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**LA THÈSE A ÉTÉ
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**Effects of Naltrexone on Nucleus Accumbens, Lateral
Hypothalamic and Ventral Tegmental Brain Stimulation Reward**

Thomas Edward George West

**A Thesis
in
The Department
of
Psychology**

**Presented in Partial Fulfillment of the Requirements
for the degree of Master of Arts at
Concordia University
Montréal, Québec, Canada**

December 1986

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ABSTRACT

Effects of Naltrexone on Nucleus Accumbens, Lateral Hypothalamic and Ventral Tegmental Brain Stimulation Reward

Thomas Edward George West

The effects of opioid receptor blockers on brain stimulation reward (BSR) have not been consistent from one report to the next. Differences in site of stimulation, stimulation parameters, and time of testing have been suggested as explanations of the inconsistencies. In the first experiment, animals were tested across a range of stimulation frequencies in order to assess the effects of naltrexone (2.5, 5.0, 10.0, 20.0 mg/kg) on responding for electrical stimulation of the nucleus accumbens, lateral hypothalamus and ventral tegmentum. Animals were tested in sessions, beginning 15 or 45 minutes after the administration of naltrexone. Naltrexone generally shifted the rate-frequency curve to the right, and the magnitude of the effect was dependent on site of stimulation and time of testing, but not on the parameters of stimulation. The greatest rightward shifts were observed with stimulation of the nucleus accumbens and the least with stimulation of the ventral tegmental area. There was a greater attenuation of responding during the second test session than during the first test session. It was not clear why naltrexone was not more effective in the first test session. It has been suggested that an animal requires a period of active responding under drug in order for naltrexone to produce significant effects, hence, a second experiment was conducted to

test this hypothesis. The time-course of naltrexone's effect on BSR was determined by measuring an animal's rate of responding over a three hour period in sessions with immediate access (five-minute delay) or delayed access (45-minute delay) to stimulation. The greatest decrease in responding was observed 45, 65, and 85 minutes after injection regardless of the time of delay. The fact that the drug was more effective 45 minutes after injection explains some of the inconsistencies in the literature and probably reflects time needed for passive diffusion of drug to its site of action.

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TABLE OF CONTENTS

ABSTRACT..... iii

ACKNOWLEDGMENTS..... v

LIST OF FIGURES..... viii

LIST OF APPENDICES..... x

INTRODUCTION..... 1

 I. The discovery of endogenous opioid peptides..... 1

 II. Search for a natural function of the
 endogenous opioid peptides..... 2

 A. The effect of opiate antagonists on
 feeding and drinking..... 3

 B. The effect of naloxone on brain stimulation
 reward..... 3

 III. The effect of opiate antagonists on brain
 stimulation reward..... 4

 A. Differences in the effect of opiate
 antagonists on brain stimulation reward..... 4

 B. Factors which may explain the differences.... 6

 1. Site of electrode implantation..... 6

 ii. Time and duration of testing..... 8

 iii. Parameters of rewarding stimulation... 11

 C. Paradigms for studying drug effects on
 self-stimulation..... 12

EXPERIMENT 1..... 19

 Method..... 20

 Results..... 27

Discussion.....	53
EXPERIMENT 2.....	60
Method.....	60
Results.....	62
Discussion.....	74
SUMMARY AND CONCLUSIONS.....	75
REFERENCES.....	77
APPENDIX I.....	90
APPENDIX II.....	91

LIST OF FIGURES

- Figure 1: The effect of naltrexone (2.5 mg/kg) on nucleus accumbens brain stimulation reward..... 29
- Figure 2: The effect on naltrexone (5.0 mg/kg) on nucleus accumbens brain stimulation reward..... 31
- Figure 3: The effect of naltrexone (10.0 mg/kg) on nucleus accumbens brain stimulation reward..... 33
- Figure 4: The effect of naltrexone (20.0 mg/kg) on nucleus accumbens brain stimulation reward..... 35
- Figure 5: The effect of naltrexone (2.5 mg/kg) on lateral hypothalamic brain stimulation reward.. 37
- Figure 6: The effect of naltrexone (5.0 mg/kg) on lateral hypothalamic brain stimulation reward.. 39
- Figure 7: The effect of naltrexone (10.0 mg/kg) on lateral hypothalamic brain stimulation reward.. 41
- Figure 8: The effect of naltrexone (20.0 mg/kg) on lateral hypothalamic brain stimulation reward.. 43
- Figure 9: The effect of naltrexone (2.5 mg/kg) on ventral tegmental brain stimulation reward..... 45
- Figure 10: The effect of naltrexone (5.0 mg/kg) on ventral tegmental brain stimulation reward..... 47
- Figure 11: The effect of naltrexone (10.0 mg/kg) on ventral tegmental brain stimulation reward..... 49
- Figure 12: The effect of naltrexone (20.0 mg/kg) on ventral tegmental brain stimulation reward..... 51
- Figure 13: Time-course of the effects of naltrexone on lateral hypothalamic brain stimulation reward beginning 5 minutes after injection..... 63
- Figure 14: Time-course of the effects of naltrexone on lateral hypothalamic brain stimulation reward beginning 45 minutes after injection..... 65
- Figure 15: Histological localization of electrode tips aimed at the nucleus accumbens..... 68
- Figure 16: Histological localization of electrode tips aimed at the lateral hypothalamic area..... 70

Figure 17: Histological localization of electrode tips
aimed at the ventral tegmental area..... 72

LIST OF APPENDICES

- Appendix I: Effects of four doses of naltrexone on nucleus accumbens, lateral hypothalamic or ventral tegmental reward thresholds..... 90
- Appendix II: Effects of four doses of naltrexone on maximal rate of responding for stimulation of the nucleus accumbens, lateral hypothalamic area, or ventral tegmental area. 91

I. The discovery of the endogenous opioid peptides.

One of the major advances of neuroscience research in the past twenty years was the discovery of high affinity, stereospecific opiate receptors in the brain (Pert & Snyder, 1973; Simon, Hiller, & Edelman, 1973; Terenius, 1973). The discovery of the opiate receptor triggered a search for endogenous opioid neurotransmitters, since as Goldstein (1976) suggested, "it seemed unlikely that such highly stereospecific receptors would have been developed by nature to interact with alkaloids from the opium poppy". By 1975, three research groups working independently were able to isolate endogenous compounds from the brain (Hughes, 1975; Pasternak, Goodman, & Snyder, 1975) and human cerebral spinal fluid (Terenius & Wahlstrom, 1975) that mimicked opiate activity. The opiate-like compounds extracted from the brain by Hughes (1975) consisted of two pentapeptides which were termed methionine-enkephalin and leucine-enkephalin (Hughes, Smith, Kosterlitz, Fothergill, Morgan, & Morris, 1975). In the same year, Teschemacher, Opheim, Cox, and Goldstein (1975) and Cox, Opheim, Teschemacher, and Goldstein (1975) discovered substances in pituitary extracts that also exhibited opiate activity. These substances, which were demonstrated to be much larger than the enkephalins, were termed endorphins. The term "endorphin", which was coined by Simon (see Goldstein, 1976), is now used to refer collectively to the enkephalins of Hughes's discovery and to the specific endorphin of Goldstein's discovery as well as additional

endogenous opioid peptides that have been subsequently discovered.

With the identification of opiate receptors in the brain came the realization that endogenous opioid peptides might serve as neurotransmitters that play a normal physiological role in brain function. It was soon demonstrated that these substances had neurotransmitter-like actions (Bloom, Rossier, Battenberg, Bayon, French, Henricksen, Siggins, Segal, Browne, Ling, & Guillemin, 1978; Elde, Hokfelt, Johansson, & Terenius, 1976; Nicoll, Siggins, Ling, Bloom, & Guillemin, 1977). A major challenge for behavioural neuroscientists has been to determine the functions of the circuits that utilize opioid peptides as chemical messengers.

II. Search for a natural function of the endogenous opioid peptides.

One approach to determine functions of the endogenous opioid peptides involves administering these agents centrally (Joyce, Koob, Strecker, Iversen, & Bloom, 1981; Yaksh & Rudy, 1978). Another approach is to pharmacologically block opiate receptors in animals that receive no exogenous opiates. If the normal behaviour of an animal depends on endogenous opioid action at opiate receptors then administration of an opiate receptor blocker should produce a behavioural disruption. Naloxone and naltrexone are selective opiate receptor blockers that have been widely used for this purpose.

A. The effect of opiate antagonists on feeding and drinking.

One of the first demonstrations that an opiate antagonist could affect the behaviour of a drug-free animal was reported by Holtzman (1974). Naloxone, when administered to food-deprived animals, was found to significantly reduce food intake. An attenuation of food intake in food-deprived animals has subsequently been observed by many other researchers (Brands, Thorngill, Hirst, & Gowdy, 1979; Holtzman, 1979; Jalowiec, Pankseep, Zolovick, Najam, & Herman, 1981; Jones & Richter, 1981). It appears that endogenous opioids are important in a physiological system which is involved in water intake as well. Holtzman (1975) reported that naloxone and naltrexone each significantly attenuated water consumption in fluid-deprived animals. These effects have also been confirmed in other studies (Brown, Blank, & Holtzman, 1980; Frenk & Rogers, 1979; Holtzman, 1979; Maickel, Braude, & Zablow, 1977). It appears that these opioid effects occur at receptors within the central nervous system, since the quaternary derivatives of naloxone and naltrexone, which do not readily pass through the blood-brain barrier, have minimal effects on fluid consumption in water-deprived animals (Brown & Holtzman, 1981).

B. The effect of naloxone on brain stimulation reward.

It has also been reported that naloxone decreases responding for brain stimulation reward (Belluzzi & Stein, 1977). This

finding is of particular interest and potential importance because brain stimulation reward (BSR) is thought to activate mechanisms that are presumed to mediate the effects of natural reinforcers. Hence, perhaps the effects of naloxone on feeding and drinking involve an action in the mechanisms that mediate food and water reinforcement.

III. The effect of opiate antagonists on brain stimulation reward.

A. Differences in the effect of opiate antagonists on brain stimulation reward.

Belluzzi and Stein (1977) were the first to demonstrate that the endogenous opioid peptides may be involved in subserving the reinforcing effect of brain stimulation reward. When naloxone was administered to rats working for brain stimulation reward, a dose-related decrease in responding was observed. The researchers reasoned that the activation of opiate receptors by electrically-induced release of opioid peptides was responsible, at least in part, for the rewarding effects of brain stimulation. The observed decrease in responding for brain stimulation reward following naloxone administration was interpreted as a result of a blocking by naloxone of the rewarding action of the electrically-released peptides. The finding of Belluzzi and Stein was soon challenged however, by van der Kooy, LePiane and Phillips (1977) who reported that

naloxone had no effect on brain stimulation reward. Their finding led them to suggest that there was "no important role of the endogenous opioid-opiate receptor mechanism" in the rewarding effects of electrical stimulation of the brain.

The results of subsequent studies investigating the effect of opiate antagonists on brain stimulation reward have been equivocal. A survey of these results reveals 21 reports of suppressive actions of opiate antagonists on BSR (Belluzzi & Stein, 1977; Belluzzi, Trujillo, Sun, & Stein, 1985; Collier & Routtenberg, 1984; Franklin & Robertson, 1982; Freedman & Pangborn, 1984; Glick, Weaver, & Meibach, 1982; Ichitani, Iwasaki, & Satoh, 1985; Katz, 1981; Kelsey, Belluzzi, & Stein, 1984; Reymann, Wulcko, Ott, & Matties, 1986; Schaefer & Michael, 1981, 1982, 1985; Stapleton, Merriman, Coogel, Gelbard, & Reid, 1979; Stein, 1985; Stein & Belluzzi, 1978; Trujillo, Belluzzi, & Stein, 1983, 1984; van Wolfswinkel, Seifert, & van Ree, 1985; van Wolfswinkel & van Ree, 1985; West, Schaefer, & Michael, 1983). Against these reports of suppressive effects are thirteen reports of no effect of opiate antagonists on self-stimulation (Bozarth & Reid, 1977; DeWitte, 1981; Esposito, Perry, & Kornetsky, 1980; Goldstein & Malick, 1977; Holtzman, 1976; Lorens & Sainati, 1978; Nazzaro, Seeger, & Gardner, 1981; Perry, Esposito, & Kornetsky, 1981; Schenk & Nawiesniak, 1985; Seeger, Carlson, & Nazzaro, 1981; Stillwell, Levitt, Horn, Irvin, Gross, Parsons, Scott, & Bradley, 1980; van der Kooy, LePiane, & Phillips, 1977; Wauquier, Niemegeers, & Lal, 1974).

