditioning, and testing.

Since the majority of the rats died due to morphine overdose in the lever press experiment and did not complete the paradigm, a second experiment was conducted. A classical conditioning experiment was designed to determine if a conditioned neural response would develop in evoked potentials elicited by a foreleg stimulus (CS+) that was paired with morphine administration during the development of dependence. A stimulus to the other foreleg (CS-) was presented explicitly unpaired with morphine delivery. Evoked potentials were recorded from association cortex, parafascicularis nucleus

of the thalamus, periventricular gray and lateral hypothalamus. After dependence was established, the rats were taken from the experimental chamber and allowed to withdraw from morphine for 6 days in their home cages. Finally, during the testing phase, the animals were returned to the experimental chamber and both of the foreleg stimuli were presented. Either morphine or saline injections were paired with the CS+ presentation.

The following results were obtained:

Conditioning

A. Sixteen out of twenty rats self-administered lethal doses of morphine during conditioning. The pulse duration of the foreleg stimuli was implicated as the critical factor in overdose behavior, with the long duration pulse (0.2 msec) resulting in more deaths than the shorter duration pulse (0.02 msec).

B. Changes in evoked potential peak-to-peak amplitudes were recorded from the cortex in both Experiment 1 and Experiment 2 due only to the presentation of increasing doses of morphine during conditioning. Similar morphine-related evoked potential changes were recorded from subcortical brain areas in the second experiment. These changes were recorded mainly in the longer latency evoked potential components (60-240 msec).

C. Conditioned neural responses were recorded in the cortical evoked potential component with a latency of approximately 100-280 msec during both experiments. In Experiment 1,

the conditioned response was a decrement in amplitude, whereas in the second experiment the conditioned response was an increase in amplitude.

Testing

A. None of the evoked potential changes due to the physiological effects of morphine persisted following withdrawal.

B. The conditioned neural response appeared to persist after withdrawal and was observed in the testing phase of both experiments. The conditioned response was apparent in the cortical evoked potential from drug-free or morphine-intoxicated animals.

C. The lever pressing behavior from the three animals tested in Experiment 1 did not appear to be influenced by the presentation of the conditioned stimulus.

Based on these results, the follwoing conclusions were made:

A. Physiological changes due to morphine administration were attributed to an effect of morphine on multi-synaptic neural pathways. These physiological changes did not persist after withdrawal and therefore did not appear to be a factor in relapse to drug use.

B. The conditioned neural response persisted after withdrawal and was present in a drug-free or morphine-intoxicated animal. However, presentation of the conditioned stimulus did not appear to elicit lever pressing behavior. These results do not support the relapse theory which states that non-extinguished conditioned responses elicit drug-seeking behavior after withdrawal.

# REFERENCES

Andry, D. K., & Luttges, M. W. Drug-induced dissociation of evoked responses and performance in mice. Behavioral Biology, 1976, 17, 17-29.

Begleiter, H., & Platz, A. Evoked potentials: Modifications by classical conditioning. Science, 1969, 166, 769-771.

Boyd, E. S., Boyd, E. H., & Brown, L. E. A cortical evoked potential that reflects the conditioned, positive incentive value of the stimulus. I. The evoked potential. Electroencephalography and Clinical Neurophysiology, 1977a, 42, 341-354.

Boyd, E. S., Boyd, E. H., & Brown, L. E. A cortical evoked potential that reflects the conditioned, positive incentive value of the stimulus. II. Manipulative motivation and the value of the reward. Electroencephalography and Clinical Neurophysiology, 1977b, 42, 364-371.

Burks, T. F., & Dafny, N. Naloxone and morphine effects on sensory-evoked responses. Neuropharmacology, 1977, 16, 681-686.

Buzsaki, G., Grastyan, E., Tveritskaya, I. N., & Czopf, J. Hippocampal evoked potentials and EEG changes during classical conditioning in the rat. Electroencephalography and Clinical Neurophysiology, 1979, 47, 64-74.

Caviedes, E., & Bures, J. Changes in cortical and tegmental evoked responses to sensory stimuli reinforced by electrical stimulation of the area of recording. Brain Research, 1970, 19, 249-261.

Chandler, S. D., & Liles, S. L. Classically conditioned eye blink and changes in activity in the precruciate cortex and thalamus in the cat. Experimental Neurology, 1977, 55, 368-380.

Cherubini, E., Bilancia, G., & Ricci, G. F. Changes of visual evoked responses to geniculate stimuli during light conditioning in the cat. Brain Research, 1976, 105, 578-582.

Chin, J. H., & Domino, E. G. Effects of morphine on brain potentials evoked by stimulation of the tooth pulp of the dog. Journal of Pharmacology and Experimental Therapeutics, 1961, 132, 74-86.

Crosby, E. C., Humphrey, T., & Lauer, E. W. Correlative Anatomy of The Nervous System. New York: MacMillan, 1962.

Dafny, N., & Burks, T. F. Neurophysiological changes in caudate nucleus and substantia nigra following morphine treatment. Neuropharmacology, 1976, 15, 547-554.

Davis, W. M., & Smith, S. G. Naloxone used to eliminate opiate-seeking behavior: Need for extinction of conditioned reinforcement. Biological Psychiatry, 1974, 9, 181-189.

Davis, W. M., & Smith, S. G. Role of conditioned reinforcers in the initiation, maintenance, and extinction of drug-seeking behavior. Pavlovian Journal of Biological Science, 1976, 11, 222-236.

Drawbaugh, R., & Lal, H. Reversal by narcotic antagonist of a narcotic action elicited by a conditional stimulus. Nature, 1974, 247, 65-67.

Erlanger, J., & Gasser, H. S. Electrical Signs of Nervous Activity. Philadelphia: University of Pennsylvania Press, 1937.

