EVOKED POTENTIAL CONDITIONING USING MORPHINE AS THE UNCONDITIONED STIMULUS IN RATS

by

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INTRODUCTION

The phenomenon of narcotic dependence has been recognized for centuries. Still, despite prolific theorizing by workers in several fields, one investigator has commented: "The hypotheses that have been advanced to explain these phenomena can be summarized briefly: Theories that have been tested have been disproven; theories now current are speculative." (Dole, 1970). While this position is somewhat overstated, it is true that there is little agreement on how best to explain the phenomena of opiate tolerance and dependence. Even more puzzling has been the repeated relapse to drug seeking behavior by human addicts who have been treated and were presumably no longer physically dependent on opiates.

Without regard to any particular theory, it is obvious that metabolic processes are involved in the development of tolerance and physical dependence. Evidence for this statement is provided by several experiments which show that metabolic inhibitors, including Actinomycin D, puromycin and cyclohexamide, will inhibit the development of tolerance to narcotic drugs without blocking the action of narcotics in nontolerant animals (Cox, Ginsburg, & Osman, 1968; Smith, Karmin, & Gavit, 1966; Way, Loh, & Shen, 1968). In addition, cyclohexamide has been shown to block the development of physical dependence on morphine (Zarofonetis, 1972). Evidence such as this has led to the development of several biochemical models of opiate dependence (Dole, 1972).

The majority of these theories invoke morphine effects on the feedback control of some neurotransmitter concentration to explain tolerance and the effects of acute withdrawal (Schuster, 1961; Goldstein & Goldstein, 1961; Goldstein & Goldstein, 1968). Dole (1970) has explained these "enzyme expansion" and derepression hypotheses as follows:

> "The general assumption of these theories is that narcotic drugs either inhibit or derepress some enzyme that synthesizes neurotransmitters, while reciprocally the synthesis of the enzyme is depressed by the accumulation of transmitter. If, for example, morphine inhibits an enzyme that synthesizes a transmitter essential for perception of pain or for sensitivity of the respiratory center, the first effect of the drug is to reduce the concentration or availability of this substance (leading to its analgetic and other narcotic effects). With continued exposure to the narcotic drug, the quantity of the enzyme expands by increase in the rate of enzyme synthesis, which is no longer depressed by the transmitter, and so restores the normal rate of transmitter production with an enlarged pool of enzyme (tolerance). If the drug is suddenly withdrawn, the excess quantity of enzyme, no longer inhibited, produces an excess of transmitter substance (abstinence)."

While these theories are provocative, they are supported principally by analogy and indirect evidence, though they fit fairly well with the effects of metabolic inhibitors cited above. In addition, they do not account for the very long persistence of tolerance and abstinence symptoms following withdrawal (Wikler, 1965; Wikler & Pescor, 1967; O'Brien, 1975). It is true that a complete understanding of narcotic dependence will require a definition of the biochemical events which mediate the development of tolerance and dependence, as well as the persistent changes which can account for relapse following withdrawal.

However, at present the most productive theoretical approach in terms of implications for both treatment and research is that which utilizes a conditioning model. As expressed in a recent review: "With the exception of conditioning theory, there is surprisingly little in the psychiatric or psychoanalytic literature that relates theory to current treatment approaches to narcotic addiction." (Khantzian, 1974).

To explain opiate addiction and relapse in terms of conditioning theory it has been postulated that drug-seeking behavior is reinforced both by the initial euphoric properties of the drug and later by the drug's ability to alleviate the dysphoric symptoms of withdrawal (Jaffe, 1970; Wikler, 1973). In this view it is hypothesized that, for the subject receiving an opiate injection, the external environment as well as interoceptive drug-produced stimuli serve as a compound conditioned stimulus. The drug presentation acts as the unconditioned stimulus, and the physiological and pharmacological effects of the drug as the unconditioned response. The conditioned response may be similar in form to the unconditioned response (Drawbaugh & Lal, 1974; Numan, Smith, & Lal, 1975; Numan, Banerjee, Smith, & Lal, 1976) or they may be in the opposite direction (Wikler, 1973). Thus, it follows that drug effects which are repeatedly produced in the presence of certain stimuli may become conditioned to those stimuli and so, elicitable by them. Since classically conditioned morphine abstinence phenomena have been shown to persist for several months following withdrawal from the drug (Wikler, 1965; Wikler & Pescor, 1967; Goldberg & Schuster, 1970), it is believed that conditioning theory is also relevant to the problem of addict relapse.

The first conditioning studies to employ opiate injections as an unconditioned stimulus were described by Pavlov:

> "A dog was given a small dose of apomorphine subcutaneously and after one or two minutes a note of a definite pitch was sounded during a considerable time. While the note was still sounding the drug began to take effect upon the dog: the animal grew restless, began to moisten its lips with its tongue, secreted saliva and showed some disposition to vomit. After the experimenter had reinforced the tone with apomorphine several times it was found that the sound of the note alone sufficed to produce all the symptoms of the drug, only in a less degree." (Pavlov, 1927, pg. 35).

Additional research by Krylov using morphine as the unconditioned stimulus, suggested that even if there is no explicit conditioned stimulus, the mere preparations for the injection of the drug could produce many of the effects of the morphine (Pavlov, 1927, pp. 35-37). Classical conditioning of the agonistic effects of morphine has been replicated subsequently in numerous studies. These studies, which are enumerated in a recent review by Lynch, Stein, and Fertziger (1976), have consistently reported that classically conditioned responses are rapidly obtained when morphine is employed as the unconditioned stimulus.

The earlier investigators who utilized morphine as the unconditioned stimulus in their experiments were not directly concerned with the implications of their research for drug addiction, or even with morphine conditioning per se. They were simply using morphine as one of many tools to explore the newly described phenomenon of conditioning. Following Wikler's original paper (1948) the emphasis in morphine conditioning studies shifted, as investigators considered the possibility that the primary factor in the relapse of human addicts following

treatment was classical conditioning of the abstinence syndrome (Lynch, Stein, & Fertziger, 1976). Thus, since 1948 almost all opiate-conditioning experimentation has been directed toward elucidating the problems of human addiction and relapse.

In these studies it has been shown that stimuli which have been paired with morphine administration acquire the ability to elicit a large array of autonomic and behavioral responses. These responses include salivation (Collins & Tatum, 1925), nausea (Wang & Glaviano, 1954), sleep (Levitt, 1964), and the alleviation of the symptoms of acute withdrawal (Drawbaugh & Lal, 1974; Numan, Smith, & Lal, 1975; Roffman, Reddy, & Lal, 1973). In addition, it has been shown that stimuli which are paired with the effects of morphine withdrawal can come to elicit a wide range of behaviors as well, in both animals (Wikler, 1965; Goldberg & Schuster, 1970), and humans (O'Brien, Testa, O'Brien, & Greenstein, 1976).

Recently, it has been suggested that tolerance to the effects of morphine is also a learned response (Siegel, 1975). In Siegel's view, narcotic tolerance is the result of learning an association between the systemic effects of the drug and those environmental cues which reliably predict drug administration. Tolerance to the analgesic effect of morphine, then, would occur because environmental cues regularly paired with drug administration come to elicit a compensatory conditioned response, hyperalgesia, which counteracts the unconditioned effects of the morphine. In support of this position Siegel has shown that: (1) it is necessary to have a consistent set of environmental

cues reliably predicting the systemic effects of morphine if rapid tolerance is to be observed; (2) experience with morphine in one environment does not facilitate the acquisition of morphine tolerance in another environment; (3) the compensatory hyperalgesic conditioned response may be directly observed in tolerant subjects when they are exposed to the drug administration ritual not followed by the central effects of the drug; and (4) presentation of those environmental cues previously associated with the narcotic, when presented with a placebo, is an effective procedure for extinguishing established morphine tolerance. More recent work by Siegel (1976) has confirmed and expanded these results.

Additional support for Siegel's position has been provided by the reported ability of drugs which are believed to inhibit memory formation to also inhibit the development of tolerance to morphine (Cox, Ginsburg, & Osman, 1968; Smith, Karmin, & Gavit, 1966; Way, Loh, & Shen, 1968). Support has also been provided by Stolerman, Bunker, Johnson, Jarvik, Krivoy, and Zimmermann (1976) who reported that electroconvulsive shock can block the development of tolerance to morphine. They administered electroconvulsive shock to mice two to three hours after each of six treatments with morphine and reported that this procedure significantly reduced the amount of tolerance which developed. Based on studies which have indicated that electroconvulsive shock can disrupt memory formation, Stolerman and his associates interpreted their results as supporting the view that the mechanisms of morphine tolerance are similar to those involved in learning and memory. While this evidence

is suggestive, it should be borne in mind that electroconvulsive shock and metabolic inhibitors affect many brain processes other than memory. In addition, the interval between morphine injection and shock delivery in the Stolerman study was much greater than that typically employed in more conventional studies of the effects of electroconvulsive shock on memory. In fact, electroconvulsive shock usually does not disrupt memory if given more than a few minutes following training.

Evidence that learning is not the only factor involved in the development of tolerance to morphine has been reported by Sklar and Amit (1978). They tested the effect of conditioning on tolerance to a lethal dose of morphine in rats and found no evidence that this tolerance was mediated by conditioning. They stated: "In contrast to the findings of Siegel (1975), these data suggest that tolerance to morphine is not due to a compensatory response, since obscuring the correlation between environmental stimuli and morphine injections did not attenuate the tolerance."

A more direct experimental test of the conditioning theories of opiate dependence which have been presented by Wikler (1972), Copeman (1975), and Goldberg (1976) has been provided by the work of Davis and Smith (1974; 1976). Davis and Smith (1974) provided important evidence that a stimulus which had been paired with morphine administration could reinstate drug-seeking behavior following the elimination of that behavior by the removal of primary pharmacological reinforcement. In this experiment rats were implanted with an indwelling cannula

and placed in chambers provided with a bar for self-injection. Each lever-press was followed by a 0.2 second buzzer presentation and a concomitant 0.2 second infusion of morphine solution. Following acquisition of the bar-press response for morphine reinforcement, extinction was carried out both with and without the buzzer present. The subjects were subsequently tested for response to the buzzer and it was found that the group which had the buzzer present during extinction showed no resumption of bar-pressing, whereas the group which was extinguished without the buzzer resumed bar-pressing when the buzzer was again presented. These results are in accord with the findings of Thompson and Ostlund (1965) that rats which were withdrawn in the same environment in which they had been addicted were less likely to exhibit relapse than rats which were withdrawn in a novel environment.

In a subsequent paper, Davis and Smith (1976) reported that: "Pavlovian pairings between a neutral environmental stimulus and a primary reinforcer were sufficient for the establishment of a secondary reinforcer which could cause an increase in the lever response." This finding indicates that a stimulus may acquire the properties of a secondary reinforcer simply through being repeatedly paired with morphine administration, in the absence of a response contingency. Davis and Smith (1976) further maintain that this conditioning was based solely on the positive reinforcement provided by morphine injections since the drug dosage they employed was not sufficient to produce dependence in their subjects. This result is interesting since it bears directly

on the important theorectical position of Wikler (1972). While stimuli which are paired with morphine abstinence in dependent subjects strongly influence subsequent behavior (Wikler, 1965; Goldberg & Schuster, 1970), Davis and Smith have shown that this is also true for stimuli which are paired with morphine administration in the absence of dependence and so, abstinence effects.

Stimuli which have been associated either with opiate self-administration (Teasdale, 1973) or with naloxone-precipitated withdrawal (O'Brien, 1976) have also been shown to be capable of eliciting behavioral effects in human patients. At least two conclusions may be drawn from this large body of literature on morphine and conditioning. First, it is abundantly clear that morphine can act as a powerful primary reinforcer such that stimuli paired either with its administration or its withdrawal from dependent subjects acquire the ability to modify and control subsequent behavior. In doing so these stimuli may act both as conditioned stimuli and as secondary reinforcers. It follows from this, that one should be able to observe conditioned neural responses within the central nervous system to stimuli which have been paired with morphine administration. The mapping of such responses within the neuraxis and an examination of their development over time could provide useful information on the role played by various brain areas in the formation of conditioned responses to stimuli which have been associated with drug administration.

To date no such study of conditioned neural responses to drug-related stimuli in various brain areas has been reported. There is,

however, a large collection of studies concerning the unconditioned effects of opiates on the nervous system, which is necessarily preliminary to any examination of neural conditioning and opiates. The effect of morphine on the firing of single neurons in various areas of the central nervous system has been examined by several investigators. In general, the results of these studies reveal that the effect which is found depends upon the region of the brain examined and the previous drug history of the subject.

In spinal neurons of naive rats, morphine iontophoresis increased the excitatory response to acetylcholine and homocysteate while reducing the depressant effect of glycine (Lodge, Headley, Duggan, & Biscoe, 1974). Bramwell and Bradley (1974) found that morphine increased spontaneous firing in 38% of the brainstem neurons they recorded, while decreasing firing rates in 18%. However, they also reported that only the morphine-produced depression was reversible by naloxone application. In naive rats morphine was found to decrease the firing rate of anterior hypothalamic neurons. In rats made morphine dependent, however, the drug caused an increase in neuronal firing in this same area (Eidelberg & Bond, 1972). Kerr, Triplett and Beeler (1974) have illustrated a reciprocal effect of morphine in two hypothalamic nuclei. They found that an intravenous injection of morphine increased the firing rate of neurons in the ventromedial hypothalamus approximately tenfold while simultaneously decreasing the firing rate in the lateral hypothalamic area by half. Both these effects were reversed by naloxone. Unlike Eidelberg and Bond (1972), these investigators found that the direction

of morphine's effect was the same in dependent subjects, although the magnitude of the effect was reduced. In the cortex it has been reported that morphine acts to depress spontaneous neuronal firing rates (Satoh, Zieglgansberger, & Herz, 1975; 1976; Satoh, Zieglgansberger, Fries, & Herz, 1974; Kerr, Triplett, & Beeler, 1974), as well as neural responsiveness to stimulation of the sciatic nerve (Biscoe, Duggan, & Lodge, 1972) and to acetylcholine or l-glutamate iontophoresis (Satoh, Zieglgansberger, & Herz, 1975). A summary of morphine effects on neuronal firing rates is presented in Table 1.

When evoked potentials, rather than single unit firing rate, have been used as the measure of neural activity, morphine has been found to depress responding in most subcortical areas that have been studied. These areas include the periaqueductal gray, medial reticular formation and entorhinal cortex (Straw & Mitchell, 1964; Nakamura & Mitchell, 1972) as well as the associative thalamic nuclei (dorsomedial, dorsolateral and lateral posterior nuclei) and the centromedian and parafascicular nuclei (Sinitsin, 1964). In contrast, morphine has been shown to increase the amplitude of auditory evoked potentials in the caudate nucleus of rats (Dafny & Burks, 1976).

The results of these studies are in fair agreement with the results of the previously cited single-unit studies. However, the effects of morphine on cortical evoked responses have been found to be excitatory, in direct contrast to its reported effects on single cortical neurons. Using rats, Jurna, Schlue and Tamm (1972) found that 2 mg/kg of morphine given intravenously increased the amplitude of both positive and negative

TABLE 1

Effects of Morphine on Single Unit Firing

Authors	Date	Species	Drug Hist.	Dose	Route	Area Studied	Stimulus	Effect
Biscoe, Duggan, & Lodge	1972	Rat	N	2-12 mg/kg	IV	Cortex	Sciatic nerve	0
Lodge, Headley, Duggan, & Biscoe	1974	Rat	N	10-30 nA	ion.	Spinal cord	V. root, ACh, DLH	+
Kerr, Triplett, & Beeler	1974	Rat	N	10 mg/kg	IV	VMH, Amygdala	Somatic	+
						LHA, Cortex, VM, ZI	Somatic	-
			D	75 mg/kg	IV	VMH, Amygdala	Somatic	+
						LHA, Cortex, VM, ZI	Somatic	-
Korf, Bunney, & Aghajanian	1974	Rat	N	10 mg/kg	IV	Locus coerulius	Somatic	-
						Dorsal raphe	Somatic	0
Eidelberg & Bond	1972	Rat	N	3-5 mg/kg	IV	Ant. Hypothalamus	Somatic	-
			D	3-5 mg/kg	IV	Ant. Hypothalamus	Somatic	+
Bramwell & Bradley	1974	Rat	N	50 nA for 60-120 s.	ion.	Brainstem	Somatic	-

TABLE 1 (cont.)

Authors	Date	Species	Drug Hist.	Dose	Route	Area Studied	Stimulus	Effect
Satoh, Zieglgansberger, Fries, & Herz	1974	Rat	N	50 nA for 120 s.	ion.	Cortex	Somatic	-
			D	50 nA for 120 s.	ion.	Cortex	Somatic	+
Satoh, Zieglgansberger, & Herz	1975, 1976	Rat	N	50 nA for 120 s.	ion.	Cortex	Somatic	-
							ACh, L-glutamate	-
			D	50 nA for 120 s.	ion.	Cortex	Somatic	+*
							ACh, L-glutamate	0
Satoh, Zieglgansberger, & Herz	1976	Rat	N	>50 nA for >120 s.	ion.	Cortex	Somatic	+*

Abbreviations used in Table 1: N=naive; D=dependent; IV=intravenous; ion.=iontophoresis; VMH=ventromedial nucleus of hypothalamus; LHA=lateral hypothalamic area; VM=ventromedial nucleus of thalamus; ZI=zona incerta; V. root=ventral root shock; ACh=acetylcholine; DLH=D, L homocysteate; 0=equivocal, or no response; +=increase in firing rate; -=decrease in firing rate; *=effect not antagonized by naloxone.

components of the response to radial nerve stimulation in somatosensory cortex without affecting peak latencies. These investigators also found, in the same experiment, that morphine increased the amplitude of the direct cortical response. In cats, Sinitsin (1964) found that 5-10 mg/kg of morphine given intravenously augmented responses to somatic, visual, and auditory stimuli in their respective primary sensory areas. It also increased the response to these stimuli in the motor cortex and increased responses to non-primary stimuli in the primary somatosensory and auditory cortex. On the basis of these results both Sinitsin (1964) and Jurna Schlue and Tamm (1972) suggested that morphine increases the excitability of cortical neurons. The effects of morphine on evoked potentials is summarized in Table 2.

It has also been shown in numerous experiments that evoked potentials are modified by classical conditioning procedures (John, 1961; Morrell, 1961; Galeano, 1963). Both aversive and appetitive stimuli have been used effectively as unconditioned stimuli in evoked potential conditioning experiments. Changes in evoked potentials recorded from several brain areas during habituation, conditioning, and extinction have been examined in a series of experiments by Robert Galambos and his associates (Hearst, Beer, Sheatz, & Galambos, 1960; Marsh, McCarthy, Sheatz, & Galambos, 1961; Galambos & Sheatz, 1962). In these studies, classical conditioning using either food, air puff, or shock presentations as the US was found to result in conditioned increases in click or light evoked potentials. Changes in these evoked potentials were found in locations along the classical sensory

TABLE 2

Effects of Morphine on Evoked Potential Amplitude

Authors	Date	Species	Drug Hist.	Dose	Route	Area Studied	Stimulus	Effect
Chin & Domino	1960	Dog	N	2-10 mg/kg	IV	MRF, MD, CM, SPf	Tooth pulp	+
						Somatosensory Cx.	Tooth pulp	0
McKenzie & Beechey	1962	Cat	N	1-6 mg/kg	IV	Midbrain	Tibial nerve	-
Straw & Mitchell	1964	Cat	N	1-4 mg/kg	IV	VPM	Tooth pulp	0
						PAG & MRF	Tooth pulp	-
Sinitsin	1964	Cat	N	1-3 mg/kg	IV	SI, AI, VI	Sciatic nerve, Flash & Click	0
				5-10 mg/kg	IV	SI, AI, AII	"	+
						Motor Cortex	"	+
						VI, VII	Sciatic nerve & Click	-
				1-3 mg/kg	IV	Assoc. Cortex	Sciatic nerve	-
						MD, LP, LD	"	-
						Assoc. Cortex	Flash & Click	+
						LG, MG, VPL	Sciatic nerve, Flash, & Click	0
				5-10 mg/kg	IV	CM & Pf	Sciatic nerve	-

TABLE 2 (cont.)

Authors	Date	Species	Drug Hist.	Dose	Route	Area Studied	Stimulus	Effect
Jurna, Schlue, & Tamm	1972	Rat	N	2 mg/kg	IV	Somatosensory Cx.	Radial nerve	+
							DCR	+
Nakamura & Mitchell	1972	Cat	N	1-4 mg/kg	IV	Entorhinal Cx. & MRF	Somatic	-
Nakamura & Mitchell	1973	Cat	N	1-4 mg/kg	IV	Entorhinal Cx.	Pyriform Cortex	0
Dafny & Burks	1976	Rat	N	50 mg/kg	IP	Caudate	Click	+
				10 & 30 mg/kg	IP	Caudate	Click	0
				10, 30, & 50 mg/kg	IP	Substantia Nigra	Click	0
Gildenberg, Murthy, Adler, & Frost	1976	Rat	N	2-8 mg/kg	IV	Pf	Sciatic nerve	-
						CG	Sciatic nerve	0
			D	2-8 mg/kg	IV	Pf	Sciatic nerve	-
						CG	Sciatic nerve	0
			WD	2-8 mg/kg	IV	Pf	Sciatic nerve	+-
						CG	Sciatic nerve	-

Abbreviations used in Table 2: WD=withdrawn; IP=intraperitoneal; MRF=mesencephalic reticular formation; MD=dorsomedial nucleus of thalamus; CM=nucleus centromedian; SPf=sub-parafascicular nucleus; VPM=nucleus ventralis posteriomedialis; PAG=periaqueductal gray; LP=lateral posterior nucleus; LD=dorsolateral nucleus of thalamus; LG=lateral geniculate; MG=medial geniculate; VPL=nucleus ventralis posteriolateralis; others as in Table 1.

pathways and in areas not normally considered to have sensory functions (Hearst et. al., 1960; Galambos & Sheatz, 1962). These authors also reported that operant conditioning procedures were ineffective in altering evoked potentials (Hearst et. al., 1960). However, more recent studies have shown that evoked potentials are subject to operant control as well (Rosenfeld, Hetzler, Birkel, Kowatch, & Antoinette, 1976). It has also been shown that evoked potentials may be altered by classical conditioning in paralyzed subjects, in the absence of overt behavioral responses (Galambos & Sheatz, 1962; Rosenblum & O'Brien, 1977).