B. Factors which may explain the differences.

There is no single factor or variable that can explain the lack of agreement of these different groups of investigators. The studies differ in a number of details that may each contribute to differences in the major findings. There has been little attempt to standardize the sites or parameters of stimulation, the dose of drug or the time of testing, the relation of testing period to the light-dark cycle, or the sex, strain or degree of training of the animals. Each of these variables is known to interact with opiates or opiate antagonists in one behavioural task or another. It is essential to the understanding of the role of endogenous opioids in the phenomenon of brain stimulation reward to determine which, if any, of these variables significantly influences the effects of opiate antagonists in brain stimulation reward paradigms. The three variables that appear most likely to be of major significance are (i) site of stimulation, (ii) time and duration of testing, and (iii) parameters of rewarding stimulation.

i. Site of stimulation.

Of the differences between these studies that are most likely to contribute to the disparity in findings, differences in the site of stimulation have been mentioned most frequently. Different workers have studied the effects of opiate antagonists

on brain stimulation reward at a variety of stimulation sites. Stimulation of the medial forebrain bundle at the levels of the lateral and posterior hypothalamic nuclei have been investigated by many researchers (Bozarth & Reid, 1977; Esposito et al., 1980; Franklin & Robertson, 1982; Freedman & Pangborn, 1984; Glick et al., 1982; Goldstein & Malick, 1977; Holtzman, 1976; Ichitani et al., 1985; Lorens & Sainati, 1978; Perry et al., 1981; Schaefer & Michael, 1981; Schenk & Nawiesniak, 1985; Stapleton et al., 1979; Stein, 1985; Stillwell et al., 1980; van der Kooy et al., 1977; Wauquier et al., 1974). The periaqueductal grey has been another popular site of stimulation (Belluzzi & Stein, 1977; Franklin & Robertson, 1982; Freedman & Pangborn, 1984; Kelsey et al., 1984; Schaefer & Michael, 1981, 1982, 1985; Stein & Belluzzi, 1978; Stillwell et al., 1980; West et al., 1983). Another site of interest to researchers has been the nucleus accumbens (Belluzzi et al., 1985; Katz, 1981; Stapleton et al., 1979; Trujillo et al., 1983, 1984). The effects of stimulation of both the ventral tegmental area (Esposito et al., 1980; Nazzaro et al., 1980; Perry et al., 1981; Seeger et al., 1981; van Wolfswinkel et al., 1985; van Wolfswinkel & van Ree, 1985) and the substantia nigra (Nazzaro et al., 1981; Stapleton et al., 1979; Stein & Belluzzi, 1978) have also been studied. Less frequently studied sites are the medial frontal cortex (Franklin & Robertson, 1982), the medial entorhinal cortex (Reymann et al., 1986), the dentate gyrus (Collier & Routtenberg, 1984), the dorsal tegmentum (Franklin & Robertson, 1982), and the nucleus paratenialis of the thalamus (Stein & Belluzzi, 1978).

Several authors (Schaefer & Michael, 1981; Stapleton et al., 1979; Trujillo et al., 1984) have suggested that differences in site of stimulation may account, at least in part, for the discrepancy in findings of the different groups of investigators. The importance of site of stimulation is not yet clear from the literature, since variations in site of stimulation have been confounded with variations in many other procedural details. However, Stapleton et al. (1979) have reported that naloxone affects periaqueductal gray self-stimulation more dramatically than it affects lateral hypothalamic or nucleus accumbens self-stimulation, at least when animals are tested in 3-minute test sessions. Even in this study, in which two stimulation sites were tested under reasonably similar conditions in the same animals, it was not possible to stimulate the different sites with identical stimulation parameters. Thus, while the effects of opiate antagonists on brain stimulation reward are generally assumed to vary as a function of the brain site stimulated, this assumption has not been conclusively tested. Until site of stimulation is shown to be an important variable within an adequately controlled study, it remains a speculation that differences in site of stimulation explain differences between studies.

ii. Time and duration of testing.

A second factor that appears to contribute to the inconsistency of results is that different investigators have

used test periods involving different delays and different durations. Stapleton et al. (1979) observed only weak effects of naloxone when they tested for 3-minute tests, 15 minutes after injection; however, they observed strong effects when they tested for the full hour following injection. Similarly, Schaefer and co-workers have reported greater effects from 60-minute tests than from 20-minute tests (Schaefer & Michael, 1981; West et al., 1983). In general, most researchers employing test sessions restricted to the first half-hour after naloxone injection have failed to observe a reduction in responding for brain stimulation reward (Bozarth & Reid, 1977; Goldstein & Malick, 1977; Lorens & Sainati, 1978; van der Kooy et al., 1977), whereas most researchers employing test sessions lasting at least one hour after injection have found reliable decreases in responding for brain stimulation reward (Belluzzi & Stein, 1977; Franklin & Robertson, 1982; Kelsey et al., 1984; Stein, 1985; Stein & Belluzzi, 1978).

One possibility that has been suggested as to why stronger effects of naloxone and naltrexone are observed in long test sessions is that opiate antagonists change the rewarding impact of stimulation, and some period of time is required for an animal to adjust its rate of responding accordingly (Trujillo et al., 1984). Trujillo et al. (1984) argue that short test sessions do not allow enough time for this to occur. In the case of the decreases in lever-pressing for brain stimulation reward that result from the dopamine antagonist, pimozide, fifteen to twenty minutes of responding are required before the average rate of

responding drops from normal to a new stable level of responding that is associated with a given dose of drug (Fouriezos & Wise, 1976). A similar progressive decline in responding has been reported with naltrexone (Trujillo et al., 1984), though in this case it takes an animal about 30 minutes from the time of injection to settle at a stable low rate of responding. Trujillo et al. (1984) suggested that the progressive decline in responding under naltrexone reflected the same process that has been argued to explain the progressive decline in responding under pimozide: a partial "extinction" of the learned lever-pressing response because of drug-induced loss of the rewarding impact of stimulation. The critical feature of this hypothesis is that responding for brain stimulation reward under naltrexone was seen to decrease only as a result of the animal's experience with the reward in the drug condition.

An alternative to the notion that active responding under naltrexone is necessary before the drug will suppress self-stimulation is the notion that passive absorption of the drug takes a half-hour or longer. While it is known that maximal brain levels of naloxone and naltrexone are reached (respectively) within about 15 (Misra, Pontani, Vadlamani, & Mule, 1976; Tepperman, Hirst, & Smith, 1983) and 30 minutes (Misra, Block, Vardy, Mule, & Verebely, 1976) of injection, it is not clear that the drugs reach and occupy the critical receptors within this period. Pimozide reaches peak concentration in the brain within the first hour after injection (Janssen, Niemegeers, Schellekens, Dresse, Lenaerts, Pinchard, Schaper, van Nueten, & Verbruggen,

1968), but it does not have peak behavioural effects until several hours later (Atalay & Wise, 1983). The same may be true for naloxone and naltrexone. Until controlled comparisons in the second half-hour are made between animals that passively absorb drug for the first half-hour and animals that actively lever-press under drug for the same period, it will not be clear why the opiate antagonists appear to suppress self-stimulation more in one-hour tests than in half-hour tests.

111. Parameters of rewarding stimulation.

The differential effectiveness of naloxone and naltrexone on brain stimulation reward may also result from differences in the stimulation parameters that are tested in different laboratories. With high frequencies or intensities and short durations of stimulation, high baseline response rates are seen, whereas with low frequencies or intensities and long durations of stimulation, low response rates can be obtained from the same animal. It is possible that the effects of naloxone and naltrexone depend on the rate of responding of the animal. Dews (1958) has argued that baseline rate of responding is a major determinate of the effects of psychomotor stimulants, and the same can be true for barbiturates (Leander & McMillan, 1974). Uncontrolled differences in stimulation parameters, causing uncontrolled differences in baseline response rate, may thus contribute to the differential effectiveness of opiate antagonists in different studies. Ichitani et al. (1985) have suggested that naloxone's

suppressive effects on self-stimulation are more pronounced when tested under stimulation parameters that produce low rates of responding.

It has also been argued that partial reinforcement schedules should be used to maximize the effectiveness of the opiate receptor blockers (Franklin & Robertson, 1983; Schaefer & Michael, 1981; West et al., 1983). In other paradigms, however, McMillan (1969) has argued that the differential effects of drugs on responding under different schedules of reinforcement are secondary to the differences in rate of responding which different schedules maintain. Whether it is the baseline rate of responding or the schedule of reinforcement that maintains it, it is clear that seemingly arbitrary decisions about the strength or density of reinforcement could contribute to differences in the apparent effectiveness of the opiate antagonists in the self-stimulation paradigm.

C. Paradigms for studying drug effects on self-stimulation.

Several paradigms have been used to study the effects of drugs on self-stimulation. Although it seems unlikely that differences in paradigms account for the fact that some experimenters report that opiate antagonists suppress self-stimulation and other experimenters report the opposite, it is still important to consider the relative strengths and weaknesses of different paradigms. The most popular self-stimulation paradigm involves determining an animal's

average rate of responding for stimulation at some arbitrary set of fixed stimulation parameters. A major problem with this type of paradigm is that different stimulation parameters have been arbitrarily chosen by different researchers to measure the reinforcing value of brain stimulation. Since, as has been described earlier, different stimulation parameters yield different rates of response, and since drugs produce differential effects depending on the baseline rate of responding, arbitrary decisions about stimulation parameters may contribute substantially to whether an effect of naloxone or naltrexone is found in a given study. A second problem with this paradigm is that when animals are tested under drug conditions, changes in response rates are difficult to interpret (Valenstein, 1964). It is difficult to decide whether a simple change in average self-stimulation rate reflects interference with the reinforcing qualities of stimulation, or whether it reflects interference with the performance capacity of an animal.

Another paradigm that has been used (Trujillo et al., 1984) to measure the effect of opiate antagonists on brain stimulation reward is the extinction paradigm. In the extinction paradigm, an animal's temporal pattern of responding, rather than the average rate of responding, is examined. Fouriezos and Wise (1976), who developed this paradigm, reasoned that if a drug selectively diminishes the reinforcing value of electrical stimulation, an animal should respond at normal rates at the beginning of a test session, but normal early responding should be followed by a gradual reduction of

responding as the session progresses. On the other hand, if a drug alters the performance capacity of an animal, its rate of responding at the beginning of a test session should be lower than normal and remain this way throughout the rest of the session or until the drug wears off. The reason for preferring the extinction paradigm to a simple rate paradigm is that it holds the promise for distinguishing drugs which decrease reward impact from drugs that decrease performance capacity. Unfortunately, in this paradigm, as with the simple rate paradigm, the strength of the reinforcing value of brain stimulation involves measuring an animal's response rate at some arbitrarily chosen set of fixed stimulation parameters.