Feeney, D. M. Evoked responses and background unit activity during appetitive conditioning in dogs. Physiology & Behavior, 1971, 6, 9-15.

Fellows, B. J. Chance stimulus sequences for discrimination tasks. Psychological Bulletin, 1967, 67, 87-92.

Fleming, D. E. Amplitude relationships between evoked potential components during trace conditioning. Electroencephalography and Clinical Neurophysiology, 1967, 23, 449-455.

Galambos, R., & Morgan, C. T. The neural basis of learning. In Field, J. (Ed.), Handbook of Physiology. Section I: Neurophysiology Volume III. Washington D. C.: American Physiological Society, 1960.

Galambos, R., & Sheatz, G. C. An electroencephalograph study of classical conditioning. American Journal of Physiology, 1962, 203, 173-184.

Galambos, R., Sheatz, G., & Vernier, V. G. Electrophysiological correlates of a conditioned response in cats. Science, 1955, 123, 376-377.

Galeano, C. Electrophysiologic aspects of brain activity during conditioning: A review. Acta Neurologica Latinoamerica, 1963, 9, 395-413.

Gildenberg, P. L., Murthy, K. S. K., Adler, M. W., & Frost, E. A. The effect of morphine on evoked potentials in naive, tolerant and withdrawn rats. In H. W. Kosterlitz (Ed.), Opiates and Endogenous Opioid Peptides. New York: North Holland Publishing Co., 1976.

Glassman, R. B. Contralateral and ipsilateral transfer of a cutaneous discrimination in normal and callosum-sectioned cats. Journal of Comparative and Physiological Psychology, 1970, 70, 470-475.

Goldberg, S. R. Relapse to opioid dependence: The role of conditioning. In Harris, R. T., McIsaac, W. M., & Schuster, C. R. (Eds.), Drug Dependence. Austin, Tx.: University of Texas Press, 1970. pp. 170-197.

Goldberg, S. R. Stimuli associated with drug injections as events that control behavior. Pharmacological Reviews, 1976a, 27(3), 325-340.

Goldberg, S. R. The behavioral analysis of drug addiction. In Glick, S. D., & Goldfarb, J. (Eds.), Behavioral Pharmacology. St. Louis, Mo.: C. V. Mosby Company, 1976b. pp. 283-316.

Goldberg, S. R., & Schuster, C. R. Conditioned morphine-induced abstinence changes: Persistence in post morphine-dependent monkeys. Journal of the Experimental Analysis of Behavior, 1970, 14, 33-46.

Goldberg, S. R., Woods, J. H., & Schuster, C. R. Morphine: Conditioned increases in self-administration in rhesus monkeys. Science, 1969, 166, 1306-1307.

Goodman, L. S., & Gilman, A. The Pharmacological Basis of Therapeutics, Fifth Edition. New York: MacMillan Publishing Co., Inc., 1975.

Goodman, R. R., Snyder, S. H., Kuhar, M. J., & Young, W. S. Light microscope autoradiographic differentiation of mu and delta opiate receptors in rat brain. Society of Neuroscience Abstracts, 1980, 6, 523.

Gotestam, K. G. Intragastric self-administration of medazepam in rats. Psychopharmacologia, 1973, 28, 87-94.

Grinberg-Zylberbaum, J., Prado-Alcala, R., & Brust-Carmona, H. Correlation of evoked potentials in the caudate nucleus and conditioned motor responses. Physiology & Behavior, 1973, 10, 1005-1009.

Hall, R. D., & Mark, R. G. Fear and the modification of acoustically evoked potentials during conditioning. Journal of Neurophysiology, 1967, 30, 893-910.

Hearst, E., Beer, B., Sheatz, G., & Galambos, R. Some electrophysiological correlates of conditioning in the monkey. Electroencephalography and Clinical Neurophysiology, 1960, 12, 137-152.

Hudspeth, W. J., & Jones, G. B. Neural models for short-term memory: A quantitative study of average evoked potential waveform. Neuropsychologia, 1978, 16, 201-212.

Irwin, W., & Seevers, M. H. Altered response to drugs in the post addict (Maccaca Mulatta). Journal of Pharmacology and Experimental Therapeutics, 1956, 116, 31-32.

Jasper, H., Ricci, G., & Doane, B. Microelectrode analysis of cortical cell discharge during avoidance conditioning in the monkey. Electroencephalography and Clinical Neurophysiology, Supp, 1960, 13, 137-155.

John, E. R. High nervous functions: Brain functions and learning. Annual Review of Physiology, 1961, 23, 451-484.

Jurna, I., Schlue, W. R., & Tamm. U. The effect of morphine on primary somatosensory evoked responses in the rat cerebral cortex. Neuropharmacology, 1972, 11, 409-415.

Khachaturian, Z. S., & Gluck, H. The effects of arousal on the amplitude of evoked potentials. Brain Research, 1969, 14, 589-606.

Kitai, S. T., Cohen, B., & Morin, F. Changes in the amplitude of photically evoked potentials by a conditioned stimulus. Electroencephalography and Clinical Neurophysiology, 1965, 19, 344-349.

Kumar, R. Morphine dependence in rats: Secondary reinforcement from environmental stimuli. Psychopharmacologia, 1972, 25, 332-338.

Kumar, R., & Stolerman, I. P. Morphine dependent behavior in rats: Some clinical implications. Psychological Medicine, 1973, 3, 225-237.

Kumar, R., Steinberg, H., & Stolerman, I. P. How rats can become dependent on morphine in the course of relieving another need. In H. Steinberg (Ed.), Scientific Basis of Drug Dependence. New York: Grune & Stratton, 1969.

Lal, H., Miksic, S., Drawbaugh, R., Numan, R., & Smith, N. Alleviation of narcotic withdrawal syndrome by conditioned stimuli. Pavlovian Journal of Biological Science, 1976, 11(4), 251-262.