In all of the classical conditioning studies cited above, the effect of the conditioning procedure was to increase the amplitude of certain components of the evoked potential waveform. In some cases these increases have been attributed to general increases in excitability of the region from which the recordings were made (Segal, 1977; Cherubini, Bilancia, & Ricci, 1976). However, other experiments have employed differential conditioning paradigms, and found the increases in evoked potential amplitude to be limited to the reinforced stimulus (Hearst et. al., 1960; Rosenblum & O'Brien, 1977). Increases are not universal, however. In a recent study on human subjects, during discrimination eyelid conditioning the late components of the visual evoked potential were found to decrease in amplitude in response to the reinforced conditioned stimulus, but not to the non-reinforced stimulus (Sugawara, Kitajima, & Kanoh, 1977). Thus, it appears that changes in neural activity produced by conditioning may be reflected

by systematic increases or decreases in the amplitude of evoked potentials. It is also not uncommon, in studies of evoked potentials and conditioning, to find that the amplitude of the response increases initially with conditioning but then returns toward baseline with over-training (Fleming, 1967).

It has been reported that the earliest components of sensory evoked potentials are relatively resistant to modification, while later components are more labile (John, 1961; Boyd, Boyd, & Brown, 1977; Sugawara, Kitajima, & Kanoh, 1977). However, even very early evoked potential components (latencies from 12-70 ms) have been successfully modified by classical (Chandler & Liles, 1977; Fleming, 1967) and operant (Rosenfeld et. al., 1976) conditioning procedures. Despite widely differing methodologies, recording sites, and stimuli employed, these studies provide a remarkable consensus. It is clear that sensory evoked potentials may be reliably altered by classical conditioning procedures utilizing a variety of CS-US combinations.

In addition to its effects on neural responding, morphine also has been shown to increase the turnover of dopamine in rat brains (Costa, Carenze, Guidotti, & Revuelta, 1973). Perez-Cruet (1976) has shown that this alteration of putative neurotransmitter metabolism by morphine can be brought under the control of a conditioned stimulus. When morphine injections were preceded by a buzzer for a minimum of ten trials spaced over at least two weeks, it was found that presentation of the buzzer and a saline injection could produce an increase in dopamine turnover. This increase was not found in animals which had

received an equivalent number of morphine injections but no previous buzzer presentations. These results suggest that brain processes, just as autonomic and behavioral responses, may be conditioned using a Pavlovian paradigm with morphine as the unconditioned stimulus.

The only other study which has investigated conditioned neural responses based on morphine as the unconditioned stimulus was recently reported by Stein, Lynch, and Ruchkin (1977). In this experiment an attempt was made to condition the cortical evoked response to an auditory stimulus by pairing the stimulus presentation with an intravenous infusion of morphine. Each experimental session consisted of two trials, separated by 15 to 30 minutes. During each trial a two-minute train of clicks and a 30 second infusion of morphine was presented. The order of presentation was: one minute of clicks, 30 seconds of clicks overlapping the morphine infusion, and 30 seconds of clicks following the termination of morphine infusion. Prior to the beginning of conditioning, each subject received five habituation sessions, during which saline was injected in place of morphine. Each subject was then given four blocks of conditioning sessions, each block consisting of from four to eight conditioning sessions followed by a probe session during which saline was injected instead of morphine.

In this experiment the unconditioned effect of morphine was to increase the amplitude and latency of the evoked potential peaks. This effect was seen primarily on the longer latency peaks. After as few as four morphine conditioning sessions, the response to click alone was altered from its habituation form. In three of the four

evoked potential peaks examined a significant change in the amplitude of the response to the conditioned stimulus occurred over trials. This conditioned effect on the evoked potentials was in the same direction as that produced by the unconditioned stimulus, morphine infusion. From this evidence the authors concluded that: "The major findings of this investigation support the hypothesis that morphine, acting as an unconditioned stimulus, can form classical conditional responses in the rat which are manifest in the behavior of evoked potentials elicited by the conditional stimulus." (Stein, Lynch, & Ruchkin, 1977).

The studies cited above, when taken together, provide good evidence that learning processes play a pivotal role in the expression of morphine's effects. It has long been recognized that morphine can act as a very effective unconditioned stimulus in a variety of classical conditioning paradigms (Pavlov, 1927; Kleitman & Crisler, 1927; Lynch, Fertziger, & Teitelbaum, 1973). In addition, a large number of experiments has demonstrated that conditioning plays a role in the development of opiate dependence (Wikler, 1965; O'Brien, 1975; Zarofonetis, 1972; Numan, Smith, & Lal, 1975; Davis & Smith, 1976) and tolerance (Way, Loh, & Shen, 1968; Siegel, 1975). Morphine has also been found to affect neural behavior at almost all levels of the central nervous system (Borison, 1971; Dafny & Burks, 1976; Biscoe, Duggan, & Lodge, 1972). Furthermore, there is evidence that, within the central nervous system, polysensory neurons may be especially affected by narcotic drugs (Biscoe, Duggan, & Lodge, 1972; Sinitsin, 1964).

This is particularly significant in view of evidence from the neural conditioning literature which suggests that polysensory neurons play an important role in conditioning processes (O'Brien, Wilder, & Stevens, 1977). Finally it has been demonstrated that morphine can be used as an unconditioned stimulus to classically condition neural responses as measured by evoked potentials (Stein, Lynch, & Ruchkin, 1977).

This last methodology has great potential for exploring the role of various brain regions in the development of morphine-reinforced conditioning. Unfortunately, Stein, Lynch and Ruchkin (1977) examined only cortical evoked responses. They also failed to explore the possibility that the conditioned evoked response persisted following withdrawal of their subjects from morphine.

Aim of the study

The objectives of the present experiment, then, were: (1) to determine the effects of intravenous morphine injections on somatosensory evoked potentials recorded from various regions of the rat's brain, and (2) to explore brain areas in addition to the cortex for their capacity to develop conditioned neural responses, in a paradigm using morphine as the unconditioned stimulus. For this purpose, recording electrodes were directed toward the periventricular gray region, the ventromedial hypothalamus, the paraventricular nucleus of the thalamus, and the cortex. These are all locations in which morphine has been shown to affect neural activity. In addition, all but the cortex have been implicated in the mediation of the analgesic

response to morphine. A further objective of the experiment was to determine the effects of withdrawal on the expression of any conditioned neural responses which were found. Finally, the experiment was particularly designed to differentiate between generalized, unconditioned effects of morphine on the central nervous system and specific, conditioned neural responses formed through association of a particular stimulus with the effects of the drug.

METHODS

Animal preparation

Surgical procedures were carried out on 50 male albino rats which weighed 220-250 grams at the time of surgery. All surgery was performed under pentobarbital anesthesia (50 mg/kg) supplemented with ether as necessary. The hair was removed from the subjects' chest and neck with electric clippers and the skin was sponged with Zephiran solution. A longitudinal incision was made over the right external jugular vein. Approximately 10 mm of the vein was exposed by blunt dissection and freed of connective tissue. The vein was tied off using 4-0 silk suture and a cut was made in its upper wall using small scissors. The cannula was filled with 5% heparin solution to prevent clotting and inserted into the vein until its tip was within approximately one millimeter of the heart. A loop of suture was passed around the vein just below the point at which the cannula was inserted and tied, being careful not to constrict the cannula lumen. A suture was taken through the muscles of the neck and tied to the PE 20 segment of the cannula to secure it in place. The tips of closed hemostatic forceps were passed under the salivary glands and opened to make a pocket into which the looped segment of the cannula could be inserted. An incision was made in the scalp and a pair of closed hemostatic forceps was inserted, passing subcutaneously behind the right foreleg to emerge from the incision over the jugular vein. The free end of the cannula was grasped with the forceps, pulled through the incision in the scalp,

cut to the proper length and plugged. The incision over the jugular vein was sutured and treated with Furacin powder. See Figure 1.

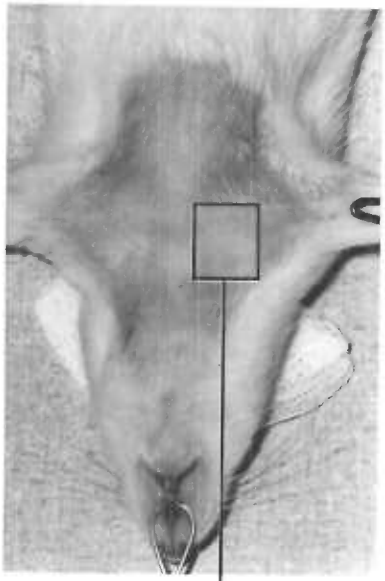
A bipolar stimulating electrode was then implanted in each foreleg. A horizontal incision about 10 mm long was made in the skin of the leg just proximal to the ankle. The skin was separated from the underlying muscle by blunt dissection and a suture was taken through the muscles of the leg at each end of the incision. Hemostatic forceps were inserted through the scalp incision and passed subcutaneously out through the incision in the leg. The electrode leads were grasped with the forceps and pulled through the scalp incision. The electrode tips, which remained in the leg, were then secured by the sutures previously placed in the leg muscles. It was necessary to insure that the electrode tips were well separated and held firmly in place. The incision in the leg was then closed and treated with Furacin.

After the stimulating electrodes were in place, the subject was mounted in a Kopf stereotaxic apparatus. The midline scalp incision, through which the cannula and stimulating electrodes were externalized, was enlarged and the surface of the skull was cleared of periosteum and allowed to dry. Using stereotaxic coordinates, small burr holes were drilled in the skull and four concentric bipolar recording electrodes were implanted. The electrodes were aimed for the cortex, the paraventricular nucleus of the thalamus (PVT), the ventromedial nucleus of the hypothalamus (VMH), and the periventricular gray region of the mesencephalon (PVG). The stereotaxic coordinates used were: PVT - AP: 3.6, L: 0.3, V: -0.5; VMH - AP: 6.2, L: 0.8, V: -3.0;

Figure 1. Sequence of steps in the cannula implantation procedure.

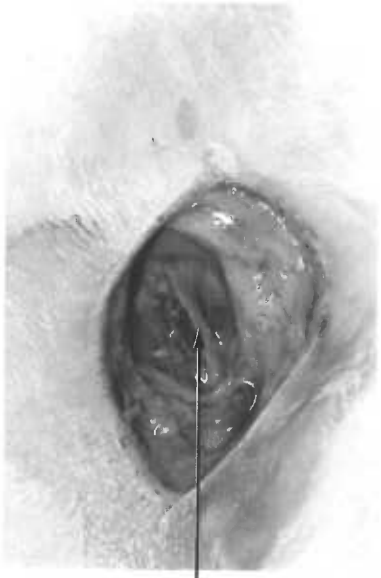
CANNULA IMPLANTATION

Subject Prepared For Surgery



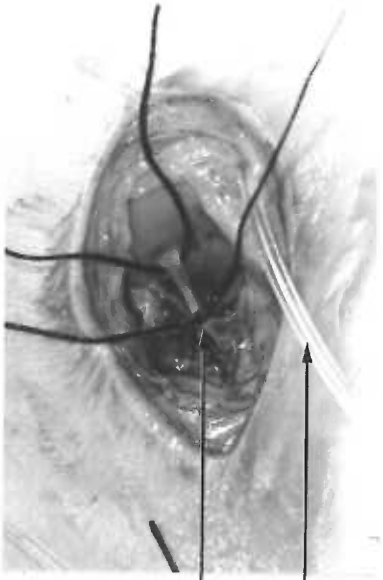
Location of Incision

Jugular Vein Exposed



External Jugular Vein

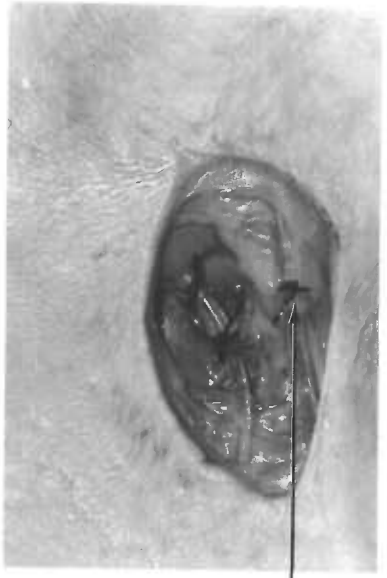
Vein Ligated Prior To Cannula Insertion



Ligature

Cannula

Cannula In Place



Suture Securing Cannula To Neck Muscles

and PVG - AP: -1.0, L: 1.2, V: -2.5 (Pelligrino & Cushman, 1967). Coordinates were referenced to stereotaxic zero and the incisor bar was set at +5 mm. The cortical electrode was placed one millimeter anterior to bregma and one millimeter lateral to the midline. It was lowered 1.0 millimeter from the surface of the dura. The cortical and VMH electrodes were placed in the left side of the brain, while the PVG and PVT electrodes were placed on the right. The electrodes were secured in place using dental acrylic. Four stainless steel screws were then placed around the perimeter of the skull and dental acrylic was built up around the electrodes and screws to form a firm base.

Leads from the recording electrodes were inserted into an Amphenol connector plug (222-12N31) which was affixed to the skull with dental acrylic. Leads from the stimulating electrodes were inserted into an Amphenol connector strip (221-1560). The connector strip and the exposed end of the jugular cannula were attached to the back of the head plug. The skin of the head was treated with Furacin powder and sutured around the head assembly. The subject was injected intramuscularly with 70,000 units of penicillin and allowed a minimum of four days to recover from surgery.

Recording and stimulating electrodes

The concentric bipolar recording electrodes consisted of teflon insulated stainless steel wire, 0.127 millimeter in diameter, inside a barrel cut from 20 ga hypodermic stock. The electrodes were insulated with four coates of Epoxylite. The insulation was scraped from the barrel in a ring one millimeter from its end and the stainless steel wire was cut so that it extended 0.5 millimeters from the barrel. The electrode

leads were soldered into male Amphenol pins (220-P02) for insertion into the head plug. Electrodes were constructed in three lengths for use in the various brain placements. Those used in the hypothalamus were 9.0 mm in length, those used in the reticular formation and thalamus were 7.0 mm, and the cortical electrodes were 4.0 mm in length.

The stimulating electrodes were made from Belden twisted pair shielded cable (8429). The shielding was removed from 2.5 cm of one end of the cable and the wires were separated. The insulation was removed from 1.5 cm of the wires and the bared wires were formed into loops and tinned. Female Amphenol connector pins (220-S02) were soldered to the other end of the wires and to the cable shield. The two cable shields were connected to ground during stimulation and recording.

Cannula construction

The jugular cannulae were made using polyethylene tubing joined to silastic rubber tubing. A 15 cm length of PE 20 tubing was welded to a 6 cm length of PE 10 using hot air. A wire was inserted into the tubing during this step to insure that the lumen of the tubing remained patent. A 5 cm length of silastic tubing was soaked in chloroform for 30 seconds and then slipped over the end of the PE 10 to produce a 3 mm overlap. A drop of Eastman 910 adhesive was placed on the junction after which a 5 mm segment of heat shrink tubing was positionedover the junction and heated. The PE 10 segment was then wrapped around a glass rod so that the heat shrink tubing was opposite the PE 20 - PE 10 junction and the cannula was held in boiling water

for 10 seconds in order to permanently set the coil in the PE 10 segment. It was discovered in our laboratory by Sheryl Beck that the cannulae were less likely to become blocked in use if the PE 10 - PE 20 junction was omitted and the PE 20 was joined directly to the silastic tubing. This modified cannula was used in approximately 15 subjects in this study.

Stimulation and recording apparatus

Stimulus presentation and data collection were controlled on-line by a Digital Equipment Corporation PDP-12 computer. During all recording sessions the subjects were placed in a plexiglass restrainer within a walk-in sound attenuating chamber (Industrial Acoustics). Stimuli were 3 electrical pulses of 0.2 msec duration delivered at a frequency of 250 Hz by a Devices MK IV isolated stimulator to one of the subcutaneous electrodes in the forelegs of the subjects. Stimulus intensity was set below the level which produced a perceptible muscle twitch in the stimulated leg (approximately 3.5 volts) and was the same for all subjects.

The recorded brain signals were led through an electro-cannular slip ring (BRS/LVE 192-32), amplified and filtered (0.2-250 Hz) by Tektronix 122 preamplifiers and further amplified by Tektronix amplifiers (2A63, 3A72, or 2A60). The final amplifier settings were adjusted so that the evoked potentials were less than 2 volts peak-to-peak in order to prevent saturation of the computer's analog to digital converter. Amplifier settings varied from subject to subject but the settings for each subject remained constant for the duration of the experiment.

Evoked potentials were stored in digital form on magnetic LINCtape under computer control and an analog record was made simultaneously on a Sangamo FM tape recorder. Computer sampling began 10 msec post-stimulus to avoid any stimulus artifact, and evoked potentials from each brain area were sampled every 2.0 msec for 512 msec. Evoked potentials were averaged over 25-trial blocks prior to storage on LINCtape by the computer.

Experimental design

A differential conditioning paradigm was employed in which shocks to one randomly selected paw were delivered only when the subject was in the morphine state, and shocks to the other paw were given only in the saline state. The experiment was conducted in three phases. In the first phase each subject received 200 stimuli to each paw, in the absence of any treatment, to establish baseline response levels to the stimuli. The stimuli were presented in four 50-minute sessions, each consisting of 100 stimulus presentations with a mean intertrial interval of 30 seconds. Throughout baseline and training sessions only one paw was stimulated during any one session.

Following the baseline sessions, each subject was given 10 training sessions. A morphine injection was delivered through the implanted jugular cannula 30 minutes prior to the beginning of 5 of the training sessions (morphine sessions). During these sessions 100 stimuli (Sm) were delivered to one of the subject's forelegs, and the evoked potentials were recorded. Stimulation of the left foreleg was designated the Sm in half the subjects and stimulation

of the right foreleg was designated the Sm in the remainder. Thirty minutes after the end of each morphine session, the subjects were given a saline injection through the cannula, equal in volume to the morphine injection given prior to the session. Morphine was injected in a single bolus prior to each Sm session, rather than in small amounts following each stimulus presentation, in order to more nearly mimic the conditions of human self-administration.

The other five training sessions were preceded by a saline injection given 30 min prior to the beginning of the session (saline sessions). During these sessions, stimuli were presented to the foreleg which was not stimulated during the morphine sessions. This stimulus was designated Ss and the parameters of Sm and Ss were identical. Ss was delivered at the same rate as Sm (100 stimuli/session) and the evoked potentials elicited by this stimulus were also recorded. Thirty min after the end of each saline session, the subjects were given a morphine injection. Saline and morphine sessions were alternated on a random schedule with the provision that one type of session could occur no more than twice in succession. The dosage of morphine given either before morphine sessions or following saline sessions was 20 mg/kg for the first five training sessions and 30 mg/kg for the second five training sessions. The initial dosage was set at a level which insured that the drug effects were sustained during the training sessions. Rectal temperature was found to remain elevated for approximately 2 hr after an intravenous injection of 20 mg/kg of morphine in drug naive rats. The dosage was increased after 5 sessions to offset the effects of tolerance. The volume of saline injected in any session was always

equal to the volume of morphine given in that session. Each subject received three training sessions per day (1 session/8 hours) for the duration of training.

After the completion of the training phase, the subjects were divided into two groups. One group continued to receive three morphine injections per day, while the other group received only saline injections. On the seventh day following the end of training, the test phase began. Half of the subjects in each group were tested in the presence of saline and half were tested in the presence of morphine. Thus, four groups were formed for the test phase: addicted and tested with morphine (A/M); addicted and tested with saline (A/S); withdrawn and tested with morphine (WD/M); and withdrawn and tested with saline (WD/S). Each test session consisted of a random series of Sm and Ss stimuli. The sessions lasted 100 min and both the Ss and Sm were presented 100 times during each session. All subjects received two test sessions, so that they were given 200 Sms and 200 Sss during the test phase. The experimental design is summarized in Table 3.