A third paradigm is the threshold paradigm (Esposito et al., 1980; Perry et al., 1981). Threshold is defined as the minimum intensity or frequency of stimulation that will sustain an arbitrary amount of responding. Arbitrary amounts of responding that have been used as a measure of threshold include, among others, 10 percent of maximal rate of responding or half-maximal rate of responding. The intensity threshold or frequency threshold is assumed to reflect the sensitivity of the neural system to the rewarding impact of stimulation. Since threshold reflects the level of stimulation for which an animal is willing to sustain responding, and not the actual rate of that responding, it is often assumed that threshold is more sensitive to drug-induced changes in the rewarding impact of the stimulation than to drug-induced changes in the response capacity of the animal. However, it must be

noted that while animals are sometimes allowed to respond for high stimulation currents in the threshold paradigm, performance at these high currents is not usually assessed. Since the baseline response rates of animals earning threshold level stimulation are usually low, the threshold paradigm would not be suitable for the exploration of drug effects which vary as a function of baseline rate of responding. The paradigm would reflect drug effects which are associated with low rates of responding, but would not reflect effects associated with high rates of responding. Threshold measurements have been used by a few researchers to examine the effects of opiate antagonists on brain stimulation reward (Esposito et al., 1980; Perry et al., 1981). While the Esposito group tested an animal's rate of responding over a range of stimulation intensities, rather than using fixed stimulation parameters, they did not assess the effect of naloxone across the entire range. In fact, they used a range of stimulation intensities to find a single value (threshold) that would produce a minimal level of responding, and then tested to see if the threshold would change under naloxone conditions.

A final paradigm of interest is the curve-shift paradigm. In the curve-shift paradigm, response rate is determined across an entire range of stimulation intensities or frequencies, including some that produce responding at asymptotic (maximal) rates, some that produce moderate levels of responding, and some that fail to sustain a minimal level of responding. By testing animals over

an entire range of stimulation intensities or frequencies, this paradigm can adequately assess whether a drug has rate-dependent effects.

In the curve-shift paradigm, the amount of stimulation that an animal receives is usually varied by changing the intensity or by changing the frequency. By changing one of these parameters and keeping the other parameter fixed, a function relating response strength to reward strength can be determined. The curve that graphically represents the function relating response strength to reward strength is the curve for which the curve-shift paradigm is named.

The curve shifts in different directions under different experimental conditions (Edmonds & Gallistel, 1974). First, the curve can shift laterally, left or right, in response to changes in the rewarding impact of the stimulation. Decreasing the rewarding impact by reducing the intensity of the stimulation causes a rightward shift in the curve. The curve is thought to shift to the right because lowering the stimulation intensity decreases the number of reward relevant fibers activated, which should thus decrease reward. In order for the stimulation to be equally rewarding, the remaining fibers have to be depolarized more often, which occurs when the stimulation frequency is increased. A leftward shift in the curve occurs when the stimulation intensity is increased. A shift in the curve to the left presumably occurs because more fibers are activated by the increase in stimulation intensity; hence these fibers have to be depolarized less

often (decreasing the stimulation frequency) for the stimulation to be equally rewarding.

The curve is shifted downwards when the performance requirements imposed on an animal are increased. For example, performance requirements can be increased by placing marbles or hurdles on the floor of a runway (Edmonds & Gallistel, 1974), by increasing the weight on a lever (Milliaressis, Rompre, Laviolette, Philippe, & Coulombe, 1986), or by making animals run an incline rather than a level runway (Edmonds & Gallistel, 1974). Each of these manipulations decreases the level of maximal performance but none of these manipulations cause a shift of the rising portion of the function.

Shifts of the curve to the left or right can be demonstrated pharmacologically. Neuroleptics, cause a rightward shift in the curve, and the magnitude of the shift is dose-dependent (Franklin, 1978; Gallistel & Karras, 1984; Milliaressis et al., 1986). Since these results parallel the effect of decreasing the current, they suggest that neuroleptics decrease the rewarding impact of self-stimulation. Amphetamine on the other hand, has been found to produce an effect opposite to that of the neuroleptics by producing leftward dose-dependent shifts in the curve (Gallistel & Karras, 1984). The fact that amphetamine shifts the curve to the left, as does increasing the level of rewarding stimulation, suggests that amphetamine increases the rewarding impact of the stimulation. A leftward shift of the curve suggests that amphetamine increases the rewarding impact of electrical stimulation. In addition, when

the appropriate dose of amphetamine and of pimozide are given together their individual effects on the shift of the curve nullify each other (Gallistel & Karras, 1984).

A shift of the curve downward has also been demonstrated pharmacologically. The performance capacity of an animal can be altered by treating animals with drugs that are known to have a debilitating effect, such as methocarbamol (Edmonds & Gallistel, 1974; Milliaressis et al., 1986) or d-tubocurarine (Edmonds & Gallistel, 1974). The fact that a downward shift of the curve is also caused by increasing the performance requirements placed on an animal suggests that a drug-induced downward shift of the curve is a reflection of a drug-induced alteration of the performance capacity of the animal.

A drug could decrease both the rewarding impact of stimulation and the performance capacity of an animal; such a drug would cause the curve to shift both down and to the right.

The curve-shift paradigm thus not only provides a method for assessing the effects of drugs across a range of stimulation parameters and baseline rates; it also provides a method for determining something about the nature of the drug-induced change in behaviour.

EXPERIMENT 1

The present experiment was designed to clarify the effects of opiate antagonists on brain stimulation reward. This seemed important given that some researchers have found that opiate antagonists suppress brain stimulation reward while others have observed no effect. Differences in results have been attributed to several factors. The factors that seem most likely to explain the differences between investigators are differences in site of stimulation, differences in time of testing, and differences in stimulation parameters and their associated baseline response rates. What is needed then, is a systematic comparison of different sites of stimulation, different times after injection, and different parameters of stimulation and rates of response.

In the present experiment, each animal was implanted with electrodes aimed at three regional sites within the medial forebrain bundle: the nucleus accumbens septi, the lateral hypothalamic area, and the ventral tegmental area. Animals were tested for brain stimulation reward across a range of stimulation frequencies in order to assess whether naltrexone had any effect on responding. Naltrexone was chosen because it has been previously shown to effectively reduce response rates for brain stimulation reward. Animals were tested 15 and 45 minutes after administration of naltrexone since these are the times that seem to differentiate positive and negative effects of earlier studies.

METHOD

Subjects

Subjects were naive male Long-Evans rats weighing 325-500 grams at the beginning of the experiment. These rats were individually housed in stainless steel cages in a colony room that was maintained on a 12-hour light and 12-hour dark cycle. Food and water were available ad libitum.

Surgery

The animals were anesthetized with sodium pentobarbital (Somnotol, 60 mg/kg, ip) and secured in a stereotaxic instrument for electrode implantation. Rats were then given 0.15 ml of atropine sulfate (0.009 mg/ml, sc) to reduce respiratory distress. The co-ordinate system of deGroot (1959) was used to implant three monopolar electrodes: one in the nucleus accumbens, one in the lateral hypothalamic area, and one in the ventral tegmental area. The stereotaxic co-ordinates for the nucleus accumbens were: 3.4 mm anterior to bregma, 1.2 to 1.5 mm lateral to the midline, and 6.2 to 6.6 mm beneath the dural surface. The co-ordinates for the lateral hypothalamic area were: 0.6 to 0.8 mm posterior to bregma, 1.6 to 1.7 mm lateral to the midline, and 8.0 to 8.5 mm beneath the dural surface. The co-ordinates for the ventral tegmental area were: 3.0 to

3.4 mm posterior to bregma, 0.0 to 0.6 mm lateral to the midline, and 7.8 to 8.5 mm beneath the dural surface.

Each monopolar electrode was composed of an 11 mm stainless steel wire (diameter of 254 μ m) soldered to a connector which was fixed to the skull such that it could be connected to the stimulation lead. The electrode was insulated with varnish except for the cross-section of its tip. Five stainless steel screws threaded into the skull served as anchors for the electrode assembly. An uninsulated wire was wrapped around two of these screws to serve as a current return. The electrode assembly was held together and secured to the skull screws with acrylic cement.

Apparatus

Animals were trained in operant chambers (78 X 28 cm) that were composed of three aluminum walls and one clear front wall. A stainless steel lever, resting 5 cm above a grid floor, protruded through one of the aluminum walls. During training, depressing the lever resulted in delivery of a 0.5-sec train of 0.1 msec square wave cathodal pulses at a frequency of 60 Hz. Stimulation was provided by a constant current stimulator (Mundl, 1980) with a 1-kohm resistor connected in series with the animal. An oscilloscope was used to monitor current intensity. To enable the animals to move freely within an operant chamber, electrical stimulation was delivered to the brain via a flexible lead which was connected to a commutator.

Procedure

Screening The animals were given a recovery period of one week following surgery. These animals were then screened for lever-pressing for stimulation at a frequency of 60 Hz and an intensity of 200 uA. Animals that did not learn to lever-press spontaneously were trained, by successive approximation, to approach and press the lever. For these animals the stimulation intensity was increased in 25-uA increments to a maximum of 800 uA. An animal that did not maintain responding at a rate of at least 20 presses per minute was excluded from the experiment. From a total of 60 animals, 22 animals responded (at least 20 presses per minute), for stimulation, while 38 animals which failed to meet this criterion of responding were excluded from the experiment. Of these 22 animals, two pressed for stimulation through each of the three electrodes, while 13 animals pressed for stimulation through two electrodes. Of these 13 animals, eight pressed for stimulation of the nucleus accumbens and lateral hypothalamus, two pressed for stimulation of the ventral tegmentum and nucleus accumbens, and three animals pressed for stimulation of the ventral tegmental and lateral hypothalamic areas. A group of seven animals which maintained a high rate of responding for lateral hypothalamic stimulation was also included in the experiment. Of the 38 animals which were excluded from the experiment, 24 animals were excluded despite the fact that they

responded for stimulation at a single site.

Following the screening period, rate-frequency curves were determined for each working electrode in each animal. A curve was determined by measuring an animal's rate of responding across a range of stimulation frequencies, beginning with a stimulation frequency that produced high rates of response and then lowering the frequency in 0.04 logarithmic steps (a constant of 9.12 percent was subtracted from each frequency to determine the next stimulation frequency) every 90 seconds until a stimulation frequency was reached for which the animal would not continue to respond. Response rates were recorded during the last minute of testing at each pulse frequency. When an animal stopped pressing, non-contingent stimulation (priming) was delivered to determine whether pressing would be re-established. If pressing was not re-established the rate-frequency curve was considered complete. The highest frequency that maintained responding at a rate of 10 percent of maximum during the last minute of a 90-second trial was defined as the frequency threshold.

The amount of current delivered through each electrode was adjusted so that a frequency threshold of 40 Hz was obtained. If the threshold of an animal's initial rate-frequency curve was higher than 40 Hz, the intensity was increased by 25 μ A; following a 20-minute break, a second rate-frequency curve was determined. This procedure was conducted as many times as required to determine the stimulation intensity associated with a frequency threshold of 40 Hz for each electrode site. If on the other hand, an animal's threshold was lower than 40 Hz for the first rate-frequency curve

the intensity was decreased by 25 uA and, after 20 minutes, a second curve was determined. The same procedure was conducted as above in order to obtain an intensity which would give a frequency threshold of 40 Hz for each electrode. Frequency thresholds were considered stable when no more than a 0.04 log step change in the threshold occurred over three consecutive test days. When this criterion was met for stimulation of each electrode site in each animal, the animals were considered ready for saline testing.

Saline Testing Using the same stimulation intensity associated with the 40 Hz frequency threshold of each electrode site in each animal, rate-frequency curves were determined for stimulation of these sites in each animal each day following intraperitoneal injections of saline. Saline injections were given 10 minutes prior to the beginning of a test session. When frequency thresholds were stable after two consecutive test sessions (this usually required between three and five test days), each rat was then tested under naltrexone conditions.