Lord, J. A. H., Waterfield, A. A., Hughes, J., & Kosterlitz, H. W. Endogenous opioid peptides: Multiple agonists and receptors. Nature, 1977, 267, 495-499.

Lynch, J. J., Stein, E. A., & Fertziger, A. P. An analysis of 70 years of morphine classical conditioning: Implications for clinical treatment of narcotic addiction. Journal of Nervous and Mental Disease, 1976, 163, 47-58.

Mackintosh, N. J. The Psychology of Animal Learning. New York: Academic Press, Inc., 1974.

Majkowski, J., & Sobieszek, A. Evolution of average evoked potentials in cats during conditioning before and after tegmental lesions. Physiology & Behavior, 1975, 14, 123-131.

Margules, D. L., & Olds, J. Identical feeding and rewarding systems in the hypothalamus of rats. Science, 1962, 135, 374-377.

Mark, R. G., & Hall, R. D. Acoustically evoked potentials in the rat during conditioning. Journal of Neurophysiology, 1967, 30, 875-892.

Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E., & Gilbert, P. E. The effects of morphine and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. Journal of Pharmacology and Experimental Therapeutics, 1976, 197, 517-532.

Marsh, J. T., McCarthy, D. A., Sheatz, G., & Galambos, R. Amplitude changes in evoked auditory potentials during habituation and conditioning. Electroencephalography and Clinical Neurophysiology, 1961, 13, 224-234.

Mayer, D. J., & Price, D. D. Central nervous system mechanisms of analgesia. Pain, 1976, 2, 379-404.

McClung, R. E., Burks, T. F., & Dafny, N. Neurophysiological assessment of site specific effects of chronic morphine administration in freely behaving rats. Archives Internationales de Pharmacodynamie, 1977, 229, 144-156.

McKenzie, J. S., & Beechey, N. R. The effects of morphine and pethidine on somatic evoked responses in the midbrain of the cat,

and their relevance to analgesia. Electroencephalography and Clinical Neurophysiology, 1962, 14, 501-519.

Meikle, T. H., Sechzer, J. A., & Stellar, E. Interhemispheric transfer of tactile conditioned responses in corpus callosum-sectioned cats. Journal of Neurophysiology, 1962, 25, 530-543.

Morrell, F. Electrophysiological contributions to the neural basis of learning. Physiological Reviews, 1961, 41, 443-494.

Moushegian, G., Rupert, A., Marsh, J. T., & Galambos, R. Evoked cortical potentials in absence of middle ear muscles. Science, 1961, 133, 582-583.

Myers, R. E., & Ebner, F. F. Localization of function in corpus callosum: Tactual information transmission in Macaca Mulatta. Brain Research, 1976, 103, 455-462.

Nakamura, J., & Mitchell, C. L. The effects of morphine, pentobarbital and saline on bioelectrical potentials recorded in limbic structures of the cat evoked by radial nerve and direct brain stimulation. Archives Internationales de Pharmacodynamie et de Therapie, 1972, 200, 70-87.

Nichols, J. R., & Davis, W. M. Drug addiction. II. Variation of addiction. Journal of American Pharmacological Association, 1959, 48, 259-262.

Nichols, J. R., Headlee, C. P., & Coppock, H. W. Drug Addiction. I. Addiction by escape training. Journal of American Pharmacological Association, 1956, 44, 788-791.

Numan, R., Banerjee, U., Smith, N., & Lal, H. Secondary reinforcement property of a stimulus paired with morphine administration in the rat. Pharmacology Biochemistry and Behavior, 1976, 5, 395-399.

O'Brien, C. P. Experimental analysis of conditioning factors in human narcotic addiction. Pharmacological Reviews, 1976, 27(4), 533-543.

O'Brien, C. P., Testa, T., O'Brien, T. J., & Greenstein, R. Conditioning in human opiate addicts. Pavlovian Journal of Biological Science, 1976, 11, 195-202.

Parker, L., Failor, A., & Weidman, K. Conditioned preferences in the rat with an unnatural need state: Morphine withdrawal. Journal of Comparative and Physiological Psychology, 1973, 82, 294-300.

Pellegrino, L. J., Pellegrino, A. S., & Cushman, A. J. A Stereotaxic Atlas of the Rat Brain. Second Edition. New York: Plenum Press, 1979.

Pert, C. B., & Snyder, S. H. Properties of opiate-receptor binding in rat brain. Proceedings of the National Academy of Sciences, 1973a, 70, 2243-2247.

Pert, C. B., & Snyder, S. H. Opiate receptor: Demonstration in nervous tissue. Science, 1973b, 179, 1011-1014.

Roffman, M., Reddy, C., & Lal, H. Control of morphine-withdrawal hypothermia by conditional stimuli. Psychopharmacologia, 1973, 29, 197-201.

Rosenblum, S. M. Cortical and thalamic correlates of differential classical conditioning: A cryogenic study. Doctoral Dissertation, University of Oregon Health Sciences Center, 1977.

Rush, M. L., Pearson, L., & Lang, W. J. Conditioned autonomic responses induced in dogs by atropine and morphine. European Journal of Pharmacology, 1970, 11, 22-28.

Saito, H., Yamamoto, M., Iwai, E., & Nakahama, H. Behavioral and electrophysiological correlates during flash-frequency discrimination learning in monkeys. Electroencephalography and Clinical Neurophysiology, 1973, 34, 449-460.

Salamy, J. G., Sands, S. F., & Dafny, N. Effects of morphine on visual evoked responses recorded in five brain sites. Life Sciences, 1979, 24, 1241-1250.

Saunders, J. C. Selective facilitation and inhibition of auditory and visual evoked responses during avoidance conditioning in cats. Journal of Comparative and Physiological Psychology, 1971, 76, 15-25.