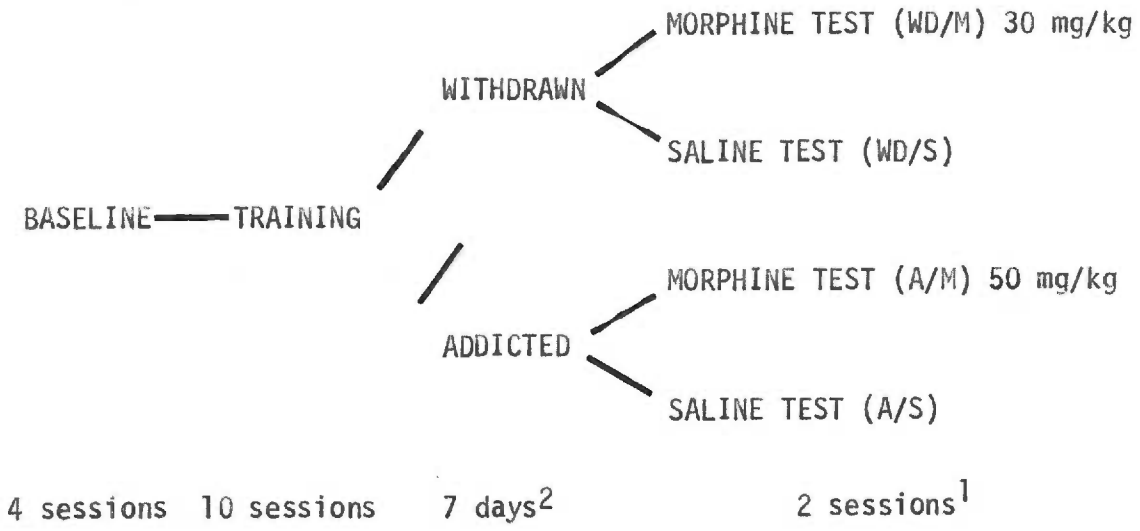
Following the test phase, subjects were sacrificed with an overdose of pentobarbital and perfused through the heart with saline followed by 20% formalin solution. The brains were then removed and preserved in formalin for histological examination.

Data analysis

The averaged evoked potentials (AEPs) from each brain area were evaluted with respect to the total area under the AEP curve, the peak-to-peak amplitude of selected components of the waveform, the latency of selected components, and the overall similarity of the

TABLE 3

Experimental Design



Order of Baseline and Training Sessions

Baseline:	BL, BR, BL, BR	
Training:	M S S M S 20 mg/kg	M S S M M 30 mg/kg

[1]During each baseline and training session, 100 stimuli were presented. Only one leg was stimulated in each session. During each test session 200 stimuli were presented, 100 to each leg, randomly interspersed.

[2]The subjects which were maintained on morphine received the following doses during the training-test interval: 30 mg/kg (days 1 & 2); 40 mg/kg (days 3-5); 50 mg/kg (days 6 & 7).

Abbreviations used in Table 4: BL=baseline session during which the left leg was stimulated; BR=baseline session during which the right leg was stimulated; M=training session preceded by a morphine injection, during which the Sm was presented; S=training session preceded by a saline injection, during which the Ss was presented.

training and testing AEPs to the baseline AEPs as measured by a correlation program. In order to compute the area under the AEP waveform, the AEP was transformed, without changing its shape, to provide equal positive and negative area around a zero baseline. The waveform was then rectified, to give all positive values, and integrated to yield the area of the AEP. The correlation values were derived by comparing the waveforms on a point-to-point basis and computing a Pearson product-moment correlation coefficent. This measure was sensitive to changes in the shape of the AEP or changes in the latency of major components, but relatively insensitive to changes in the size of the AEP. The area measure, on the other hand, was sensitive only to changes in the size of the AEP. Both these measures were applied to the entire AEP, and thus, were relatively gross. The peak-to-peak and latency analyses were more useful since they could be applied to individual components of the AEP waveform. It was necessary to consider certain intervals of the responses individually because the changes in the AEPs were complex, often involving decreases in some components simultaneous with increases in others. The components selected for analysis were those which showed alterations in size, polarity, or latency over training. The selection was made independently for Sm and Ss responses.

RESULTS

Histology

The cortical electrode placements were not verified histologically because these electrodes were placed in a constant relationship to skull landmarks and their tips penetrated only 1.0 mm below the dura. Each brain was examined grossly for evidence of hemorrhage or necrosis due to infection at the entry point of the cortical electrode. No gross abnormalities were found in any of the brains from which cortical records were obtained. Placement of the three subcortical electrodes was verified histologically. The following anterior and lateral coordinate values describe the center of the electrode track; the vertical coordinates describe the tip of the electrode at its deepest penetration. The coordinates were determined by comparing light projected serial sections with the atlas of Pellegrino and Cushman (1967).

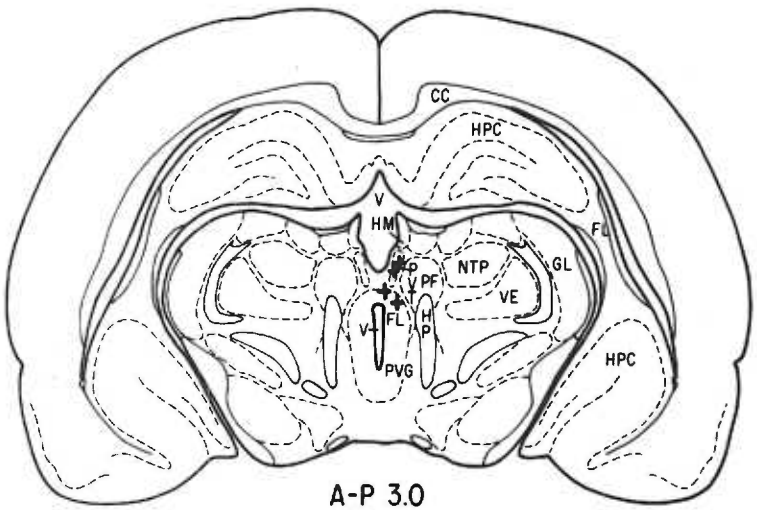
Of the nineteen electrodes directed at the PVT from which usable AEP records were obtained, fifteen (79%) were found to have been within the nucleus. These electrodes ranged from A 3.0 mm through A 3.8 mm, with a mean value of A 3.5 mm. The lateral placement ranged from 0.0 mm through 0.7 mm, with a mean of 0.3 mm; and the vertical placement ranged from 0.8 mm through -1.1 mm with a mean of -0.3 mm. The four electrode placements which were not in the PVT were in the midline thalamus, ventral to the PVT; in the ventricle, dorsal to the medial habenula; and in the dorsal longitudinal fasciculus (2). The electrodes penetrated the cortex, the corpus callosum, the medial hippocampus,

the third ventricle and the medial habenula, causing damage to each of these structures. The location of each of these placements is shown in Figure 2 and the stereotaxic coordinates of each of the placements are presented in Table 4.

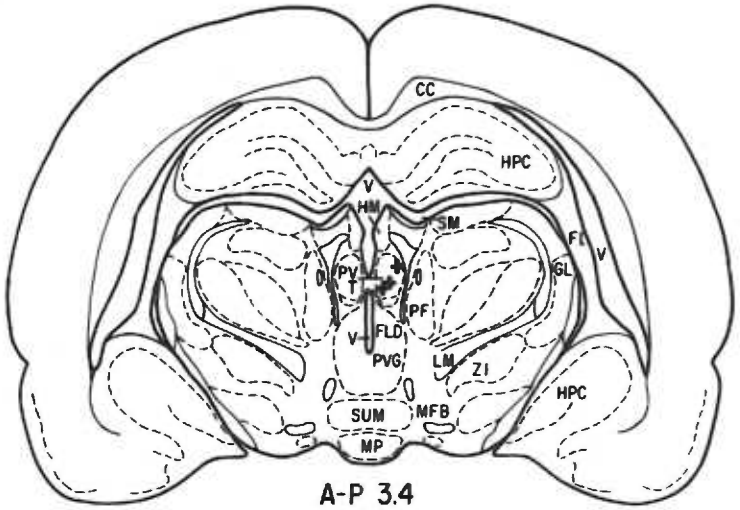
Twenty-one electrodes directed at the VMH provided usable AEP records. Of these, ten (47%) were found to have been within the nucleus; nine electrodes (43%) were found in other hypothalamic nuclei; and two electrodes were not within the hypothalamus (10%). The placements ranged from A 5.2 mm to A 6.5 mm with a mean of A 6.2 mm. The lateral coordinates ranged from 0.2 mm through 2.3 mm with a mean of 1.0 mm; and the vertical placements ranged from -2.5 mm to -4.2 mm with a mean value of -3.4 mm. Those placements which were within the hypothalamus, but not in the ventromedial nucleus were found in the lateral hypothalamic area (LHA, 5), the arcuate nucleus (ARH, 3), and the anterior hypothalamic area (AHA, 1). The remaining two electrodes were in the anterior portion of the lateral preoptic area and in the optic chiasm. The structures most commonly damaged by the insertion of the hypothalamic electrodes were the corpus callosum, the lateral septal nucleus, fornix, hippocampal commisure, anterior midline thalamic nuclei, and dorsal hypothalamic nuclei. The placement of these electrodes is shown in Figure 3 and their stereotaxic coordinates are given in Table 4.

Usable AEP records were obtained from eighteen electrodes directed at the PVG. Of these, one was found to be within the borders of the PVG. The majority of the placements (15) were found to be in the dorsal

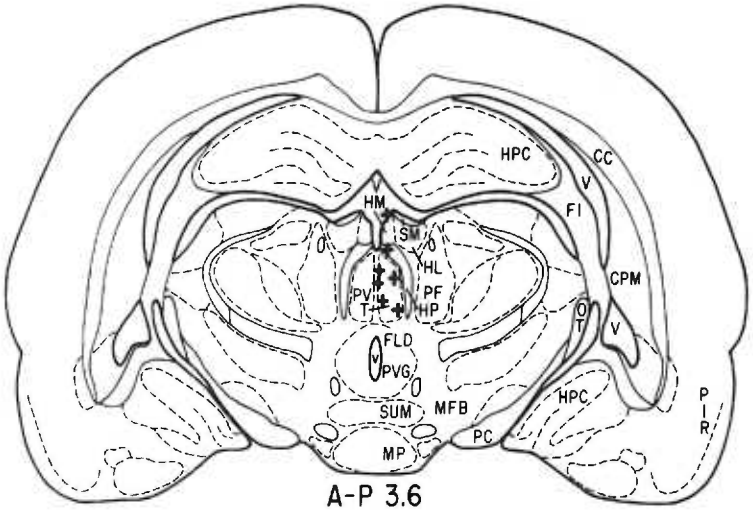
Figure 2. Thalamic electrode placements. Electrode tip locations are marked by the dark crosses. Each cross represents the location of the thalamic electrode in one subject. The anterior-posterior plane is shown under each drawing. Abbreviations: CC=corpus callosum; HPC=hippocampus; HM=medial habenula; FI=fimbria of hippocampus; FLD=dorsal longitudinal fasciculus; CI=internal capsule; CPM=caudate, putamen; V=ventricle; PVT=paraventricular nucleus of thalamus; PF=parafascicular nucleus of thalamus; NTP=posterior nucleus of thalamus; VE=ventral nucleus of thalamus; GL=lateral geniculate; PVG=central gray; HP=habenulo-interpeduncular tract; MP=posterior mamillary nucleus; LM=medial lemniscus; ZI=zona incerta; MFB=medial forebrain bundle; SM=stria medullaris; OT=optic tract; PIR=piriform cortex; PC=cerebral peduncle; HL=lateral habenular nucleus; RE=nucleus reuniens.



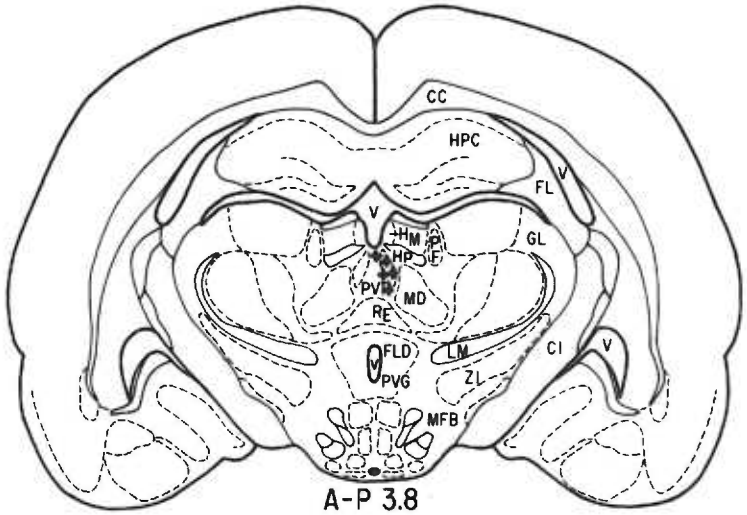
A-P 3.0



A-P 3.4

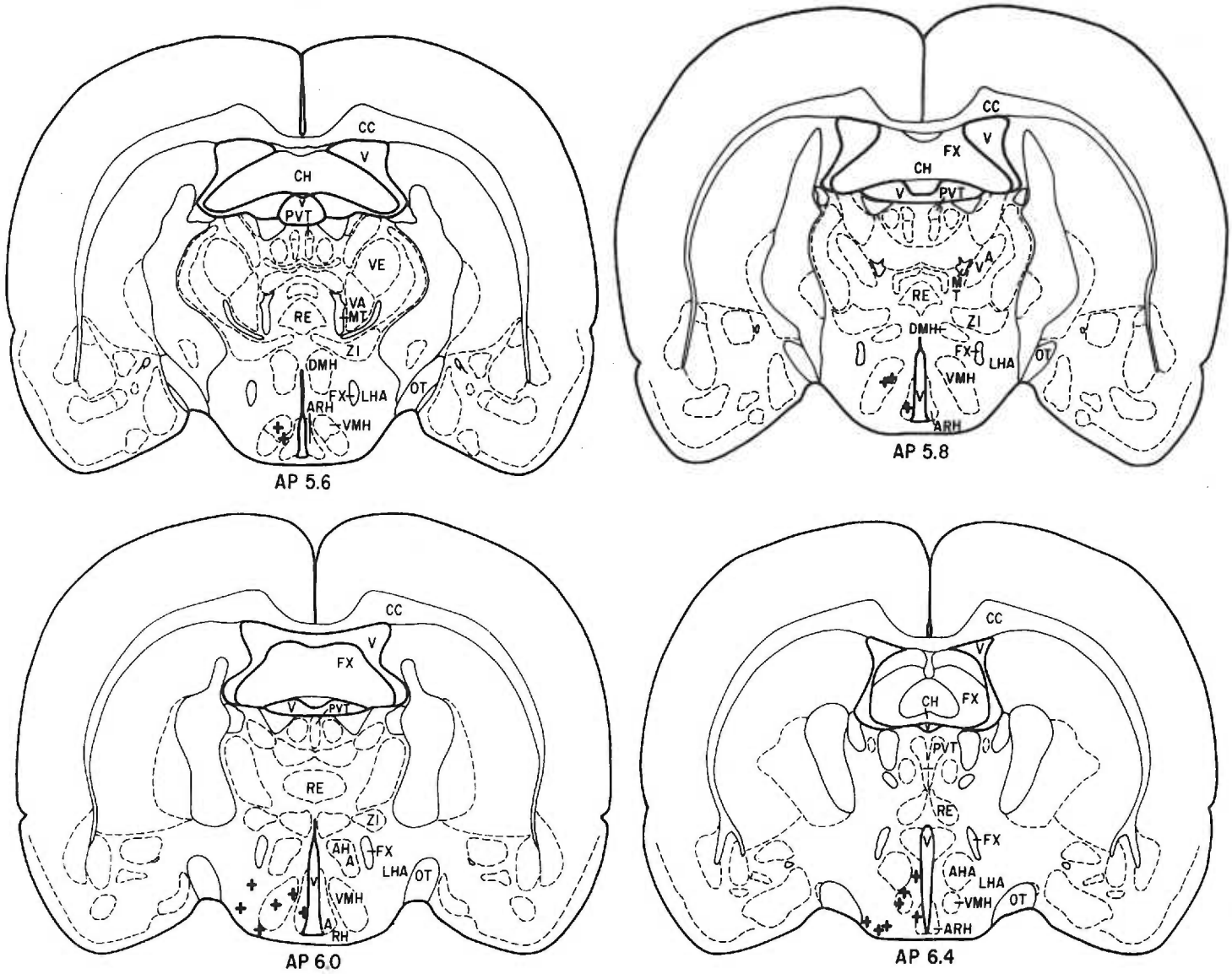


A-P 3.6



A-P 3.8

Figure 3. Hypothalamic electrode placements. Electrode tip locations are marked by the dark crosses. Each cross represents the location of the hypothalamic electrode in one subject. The anterior-posterior plane is shown under each drawing. Abbreviations: CC=corpus callosum; V=ventricle; CH=hippocampal commissure; PVT=paraventricular nucleus of the thalamus; VE=ventral nucleus of the thalamus; VA=anterior ventral nucleus of the thalamus; MT=mamillothalamic tract; RE=nucleus reuniens; DMH=dorsomedial nucleus of the hypothalamus; ZI=zona incerta; FX=fornix; LHA=lateral hypothalamic area; ARH=arcuate nucleus of the hypothalamus; VMH=ventromedial nucleus of the hypothalamus; OT=optic tract.


CC
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OT
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AP 5.8
CC
V
FX
V
PVT
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AH
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RH
AP 6.0
CC
V
CH
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PVT
RE
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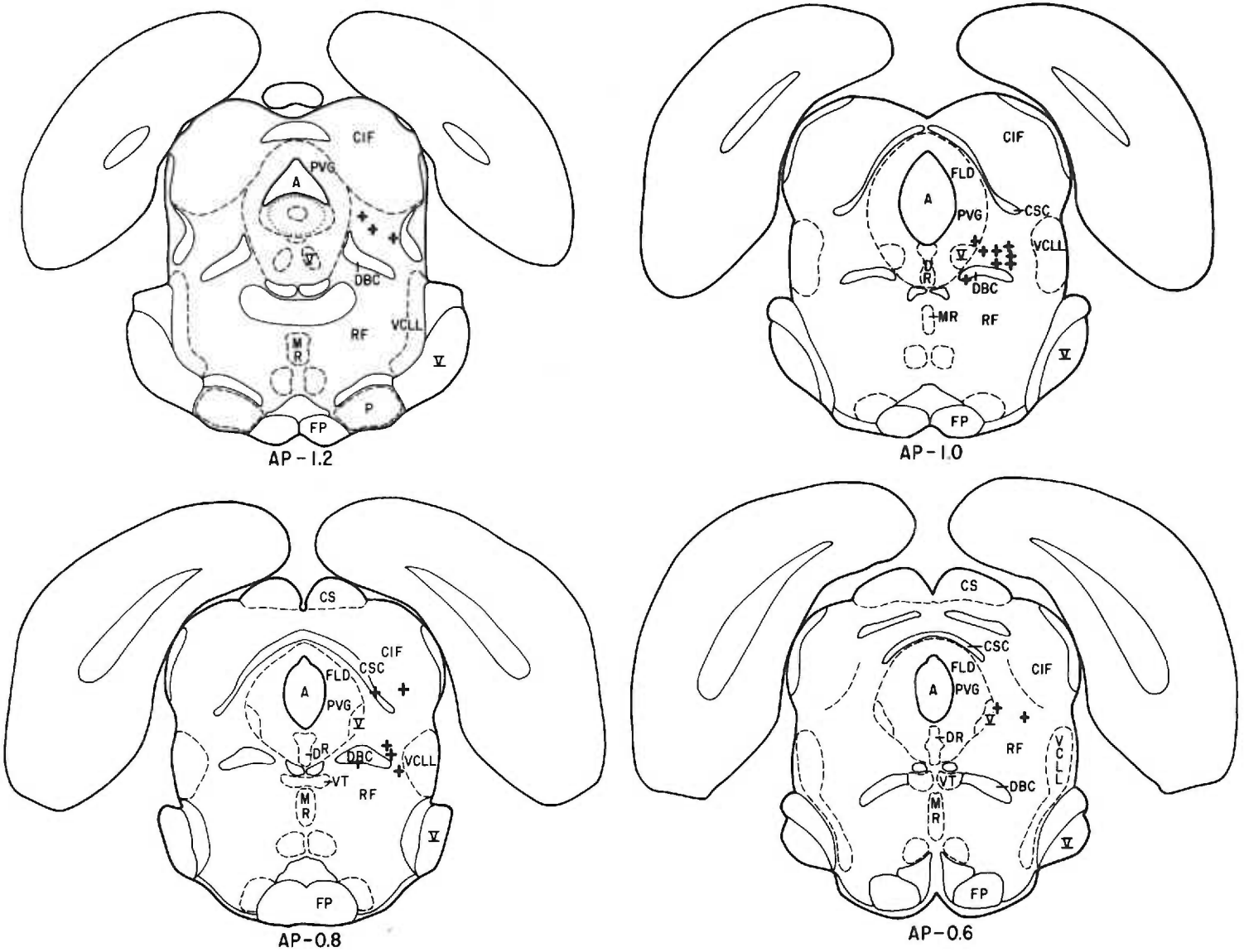
FX
AHA
LHA
VMH
OT
ARH
AP 6.4

pontine reticular formation (PRF). They were grouped in an area bounded medially by the PVG, dorsally by the inferior colliculus, laterally by the ventrocaudal nucleus of the lateral lemniscus and ventrally by the decussation of the brachium conjunctivum. One electrode was found to be in the inferior colliculus and one was in the commisure of the superior colliculus. The placements ranged from A -0.6 mm through A -1.2 mm, with a mean of A -0.9 mm. The lateral coordinates ranged from 1.0 mm to 2.5 mm, with a mean of 2.0 mm; and the vertical placements ranged from -1.3 mm to -3.5 mm, with a mean value of -2.6 mm. The structures damaged by the insertion of these electrodes were the inferior colliculus and the lateral portion of the commisure of the superior colliculus. The placement of these electrodes is illustrated in Figure 4, and their stereotaxic coordinates are given in Table 4.