Naltrexone Testing Each rat received intraperitoneal injections of naltrexone hydrochloride (Endo Laboratories) in doses of 2.5, 5.0, 10.0 or 20.0 mg/kg 15 minutes prior to a test session. In each test session animals were tested twice, once 15 minutes after each injection and once 45 minutes after injection. During each of these two tests, a rate-frequency curve was determined for each animal. The doses of naltrexone were administered in a counterbalanced order; each naltrexone

injection was followed by at least two days of saline testing. Each animal was tested for its response to stimulation of each site on each day, but only one of the tests was made under drug. The animal was tested under drug conditions after completion of the non-drug tests for that day.

Statistical Analyses.

A three-way analysis of variance, corrected for unequal sample sizes, was used to compare the effects of naltrexone on reward thresholds determined from stimulation sites in the nucleus accumbens, lateral hypothalamus, and ventral tegmental area. Individual reward thresholds were determined from the rate-frequency function by graphic interpolation. The reward threshold for a given test was defined as the "X" co-ordinate of the point at which the rate-frequency function crossed the "Y" value corresponding to 10 percent of the maximum rate of responding for that test. The three factors were "Site", "Dose" and "Time". The three brain regions were considered to be independent of one another, therefore in the three-way analysis of variance, Site was considered a between subjects factor while Dose and Time were both considered within subjects factors. Each of the four drug doses was considered a level of the factor of Dose. There were three levels to the factor of Time. Since baseline reward thresholds for individual rats were obtained prior to determining reward thresholds for the two experimental test sessions for each drug dose, baseline reward thresholds were

considered a level of the factor of Time. Scheffé's test was performed on comparisons of interest.

A three-way analysis of variance, corrected for unequal sample sizes, was also conducted on the change of asymptotic responding from baseline responding for each drug dose. The asymptotic response rate was considered the fastest rate of responding that occurred at any frequency during the determination of the rate-frequency function. The three factors were Site, Dose and Time. Site was considered to be a between factor and Dose and Time were considered to be repeated factors. Comparisons of interest were analyzed using Scheffé's test.

RESULTS

Naltrexone caused reliable and robust decreases in self-stimulation. Stronger effects were seen 45 minutes after injection; only minimal effects were seen 15 minutes after injection. Naltrexone most strongly affected responding for nucleus accumbens stimulation; it had much less, but still reliable effects on responding for lateral hypothalamic stimulation; and it had minimal, statistically nonsignificant effects on responding for ventral tegmental stimulation. Naltrexone caused minor decreases in maximal response rate for nucleus accumbens self-stimulation but these decreases were not reliable across doses. In those cases where naltrexone had a statistically significant effect, the dominant effect was an increase in the stimulation frequency required to produce normal rates of responding; naltrexone, when effective, shifted the rate-frequency curves predominantly to the right. When rate-frequency curves were shifted, the shifts were reasonably parallel; the magnitude of the shift was reasonably constant, for a given dose and stimulation site, across the range of effective stimulation frequencies. That is, there was no evidence of differential effectiveness of naltrexone on low-rate or high-rate performance.

Reward Thresholds

Naltrexone increased reward thresholds in some but not

all conditions. Analysis of Variance revealed a significant interaction of Site and Time ($F(4,72)=5.239, p<0.001$). Scheffé tests revealed no statistically significant effects of naltrexone in the first test period, beginning 15 minutes after naltrexone injections. In the second test period, beginning 45 minutes after injection, naltrexone caused a large (Figures 1, 2, 3, 4) increase in nucleus accumbens thresholds ($F(4,60)=30.003, p<0.05$) and a smaller (Figures 5, 6, 7, 8), but equally reliable ($F(4,60)=12.50, p<0.05$) increase in lateral hypothalamic reward thresholds. Naltrexone had no effect on ventral tegmental reward thresholds (Figures 9, 10, 11, 12). Variability data appear in Appendix I.

Maximal rate of responding

Naltrexone produced minor decreases in asymptotic responding in some but not most conditions. Analysis of Variance revealed a significant Site x Time interaction ($F(2,72)=8.979, p<0.001$). Further analysis of this interaction using Scheffé tests showed no statistically significant effects of naltrexone in the first test session. In the second test session, naltrexone decreased maximal rate of responding of animals pressing for stimulation of the nucleus accumbens ($F(4,60)=6.25, p<0.05$), but this effect was not dose dependent. Naltrexone had no effect on asymptotic responding of animals pressing for stimulation of the lateral hypothalamic and ventral tegmental areas. Variability data appear in Appendix II.

Figure 1. Effects of naltrexone (2.5 mg/kg) on responding for nucleus accumbens stimulation in test sessions beginning 15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.

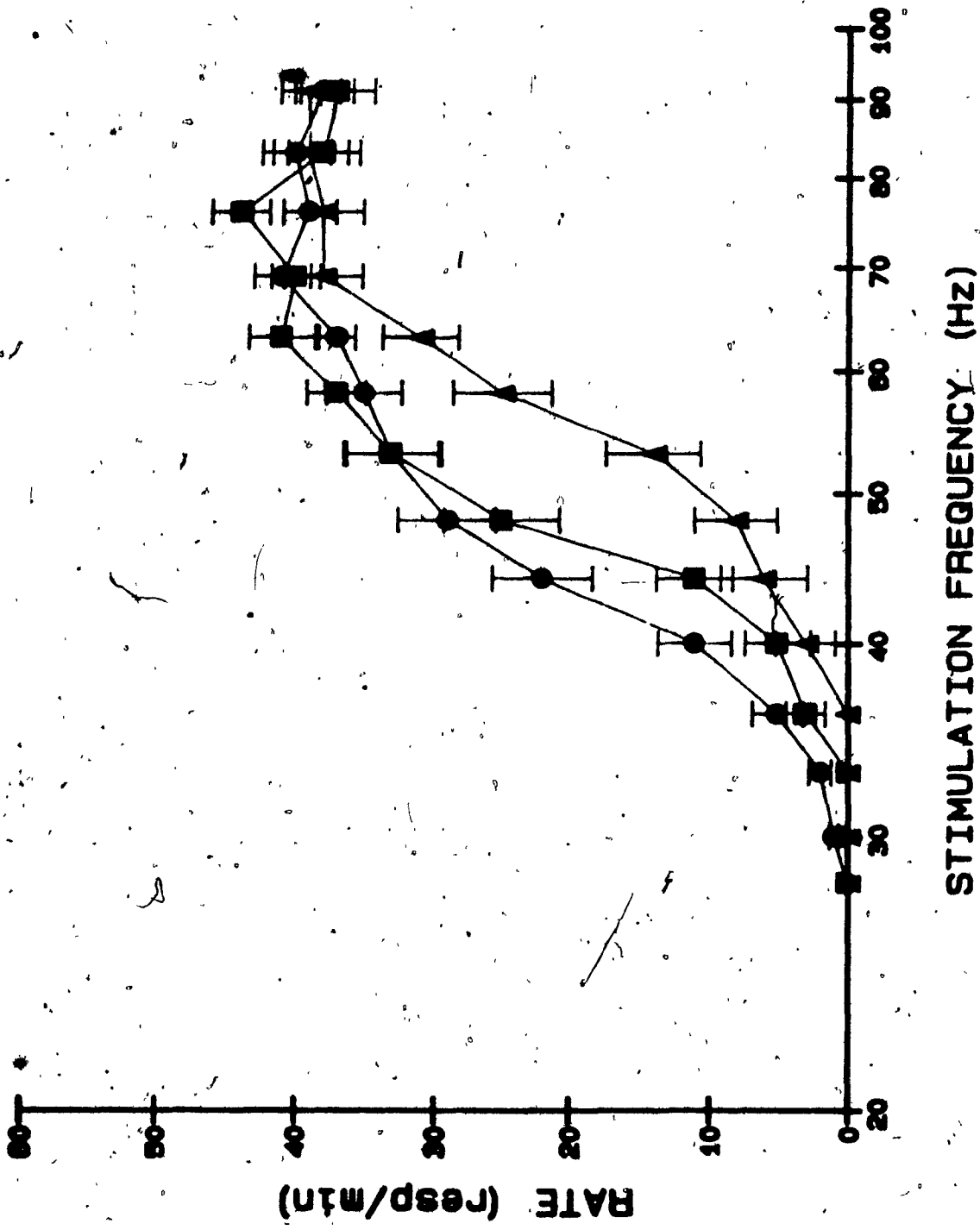


Figure 2. Effects of naltrexone (5.0 mg/kg) on responding for nucleus accumbens stimulation in test sessions beginning 15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.

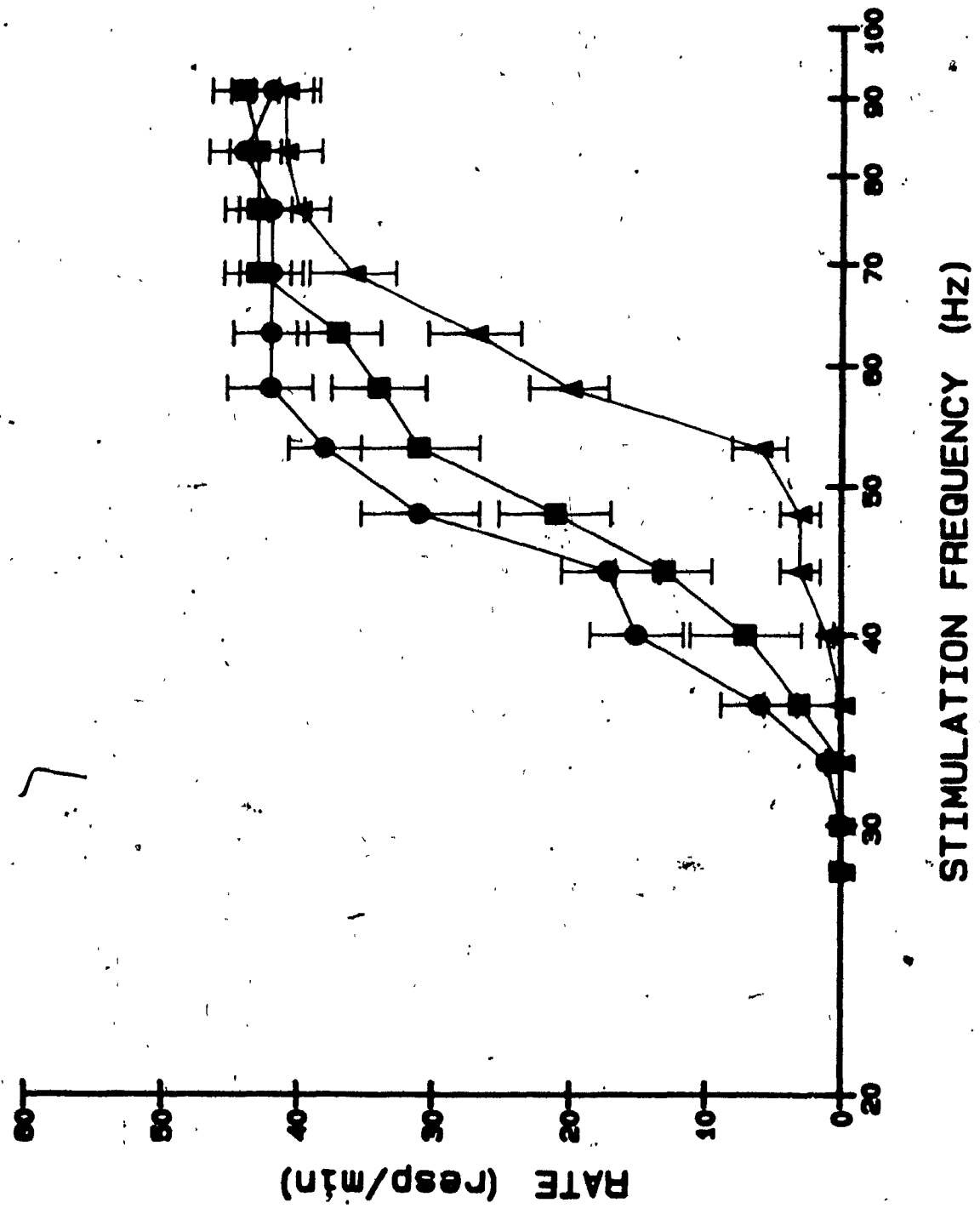


Figure 3. Effects of naltrexone (10.0 mg/kg) on responding for nucleus accumbens stimulation in test sessions beginning 15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.