Shoenfeld, W. N., Antonitis, J. J., & Bersh, P. J. A preliminary study of training conditions necessary for secondary reinforcement. Journal of Experimental Psychology, 1950, 40, 40-45.

Schuster, C. R. Discussion of symposium on conditioning and addiction. Pavlovian Journal of Biological Sciences, 1976, 11, 263-266.

Schuster, C. R., & Woods, J. H. The conditioned reinforcing effects of stimuli associated with morphine reinforcement. International Journal of Addiction, 1968, 3, 223-230.

Segal, M. Excitability changes in rat hippocampus during conditioning. Experimental Neurology, 1977, 55, 67-73.

Semmes, J., & Mishkin, M. Somatosensory loss in monkeys after ipsilateral cortical ablation. Journal of Neurophysiology, 1965, 28, 473-486.

Siegel, S. Evidence from rats that morphine tolerance is a learned response. Journal of Comparative and Physiological Psychology, 1975, 89(5), 498-506.

Siegel, S. Morphine analgesic tolerance: Its situation specificity supports a Pavlovian conditioning model. Science, 1976, 193, 323-325.

Siegel, S. Morphine tolerance acquisition as an associative process. Journal of Experimental Psychology: Animal Behavior Processes, 1977, 3(1), 1-13.

Siegel, S. A Pavlovian conditioning analysis of morphine tolerance (and opiate dependence). Paper prepared for National Institute on Drug Abuse Symposium on Behavioral Tolerance, 1977.

Siegel, S. Tolerance to the hyperthermic effect of morphine in the rat is a learned response. Journal of Comparative and Physiological Psychology, 1978a, 92, 1137-1149.

Siegel, S. A Pavlovian conditioning analysis of morphine tolerance. In W. A. Krasnegor, (ed.), Behavioral Tolerance: Research and Implications. NIDA Research Monograph No. 18, Washington, D.C.: Government Printing Office, 1978b.

Siegel, S., Hinson, R. E., & Krank, M. D. The role of predrug signals in morphine analgesic tolerance: Support for a Pavlovian conditioning model of tolerance. Journal of Experimental Psychology: Animal Behavior Processes, 1978, 4, 188-196.

Simon, E. J., Hiller, J. M., & Edelman, I. Stereospecific binding of the potent narcotic analgesic $^{3}$H-etorphine to rat-brain homogenate. Proceedings of the National Academy of Science, 1973, 70, 1947-1949.

Sinitsin, L. N. Effect of morphine and other analgesics on brain evoked potentials. International Journal of Neuropharmacology, 1964, 3, 321-326.

Smith, S. G., Werner, T. E., & Davis, W. M. Effect of unit dose and route of administration on self-administration of morphine. Psychopharmacology, 1976, 50, 103-105.

Sommer, Smith, J. A., & Morocutti, C. Cortical and subcortical evoked potentials during conditioning. Electroencephalography and Clinical Neurophysiology, 1970, 29, 383-391.

Stein, E. A. Evoked potential and heart rate changes during classical conditioning of morphine in the rat. (Doctoral dissertation, University of Maryland, 1976). Dissertation Abstracts International, 1978 (University Microfilms #76-28336).

Stein, E. A., Lynch, J. J., & Ruchkin, D. S. Evoked potential changes during classical conditioning of morphine in the awake, freely behaving rat. Experimental Neurology, 1977, 55, 505-519.

Sugawara, M., Kitajima, S., & Konoh, M. Enhancement and diminution of the evoked responses to conditioned stimuli during discrimination conditioning. Neuropsychologia, 1977, 15, 243-248.

Straw, R. N., & Mitchell, C. L. The effects of morphine, pentobarbital, pentazocine and nalorphine on bioelectrical potentials evoked in the brain stem of the cat by electrical stimulation of the tooth pulp. Journal of Pharmacology and Experimental Therapeutics, 1964, 146, 7-15.

Teitelbaum, H., Catravas, G. N., & McFarland, W. I. Reversal of morphine tolerance after medial thalamic lesions in the rat. Science, 1974, 185, 449-451.

Terenius, L. Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex.

Acta Pharmacologica et Toxicologica, 1973, 32, 317-320.

Weeks, J. R. Experimental morphine addiction: Method for automatic intravenous injections in unrestrained rats. Science, 1962, 138, 143-144.

Weeks, J. R., & Collins, R. J. Factors affecting voluntary morphine intake in self-maintained addicted rats. Psychopharmacologia, 1964, 6, 267-279.

Weeks, J. R., & Collins, R. J. Dose and physical dependence as factors in the self-administration of morphine by rats. Psychopharmacology, 1979, 65, 171-177.

Wei, E., Loh, H. H., & Way, E. L. Neuroanatomical correlates of morphine dependence. Science, 1972, 177, 616-617.

Wikler, A. Recent progress in research on the neurophysiologic basis of morphine addiction. American Journal of Psychiatry, 1948, 105, 329-338.

Wikler, A., Pescor, F. T., Miller, D., & Norrell, H. Persistent potency of a secondary (conditioned) reinforcer following withdrawal of morphine from physically dependent rats. Psychopharmacologia, 1971, 20, 103-117.

Wilder, M. B. Evoked potential conditioning using morphine as the unconditioned stimulus in rats. Doctoral Dissertation, University of Oregon Health Sciences Center, 1978.

Wilder, M. B., & O'Brien, J. H. Evoked potential conditioning using morphine as the unconditioned stimulus in rats. Experimental Neurology, 1980, 67, 539-553.

Woods, J. H., & Schuster, C. R. Opiates as reinforcing stimuli. In Thompson, T., & Pickens, R. (Eds.), Stimulus Properties of Drugs. New York: Appleton-Century-Crofts, 1971.