Morphine effects on cortical AEPs

The AEPs recorded from the cortical placement varied in form from subject to subject, but some general characteristics were typical of the majority of responses. The baseline AEPs, recorded prior to any drug injections, commonly exhibited three early peaks (usually negative-positive-negative) within the first 100-150 ms post-stimulus. Of these, the second negative peak was most often the largest. The remainder of the baseline AEPs (150-512 ms) was generally made up of smaller peaks of longer duration which were much more variable in latency and amplitude. This common AEP form is illustrated in Figure 5. Variations from this basic form consisted principally of

Figure 4. Reticular electrode placements. Electrode tip locations are marked by the dark crosses. Each cross represents the location of the reticular electrode in one subject. The anterior-posterior plane is shown under each drawing. Abbreviations: A=aqueduct of Sylvius; CIF=inferior colliculus; PVG=central gray; V=trigeminal nucleus; DBC=deccusation of brachium conjunctivum; MR=medial raphe; DR=dorsal raphe; FP=pyramidal fibers; RF=reticular formation; P=pons; VCLL=ventrocaudal nucleus of the lateral lemniscus; FLD=dorsal longitudinal fasciculus; CSC=commissure of the superior colliculus; VT=ventral tegmental nucleus.


CIF
PVG
A
V
DBC
RF
VCLL
MR
V
P
FP
AP - 1.2
CIF
FLD
A
PVG
CSC
VCLL
V
DR
DBC
MR
RF
V
FP
AP - 1.0
CS
CIF
CSC
FLD
A
PVG
V
DR
DBC
VCLL
VT
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V
FP
AP-0.8
CS
CSC
CIF
FLD
A
PVG
V
DR
RF
VCLL
VT
DBC
MR
V
FP
AP-0.6

TABLE 4

Stereotaxic Coordinates for Electrode Placements

PVT

Subject	AP	L	V
5-12	3.55	0.20	-0.70
4-13	3.55	0.10	0.10
12-13	3.00	0.20	0.10
14-13	3.00	0.30	0.00
13-13	2.70	0.20	-0.70
4-12	3.90	0.30	-0.60
9-13	3.75	0.10	0.10
7-13	3.85	0.20	0.20
7-12	3.20	0.30	-1.10
11-13	3.55	0.50	-0.30
2-10	3.40	0.70	-0.20
3-10	3.45	0.40	-0.80
5-11	3.65	0.30	0.40
2-11	3.80	0.40	-0.50
6-11	3.35	0.50	-0.70
8-11	3.60	0.00	-0.20
4-10	3.90	0.40	-0.40
7-11	3.55	0.40	0.80
9-11	3.55	0.50	-0.90
$\bar{X}$	3.50	0.30	-0.30

TABLE 4 (cont.)

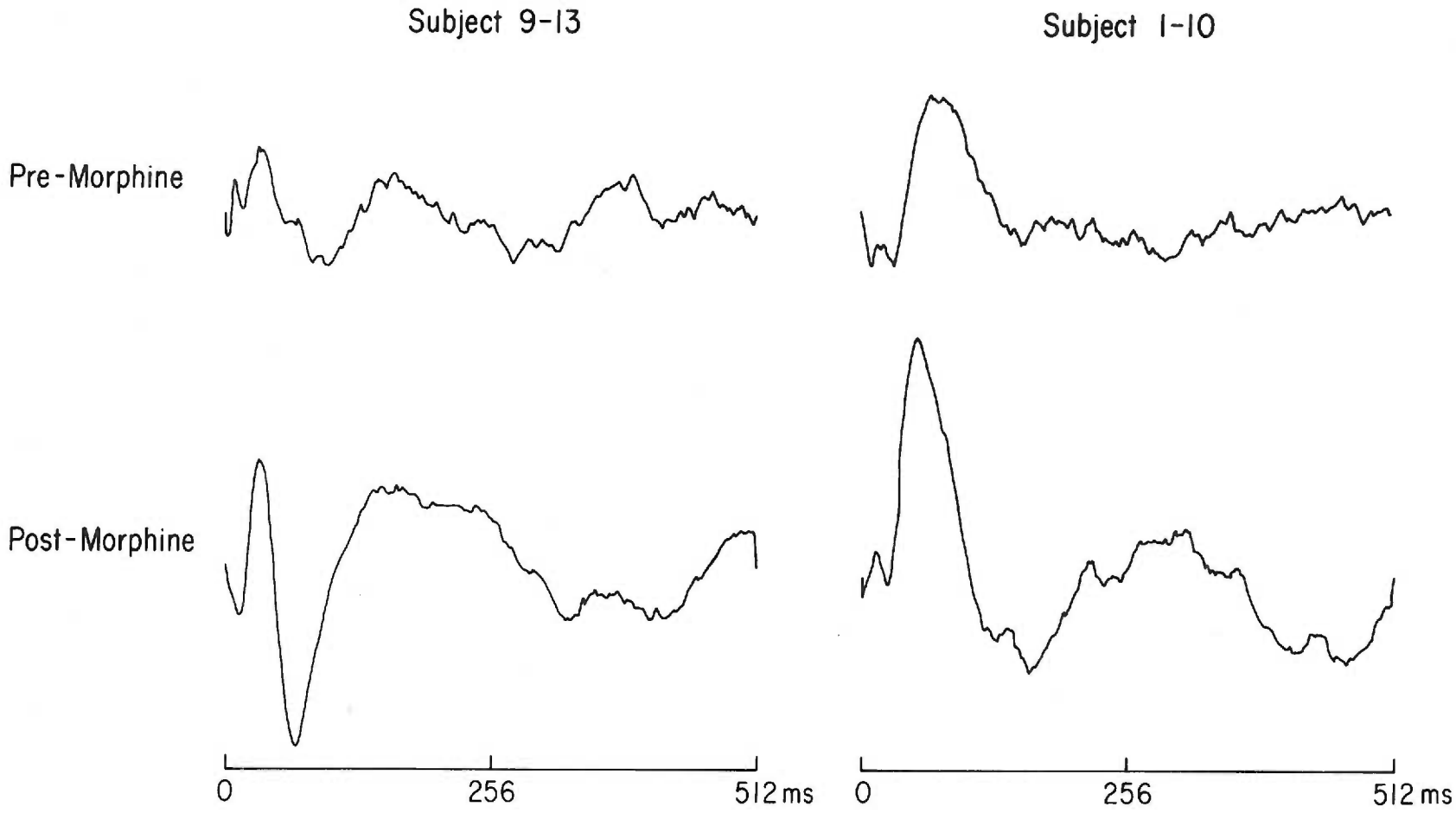
		VMH	
Subject	AP	L	V
13-13	5.85	1.00	-3.20
5-12	7.05	1.80	-3.20
4-12	6.40	1.25	-3.90
14-13	6.25	1.50	-4.00
12-13	5.20	0.80	-3.50
9-13	6.05	1.10	-3.60
4-13	6.05	1.90	-3.00
7-13	5.95	1.40	-4.20
3-12	7.60	2.30	-2.60
7-12	6.50	0.20	-2.50
10-13	5.50	0.70	-3.70
2-11	6.40	0.30	-3.60
5-11	6.05	0.60	-3.40
3-10	5.80	0.90	-3.10
6-11	5.85	0.40	-3.90
8-11	6.45	0.60	-3.00
4-10	6.10	2.00	-3.80
9-11	6.35	0.50	-3.20
7-11	5.50	0.70	-3.20
1-10	6.10	0.20	-3.70
6-10	6.50	1.50	-3.80
$\bar{X}$	6.20	1.00	-3.40

TABLE 4 (cont.)

	PRF		
Subject	AP	L	V
5-12	-0.65	2.50	-2.40
12-13	-0.80	2.50	-1.30
3-12	-0.80	2.20	-3.00
4-12	-0.75	1.30	-3.00
7-13	-1.00	2.30	-3.00
14-13	-1.05	1.00	-3.50
13-13	-1.05	2.50	-2.50
9-13	-1.00	2.00	-3.00
10-13	-0.95	2.20	-2.90
7-12	-0.75	1.70	-1.60
11-13	-0.75	2.50	-3.60
9-11	-1.15	1.70	-1.90
5-11	-1.05	1.30	-2.60
3-10	-0.80	2.00	-2.80
7-11	-0.65	1.60	-2.20
2-10	-1.20	2.00	-2.10
4-10	-0.95	1.50	-2.80
6-11	-1.00	2.00	-2.80
8-11	-1.15	2.50	-2.60
$\bar{X}$	-0.90	1.95	-2.60

Figure 5. Effect of the initial morphine injection on cortical AEPs. Responses to the Sm recorded from two subjects during the final baseline session (pre-morphine) and the first training session (post-morphine) are shown. Note the large positive peaks which develop in the late portion of each of the post-morphine responses.

Initial Morphine Effect on Cortical AEPs



differences in the number of early peaks. Following the initial injection of morphine, the form of the cortical AEPs was dramatically altered.

The most striking alteration in the cortical AEPs following morphine injection took place in the later portion of the response (generally 200-300 ms post-stimulus). Typically a very large positive wave appeared in this region of the AEP, where only small peaks had been during baseline trials. These enlarged peaks were also of longer duration (broader) than those which were present in baseline, often extending for 250 ms, or approximately half the duration of the total AEP. The appearance of this large post-morphine peak had the effect of compressing both the earlier and later portions of the AEP. Thus, the latency of peaks just preceding the enlarged peak was decreased while that of peaks which followed it was increased. These changes may be seen by comparing the baseline and post-morphine AEPs in Figure 5.

In most instances the size and latency of the earliest peaks were affected very slightly, if at all, by morphine. Occasionally the early peaks also increased in size and new peaks appeared at very long latencies (> 400 ms). In only two cases were the post-morphine AEPs smaller in size than the baseline AEPs. This observation is substantiated by a comparison of the area of the AEPs recorded during the final baseline session with those obtained following the first morphine injection. When this comparison was made using a t-test for paired values, the post-morphine cortical AEPs were found to be

significantly larger than baseline ($\overline{D}$ = 59.7; t = 3.84, df = 20, $p < .005$). This increase in AEP area found following the first morphine injection was transient, not persisting beyond the first morphine session in most animals. Because the morphine effect appeared immediately and was not maintained, it should not be confused with the changes in amplitude of certain AEP peaks discussed below which developed gradually over the course of training.

Morphine effects on thalamic AEPs

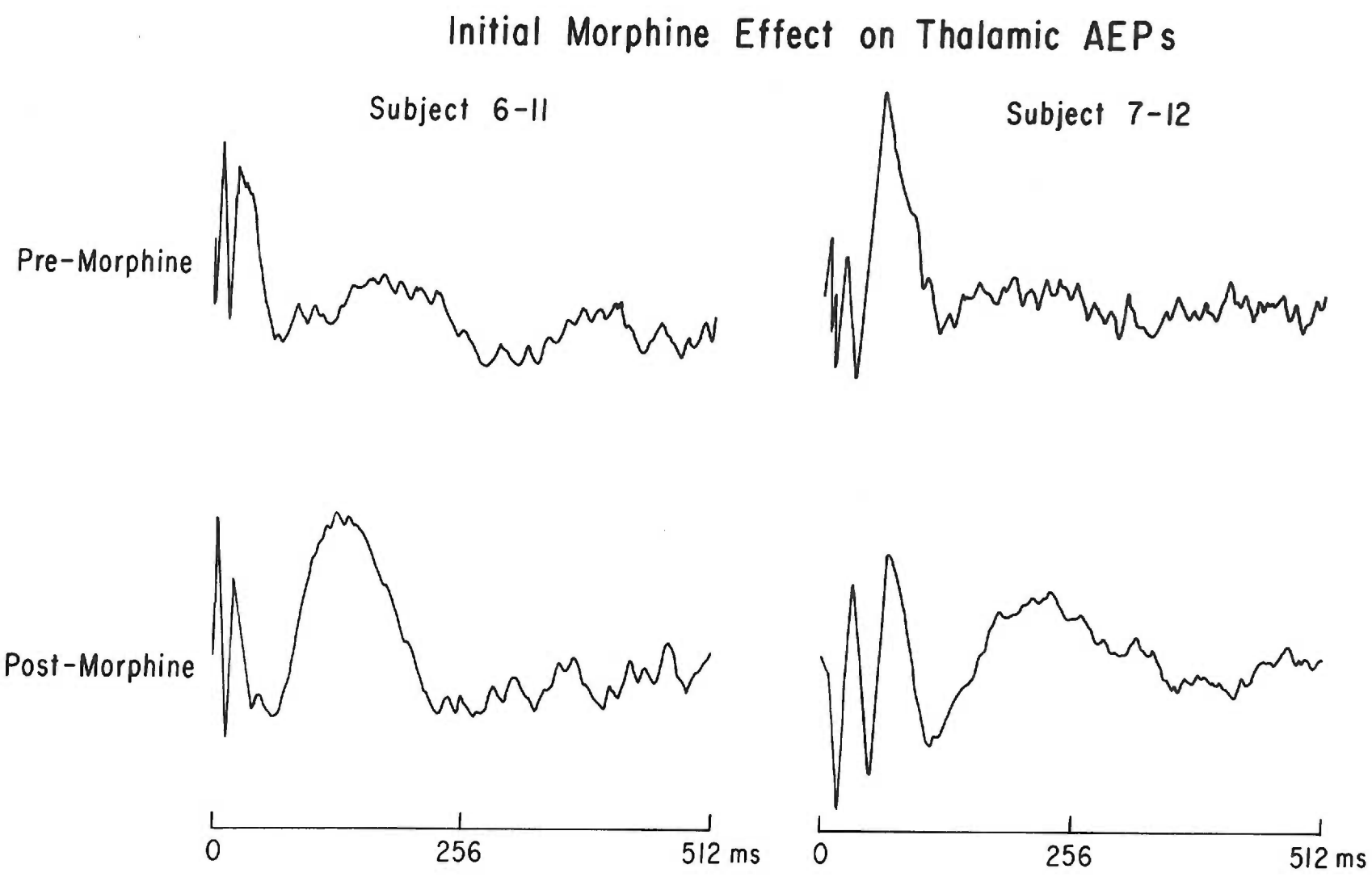
Inter-subject variability in baseline AEP form was sufficient that the records from those electrodes which were not in the PVT could not be separated reliably from those which were, on the basis of their AEPs. Nonetheless, the following descriptions are based on data from only those electrodes found to be within the PVT. The AEPs recorded from the PVT were somewhat more variable in form that the cortical AEPs. However, they exhibited general characteristics which were common to most responses. During baseline sessions, the typical thalamic AEP consisted of two or three positive, and as many negative, peaks within the first 120 ms post-stimulus. These peaks were sharper and their inter-peak interval was shorter than was the case for the cortical responses. The first peak usually had a very short latency, less than 20 ms in most cases. In many thalamic AEPs there were no peaks in the later portion of the response (> 150 ms post-stimulus). However, a number of responses resembled the cortical pattern in which later peaks were present, but smaller and variable in both size and latency. An example of each of these types of response is

shown in Figure 6. As was the case for the cortical AEPs, those recorded from the PVT varied primarily in the number of early peaks.

The most striking change in the thalamic response following the initial morphine injection was the appearance of a large positive wave in the later portion of the AEP (150-225 ms post-stimulus). This peak was typically broad, with a duration of 200-250 ms and often appeared at a latency at which no peaks were present during baseline. Despite the appearance of this broad peak, the latencies of the earlier peaks were largely unaffected. These features may be noted in the post-morphine AEPs shown in Figure 6. The size and number of early peaks were occasionally altered following the initial morphine administration. However, these changes were not common and they generally were transient. When these changes did occur, they were in the direction of fewer and larger early peaks following morphine injection.

An increase in the overall size of the AEPs was also noted after morphine injection. This effect was not found as consistently as was the case in the cortical responses. In some cases, the AEPs recorded in the first morphine session were, in fact, smaller than those from the final baseline session. Nonetheless, the mean area of the AEPs recorded following the initial morphine injection was found to be significantly greater than that of the baseline responses ($\overline{D} = 37.4$; $t = 2.01$; $df = 14$; $p < .05$). As was the case for the cortical AEPs, this increase in area was seldom seen in subsequent morphine sessions.

Figure 6. Effect of the initial morphine injection on thalamic AEPs. Responses to the Sm recorded from two subjects during the final baseline session (pre-morphine) and the first training session (post-morphine) are shown. Morphine injection results in a large increase in a late component of each of the AEPs. The latency of the affected peaks in these responses is somewhat less than was the case in the cortical responses shown in Figure 5.


Initial Morphine Effect on Thalamic AEPs
Subject 6-11
Subject 7-12
Pre-Morphine
Post-Morphine
0
256
512 ms
0
256
512 ms

Morphine effects on hypothalamic AEPs

The evoked potentials recorded from the VMH were quite variable in form between subjects during baseline. This variability could not be ascribed to the variation in electrode placement since the variability between records from electrodes within a nucleus was approximately as large as that between records from electrodes in different nuclei. The evoked potentials from all hypothalamic placements could be divided into two general types. The more common type had only one or, at most, two positive or negative peaks in the early portion of the wave (0-150 ms). In these AEPs the peaks were typically rather broad, with durations of approximately 100-150 ms. The other type of hypothalamic AEP had a large number of early peaks which were of shorter duration, typically about 50 ms. The latency of the initial peak was similar for both types of response, generally between 15 and 25 ms. Also, both types of AEP usually had one or more smaller peaks in the late portion of the response (> 200 ms post-stimulus). As was typical of both the thalamus and cortex, the later peaks were variable in both size and latency, and were smaller than the earlier peaks. Both types of baseline responses were recorded from both medial and lateral hypothalamic placements.

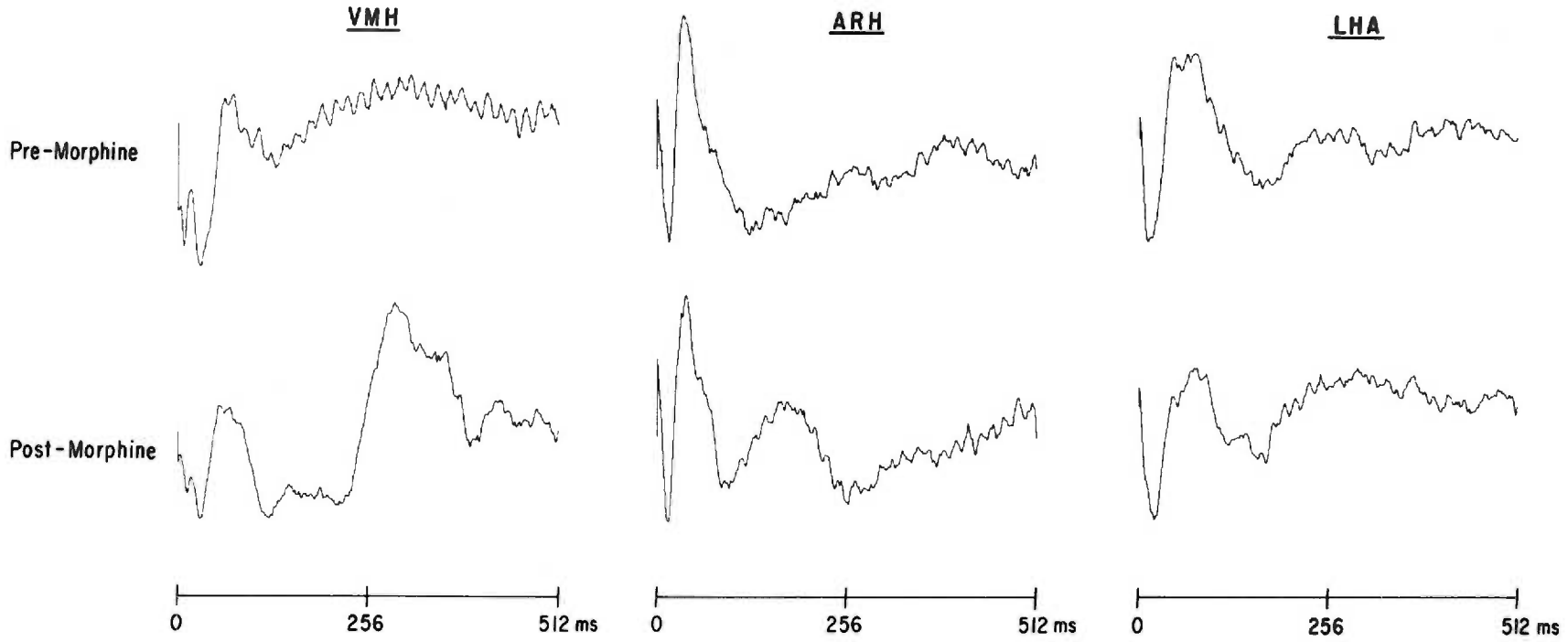
Following the initial morphine injection, a late positive peak, similar to that seen in thalamic and cortical responses, appeared in the AEPs recorded from slightly less than half the hypothalamic responses (9 of 21). It is interesting to note that all of the electrode placements from which this late positive post-morphine

response was recorded were in medial structures. None of the records from the electrodes in LHA or the lateral pre-optic area showed this phenomenon, while it was present in nine of the fifteen medial placements. When it was present, this late peak typically appeared at a latency of approximately 200 ms. However, the latency of this peak was much more variable than was the case in thalamus or cortex and it occurred both earlier than 200 ms and later than 300 ms. The late peaks recorded from hypothalamus tended to be smaller in amplitude and of shorter duration than those recorded from thalamus and cortex. The early peaks (< 150 ms post-stimulus) of the hypothalamic AEPs were quite stable, not changing appreciably in either latency or amplitude following morphine injection. These features are illustrated by the AEPs from VMH and ARH which are shown in Figure 7. These AEPs also illustrate the large variability in the latency of the late post-morphine peaks recorded from the hypothalamus.