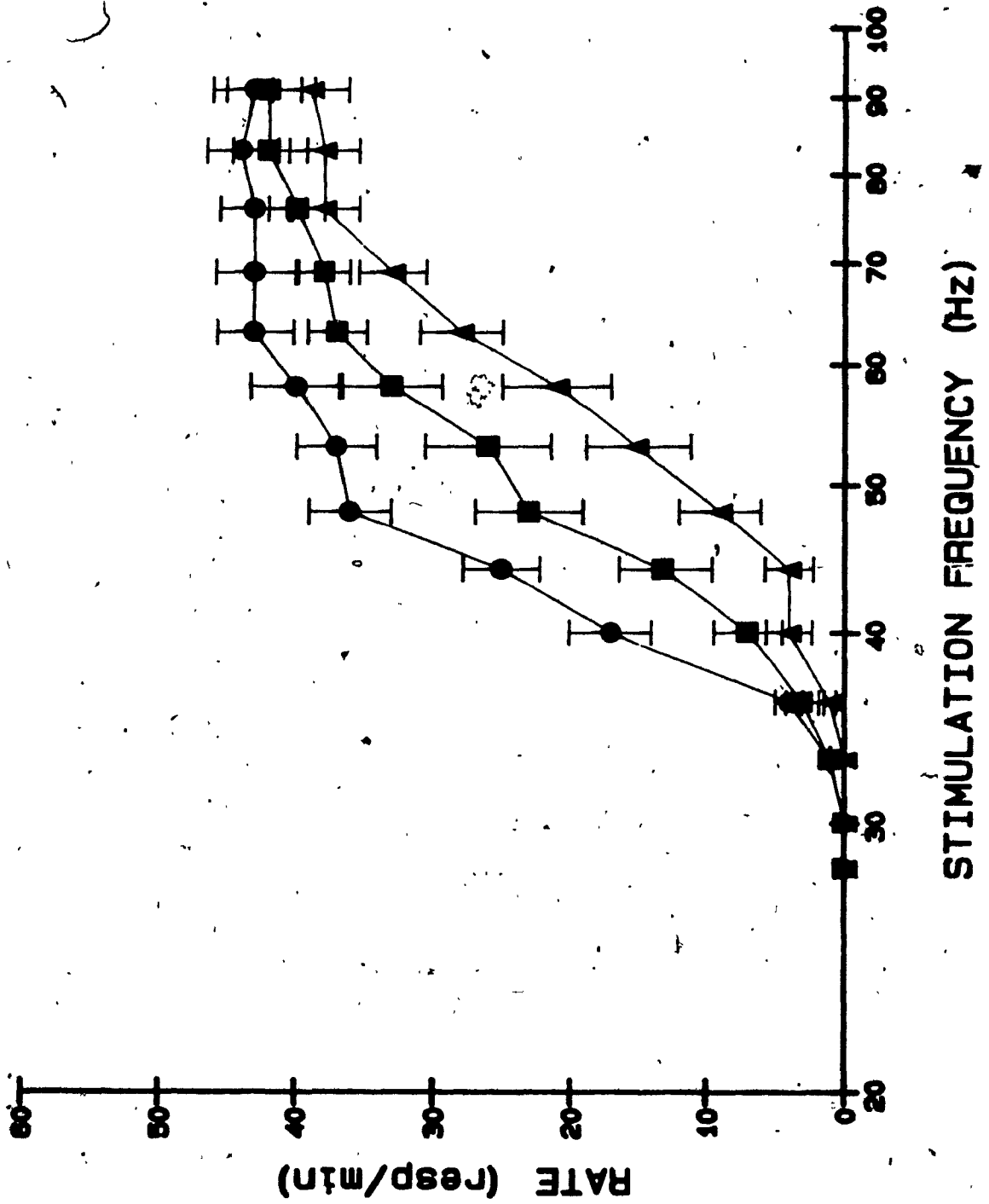


Figure 4. Effects of naltrexone (20.0 mg/kg) on responding for nucleus accumbens stimulation in test sessions beginning 15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.

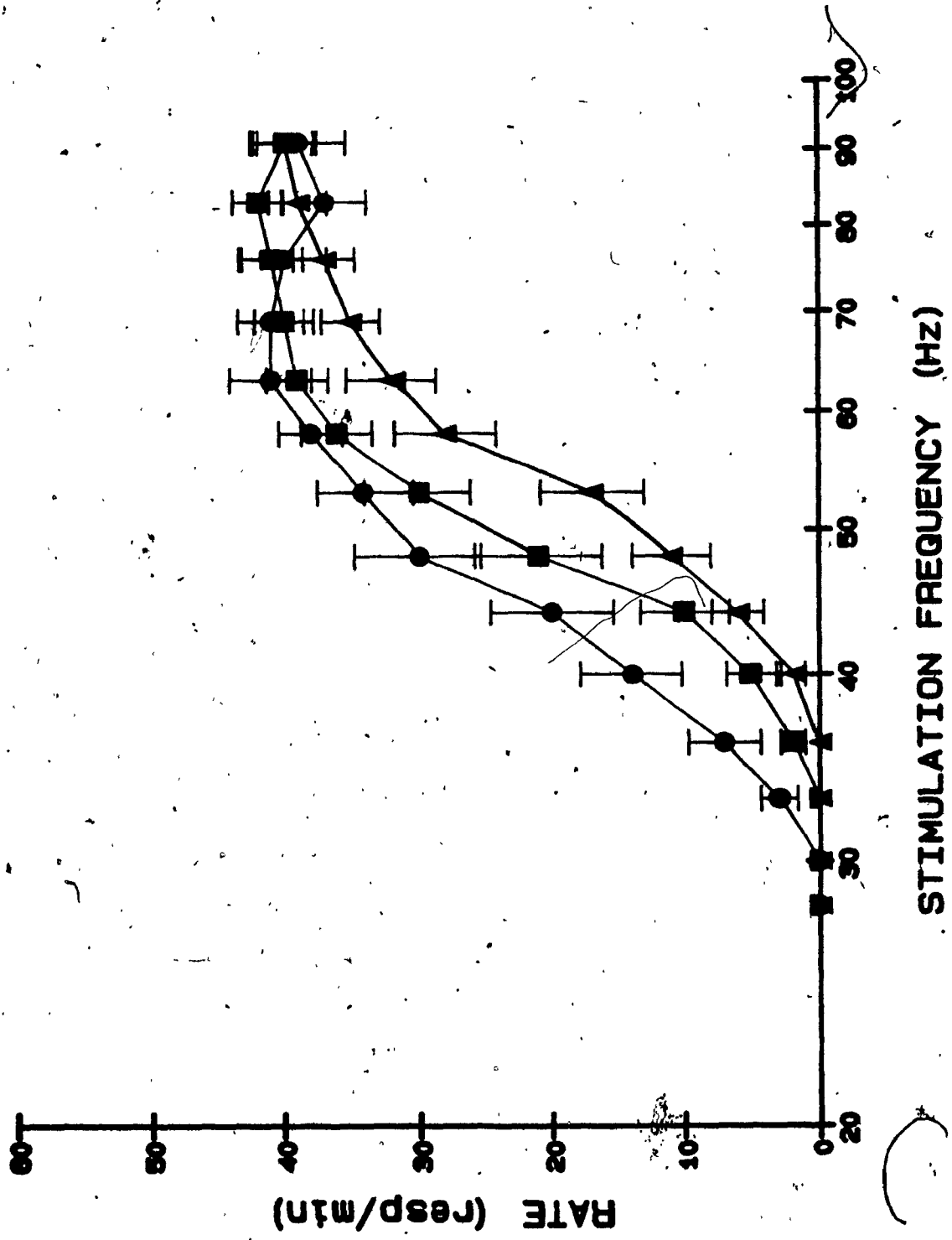
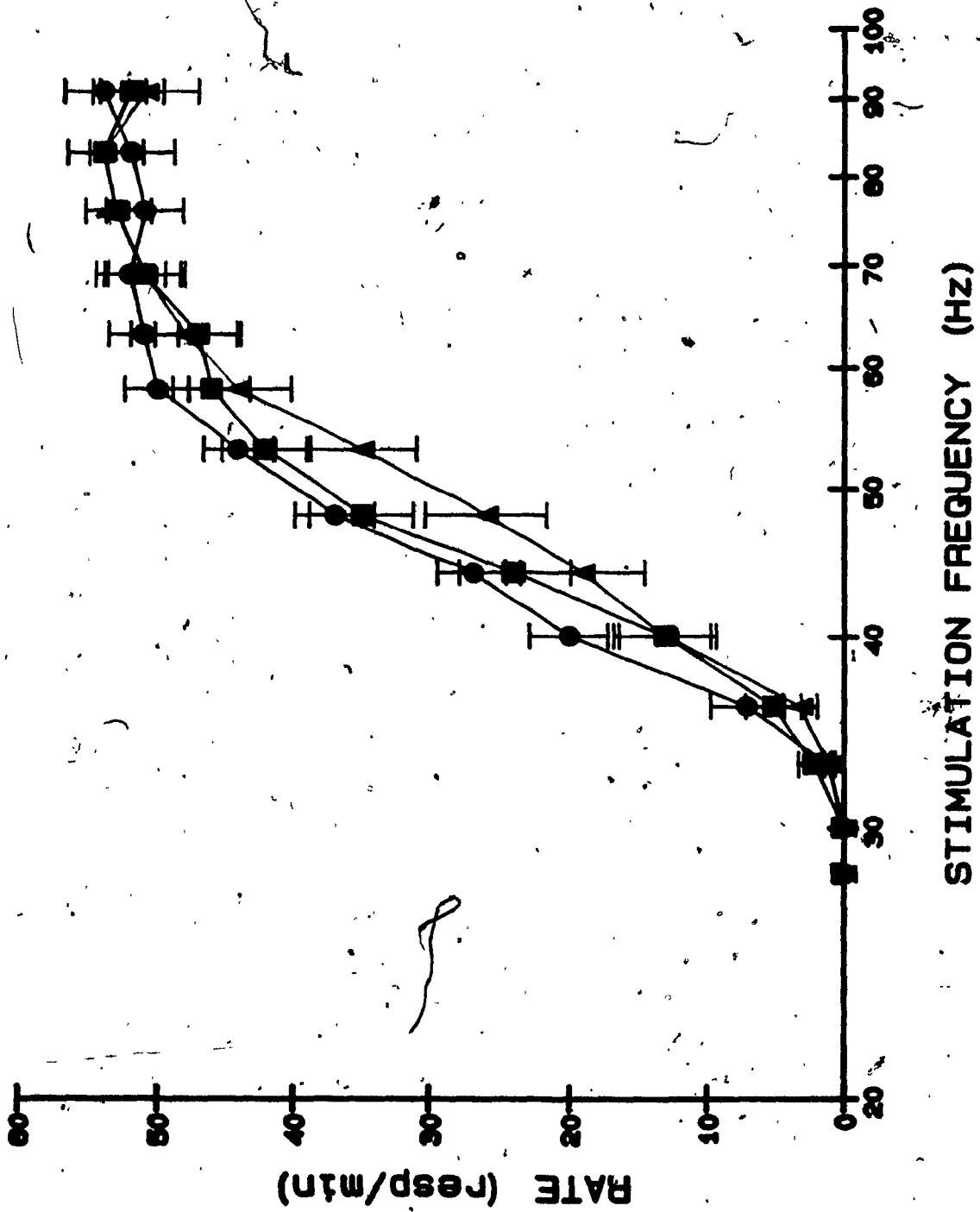


Figure 5. Effects of naltrexone (2.5 mg/kg) on responding for lateral hypothalamic stimulation in test sessions beginning 15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.