Yaksh, T. L., & Rudy, T. A. Narcotic analgesics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. Pain, 1978, 4, 299-359.

# APPENDIX A

## Conditioned Effects on Evoked Potentials

Several review articles have been written on the effects of classical and instrumental conditioning in evoked potentials (Galambos & Morgan, 1960; Galeano, 1963; John, 1961; Morrell, 1961). Tables 8 and 9 summarize most of the studies that have analyzed changes in evoked potentials using classical and instrumental procedures. Included in the tables is information concerning the nature of the stimuli, the areas recorded from, and the reported changes in the evoked potential due to conditioning.

## Classical Conditioning

The majority of the classical conditioning experiments have reported an overall increase in evoked potential amplitude, or an increase in the amplitude of a specific peak-to-peak component of the evoked potential, regardless of whether aversive or appetitive conditioning methods were used (see Table 8). In order to differentiate nonassociative from associative changes occurring in evoked potential recordings, pseudoconditioning or differential conditioning procedures must be used. Most experiments investigating changes in evoked potentials during conditioning procedures have failed to use these necessary control groups (Boyd, Boyd, & Brown, 1977b; Buzsaki, Grastyan, Tveritskaya, & Czopf, 1979; Chandler & Liles, 1977; Feeney, 1971; Fleming, 1967; Galambos & Sheatz, 1962; Galambos, Sheatz, & Vernier, 1955; Hudspeth & Jones, 1978; Kitai, Cohen, & Morin, 1965; Marsh, McCarthy, Sheatz, & Galambos, 1961; Moushegian, Rupert, Marsh, & Galambos, 1961). The changes in evoked potential amplitude reported to be a result

Table 8

Classical Conditioning Effects on AEPs

| Author | Subjects | CS+ | CS- | Probe | US | Area | CR |
|---|---|---|---|---|---|---|---|
| Begleiter & Platz (1969) | 16 H | Arrows | Arrows | --- | Clicks | Assoc. Cx. | ↑ Amp to CS+ 155-160 msec |
| Boyd, Boyd & Brown (1977b) | 3 M | Tone | --- | --- | Food | Parietal Cx<br>Postcentral Cx<br>Prearcuate Cx<br>Postarcuate Cx | ↑ Amp 100-200 msec |
| Buzsaki, Grastyan, Tveritskaya, & Czopf (1979) | 23 R | Tone | --- | --- | Water | Dorsal Hippo<br>Ventral Hippo | ↓ Amp 100-150 msec |
| Caviedes & Bures (1970) | 5 R | Clicks | --- | --- | ES-SM Cx | SM Cx | ↑ Amp 30-40 msec |
| | 10 R | Clicks | --- | --- | ES-Teg | Tegmentum | ↑ Amp 30-40 msec |
| Chandler & Liles (1977) | 6 C | Clicks | --- | --- | Glabellar Tap | Pericruciate Cx<br>RF, CM, VPM, VPL | ↑ Amp<br>4-6 msec<br>10-12 msec<br>30 msec |
| | | | | | | Caudate | No change |
| | | | | | | Aud Nucleus | No change |
| Feeney (1971) | 9 D | Tone or Light | --- | ES-thal | Food | Vis Cx | ↑ Amp to probe during CS+ 4-6 msec |
| | | | | | | Aud Cx | ↓ Amp prior to US to probe during CS+ |

| Author | Subjects | CS+ | CS- | Probe | US | Area | CR |
|---|---|---|---|---|---|---|---|
| Fleming (1967) | 6 C | Light Flash | --- | --- | Shock | Vis Cx | ↑ Amp 30-63 msec 12-24 msec 21-38 msec |
| Galambos & Sheatz (1962) | 8 M<br>2 C | Click and/or Flash | --- | --- | Airpuff | Brain-stem core, Thal, Hypo, Assoc Cx, Paleocortex | ↑ Amp 50-100 msec |
| Galambos, Sheatz & Vernier (1955) | 10 C | Clicks | --- | --- | Shock to chest | Coch Nuc<br>Aud Cx<br>Hippo | ↑ Amp |
| Hearst, Beer, Sheatz & Galambos (1960) | 1 M | Tone series | --- | --- | Food | Hippo | ↑ Amp |
| | 4 M | Tone | Tone | --- | Shock | Aud Cx<br>Hippo<br>Caudate<br>Med Gen | ↑ Amp to CS+ |
| Hudspeth & Jones (1978) | 6 Rb | Tone and/or Clicks | Clicks | Flashing checker board | ES-ear | Post thal<br>Med Gen<br>Limbic Cx<br>RF | Unique AEP waveform to probe during CS+ |
| Khachaturian & Gluck (1969) | 5 C | Tone | --- | Light flash | Shock to thigh | Vis Cx<br>Par Cx<br>Thal | ↑ Amp to probe during CS+<br>New Component |
| Kitai, Cohen & Morin (1965) | 4 C | Tone | --- | Light flash | Food | Post hypo<br>RF, Aud Cx<br>Vix Cx | ↓ Amp to probe during CS+ |

| Authors | Subjects | CS+ | CS- | Probe | US | Area | CR |
|---|---|---|---|---|---|---|---|
| Marsh, McCarthy, Sheatz & Galambos (1961) | 5 C | Clicks | --- | --- | Airpuff | Coch Nuc<br>Inf Olive<br>Optic Rad<br>Aud Cx, Hippo<br>Caudate | ↑ Amp<br>200 msec |
| Moushegian, Rupert, Marsh, & Galambos (1961) | 4 C | Clicks | --- | --- | Airpuff | Aud Cx | ↑ Amp |
| Rosenblum (1977) | 22 C | Forepaw Shock | Forepaw Shock | --- | Hindpaw Shock | CM | ↑ Amp to CS+ 1st 25 trials, then:<br>↓ Amp to CS+ and CS- |
| Segal (1977) | 13 R | Tone | --- | ES-hippo | Water | Hippo | ↑ Amp to probe during CS+ |
| | 8 R | Tone | --- | ES-hippo | Shock to Shoulder | Hippo | ↑ Amp 3/8 to probe during CS+<br>↓ Amp 3/8 to probe during CS+ |
| Sommer-Smith & Morocutti (1970) | 10 C | Tone | --- | --- | ES-paw | Aud Cx, Vis Cx, Sm Cx, Hippo, Med Gen, CM, RF | No change |