The evoked potentials in which no late positive peak appeared following the initial morphine injection were often smaller and simpler than those in which the late peak was found. These responses were typically unaltered following morphine injection, except for a slight decrease in the overall size of the response which occurred in several cases. This type of response is illustrated by the AEP from LHA, also shown in Figure 7.

The area of the responses recorded following the initial morphine injection was compared to that of those recorded during the final baseline session using a t-test for paired values. It was found that the

Figure 7. Initial morphine effect on hypothalamic AEPs. The responses to the Sm recorded from three hypothalamic areas during the final baseline session (pre-morphine) and the first training session (post-morphine) are shown. Note the large increase in amplitude in the late components of the responses recorded from medial structures (VMH & ARH) and the absence of such an increase in the LHA response. Abbreviations: VMH=Ventromedial nucleus of hypothalamus; ARH=Arcuate nucleus; LHA=Lateral hypothalamic area.


Initial Morphine Effect on Hypothalamic AEP's
VMH
ARH
LHA
Pre-Morphine
Post-Morphine
0
256
512 ms
0
256
512 ms
0
256
512 ms

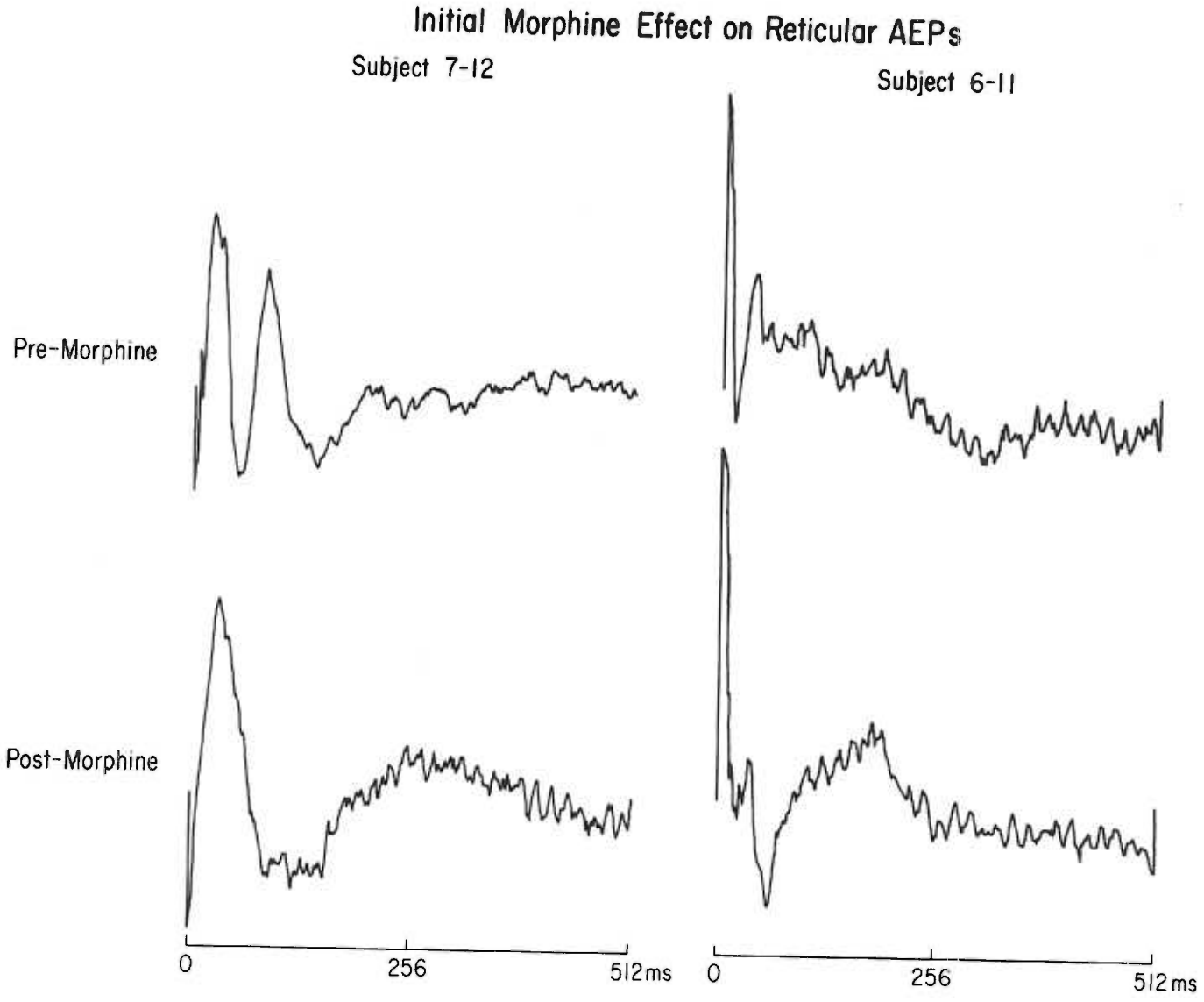
post-morphine responses were larger in area, however, this difference was not statistically significant. The magnitude of this difference in area was much less than that found for the cortical and thalamic area. It appears that the large increases in area found in those responses in which a late positive post-morphine wave appeared were sufficient to offset the much smaller decreases in area which occurred in many of the remaining AEPs.

Morphine effects on reticular AEPs

The evoked potentials recorded from the pontine reticular formation had shorter latency peaks than those from any of the other areas recorded. The first peak in these AEPs usually occurred within 15 ms post-stimulus. These peaks were also of short duration, most being in the range of 20 ms, with the earliest peaks being the sharpest. Most of the reticular AEPs had several peaks within the first 100 ms of the response. There were seldom less than four peaks present and there were as many as seven in some records. The later portions of the responses were typically quiet. Very few responses had any peaks with latencies greater than 150 ms. The characteristic form of the reticular formation responses is shown in the pre-morphine AEPs of Figure 8.

Morphine administration had minimal effects on the majority of the responses recorded from the reticular formation. In those responses which were altered following morphine administration, the changes were of two types. In a small number of cases, a broad positive wave was present at a latency of about 180 ms in the post-morphine responses. This effect, while quite rare, was apparently analogous to the morphine-produced alterations seen in the AEPs from the other brain regions.

Figure 8. Effect of the initial morphine injection on reticular AEPs. Responses to the Sm recorded from two subjects during the final baseline session (pre-morphine) and the first training session (post-morphine) are shown. Morphine effects were more variable in the PRF than in other areas. Note the loss of the second large positive peak in the response from subject 7-12 and the appearance of a positive wave at a latency of about 250 ms in the response from subject 6-11.


Initial Morphine Effect on Reticular AEPs
Subject 7-12
Subject 6-11
Pre-Morphine
Post-Morphine
0
256
512ms
0
256
512ms

This effect may be seem by comparing the pre-morphine and post-morphine responses from subjects 6-11 in Figure 8. A more common effect of the initial morphine injection on the responses from the reticular formation was an overall decrease in the size of the AEPs. This effect was often accompanied by a decrease in the number of AEP peaks and an increase in peak latencies. This type of response alteration is illustrated by the response of subjects 7-12 shown in Figure 8.

The general tendency for the reticular responses to decrease in size following the initial morphine injection is reflected in the results of the analysis of the AEP areas. It was found that the area of the post-morphine responses was less than that of the responses recorded during the final baseline session, but the difference did not reach significance. This post-morphine decrease in AEP area was unique among the brain areas investigated in this study.

The magnitude of the increase in AEP area following the initial morphine injection was found to be a good predictor of the success of the conditioning procedure used in this study. The most reliable evidence of conditioning was found in the cortex, followed by PVT. Thus, the area in which morphine produced the greatest increase in AEP area (cortex) showed the best conditioning, while less reliable evidence of conditioning was found in PVT, which had the next largest increase in area. No evidence of conditioning was found in VMH, where morphine produced only a small increase in AEP area, and the results from PRF were equivocal. The results of the conditioning procedure are discussed fully below.

Conditioning: Cortex

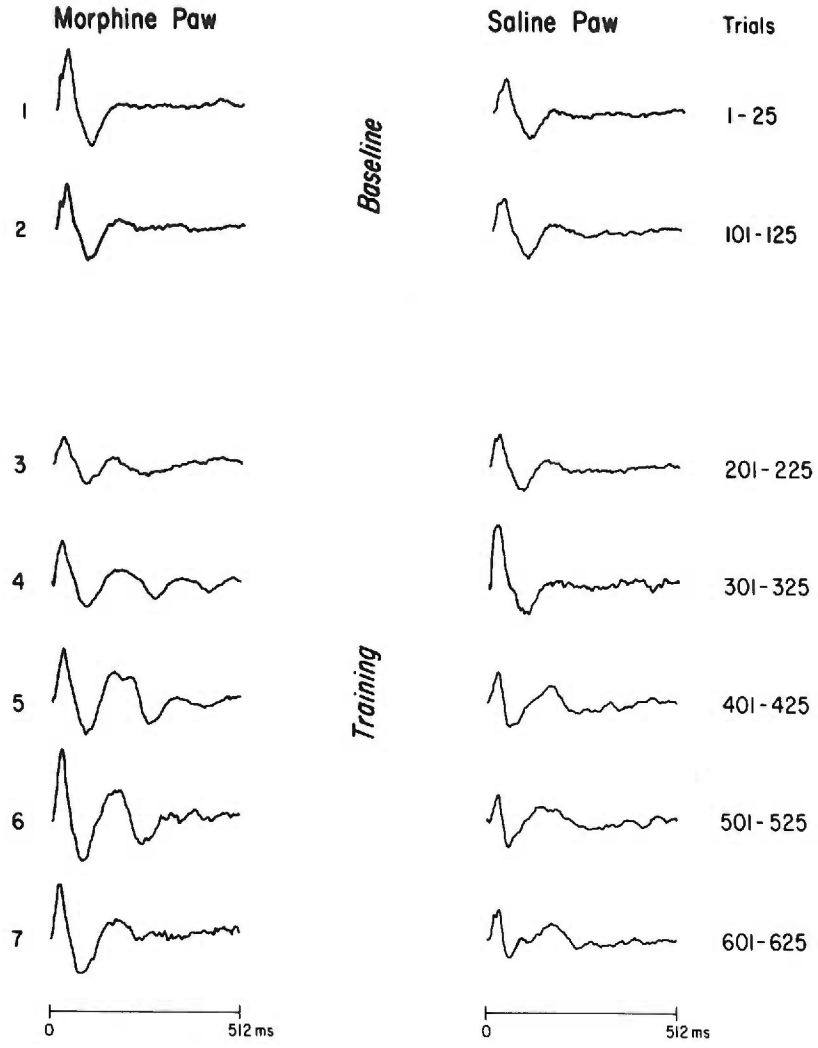
Complete cortical AEP records were obtained from twenty-one subjects. In fourteen of these subjects (67%) the conditioning procedure resulted in an increment over trials in the peak-to-peak amplitude of selected components of the evoked response to the Sm. This increment in response amplitude did not occur in the AEPs elicited by Ss presentations. Thus, the responses to the Sm and Ss, which were initially similar, became progressively differentiated over the course of training. In the remaining seven subjects the response to the Sm did not contain a component which showed a developmental response increment over training, or there was no differentiation between the responses to the Sm and the Ss. All statistical tests and grouped data curves, however, include the data from all twenty-one subjects.

Baseline and training data from a subject which did respond differentially to the Sm and Ss are shown in Figure 9. In the top section of the figure, AEPs elicited by stimulation of the morphine-paired paw (Sm) and the saline-paired paw (Ss) are shown from each block of baseline and training. The AEPs are similar in form during baseline, but during training a large positive peak develops in the response to the Sm at a latency of approximately 175 ms. The response to the Ss shows a slight development at this same latency but it is not as great and does not increase over training. In the graphs at the bottom of Figure 9 the peak-to-peak amplitude of this component is plotted. The progressive development of this peak in the response to the Sm is

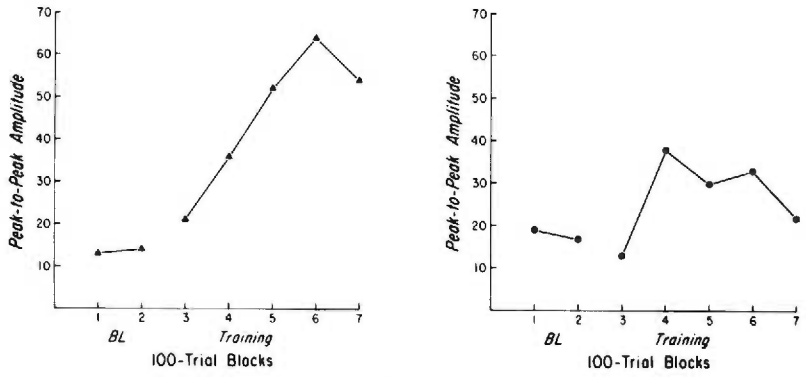
Figure 9. Cortical evoked responses to Sm (morphine paw) and Ss (saline paw). The upper portion of the figure shows the average of the first 25 evoked potentials recorded from subject 4-10 during each block of baseline and training. Note the progressive development of the late positive peak during training in the Sm responses. No such progressive increase is present in the components of the Ss response. The peak-to-peak amplitude for corresponding components of the Sm and Ss responses is plotted in the graphs at the bottom of the figure.

Average Evoked Potentials (Cortex)

Subject 4-10



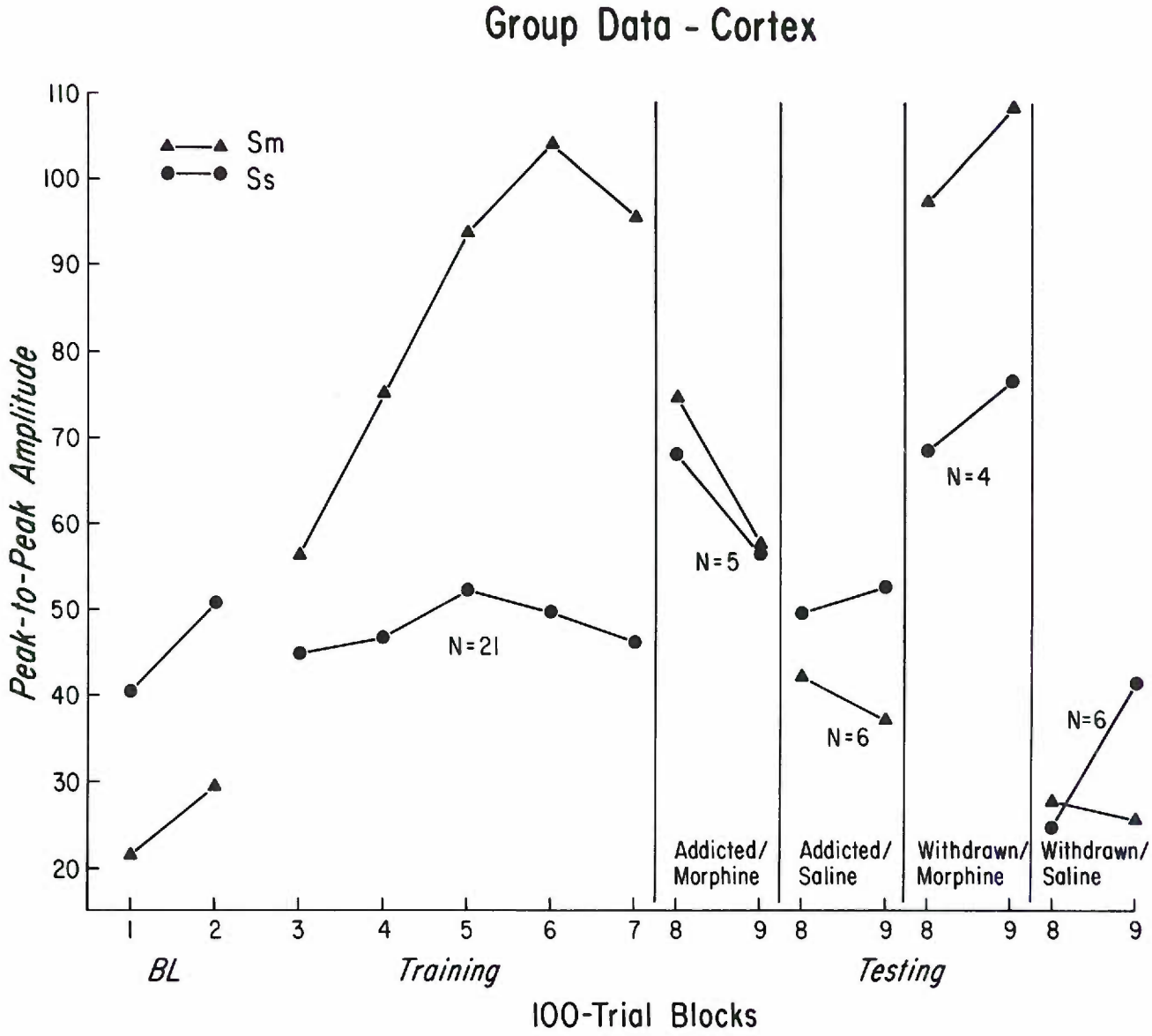
Peak N3 - P4 (120-175 ms)



clearly shown. It is interesting to note that the response to the Ss increases initially, as well, before falling back toward baseline levels in the later training sessions. This early increase, followed by a subsequent decrease in the Ss response could be attributed to stimulus generalization in the initial training sessions followed by differentiation as training progressed.

The combined data from all the cortical placements are shown in Figure 10. The responses to the Sm and Ss are again seen to diverge in training as the amplitude of the Sm response increases progressively, while that of the Ss response does not. The large increase in peak-to-peak amplitude of the Sm response between the end of baseline and the first training session is attributable to the effects of the initial morphine injection, which were discussed above. However, the progressive increase in peak-to-peak amplitude across training in the Sm response cannot be attributed to a simple drug effect. Considering only the baseline and training segments of this figure, the data are consistent with the hypothesis that a differential conditioned response developed to the Sm over the course of training. A two-way repeated measures analysis of variance was carried out on the cortical data from the training sessions. This analysis revealed a significant effect of training sessions ($F = 5.13$; $df = 4/180$; $p < .01$). The difference in mean peak-to-peak amplitude between the Sm response and the Ss response was also significant ($F = 75.5$; $df = 1/180$; $p < .001$). The groups by sessions interaction was significant as well ($F = 3.6$; $df = 4/180$; $p < .05$), indicating that the change in the Sm response over training was greater than the change in the Ss response.

Figure 10. Grouped cortical data. The pooled data from all cortical placements are shown for baseline, training, and testing. Note the differential development of the Sm and Ss responses during training. See text for a discussion of the test results.

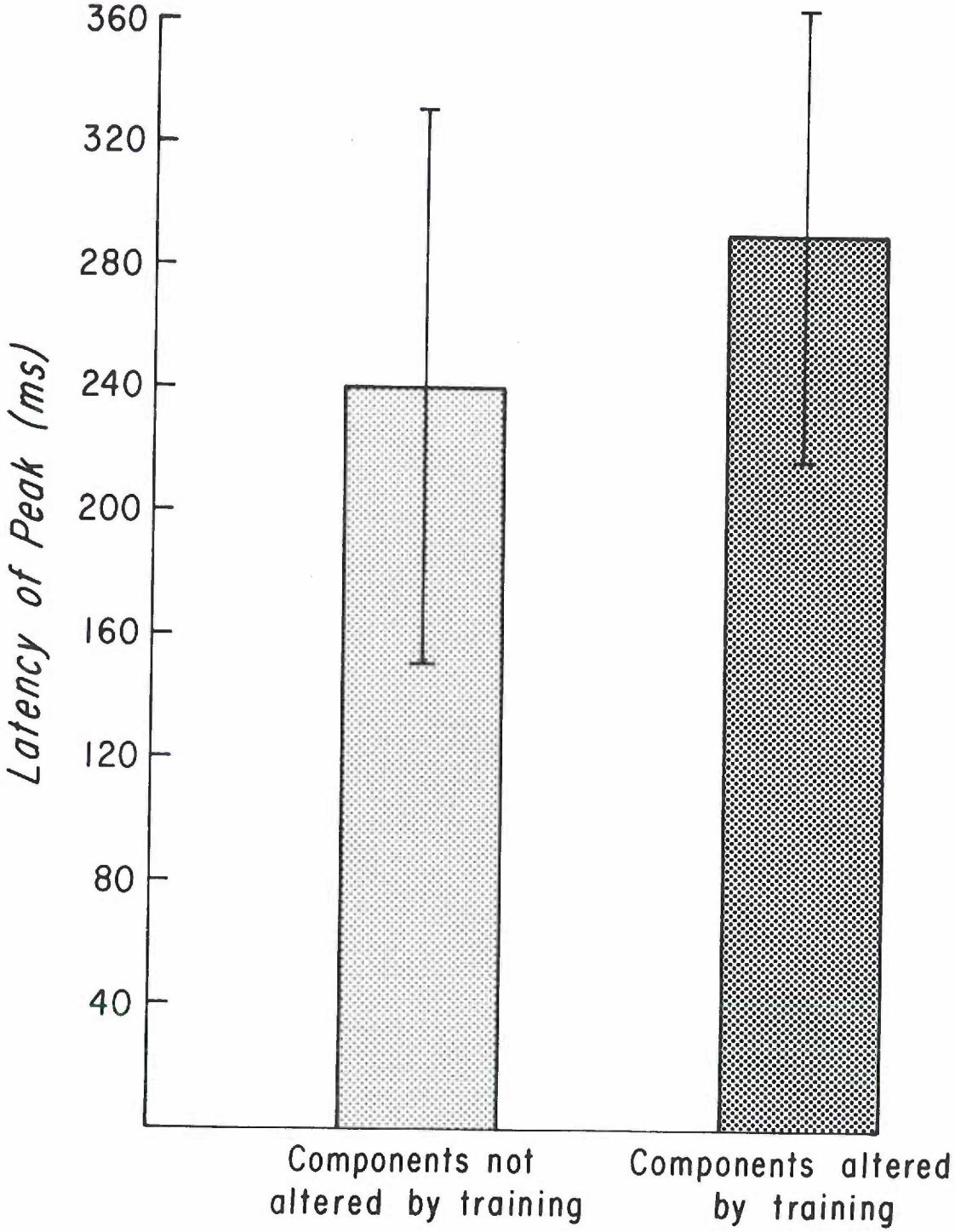

Group Data - Cortex
Peak-to-Peak Amplitude
110
100
90
80
70
60
50
40
30
20
Sm
Ss
N=21
N=5
N=6
N=4
N=6
Addicted/ Morphine
Addicted/ Saline
Withdrawn/ Morphine
Withdrawn/ Saline
1
2
3
4
5
6
7
8
9
8
9
8
9
8
9
BL
Training
Testing
100-Trial Blocks

Generally, at least two components of the cortical AEPs were selected for analysis from each subject. These components were divided into a group which responded differentially to the Ss and Sm, and a group which did not. This separation was based on a visual examination of the peak-to-peak amplitude plots made for each component. The mean latency of the peaks making up each group are shown in Figure 11. The mean latency of the peaks which responded differentially to the Ss and Sm was 291.4 ms, while the mean latency of the peaks making up the other group was 240.8 ms. This difference in latency was found to be significant using a t-test for paired values ($t = 2.37$; $df = 20$; $p < .05$). Thus, it appears that the peaks from the later portion of the cortical evoked response were more likely to respond differentially to the Sm and Ss. It should be recalled that the longer latency peaks were also those which were more affected by the initial morphine injection, as was discussed above.