Figure 6. Effects of naltrexone (5.0 mg/kg) on responding for lateral hypothalamic stimulation in test sessions beginning 15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.

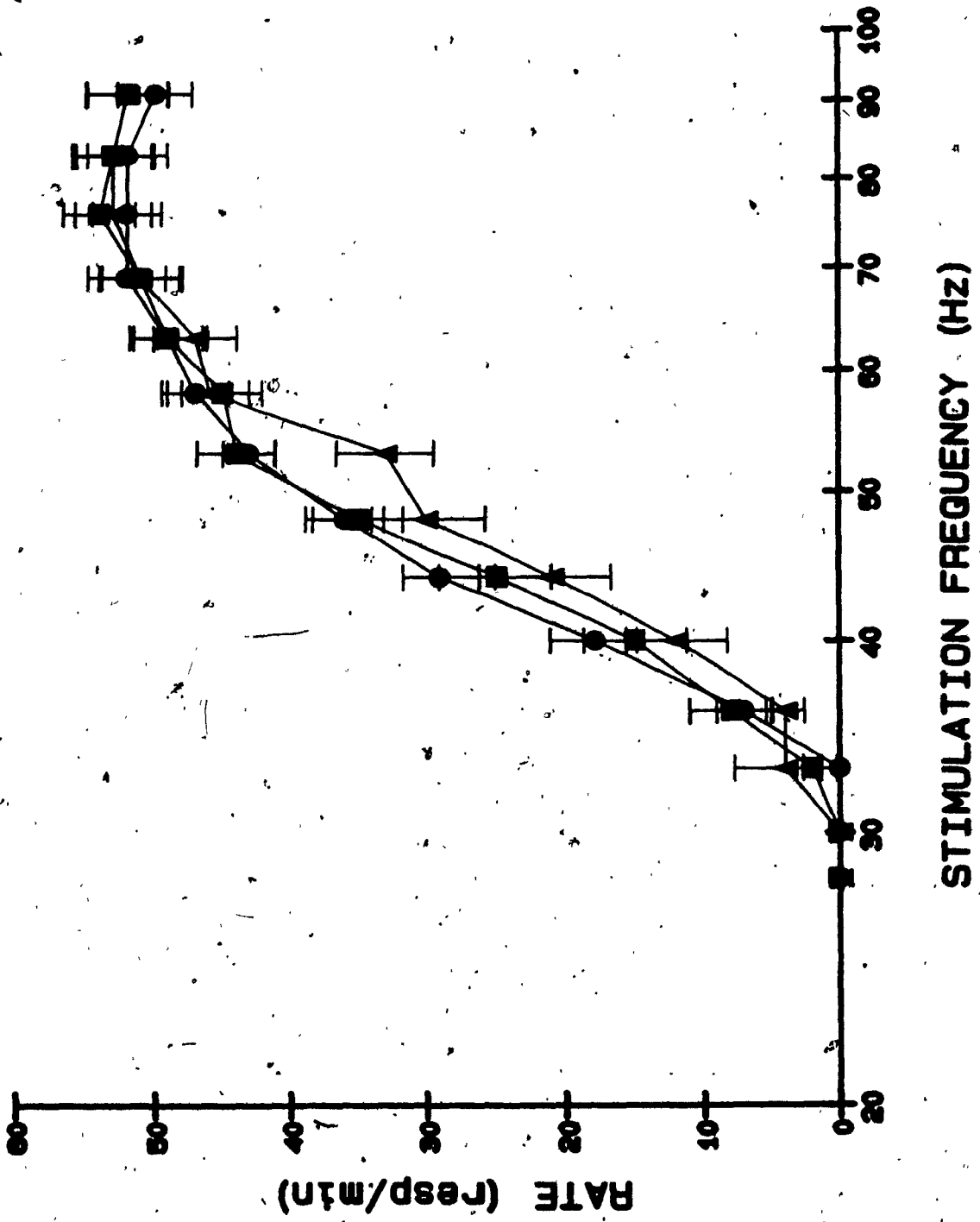


Figure 7. Effects of naltrexone (10.0 mg/kg) on responding for lateral hypothalamic stimulation in test sessions beginning 15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.

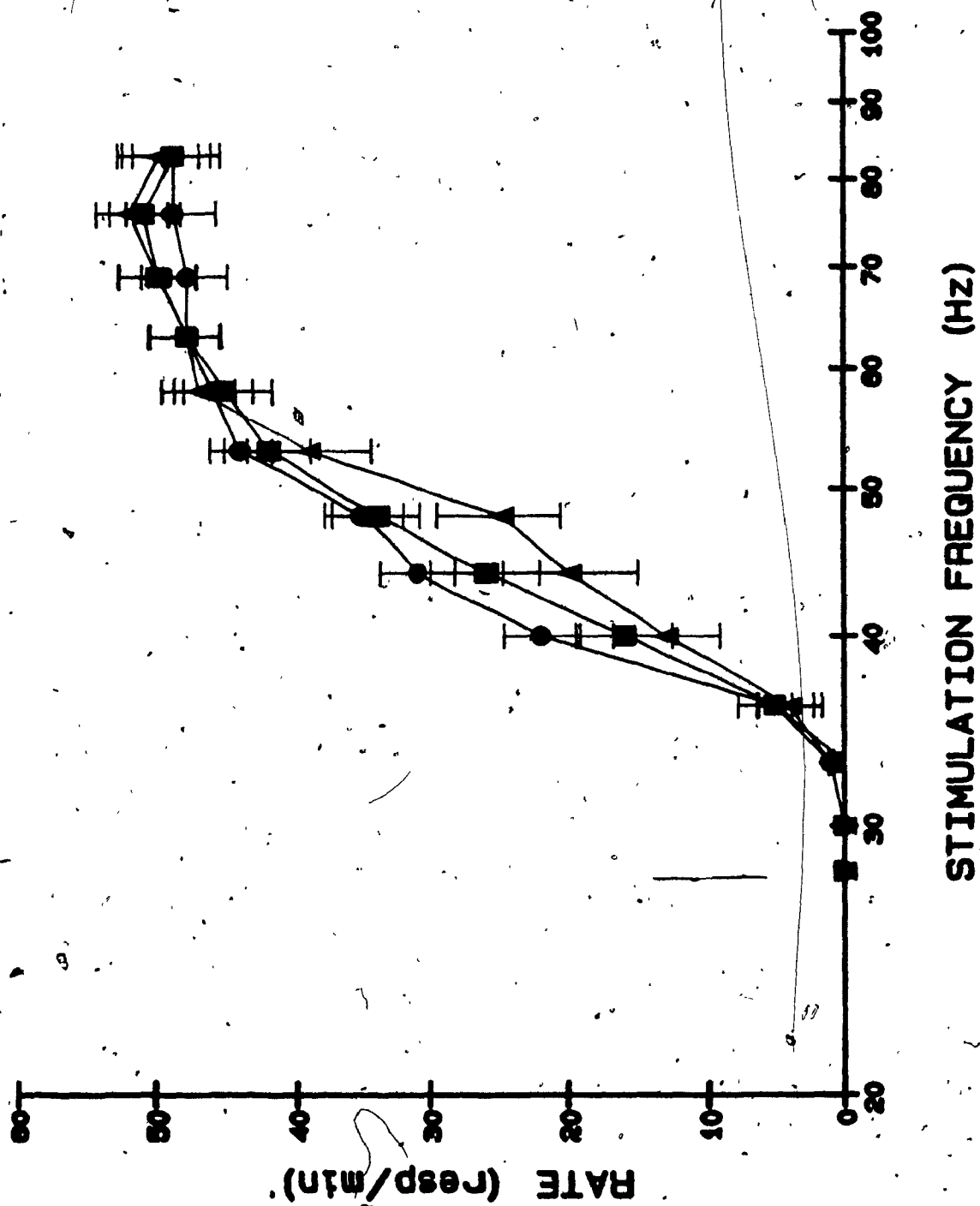
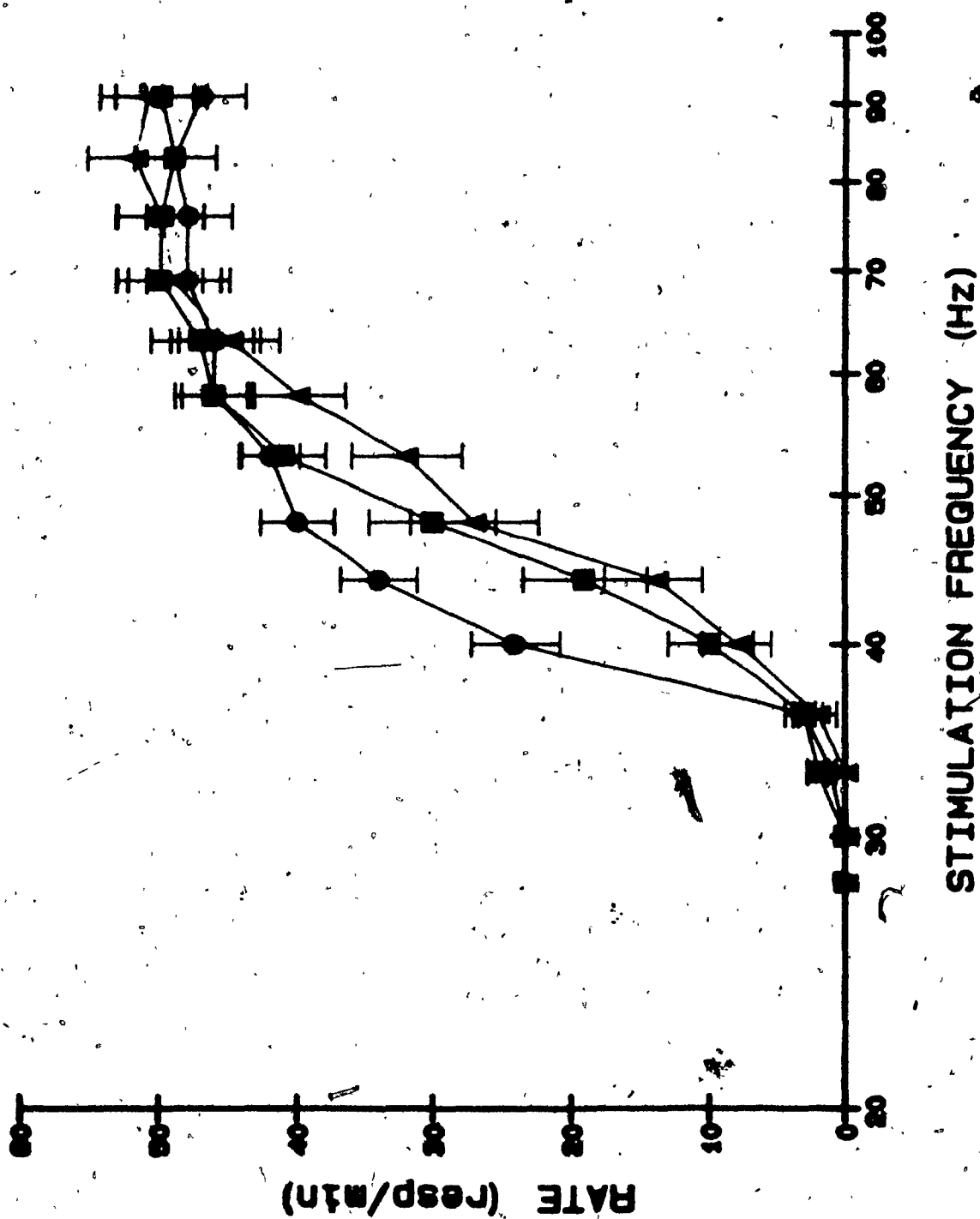


Figure 8. Effects of naltrexone (20.0 mg/kg) on responding for lateral hypothalamic stimulation in test sessions beginning 15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.