| Author | Subjects | CS+ | CS- | Probe | US | Area | CR |
|---|---|---|---|---|---|---|---|
| Sugawara, Sitajima & Konoh (1977) | 8 H | Triangle or Disk | Triangle or Disk | --- | Airpuff or Tone | Vis Cx | ↓ Amp to CS+ 100-180 msec 180-250 msec |

Abbreviations for Table 5

H--Humans; C--Cats; R--Rats; Rb--Rabbits; M--Monkeys; D--Dogs;

ES--Electrical Stimulation

Coch Nuc--Cochlear nucleus; Hippo--Hippocampus; Teg--Tegmentum; RF--Reticular formation; CM--centromedian; VPM--Ventralis posterior medialis of thalamus; VPL--Ventralis posterior; SM--Sensorimotor Cortex; Vis Cx--Visual cortex; Hypo--Hypothalamus; Assoc Cx--Association Cortex; Med Gen--Medial geniculate; Inf Olive--Inferior olive; Inf Coll--Inferior colliculus; Par Cx--Parietal cortex
CS--Conditioned stimulus; US--Unconditioned stimulus; CR--Conditioned response

of classical conditioning procedures by these studies must be questioned. The reported evoked potential changes could be attributed to general excitatory or motivation changes due to the presentation of the US. This type of general excitatory changes was found in a study by Caviedes and Bures (1970), who reported an increase in amplitude of the sensorimotor cortical evoked potential during both pseudoconditioning and appetitive conditioning. Other experiments have reported amplitude changes in evoked potentials to stimuli presented randomly throughout conditioning (Khachaturian & Gluck, 1969).

Studies which have used pseudoconditioning control groups have found that amplitude changes develop in evoked potentials elicited by the CS+ during either appetitive (Caviedes & Bures, 1970; Segal, 1977) or aversive (Khachaturian & Gluck, 1969; Segal, 1977) conditioning procedures. Evoked potential recordings were from occipital and parietal cortex, thalamus, hippocampus, and tegmentum. Analogous evoked potential changes were not apparent in recordings elicited by the stimulus in the unpaired control group. Segal (1977) found ambiguous results in hippocampal recordings. With appetitive conditioning procedures, 46% of the rats demonstrated a generalized increase in evoked potential amplitude. Using aversive conditioning, 38% of the rats demonstrated an increase in evoked potential amplitude, 38% demonstrated a decrease in amplitude, and the remaining animals showed no change due to conditioning.

When differential conditioning procedures were used, overall amplitude changes have been reported to develop in evoked potentials elicited by the CS+ but not in the evoked potentials elicited by the

CS-. Hearst, Beer, Sheatz, and Galambos (1960) found an overall increase in evoked potential amplitude (in recordings from four brain areas) to the CS+, but no amplitude change in the evoked potential elicited by the CS-. Using somatic stimulation as CS+ and CS-, Rosenblum (1977) reported an initial increase in overall evoked potential amplitude to the CS+, but not the CS-. During succeeding trials, however, the amplitude of the centromedian evoked potentials elicited by both the CS+ and CS- decreased. Other experimenters have measured changes in specific peak-to-peak amplitudes during differential conditioning. Sugawara, Kitajima, and Konoh (1977) found decreases in evoked potential components with a latency of 100-180 msec and 180-250 msec. The progressive decrease in amplitude developed in the cortical evoked potentials elicited by the CS+ during both appetitive and aversive conditioning procedures. No change was reported in the CS- elicited evoked potential. Begleiter and Platz (1969) found an increase in the amplitude of the long latency cortical evoked potential component (150-160 msec) elicited by the CS+ during differential sensory-sensory conditioning. Hudspeth and Jones (1978) reported the appearance of a new long latency (150-200 msec) component during aversive conditioning procedures in CS+ elicited evoked potentials from nuclei in the thalamus, reticular formation, and cortex.

Based on the studies cited above, it can be concluded that classical conditioning procedures elicit changes in auditory, visual, or somatic evoked potentials in cortical and subcortical brain areas. The predominant effect of conditioning appears to be an augmentation of evoked potential amplitude for aversive, appetitive, or sensory

classical conditioning procedures. However, a decrease in evoked potential changes has primarily been in the late components. The changes correlated with conditioning have been recorded in most areas of the brain.

Changes in evoked potential amplitude have also been studied during instrumental conditioning procedures (see Table 9). Generally, animals were taught the instrumental task, and then the discriminative stimuli were introduced. Changes in evoked potentials were monitored during discriminative conditioning of the experimental task. As with the classical conditioning experiments, increases, decreases, and no changes in evoked potential amplitude were reported from recordings taken from various regions of the brain during appetitive or aversive conditioning. Most recordings have been from cortical areas, but some subcortical recordings were reported for cochlear nucleus, optic chiasma, centromedian, reticular formation, medial geniculate, hippocampus, lateral lemniscus, and internal capsule (see Table 9).