The data from the testing sessions, shown in Figure 10, were much more complex than the baseline and training data. The results of the testing sessions may be summarized by pointing out the following general outcomes. The responses to the Sm decreased dramatically when the subjects were tested in the saline condition. The responses to the Ss were increased when testing was carried out in the morphine condition. Both of these results were present whether the subjects were withdrawn or maintained on morphine during the training-testing interval. The other result seen in the testing sessions which is of interest is that the Sm response in subjects which were maintained on morphine decreased

Figure 11. Mean latencies of stable and modifiable peaks in the cortical average evoked potentials. Mean latency plus and minus one standard deviation is shown.

Latencies of Stable and Modifiable Peaks in the Cortical AEP



substantially from the end training levels when tested under morphine while the response of those subjects which were withdrawn did not. The implications of the testing results for the interpretation of the conditioning data are discussed fully below.

Conditioning: Paraventricular thalamus

Complete AEP records were obtained from nineteen of the electrodes which were directed at the paraventricular thalamus. Histological examination revealed that these electrodes were within the paraventricular thalamus in fifteen subjects. In six of these subjects (40%), the conditioning procedure resulted in an increment over training in the peak-to-peak amplitude of selected components of the evoked response to the Sm, without a corresponding increment in the Ss response. In these six subjects the differential response which developed to the Sm over training was analogous to that found in the cortex, although the differentiation between the Sm and Ss responses was not as clear as was the case in the cortex. In the remaining nine subjects with accurately placed paraventricular thalamus electrodes, the responses to the Sm did not contain a component which showed a developmental response increment, or there was no differentiation between the Sm and Ss responses. The AEP record from one of the four electrodes which were not within the paraventricular thalamus showed differential responding to the Sm and Ss over training. This electrode was placed ventral to the paraventricular thalamus within the medial thalamus, just dorsal to the nucleus reuniens. The records from the two electrodes which were in the dorsal longitudinal fasciculus and the one in the third ventricle

showed no evidence of differential responding to the Ss and Sm. It is interesting to note that all seven of the subjects in which the thalamic records showed differential responding to the Ss and Sm also showed differential responding in their cortical records.

Baseline and training data from a subject which showed differentiation during training are shown in Figure 12. AEPs from each block of baseline and training trials are shown in the upper portion of the figure. The AEPs elicited by the Ss and Sm are similar during baseline trials and the form of their earliest peaks remains so during training. However, a broad positive peak with a latency of approximately 150 ms develops in the Sm response over the first 300 training trials, and is then maintained for the remaining 200 trials - although not at the peak level. No comparable, consistent development is seen in the AEPs elicited by the Ss. The differential development of the response to the Sm over training is clearly shown by a comparison of the peak-to-peak amplitude graphs at the bottom of Figure 12. It may also be seen that the response to the Sm reached its maximum during the third block of training trials, decreasing somewhat thereafter. This was typical of the paraventricular thalamus results and one way in which they differed from the cortical responses which usually attained their maximum during the fourth training block. The latency of the component which was modified during training was also less than that usually observed in the cortical responses.

The combined data from all placements which were within the paraventricular thalamus are shown in Figure 13. It is obvious from an

Figure 12. Thalamic evoked responses to Sm (morphine paw) and Ss (saline paw). The upper portion of the figure shows the average of the first 25 evoked potentials recorded from subject 8-11 during each block of baseline and training. Note the development in the later portion of the Sm response which is not present in the Ss response. The peak-to-peak amplitude for corresponding components of the Sm and Ss responses is plotted in the graphs at the bottom of the figure.

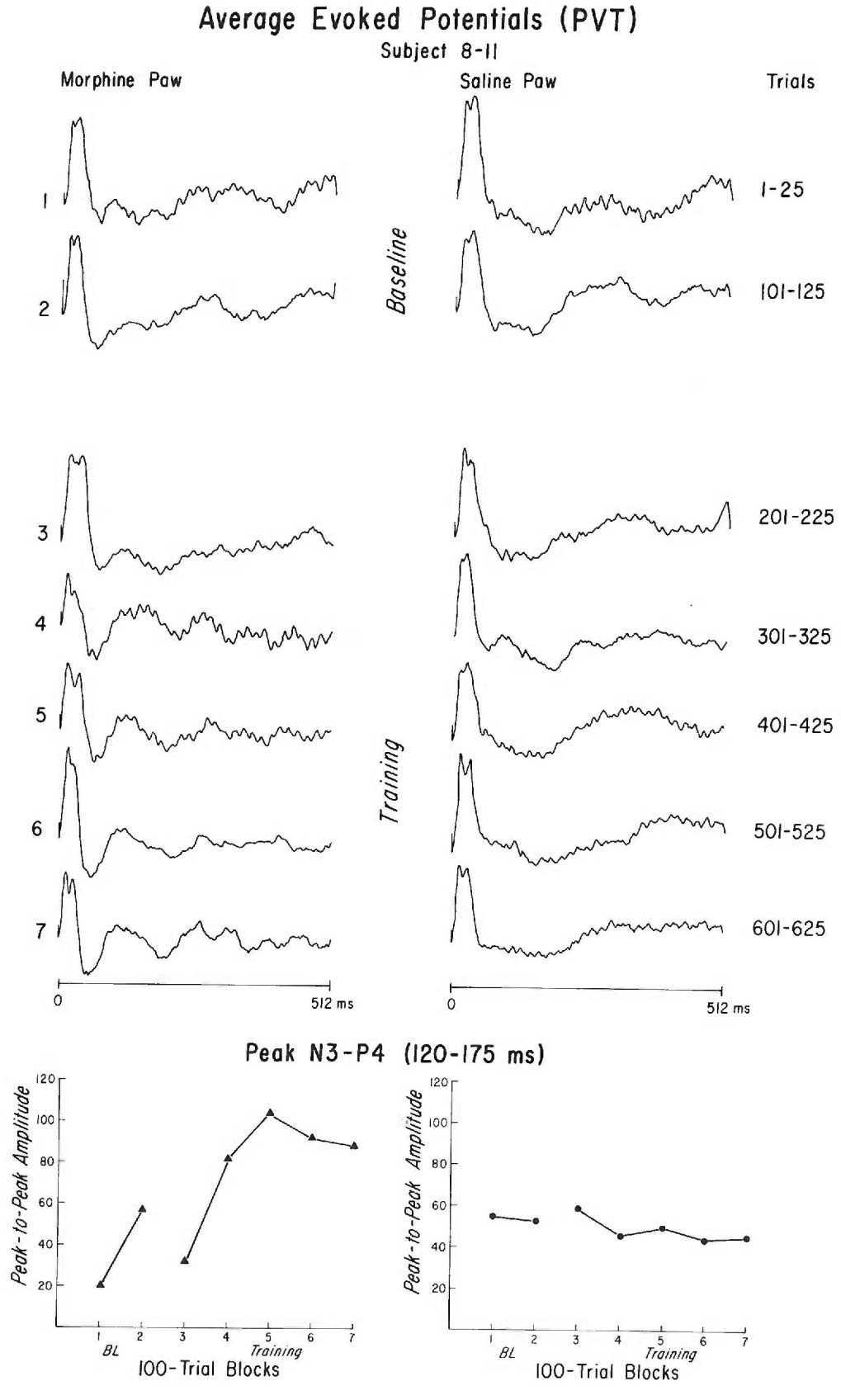
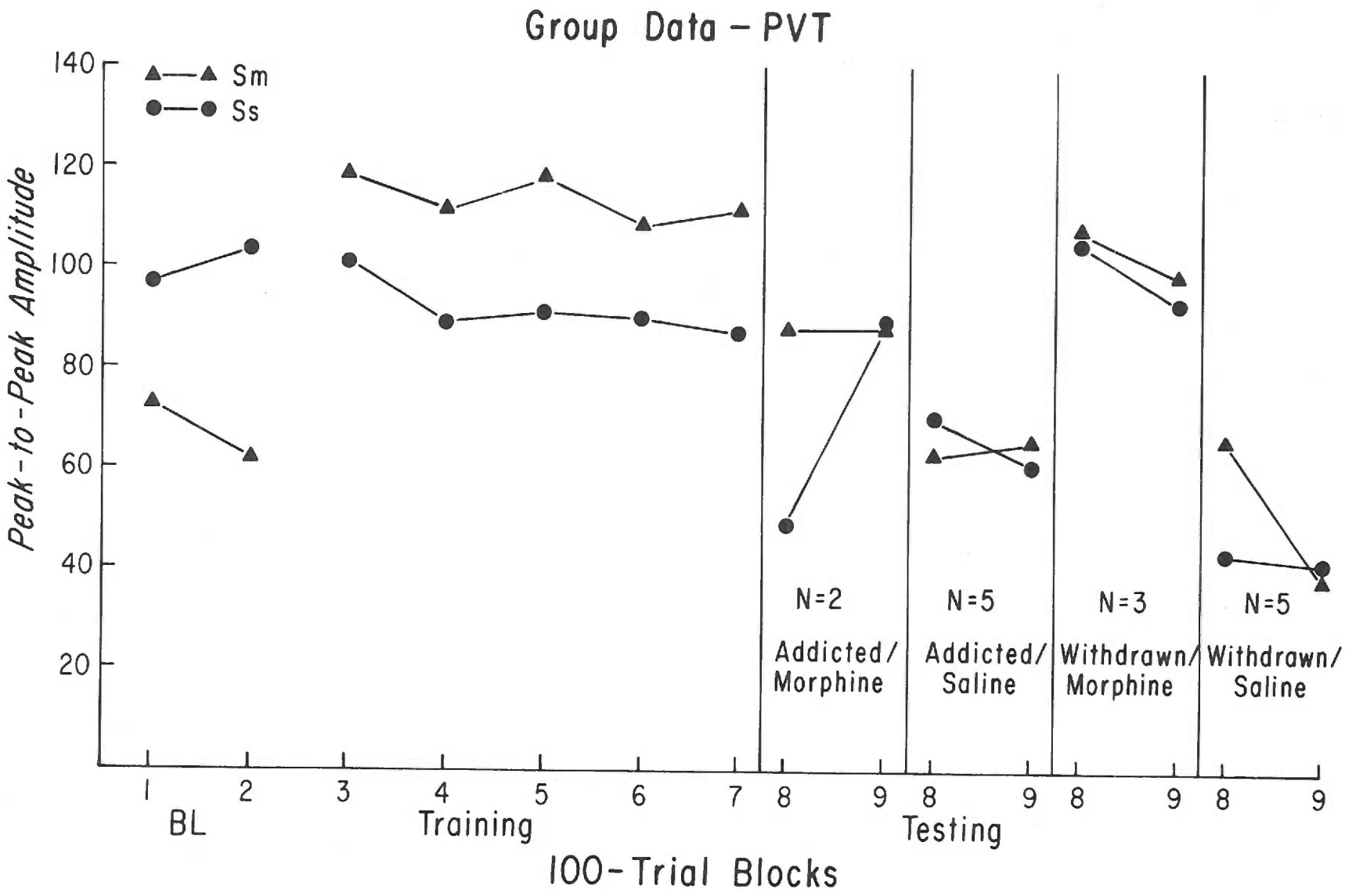

Average Evoked Potentials (PVT)
Subject 8-11
Morphine Paw
Saline Paw
Trials
1
2
Baseline
1-25
101-125
3
4
5
6
7
Training
201-225
301-325
401-425
501-525
601-625
0
512 ms
0
512 ms
Peak N3-P4 (120-175 ms)
Peak-to-Peak Amplitude
120
100
80
60
40
20
1
2
3
4
5
6
7
BL
Training
100-Trial Blocks
Peak-to-Peak Amplitude
120
100
80
60
40
20
1
2
3
4
5
6
7
BL
Training
100-Trial Blocks

Figure 13. Grouped thalamic data. The pooled data from all electrodes which were within the paraventricular nucleus of the thalamus are shown for baseline, training, and testing. The response to the Sm reveals the unconditioned effect of morphine by the large increase in amplitude in the first training session. No progressive development during training occurs in the response to either stimulus. See text for a discussion of the test results.


Group Data – PVT
Sm
Ss
Peak-to-Peak Amplitude
140
120
100
80
60
40
20
N=2
Addicted/
Morphine
N=5
Addicted/
Saline
N=3
Withdrawn/
Morphine
N=5
Withdrawn/
Saline
1
2
3
4
5
6
7
8
9
8
9
8
9
8
9
BL
Training
Testing
100-Trial Blocks

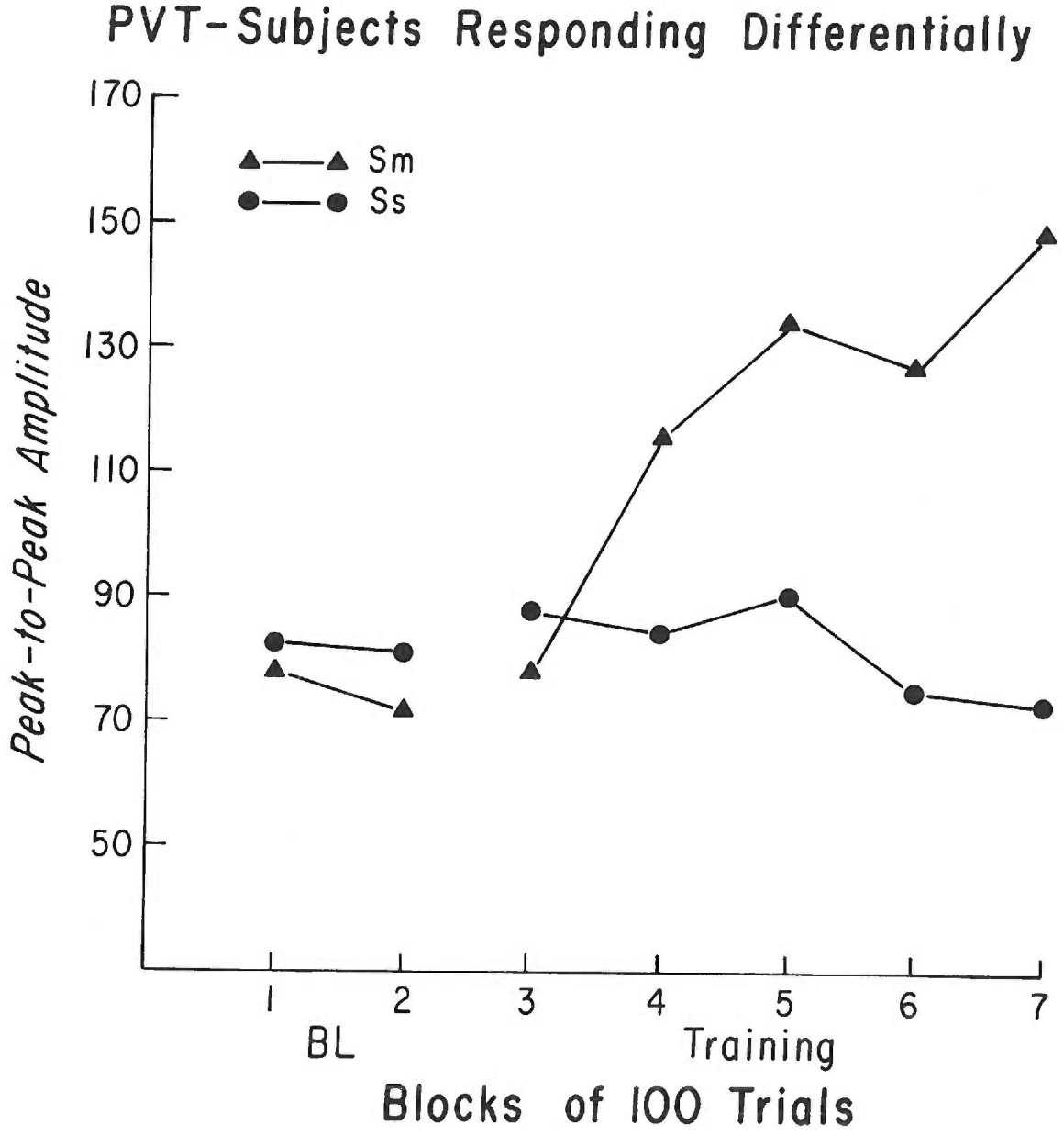
inspection of the baseline and training data shown in this figure that there is no development over training in the response to either the Sm or the Ss. This was confirmed by the lack of a significant trials effect in the analysis of variance ($F = 0.41$; $df = 4/162$; $p > .05$). However, the amplitude of the responses to the Sm was significantly greater than that of the Ss responses ($F = 19.2$; $df = 1/162$; $p < .001$). The trials X groups interaction was not significant ($F = 0.26$; $df = 4/162$; $p > .05$), indicating that the responses to the Sm and Ss did not diverge over the course of training. It was shown above that the immediate effect of morphine on thalamic AEPs was to increase the amplitude of their peaks. Thus, the immediate increase in the Sm response seen in the first training session, and the difference in amplitude between the Sm and Ss responses is probably due to the effect of morphine rather than to an association between morphine and the Sm.

The number of subjects in the groups tested with morphine was so small for paraventricular thalamus that it would be unwise to draw conclusions from that data. Nonetheless, the results were similar to those from the cortical placements. The Sm response was decreased somewhat in the addicted/morphine group and was maintained in the withdrawn/morphine group. The Ss responses were not elevated in the groups tested under morphine, however. The groups tested with saline were somewhat larger, and here the pattern was also similar to that seen in the cortical recordings. The response to the Sm was decreased greatly when tested in the saline condition whether the subjects were addicted or withdrawn at the time of testing.

The data from the seven subjects which did develop differential responding to the Sm and Ss are obscured in the group curve shown in Figure 13. The data from these subjects alone are shown in Figure 14. In these subjects the baseline thalamic responses to the Sm and Ss were similar, as were the responses during the initial training session. However, over the course of training the Sm response increased systematically while the Ss response did not. The results of the conditioning procedure on the paraventricular thalamus responses of these subjects were analogous to the results obtained from the cortical placements. An analysis of variance on the training data from these seven subjects revealed a significant groups effect ($F = 23.9$; $df = 1/54$; $p < .001$), and a significant groups by trials interaction ($F = 3.02$; $df = 4/54$; $p < .05$). The main effect of trials was not significant. However, a Schefe analysis showed that the second point on the Sm curve was significantly greater than the first ($F = 4.35$; $df = 1/54$; $p < .05$). This indicates that the Sm response did increase significantly over training and that the absence of a trials effect in the analysis of variance was due to the slight decrease in the Ss response during training. Testing data for these subjects is not shown because all test conditions were not represented among them.

As was pointed out above, in all seven subjects in which thalamic AEP components responded differentially to the Sm and Ss during training, differential responding was also found in the cortex. The mean latency of the thalamic peaks which were modified was 247.1 ms. The mean latency of the cortical peaks which were altered in these same subject was

Figure 14. Grouped data from the seven subjects in which the thalamic responses developed differentially to the Sm and Ss during training. Test data is not shown because all groups were not represented among these subjects.