Figure 9. Effects of naltrexone (2.5 mg/kg) on responding for ventral tegmental stimulation in test sessions beginning 15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.

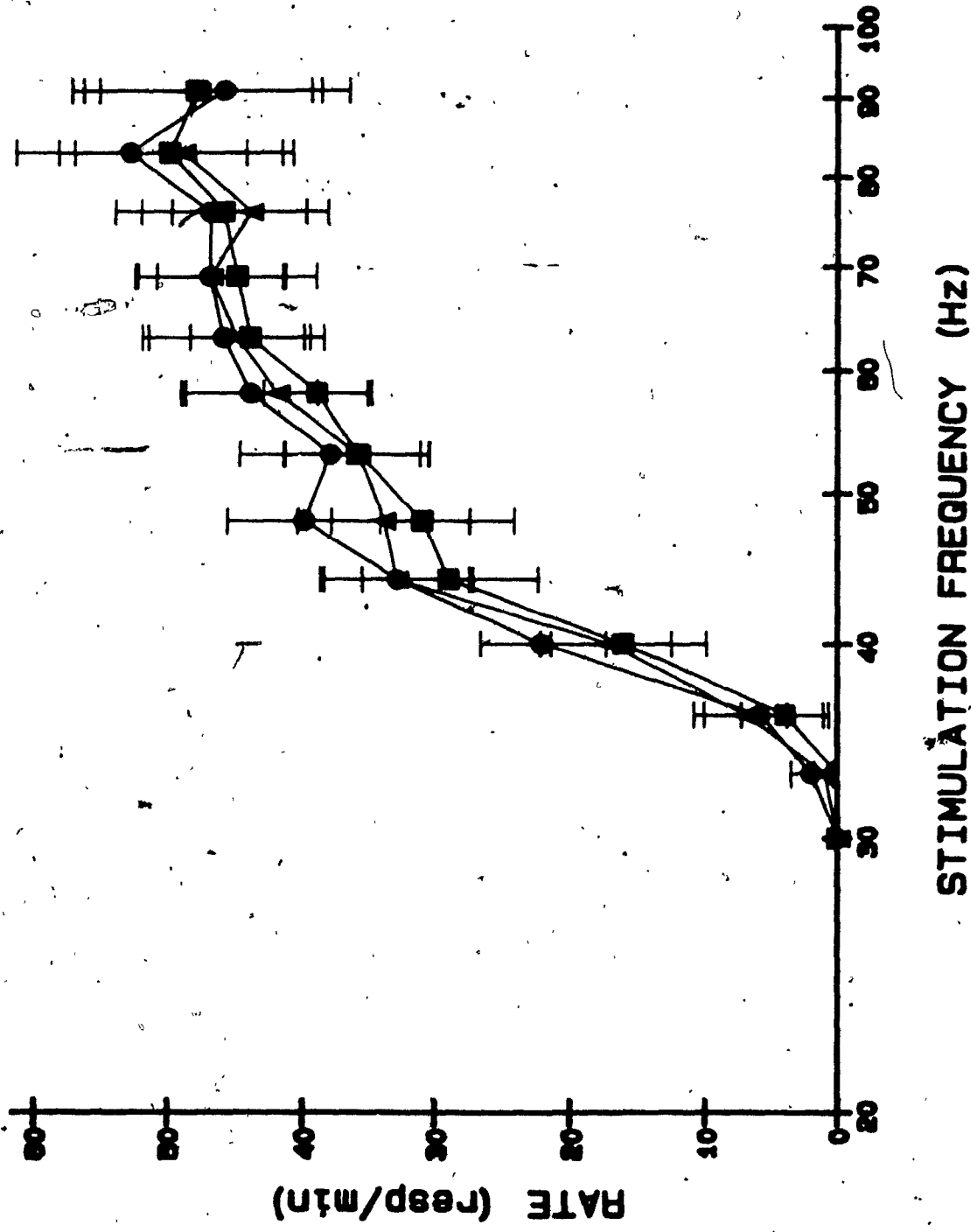


Figure 10. Effects of naltrexone (5.0 mg/kg) on responding for ventral tegmental stimulation in test sessions beginning 15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.

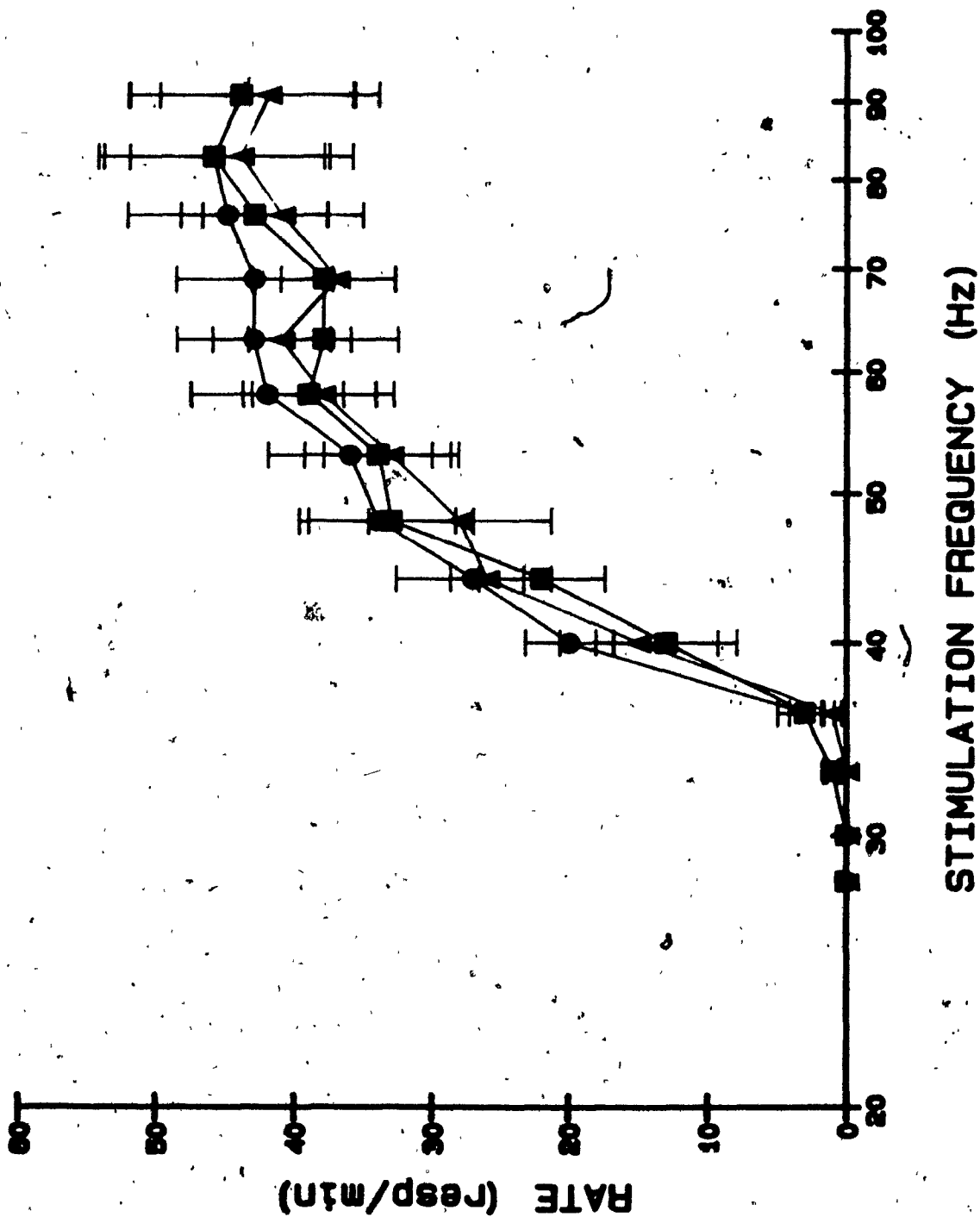


Figure 11. Effects of naltrexone (10.0 mg/kg) on responding for ventral tegmental stimulation in test sessions beginning .15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.