The majority of experiments using one discriminative stimulus (S+) have reported an increase in evoked potential amplitude during both appetitive and shock avoidance paradigms (Andry & Luttges, 1976; Boyd, Boyd, & Brown, 1977a; Grinberg-Zylberbaum, Prado-Alcala, & Brust-Carmona, 1973). Both Andry and Luttges (1976) and Grinberg-Zylberbaum et al. (1973) found that the increase in evoked potential amplitude occurred only when the animal made a correct response. The evoked potential components which increased in amplitude were reported to be both early and late, with latencies around 25-100 msec and 100-250 msec (Andry & Luttges, 1976; Boyd, Boyd, & Brown, 1977a).

Only one study reported a decrease in evoked potential amplitude during both shock avoidance and appetitive conditioning when using one discriminative stimulus (Hearst, Beer, Sheatz, & Galambos, 1960). The appearance of the conditioned evoked potential response was correlated with the elicitation of the correct behavioral response. The latency of the evoked potential component that contained the conditioned response was not reported.

Studies which have used both an S+ and S- for discriminative conditioning have reported the development of differential changes in evoked potentials elicited by the S+ and the S- (Cherubini, Bilancia, & Franco-Ricci, 1976; Majkowski & Sobieszek, 1975; Saito, Yamamoto, Iwai, & Nakahama, 1973; Saunders, 1971). Both Saito et al. (1973) and Cherubini et al. (1976) used trains of flashing lights at different frequencies or intensities for the S+ and S-. Cherubini et al. (1976) used a probe stimulus to elicit evoked potentials during the presentation of the S+ and S-, whereas Saito et al. (1973) recorded evoked potentials in response to the S+ and S- presentations. For both studies evoked potential changes, as compared to baseline evoked potentials, occurred to the probe stimulus during the S+ or S- presentation or to both the S+ and S- at the onset of the stimulus train. As the stimulus train continued, changes in amplitude occurred only during the S+ presentation in the probe-elicited evoked potentials or in the S+ elicited evoked potentials, just prior to the initiation of the appetitive instrumental response. Cherubini et al. (1976) reported a decrease in the probe elicited evoked potential amplitude, whereas Saito et al. (1973) reported an increase in amplitude in the S+ elicited evoked potentials recorded from the visual cortex. Both

studies reported that the evoked potential components which were modified were long latency, approximately 100-300 msec. Only a study by Jasper, Ricci, and Doane (1960) reported no differentiation in the decrease in evoked potential amplitude elicited by the S+ or S- during shock avoidance conditioning. Majkowski and Sobieszek (1975) have also reported that a differentiated decrease in evoked potential amplitude was not correlated with the conditioned avoidance response.

Hall and Mark (1966) attempted to determine the underlying factors in the evoked potential changes elicited during instrumental conditioning. The experiments included shock avoidance, appetitive, and conditioned emotional response conditioning. In order to rule out movement as the underlying factor for the increase amplitude changes, the animals were trained to lever press at equal rates under appetitive or aversive reinforcement, or required to sit still for food reinforcement. The changes in evoked potential components were larger, with the aversive reinforcement task that was shock avoidance. In all cases, the change in evoked potential amplitude was correlated with the elicitation of the behavioral response. The changes seen in the long latency components of the evoked potential were concluded to be related to an increased arousal or fear, not to movement or acquired conditional properties of the stimulus (Hall & Mark, 1966). This interpretation, as previously discussed, has also been presented as an explanation for the evoked potential changes found during classical conditioning procedures.

Other experimenters have argued, however, that the evoked potential changes which develop during both classical and instrumental conditioning procedures cannot be accounted for exclusively by

nonspecific arousal or fear (Begleiter & Platz, 1969; Boyd et al., 1977a; Saunders, 1971). The changes in the evoked potentials that develop during conditioning were stated to be fundamental to the acquired incentive value of the stimulus due to conditioning. This interpretation was based on the results obtained from differential conditioning and pseudoconditioning procedures used during classical and instrumental conditioning. As reviewed above, evoked potential amplitude changes occurred to the CS+ or S+ and not to the unpaired control stimulus, the CS- or S-. If the changes were due only to arousal mechanisms, the evoked potential changes should have developed to all stimuli.

Table 9

Instrumental Conditioning Effects on EPs

| Authors | Subjects | S+ | S- | Probe | IR | Reinf. | Area | CR |
|---|---|---|---|---|---|---|---|---|
| Andry & Luttges (1976) | 64 mice | Tones or LF | --- | --- | Turn wheel | Avoid shock | Cx | ↑ Amp 25-100 msec 101-250 msec |
| Boyd, Boyd & Brown (1977a) | 12 M | Tone | --- | --- | Lever press | Food | Post-arcuate Cx Post-central Cx | ↑ Amp 100-200 msec |
| Cherubini, Bilancia & Ricci (1976) | 5 C | Strong light | Dim light | ES-LGN | Lever press | Food | Vis Cx | ↑ Amp to probe during S+ & S- ↓ Amp to probe during S+ prior to Resp |
| Grinberg-Zylberbaum, Prado-Alcala & Brust-Carmona (1973) | 8 C | Clicks | --- | --- | Sitting | Milk | CN CM | ↑ Amp |
| | 5 C | LF | --- | --- | Walk to feeder | Milk | CN CM | ↑ Amp |
| Hall & Mark (1966) | 6 R | LF | --- | Click ES-cochlear | CER | Shock | Aud Cx IC Lat Lem | ↑ Amp 25-45 msec |
| | 6 R | LF | --- | Click | Bar press | Avoid shock/ food | Aud Cx MGN RF | ↑ Amp |