PVT-Subjects Responding Differentially
Peak-to-Peak Amplitude
170
150
130
110
90
70
50
Sm
Ss
1
2
3
4
5
6
7
BL
Training
Blocks of 100 Trials

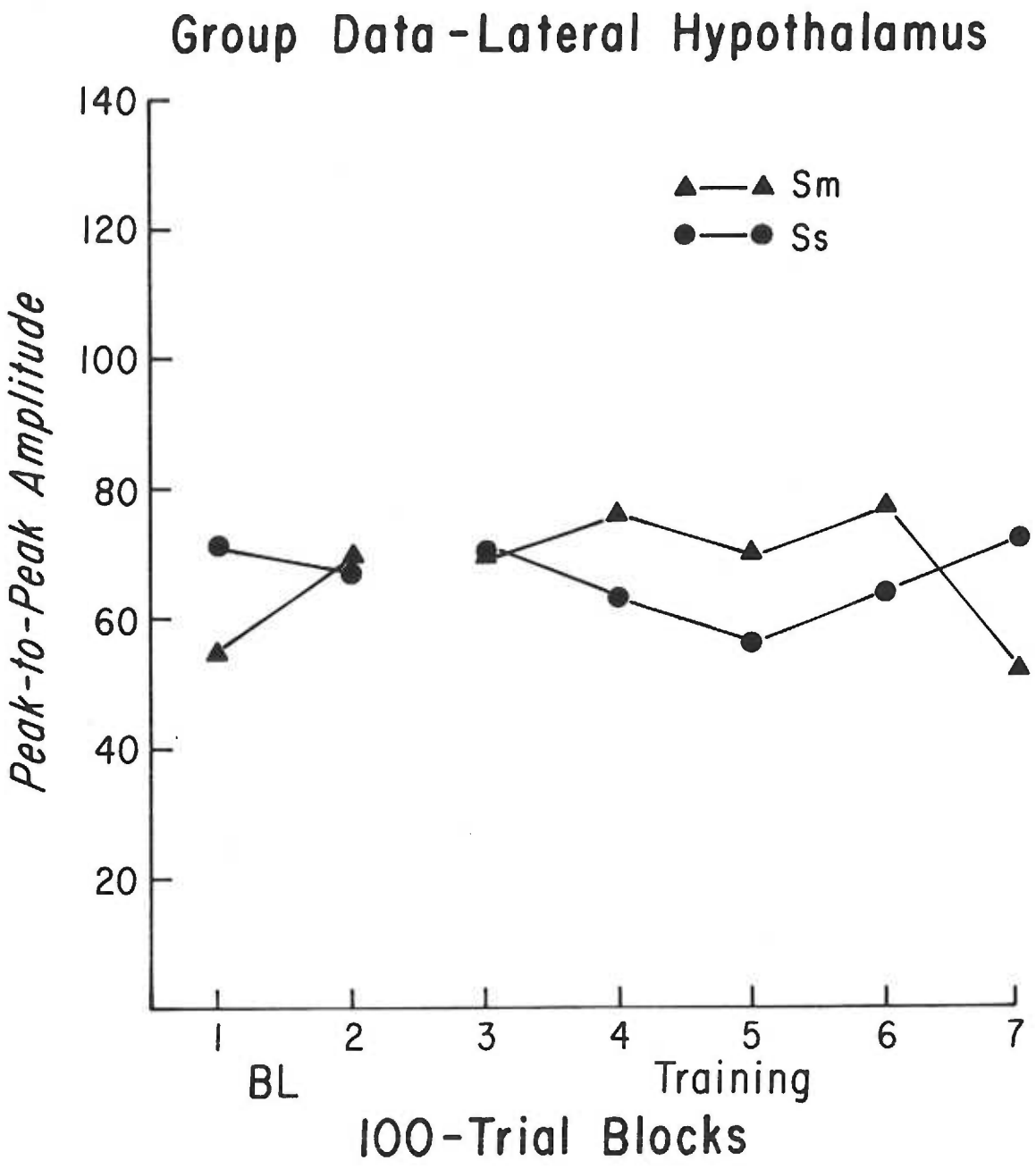
341.4 ms. A t-test for paired data revealed that the latency to the affected peak was significantly greater in the cortex than in the thalamus ($\overline{D}$ = 94.3; t = 2.44; df = 6; $p < .05$). As was true for the cortex, the thalamic peaks which developed differentially during training were within the latency range in which the initial morphine injection exerted its greatest unconditioned effect on the somatosensory AEPs.

Conditioning: Hypothalamus

Complete AEP records were obtained from twenty-one electrodes which were directed at VMH. Histological examination revealed that the electrodes were within VMH in ten subjects. In ten other subjects the electrodes were within hypothalamic nuclei other than VMH. No evidence of response alterations which could be attributed to conditioning was found in the records from any of these subjects. The responses recorded from the medial hypothalamic placements, however, did differ from those recorded from the lateral placements.

Figure 15 shows the responses recorded from the lateral hypothalamic placements. Included in this figure are data from five electrodes which were in LHA and one which was in the lateral pre-optic area. The data from these areas were similar and were combined. Examination of the figure reveals that there was no systematic change over training in the AEP response to either the Sm or the Ss. Nor was there any effect of morphine administration, since the Sm response during training did not differ from its baseline level.

Figure 15. Pooled data from lateral hypothalamic placements. Note the absence of a drug effect on the Sm response as well as the failure of the response to either stimulus to change during training.

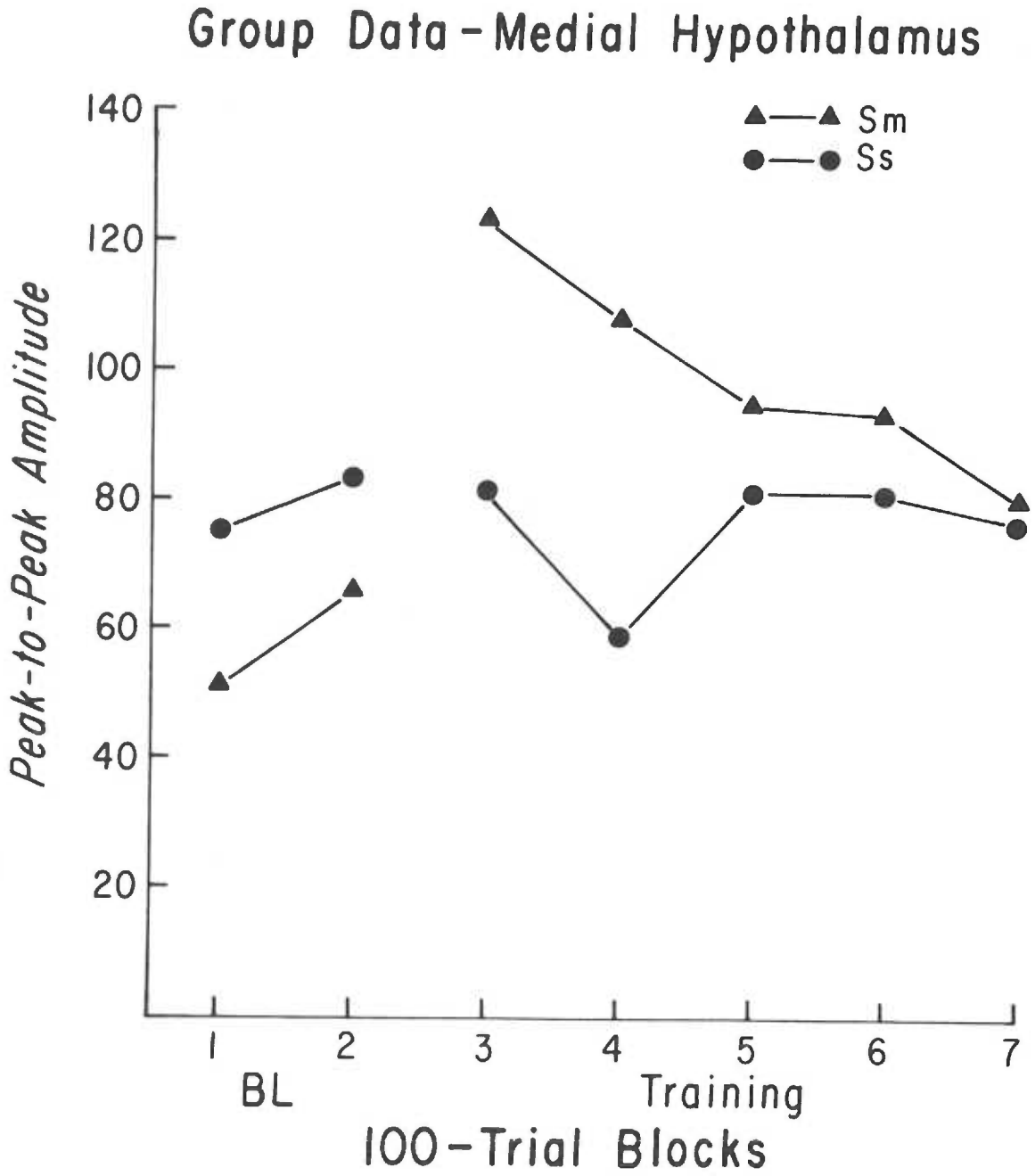

Group Data - Lateral Hypothalamus
140
120
100
80
60
40
20
Peak-to-Peak Amplitude
Sm
Ss
1
2
3
4
5
6
7
BL
Training
100-Trial Blocks

The data from the medial hypothalamic placements were also similar and the combined results from ten VMH, three ARH, and one AHA placements are shown in Figure 16. The AEPs from these areas did not develop differentially to the Sm and Ss over training. However, in these areas there was a large effect of morphine administration which is shown by the large increase in the Sm response in the first block of training. This drug effect lessens progressively over the course of training, possibly due to increasing tolerance to morphine, so that the Sm response at the end of training is approximately equal to the baseline response. There were no systematic changes in the responses to the Ss over training. The test sessions were intended to aid in the interpretation of possible conditioning effects and would serve no purpose in the absence of any such effects. For this reason the results of the test sessions were not analyzed for the hypothalamic placements.

Conditioning: Pontine reticular formation

The results described below were obtained from the 15 electrodes which were placed in the dorsal pontine reticular formation and the one electrode which was within the PVG. The data from the electrodes in the inferior colliculus and the commisure of the superior colliculus were not included. Differential responding to the Sm and Ss developed over training in five of these subjects (31%). In six subjects the response to the Sm did not change over the course of training, and in the remaining five subjects the Sm response actually decreased during training. In some cases the Ss response showed increases over

Figure 16. Grouped data from the medial hypothalamic placements. In this region there was a large increase in the Sm response in the first training session, followed by a progressive return toward baseline response levels. This is apparently due to an initially large drug effect which was attenuated by the development of tolerance to morphine as training continued.


Group Data - Medial Hypothalamus
Sm
Ss
Peak-to-Peak Amplitude
140
120
100
80
60
40
20
1
2
3
4
5
6
7
BL
Training
100-Trial Blocks

training in those subjects in which the Sm response decreased. These were the only cases found in this experiment in which the Ss responses increased during training sessions.

The data from the subjects which showed differential responding to the Sm and Ss are shown in Figure 17. The responses to the Sm and Ss were quite similar during baseline sessions. However, an orderly increase in the amplitude of the response to the Sm over training is clearly shown. The response to the Ss increases from baseline in the first training session, after which it returns to the baseline level. With the exception of the initial Ss training point, which is unusually elevated, the data from these five subjects are quite similar to the data from the cortical recording sites.

The data from the remaining PRF electrodes are shown in Figure 18. The Sm responses from these subjects, in contrast to those shown in Figure 17, decreased progressively during training. However, all training points remained within the range of the baseline data. The Ss responses were also quite different from those shown in Figure 17. In these subjects, the Ss responses were much greater in amplitude during the baseline sessions than during training.

The most interesting observation to be made about these two groups of subjects was the very large difference in amplitude of their baseline responses. In those subjects in which the Sm response increased over training, the mean Ss baseline response amplitude was 88. For the other subjects, the mean Ss baseline response amplitude was 163. The corresponding values for the Sm response during baseline were 79 and

Figure 17. Grouped data from those subjects in which the response to the Sm, recorded from the PRF, incremented over training.

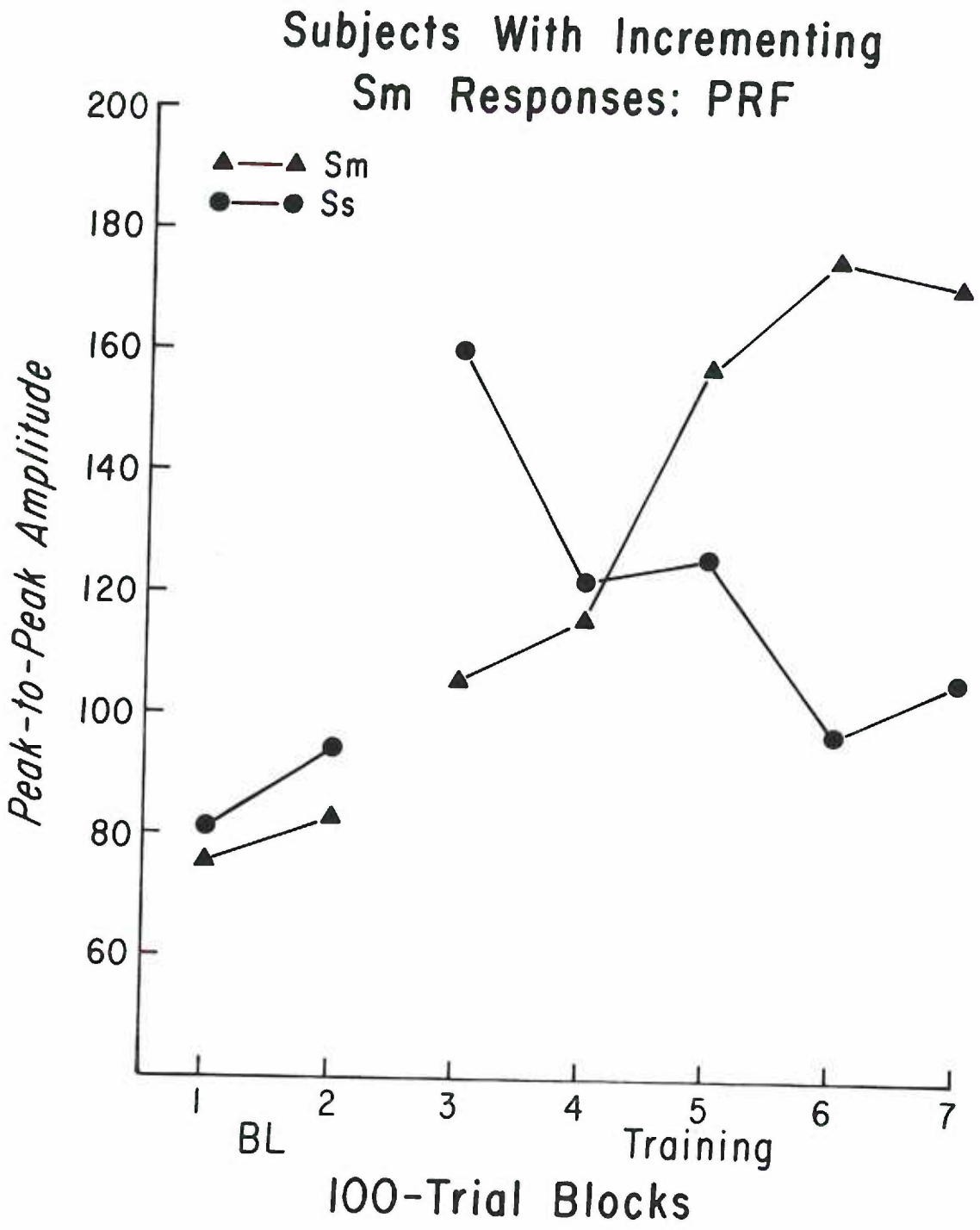
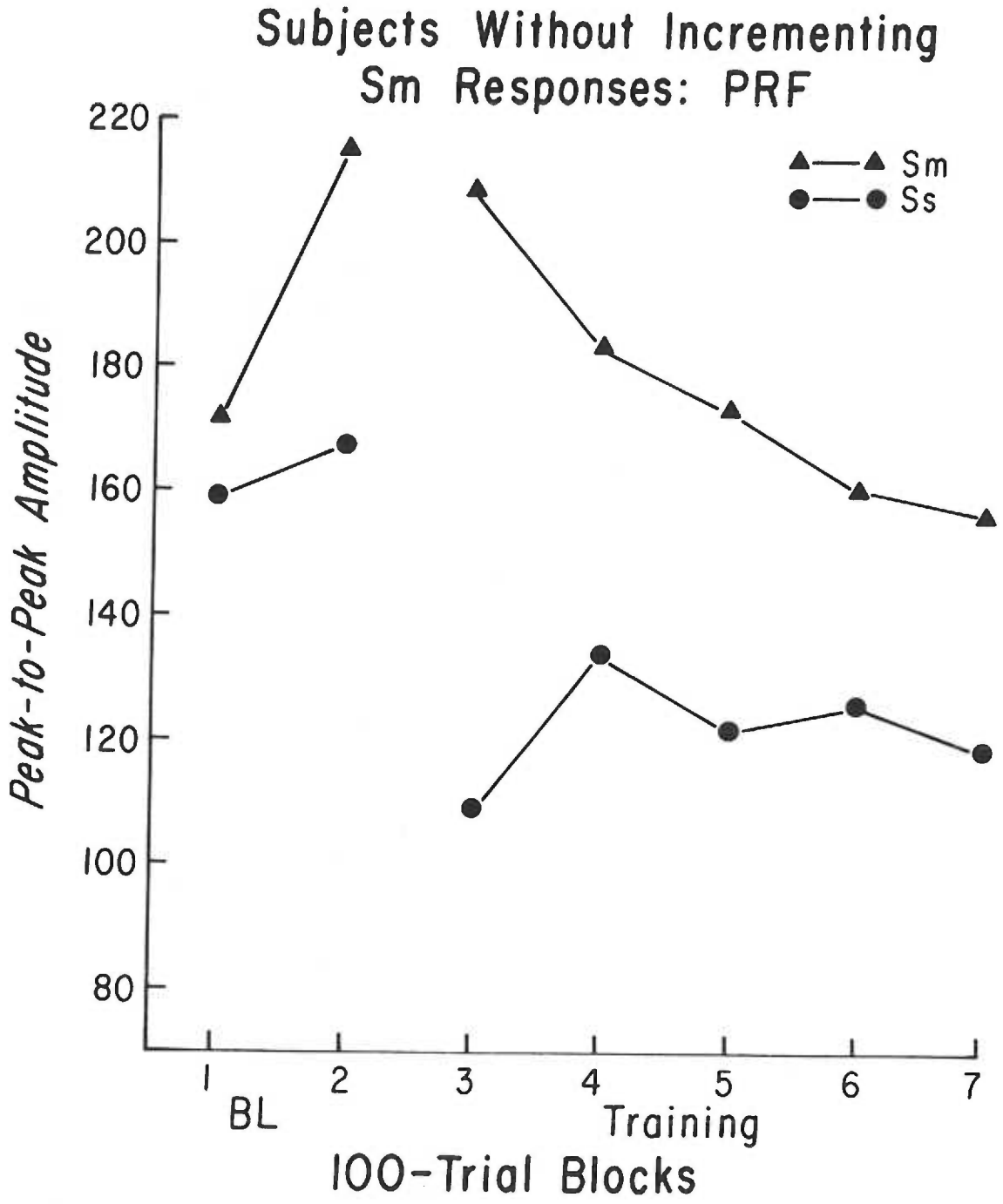

Subjects With Incrementing
Sm Responses: PRF
Sm
Ss
200
180
160
140
120
100
80
60
Peak-to-Peak Amplitude
1
2
3
4
5
6
7
BL
Training
100-Trial Blocks

Figure 18. Grouped data from those subjects in which the Sm response recorded from the PRF did not increment during training. Note that the baseline response amplitude for both the Ss and Sm responses are much greater than was the case for the subjects shown in Figure 17.


Subjects Without Incrementing
Sm Responses: PRF
Sm
Ss
Peak-to-Peak Amplitude
220
200
180
160
140
120
100
80
1
2
3
4
5
6
7
BL
Training
100-Trial Blocks

192. For both stimuli the difference between these pairs of values was significant (Sm: $t = 2.06$; $df = 30$; $p < .025$; Ss: $t = 2.19$; $df = 30$; $p < .025$). It is possible, although not certain, that the failure of the Sm response to increment during training in those subjects with larger baseline responses can be attributed to a ceiling effect.

DISCUSSION

The effects of morphine on cortical somatosensory evoked potentials found in this study are in general agreement with previously reported results. The initial morphine injection resulted in increases in the amplitude of the late components of the cortical AEPs (Figure 5). Similar results were obtained by Jurna, Schue, and Tamm (1972). They found that evoked responses to radial nerve stimulation, recorded from somatosensory cortex in rats, were increased following morphine injection. In cats, morphine has been found to increase the amplitude of evoked potentials in primary somatosensory cortex, primary auditory cortex, and motor cortex (Sinitsin, 1964). However, morphine-produced decreases in somatosensory evoked potentials have also been reported in the association cortex of cats (Sinitsin, 1964), and primary somatosensory cortex of dogs (Chin & Domino, 1961). In addition, both Sinitsin (1964) and Straw and Mitchell (1964) have reported that morphine had no effect on the amplitude of evoked responses to somatic stimulation recorded from the primary thalamic relay nuclei. These latter findings fit well with the lack of change in the short-latency components of the cortical evoked potentials seen in the present study.

Previous studies of morphine effects on evoked potentials recorded from mid-line thalamic nuclei, however, have most commonly reported decreases in evoked potential amplitude following morphine administration. This is in contrast to the large increases in the amplitude of the late components of the thalamic AEPs found in this study. Increases were also

found in the centromedian, parafascicular and dorso-medial nuclei of the thalamus in dogs by Chin and Domino (1961). Sinitsin (1964), however, found that morphine injection resulted in decreased evoked potential amplitude in the centromedian, parafascicular, dorso-medial, dorso-lateral, and posterior lateral nuclei of the thalamus in cats. Gildenberg, Murthy, Adler, and Frost (1976) studied the effect of morphine on the evoked response to sciatic nerve stimulation in the nucleus parafascicularis of rats. They found decreases in the amplitude of the evoked potentials following morphine injection. Furthermore, these decreases were most pronounced in the later components of the evoked potentials at approximately the latency at which the largest increase in AEP amplitude were found in the present study. This difference appears somewhat puzzling since the parafascicular nucleus is adjacent to the recording site employed in this experiment, less than one millimeter lateral. The difference in results could be due to the different dose levels of morphine employed. It is more likely, however, that it is due to the differences in anatomic interconnections between the nucleus parafascicularis and the paraventricular nucleus. The nucleus parafascicularis, recorded by Gildenberg et al. (1976), receives major input from the spino-thalamic system (Crosby, Humphrey, & Lauer, 1962). Therefore, it is not surprising that an analgesic dose of morphine would result in decreased evoked potentials in this area. The paraventricular nucleus, on the other hand, has interconnections with medial hypothalamic areas and possibly connections with the cortex (Crosby, Humphrey, & Lauer, 1962). Both of these areas

showed increases in AEP amplitude following morphine injection in the present study.