| Authors | Subjects | S+ | S- | Probe | IR | Reinf. | Area | CR |
|---|---|---|---|---|---|---|---|---|
| Hall & Mark (cont.) | 6 R | Light | --- | Click | Sitting still | Food | Aud Cx<br>IC<br>MGN | ↑ Amp to probe during S+<br>Late lat |
| Hearst, Beer, Sheatz & Galambos (1960) | 1 M | Clicks | --- | --- | Lever press | Food | CN<br>Hippo<br>MGN<br>Coch N | ↓ Amp |
| | 1 M | Clicks | --- | --- | Lever press | Avoid shock | Aud Cx<br>Hippo<br>MGN<br>Coch N | ↓ Amp |
| Jasper, Ricci & Doane (1960) | 4 M | LF | LF | --- | Paw flex | Avoid shock | Vis Cx | ↓ Amp to both S+ and S-<br>Short lat |
| Majkowski & Sobieszek (1975) | 8 C | LF or Clicks | LF or Clicks | --- | Leg flex | Avoid shock | Vis Cx | New AEP component<br>↓ Amp overall |
| | | | | | | | Aud Cx | ↓ Amp |
| | | | | | | | RF | No effect |
| Mark & Hall (1966) | 3-8 R | LF | Clicks | --- | CER | Shock | Aud Cx | ↑ Amp 25-45 msec |
| | | | | | | | RF | ↑ Amp |
| | | | | | | | MGN | ↑ Amp 5-20 msec<br>22-57 msec |

| Authors | Subjects | S+ | S- | Probe | — | Reinf. | Area | CR |
|---|---|---|---|---|---|---|---|---|
| Saito, Yamamoto, Iwai & Nakahama (1973) | 4 M | LF | LF | --- | Pull lever | Food | Vis Cx | ↓ Amp - S+ & S- at beginning |
| | | | | | | | | ↑ Amp - S+ before R |
| | | | | | | | LGN | ↑ Amp - S+ & S- at beginning |
| | | | | | | | | ↑ Amp - S+ only before R |
| Saunders (1971) | 5 C | Clicks LF | Clicks | --- | Tilt box | Avoid shock | Vis Cx | ↑ Amp - S+ ↓ Amp - S- |
| | | | | | | | Aud Cx | ↑ Amp - S+ only |
| | | | | | | | Coch N | No effect |
| | | | | | | | OC | No effect |

Abbreviations: R--rat; M--monkey; C--cat; LF--light flash; CER--conditioned emotional response; ES--electrical stimulation; CX--cortex; CN--caudate nucleus; CM--centromedian; IC--internal capsule; Lat Lem--laternal lemniscus; MGN--medial geniculate nucleus; RF--reticular formation; Vent coch--ventral cochlear nucleus; Hippo--hippocampus; LGN--laternal geniculate nucleus; OC--optic chiasma
IR--Instrumental response; CR--Conditioned response; S--Discriminative stimulus

# APPENDIX B

## Three Sequences of CS+ and CS- Presentations

| I | | II | | III | |
|---|---|---|---|---|---|
| Trial Type | ITI (min) | Trial Type | ITI (min) | Trial Type | ITI (min) |
| CS- | 7 | CS+ | 5 | CS- | 7 |
| CS+ | 7 | CS+ | 7 | CS- | 13 |
| CS- | 5 | CS- | 11 | CS+ | 5 |
| CS- | 9 | CS- | 5 | CS+ | 11 |
| CS+ | 13 | CS- | 13 | CS- | 11 |
| CS+ | 9 | CS+ | 13 | CS+ | 7 |
| CS+ | 5 | CS+ | 11 | CS+ | 9 |
| CS= | 13 | CS- | 9 | CS- | 9 |
| CS- | 5 | CS+ | 13 | CS- | 5 |
| CS- | 7 | CS+ | 9 | CS- | 7 |
| CS+ | 5 | CS+ | 9 | CS+ | 13 |
| CS+ | 11 | CS- | 7 | CS+ | 7 |
| CS- | 11 | CS- | 13 | CS+ | 5 |
| CS+ | 9 | CS- | 5 | CS- | 5 |
| CS+ | 13 | CS+ | 11 | CS- | 11 |
| CS+ | 11 | CS+ | 7 | CS+ | 11 |
| CS- | 9 | CS- | 7 | CS+ | 13 |
| CS- | 13 | CS+ | 5 | CS- | 13 |
| CS+ | 7 | CS- | 9 | CS- | 9 |
| CS- | 11 | CS- | 11 | CS+ | 9 |
| $\bar{X}$ = | 9 | $\bar{X}$ = | 9 | $\bar{X}$ = | 9 |

Average ITI between morphine delivery and CS+ or CS- presentation

| | | |
|---|---|---|
| US - CS- = 9.8 | US - CS- = 8.6 | US - CS- = 9.0 |
| US - CS+ = 9.8 | US - CS+ = 8.6 | US - CS+ = 9.0 |

# APPENDIX C

## Vincentized Trial Blocks for Each Animal

| Rat | | No. of Trials/Block | | No. of Hours (Days) to Obtain 10 Blocks |
|---|---|---|---|---|
| 0.02 msec stimulation | | | | |
| F41 | | 20 | | 116 (4.83) |
| F43 | | 20 | | 108 (4.5) |
| F44 | | 20 | | 186 (7.75) |
| F45 | | 20 | | 130 (5.42) |
| | $\bar{X}$ | 20 | $\bar{X}$ | 125 (5.60) |
| | SEM | 0 | SEM | 35 (1.47) |
| 0.2 msec stimulation | | | | |
| F16 | | 40 | | 74 (3.08) |
| F20 | | 60 | | 120 (4.5) |
| F22 | | 40 | | 45 (1.88) |
| F28 | | 10 | | 54 (2.25) |
| F29 | | 30 | | 108 (4.5) |
| F30 | | 20 | | 29 (1.21) |
| F38 | | 40 | | 90 (3.75) |
| | $\bar{X}$ | 34.3 | $\bar{X}$ | 74 (3.0) |
| | SEM | 16.2 | SEM | 33 (1.30) |