It is interesting to note that the alterations in both the cortical and thalamic AEPs following morphine administration occurred principally in the late portion of the response (thalamus: 150-225 ms; cortex: 200-300 ms). The long latency of the affected peaks indicates that morphine's major effect was exerted on the slowly conducting, polysynaptic pathways. The rapidly conducting primary sensory system appeared to be relatively unaffected by morphine. This fits with previous work which found that evoked potentials recorded from primary relay nuclei of the thalamus were not altered by morphine (Sinitsin, 1964). The later portion of the AEP, which was preferentially altered by morphine, has also been reported to be more susceptible to modification by conditioning procedures (John, 1961; Morrell, 1961).

No previous studies on the effect of morphine on evoked potentials recorded from the hypothalamus have been reported. The most striking finding regarding this area in the present study was the great difference in the effect of morphine on evoked potentials between medial and lateral hypothalamic recording sites (see Figure 7). Similar differentiation of morphine effects between medial and lateral hypothalamic nuclei has been reported in a study in which single hypothalamic neurons were recorded in rats (Kerr, Triplett, & Beeler, 1974). These investigators found that, following morphine injection, the firing rate of neurons from the ventromedial nucleus of the hypothalamus was increased, while that of neurons in the lateral hypothalamic area was decreased. The

reciprocal effect of morphine on these two hypothalamic areas is not surprising in view of their well documented reciprocal interaction in other functions (i.e., hunger - Anand & Brobeck, 1951; reward - Olds, 1962). Kerr, Triplett, and Beeler (1974) hypothesized, on the basis of their data, that the reciprocal effects of morphine on VMH and LHA were due to a direct inhibition of neurons in LHA which released those in VMH from tonic inhibition. No conclusions concerning this hypothesis can be reached based on the data from the present study. Decreases in AEP amplitude were found in some post-morphine records from LHA placements in the present study, however, these decreases were quite small and did not occur in all cases. The critical difference between these two studies is that Kerr, Triplett, and Beeler (1974) studied spontaneous neural activity, while evoked activity was recorded in the present study.

The most common result of morphine administration on reticular AEPs in the present experiment was a decrease in peak amplitude and an increase in peak latency. This morphine-produced decrease in evoked potential amplitude has been found by numerous investigators in the mesencephalic reticular formation (Straw & Mitchell, 1964; Nakamura & Mitchell, 1972) and the central gray region (Straw & Mitchell, 1964; McKenzie & Beechey, 1962). The only report of increases in evoked potentials following morphine in the reticular formation is from a study on dogs using tooth pulp stimulation (Chin & Domino, 1960). Depression of evoked potential responses in the mesencephalic and rostral pontine reticular formation by analgesic doses of morphine

would be expected because the multi-synaptic spinothalamic system, which has been implicated in the mediation of pain (Melzack, 1973; Wall, 1960; Wall, 1970), passes through this region. It has been shown that morphine depresses neural activity in the spinal regions which supply afferents to this system (Borison, 1971).

One relationship which was noted in the results of this experiment was that between the magnitude of the initial morphine effect and the success of the conditioning paradigm. Those brain regions in which morphine injection resulted in a large increase in AEP area (Cx and paraventricular thalamus) responded differentially to the Sm and Ss during training more frequently than those regions which were less affected by morphine (VMH and PRF). A positive relationship between the intensity of the unconditioned stimulus (US) and the frequency of the conditioned response (CR) has been found in a number of classical conditioning studies (Gormezano & Moore, 1962, 1969; Smith, DiLollo, & Gormezano, 1966). The present case is not precisely analogous to these studies, however, in that the unconditioned stimulus was the same for all brain areas. It was the unconditioned response (UR) to the morphine injections which differed among the areas. Very few of the experiments in which the effect of US intensity on CR development has been examined have included explicit measurements of UR magnitude. In one study in which UR magnitude was measured (Fitzgerald & Teyler, 1969), it was found to be correlated positively with the CR magnitude.

In previous studies involving the conditioning of neural responses, it has been found that the most successful conditioning is obtained when

both the US and the CS produce a response in the neural structure being recorded (Rosenblum & O'Brien, 1977; O'Brien, Wilder, & Stevens, 1977). In one of these studies (O'Brien, Wilder, & Stevens, 1977), cortical neurons were conditioned using a somatic CS and antidromic activation via a shock to the pyramidal tract as the US. A control group of neurons was run using the same CS and US parameters as the experimental group. However, these neurons did not respond to the US (no UR) and they showed no evidence of conditioning. Thus, it appears reasonable to expect a higher percentage of subjects to show evidence of conditioning in those brain areas on which the US exerts a large excitatory effect. Certainly this relationship held in the present experiment. The brain regions in which morphine had only a slight excitatory effect or resulted in inhibition of responding did not develop differential responding to the Ss and Sm. Furthermore, within the cortex and thalamus, changes over the course of training sessions were found primarily in the later peaks of the AEPs, at the latencies most affected by morphine. The early peaks which were seldom affected by morphine were rarely altered by the training procedure. This raises the possibility that only those brain areas in which morphine increases neural excitability can be conditioned utilizing morphine as the US. However, in the present study, morphine produced large changes in the AEPs from the medial hypothalamus, but no evidence of conditioning was seen in the records from that area. In addition, morphine resulted in a slight depression of reticular AEPs, but differential responding to the Sm and Ss during training was found in a few of the recordings

from the PRF. Thus, suggestions concerning a relationship between morphine-produced increases in neural excitability and conditioning must be regarded as speculative.

Conditioning

The major result of the conditioning procedure used in this experiment was the progressive development of differential responding to the Sm and Ss over the course of training. This differentiation was manifested by an increment in the AEP response to the Sm which did not occur to the Ss. Differential responding was found most frequently in the cortex and thalamus, although five subjects did show evidence of differential responding in the reticular formation (see Figures 10, 14, and 17). The principal findings from the testing sessions (shown in Figures 10 and 13) were: (1) a very large decrease in the Sm response when testing was done under saline; (2) an increase in the Ss response when testing was done under morphine, and (3) a decrease in the Sm response in the addicted/morphine group but not in the withdrawn/morphine group.

The abrupt decrease in the Sm response when tested in the saline condition seems to suggest that the increase in Sm responding during training must be attributed to something other than conditioning. For if a conditioned response to the Sm had developed during training, one would expect it to be maintained, at least initially, during the saline testing sessions. The increment in the AEP response to the Sm over training cannot be attributed to an increase in neural excitability due to repeated drug injections or any other general cause,

since the Ss response did not increase. The gradual development of tolerance to morphine could conceivably result in a progressive change in the response to a stimulus which is presented following morphine injection. However, tolerance is said to have developed when, after repeated administrations, the effect of a given dose of a drug is less than it was originally. The initial effect of morphine administration on cortical and thalamic AEPs in the present experiment was an increase in AEP amplitude. Therefore, if only the development of morphine tolerance over training were operating, one would expect to find the initial drug-produced increase in AEP amplitude, followed by a progressive return toward baseline response levels. The results from the medial hypothalamus, shown in Figure 16, fit this pattern and very likely reflect increasing tolerance to morphine. However, it is clear that tolerance cannot account for the progressive increase in the Sm response which actually was found in the cortex and, to a lesser extent, in the thalamus.

A more plausible alternative hypothesis which could account for the results of training is to postulate a progressive augmentation of the initial drug effect. This could be attributed to build-up of morphine in the subjects over time. Morphine was administered once/8 hours throughout the course of training. It is likely that the previous dose was incompletely metabolized at the time of each subsequent dose. Thus, each dose after the first would be increased by some small amount. Still, it does not seem likely that this could account for the large changes in the Sm response. Any morphine accumulation would also be

present during Ss training sessions, yet no increase in Ss responses was found over training. Furthermore, tolerance to the effects of morphine develops rapidly in rats (Siegel, 1975) and should offset any increase in effective drug dosage from session to session. The morphine dosage was increased once during training to offset the effects of tolerance. However, the increase in Sm response from the first training session to the second (prior to the dosage increase) was as large as that from the second to the third (following the dosage increase). Thus, while postulating a drug effect would account for the decreases in Sm responding under the saline test condition, it would not account for the results of training.

One may account for the results of training, however, by assuming that a conditioned response developed to the Sm but not to the differential stimulus, the Ss. Several characteristics of the response changes suggest that they are due to classical conditioning:

(1) Pairing the Sm with morphine administration resulted in an orderly, incremental development of the response (Figures 10 and 14).

(2) The form of the acquisition curve is comparable to that for behavioral measures of conditioning (Pennypacker, 1967; Fig. 8.2), and to previous evoked potential conditioning results (Galambos & Sheatz, 1962).

(3) Differential responsiveness to stimuli, particularly if these stimuli excite the same sensory modality, is often considered sufficient evidence to demonstrate conditioning (Seligman, 1970; Kimble, 1967).

In addition, the decrease in the response to the Sm in the addicted/morphine group during testing (Fig. 10) may be explained within a conditioning context. The subjects which were maintained on morphine during the training-test interval received three morphine injections per day throughout this seven-day period. These injections were administered in the animal colony room, without Sm or Ss presentations. The regular administration of morphine injections has been shown to be sufficient to produce conditioned responses to the cues associated with the injection ritual (Pavlov, 1927; Lynch, Stein, & Fetziger, 1976). Thus, the maintenance procedure could have allowed the subjects to develop conditioned responses to the set of stimuli regularly associated with the morphine injections (i.e., the colony room, the experimenter). It is conceivable that the development of conditioning to this new set of stimuli could have interfered with the previously acquired association between the Sm and the morphine state. This would account for the decreased Sm response of the addicted subjects when tested with morphine. The withdrawn subjects, on the other hand, received only saline injections during the training test interval. Thus, they had no opportunity to form competing conditioned responses, and the Sm response in the withdrawn subjects tested under morphine is not decreased from the level attained at the end of training (Figure 10).

The increases in response to the Ss in the groups tested with morphine (Figure 10) are almost certainly due to the unconditioned excitatory effect of morphine on the cortex. These increases appear to be analogous to those found in the Sm response between the end of

baseline and the first training session. If this is the case, then the testing results for the withdrawn/morphine group shown in Figure 10 are very instructive. The amplitude of the Ss response provides an estimate of the unconditioned effect of morphine on the cortical AEP. However, the Sm response in these subjects is significantly greater than that to the Ss ($t = 2.42$, $df = 3$, $p < .05$). During testing, both the Ss and Sm were presented during each session, under the same drug condition. Thus, any difference in the responses to these stimuli must be attributed to the subjects' previous experience. In this regard, they differ only with respect to the training procedure, in which the Sm was presented while the subjects were morphine-intoxicated and the Ss was presented when they were not. This training procedure, then, appears to have resulted in a differential increment in the cortical AEP response to the Sm over and above the unconditioned excitatory effect of morphine. This differential increment persisted for at least seven days following the end of training when no additional drug exposure intervened. It is also interesting to note that in the thalamus, where the grouped data from all subjects did not show evidence of conditioning during the training sessions, the Sm and Ss responses of the withdrawn/morphine group did not differ during testing (Figure 13).

Still, in order to have confidence in the hypothesis that the increment in the Sm response over training was due to conditioning, we must be able to explain the dramatic decrease in the Sm response of those groups tested with saline (Figure 10). It is possible that this loss of responding under the saline condition was due to one of

the methodological problems of experiments which study drugs and conditioning:drug-dissociated learning or state dependency. Drugs which act on the brain can serve as discriminative stimuli. A rat may learn to make a choice in a T-maze based only on its drug state, no other discriminative conditions are necessary (Overton, 1968). In some cases, after a response has been learned in a particular drug state, it is not performed when the subject is no longer in that drug state (Overton, 1968; Bliss, 1974). Narcotic drugs, including morphine, have been shown to produce state dependent learning (Belleville, 1964; Charney & Reynolds, 1967). It has also been found that dissociation is dose dependent, with higher doses yielding less transfer between states (Overton, 1964). The doses of morphine used in the present study were moderately high, being well above the level necessary to produce analgesia, and sufficient to produce a semi-catatonic state. Doses of this magnitude have been shown to be an effective discriminative stimulus in both avoidance (Hill, Jones, & Bell, 1971) and food motivated (Gianutos & Lal, 1975) tasks. In a shock-avoidance task, Shannon and Holtzman (1976) have shown that morphine is an effective discriminative stimulus in doses as low as 3 mg/kg and produces dose-related discriminative effects over a 100-fold dose range. Thus, it seems quite possible that the decrease in the Sm response during testing under saline was due to the dissociative effect of the change in drug state from training to testing.

In summary, it appears that the best interpretation of the results obtained in this experiment is as follows:

(1) The training procedure resulted in the development of a differential conditioned response which was reflected by a developmental increment in the cortical AEP elicited by the Sm but not the Ss.

(2) Repeated morphine injections in the presence of cues other than the Sm result in a decrement in the Sm response.

(3) The conditioned response was subject to drug-dissociation effects and, therefore, was not expressed in the non-drug state.

(4) The conditioned response was maintained for at least one week after training under certain conditions.

It is possible that the differential resonding to the Sm and Ss which developed during training in 40% of the thalamic recordings and in 30% of the reticular recordings also should be attributed to conditioning. The grouped data from those subjects which did respond differentially in these areas (Figures 14 and 17) show a response topography quite similar to that found in the cortical data. However, all test conditions were not represented among the animals which showed differential responding in these areas. When the data from all subjects was pooled, the contribution of those subjects which responded differentially was obscured by that from those which did not. Thus, the differential responding to the Sm and Ss which developed during training in paraventricular thalamus and PRF cannot definitely be ascribed to conditioning, despite the similarity to the cortical response.

It is certain, however, that the development of differential responding in the cortex was not dependent on differential responding in any of the subcortical areas recorded in this study. Seven of the subjects which responded differentially to the Sm and Ss in the cortex did so in none of the other areas recorded. It is possible, in fact, that the differentiation between Sm and Ss found in the paraventricular thalamus was based on some type of descending cortical influence, since all subjects which responded differentially in the thalamus also did so in the cortex. The thalamic response was not simply projected from the cortex, however, since the latency of the modified components in the thalamic AEPs was typically less than that found in the cortex.

The only previous attempt to produce conditioned alterations in evoked potentials using morphine administration as the unconditioned stimulus is that of Stein, Lynch, and Ruchkin (1977). These authors reported conditioned changes in the cortical auditory evoked potential elicited by clicks which had been paired with morphine injections. A differential conditioning paradigm was not employed in this experiment, however. The evoked potentials recorded from subjects given morphine were compared to those from subjects which received only saline. Thus, changes in the evoked potentials due to conditioning could not be differentiated from those resulting from general changes in the subjects' state, produced by repeated morphine injections. Nor did these experimenters attempt to ascertain whether the putative conditioned responses found in their experiment persisted following withdrawal of the subjects from morphine.

Despite these shortcomings, the results reported by Stein, Lynch, and Ruchkin (1977) were quite similar to those found in the present study. In both studies the effect of morphine was to increase the amplitude of late components of the evoked responses. Also in both cases, the conditioned response which developed was in the same direction as the unconditioned morphine effect. The principal differences in results between the two studies are in the latency of the evoked potential components which were modified by the conditioning procedures and in the number of trials necessary to produce a conditioned response. Stein, Lynch, and Ruchkin (1977) found conditioned changes in the early components of the auditory evoked potential. In fact, all the changes which they found occurred within the first 75 ms of the evoked response. In contrast, the changes in the cortical evoked potentials found in the present study occurred most frequently at latencies approximating 200 ms (Figure 11), while the early evoked potential components were unaltered by training. This difference could be due to the different stimulus modalities employed, or to the fact that Stein, Lynch, and Ruchkin (1977) recorded only the first 250 ms of the auditory evoked response, thus missing any changes which may have occurred at greater latencies. Changes were found in the cortical AEPs at latencies greater than 300 ms in the present study.

Despite the differences between these two studies in design, both found evidence that morphine, acting as an unconditioned stimulus, can reinforce the formation of classically conditioned responses in the rat which are reflected by alterations in the cortical evoked potentials

elicited by a stimulus which is paired with morphine administration. The agreement between these experiments, despite their procedural differences, indicates that the effect under study may be quite general. It has been shown that morphine affects spontaneous activity in numerous areas of the brain as well as activity evoked by a variety of stimuli (Tables 1 & 2). Based on the findings of Stein, Lynch, and Ruchkin (1977) and the present experiment, it appears that the neural response to various sensory stimuli may be altered as well, at least in the cortex, when these stimuli are associated with morphine injections. Furthermore, in the present experiment, the changes produced in the cortical response were found to persist following withdrawal from morphine. These findings of long-lasting changes in the neural response to stimuli which have been paired with morphine intoxication have a direct bearing on conditioning theories of narcotic addiction and relapse (Wikler, 1948; 1972; 1973; Copeman, 1975; Goldberg, 1976).

One of the most clearly formulated conditioning theories of narcotic addiction is that proposed by Wikler (1948; 1972; 1973). The keystone of Wikler's formulation is the suggestion that cues which are associated with morphine administration come to elicit compensatory conditioned responses. Thus, these cues, when presented in the absence of the drug, would produce responses opposite to the direct drug effect (i.e. hyperalgesia, anxiety, tachycardia). There is no doubt that some autonomic responses to stimuli which have been paired with morphine injection are opposite to the unconditioned responses elicited by drug injection. Morphine causes bradycardia in dogs, however, the

cardiac conditioned response formed when morphine is used as the US is typically tachycardia (Rush, Pearson, & Long, 1970; Lynch, Fertziger, & Teitelbaum, 1973). Similarly, Siegel (1975) has reported conditioned hyperalgesia to a stimulus complex which was paired with morphine injections. Wikler maintains that the sum of these compensatory responses is similar to the effect of acute morphine withdrawal. Thus, the presentation of stimuli previously associated with morphine intoxication, even in the withdrawn subject, could result in a conditioned abstinence response which would lead to drug seeking behavior. Compensatory conditioned responses have also been invoked to explain the development of tolerance (Siegel, 1975; 1976). The conditioned neural responses found in the present study and by Stein, Lynch, and Ruchkin (1977), however, were not compensatory. Rather, they were in the same direction as the unconditioned response to the drug. Thus, these results appear to be more similar to those of Davis and Smith (1976), Lal, Miksic, Drawbaugh, Numan, and Smith (1976), and Copeman (1975) as well as Pavlov (1927) who have reported conditioned responses reinforced by morphine which are much like the direct effects of the drug.

The results of the present study differed from those of previously reported experiments on morphine-reinforced conditioning in that the conditioned response found was state-dependent. This could be due to the somewhat unusual conditioning procedure of presenting the drug-paired stimulus (Sm) following the morphine injections. This limited the subjects' experience with the Sm to the drug condition prior to testing.

However, this same procedure was employed in Pavlov's laboratory and the conditioned response which was formed was reliably elicited from the subject when in the non-drugged state, In any case, since the conditioned response formed in the present experiment was expressed only when the subjects were in the drugged state, it cannot reflect the processes postulated by Wikler and others to trigger relapse in drug-free addicts.

SUMMARY AND CONCLUSIONS

The effects of intravenous morphine administration on somatosensory evoked potentials were investigated in rats. Evoked potentials were collected from the association cortex, paraventricular thalamus, hypothalamus, and the pontine reticular formation. The most striking effect of morphine on the average evoked potentials was the frequent appearance of a broad, late positive peak at a latency of 200-300 ms. This effect was most pronounced in the cortical and thalamic responses, found only in the medial hypothalamic responses and quite rare in those recorded from the pontine reticular formation.

In addition, an attempt was made to produce conditioned changes in the somatosensory evoked responses by explicitly associating stimulus presentations with the systemic effects of intravenous morphine administrations. A differential conditioning paradigm was employed, in which shocks to one randomly selected forepaw were delivered only when the subject was morphine-intoxicated. Shocks to the other paw were delivered only when the subject was not intoxicated. This procedure resulted in alterations in the cortical response to the stimulus which was associated with the morphine state. These changes did not occur in the responses to the differential stimulus. It was possible to differentiate these putative conditioned changes in the cortical AEPs from the unconditioned sensory effects of the drug, and from the changes produced by the development of tolerance to morphine. Analogous results were found in the thalamic and reticular records of some subjects but, as a group,

the AEPs from these areas did not show significant changes attributable to conditioning effects. No evidence of conditioning was found in the hypothalamic data.

The conditioned cortical response was found to persist following withdrawal of the subject from morphine. However, the response was found to be state-dependent and was expressed only when the subjects were in the acquisition state; that is, morphine-intoxicated. It was concluded that the conditioning paradigm employed resulted in the formation of conditioned responses which were reflected by alterations in the cortical evoked potentials elicited by the morphine-paired stimulus. However, since the conditioned response was state-dependent, it could not reflect the conditioning processes presumed by many investigators to underlie relapse in drug-free, withdrawn addicts.

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