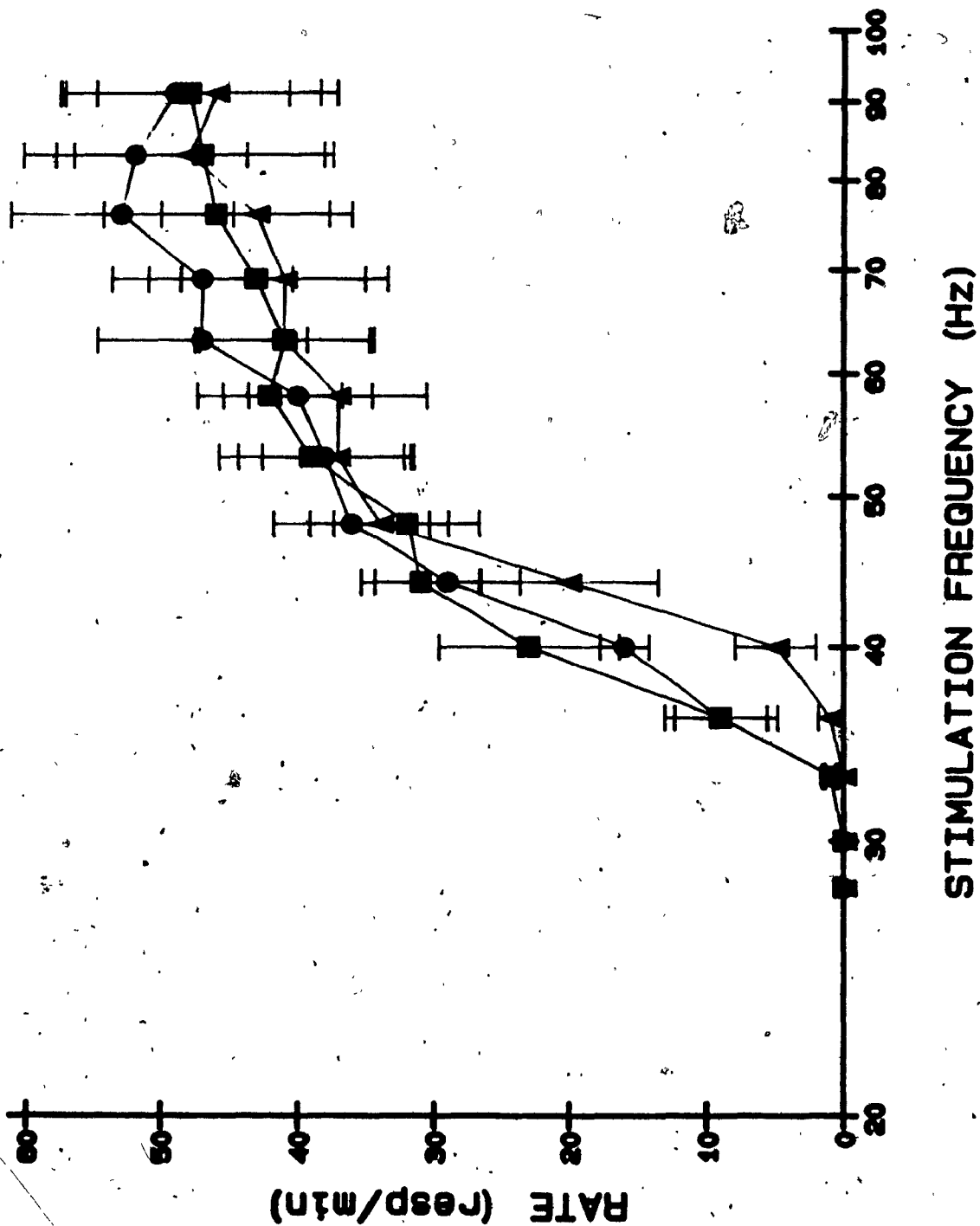
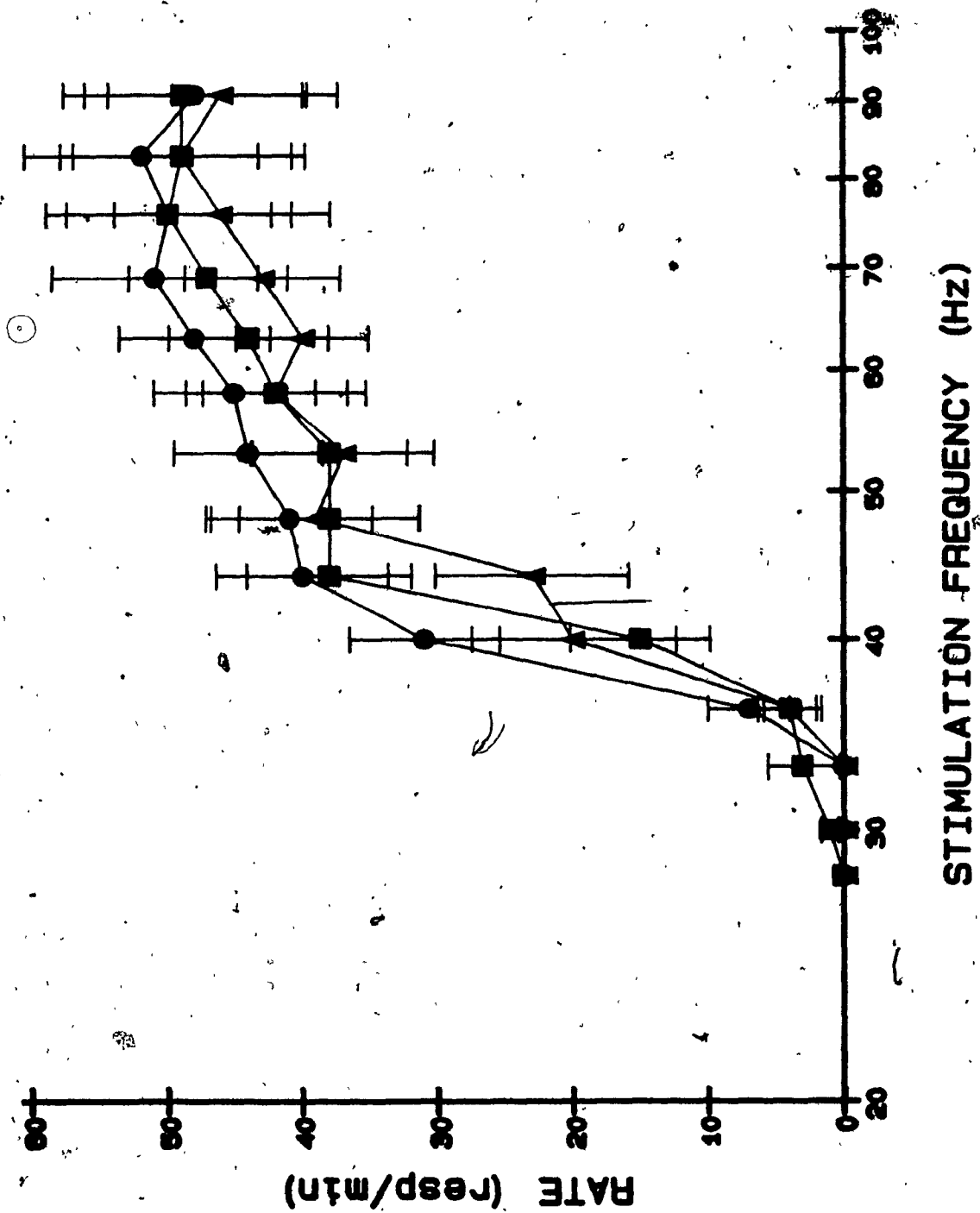


Figure 12. Effects of naltrexone (20.0 mg/kg) on responding for ventral tegmental stimulation in test sessions beginning 15 minutes (squares) or 45 minutes (triangles) after injection or under saline conditions (circles). Experiment 1.



DISCUSSION

Naltrexone caused significant decreases in responding for brain stimulation reward. The effectiveness of the drug depended on the site of stimulation and the time of testing after injection, but not on the parameters of stimulation. The relationship between the findings and each of these three factors is discussed below.

Site of stimulation

The greatest effect of naltrexone was observed with electrical stimulation of the nucleus accumbens and the least with stimulation of the ventral tegmental area. Other investigators have also reported that opiate antagonists have different effects on brain stimulation reward involving different stimulation sites (Freedman & Pangborn, 1984; Schaefer & Michael, 1981; Stapleton et al., 1979), and it has been suggested by Stapleton et al. (1979) that self-stimulation involving the nucleus accumbens is particularly sensitive to naloxone. The present study confirms this sensitivity.

Why is the reinforcing effect of electrical stimulation of the nucleus accumbens more sensitive to opiate receptor blockade than is the reinforcing effect of stimulation of either the lateral hypothalamus or the ventral tegmentum? At the very least it would appear that electrical stimulation of the nucleus accumbens activates a reward mechanism that is

different in some important way than the mechanism that is activated by electrical stimulation of either the lateral hypothalamic area or the ventral tegmental area. Several lines of independent evidence are consistent with this suggestion.

First, psychophysical studies show that the neurons directly activated by rewarding nucleus accumbens stimulation (Bielajew, Walker, & Fouriez, 1983) have different refractory periods than those directly activated by rewarding lateral hypothalamic or ventral tegmental stimulation (Bielajew & Shizgal, 1982, 1986; Rompré & Shizgal, 1986; Shizgal, Bielajew, Corbett, Skelton, & Yeomans, 1980; Yeomans, 1975, 1979). Thus different populations of cells or fibers are directly activated by rewarding nucleus accumbens stimulation and rewarding medial forebrain bundle (lateral hypothalamic and ventral tegmental) stimulation. It is possible that the elements activated at these different sites are in synaptic communication with one another, but it is clear that different elements are activated at the electrode tips.

Second, neuroleptics and lesions differentially affect nucleus accumbens and medial forebrain bundle self-stimulation. Pharmacological blockade and neurotoxin lesions of one or more of the dopamine systems disrupt medial forebrain bundle (both at the level of the lateral hypothalamic area and the ventral tegmental area) self-stimulation (Corbett, 1986; Koob, Fray, & Iverson, 1978) but appear to be less effective against nucleus accumbens self-stimulation (Simon, Stinus, Tassin, Lavielle,

Blanc, Thierry, Glowinski, & LeMoal, 1979, but see Mogenson, Takigawa, Robertson, & Wu, 1979).

Note, however, that the fact that naltrexone differentially affects self-stimulation involving different stimulation sites does not, in and of itself, require the conclusion that different mechanisms are involved. While the possibility that nucleus accumbens self-stimulation and medial forebrain bundle self-stimulation involve different mechanisms is confirmed by independent evidence, such evidence does not exist to confirm a difference between the mechanisms of lateral hypothalamic and ventral tegmental stimulation. In fact, as suggested above, the electrophysiological characteristics of the neurons directly activated by rewarding lateral hypothalamic stimulation appear identical to the electrophysiological characteristics of the neurons directly activated by rewarding ventral tegmental stimulation (Bielajew & Shizgal, 1982, 1986; Rompré & Shizgal, 1986; Shizgal, Bielajew, Corbett, Skelton, & Yeomans, 1980; Yeomans, 1975, 1979). Moreover, collision tests have demonstrated that a significant number of the fibers directly activated by stimulation at one of these sites also pass near the other, without an intervening synapse (Bielajew & Shizgal, 1982). This evidence indicates that rewarding stimulation of each of these areas directly activates the same set of medial forebrain bundle axons at different points along their length. If the same set of reward fibers is activated with lateral hypothalamic and ventral tegmental stimulation, some other explanation must be sought for the differential effects of

naltrexone in these two cases.

One possibility is that the opioid neurons which contribute to the reward messages initiated at either the lateral hypothalamus or ventral tegmental area pass near to the latter, innervating the ventral tegmental area itself. If so, then electrical stimulation of the ventral tegmental area may spread to the opioid terminals directly altering their contribution to the net reward signal. This seems a likely possibility, since opioid neurons do innervate the area (Khachaturian, Watson, Lewis, Coy, Goldstein, & Akil, 1982), and it is local injections of morphine in this area (but not others) that facilitate brain stimulation reward (Broekkamp, van den Bogaard, Heijnen, Rops, Cools, & van Rossum, 1976). Electrical stimulation of the hypothalamus would presumably have no effect on the normal firing of the same opioid terminals because of the distance between these terminals and the locus of stimulation.

A second possibility is that, while the same fiber tract has been implicated in lateral hypothalamic and ventral tegmental self-stimulation (Bielajew & Shizgal, 1982), the same exact elements of that tract may not have been activated in the present study. If an electrode aimed at the lateral hypothalamic area were not aligned in the same segment of the fiber bundle as an electrode aimed at the ventral tegmental area, then different neural sub-populations of the same system could be activated by stimulation of the two sites. In this case, naltrexone could be differentially effective because of differential opioid innervation of different regions of the ventral tegmental area.

Differential opioid innervation of the dopaminergic cells of this region has been established in electrophysiological studies (Matthews & German, 1984).

A final possibility is that electrical stimulation of the ventral tegmental area, is accompanied by different motoric or emotional side effects than accompany stimulation of the lateral hypothalamic area. Even if exactly the same reward fibers were activated by stimulation at the two sites, naltrexone could interact differentially because of differential activation of other nearby fibers--fibers involved in functions other than reward. It is important to remember that the net behaviour of an animal reflects more than simply the interaction of the drug with the rewarding effects of the stimulation per se; it also reflects side effects of stimulation which may be different even when the rewarding effects remain the same.

Parameters of rewarding stimulation

In the present investigation, the effectiveness of naltrexone was not dependent on an animal's rate of responding. Although it has been suggested that the effect of opiate antagonists on brain stimulation reward are more pronounced when animals are tested under stimulation parameters that produce low rates of response (Ichitani et al., 1985), the results of the present experiment fail to support this suggestion. There was no difference between the effectiveness of naltrexone when animals were tested at high frequencies (which produced

high rates of response) and low frequencies (which produced low rates of response). Naltrexone caused parallel shifts of the rate-frequency curve, increasing the amount of stimulation required to produce maximal responding by the same number of log units that increased the stimulation required to produce threshold-level responding. Hence, uncontrolled differences in stimulation parameters, causing uncontrolled differences in baseline response rate, would not seem to contribute significantly to the varied effectiveness of opiate antagonists which is seen in different studies.

Time and duration of testing

Naltrexone shifted the rate-frequency curve to the right, and the magnitude of the shift was dependent on the time of testing. Greater rightward shifts of the curve were seen during the second test session, which began 45 minutes after injection, than during the first session, which began 15 minutes after injection. These results are consistent with those of Schaefer and co-workers who reported stronger effects from 60-minute tests than from 20-minute tests (Schaefer & Michael, 1981; West et al., 1983) and with those of Trujillo et al. (1984) who observed greater effects in the second half-hour period after injection. These findings offer an explanation as to why researchers generally fail to observe effects when test sessions are restricted to the first half-hour after injection.

It is not clear why naltrexone was not more effective in

the first test session. The literature suggests that the drug reaches the brain in this period, but perhaps it takes longer for the drug to diffuse to its site of action. On the other hand, it may be that some period of time is required for an animal to react to a naltrexone-induced change in the reinforcing value of the stimulation. In order to determine whether active responding under the drug is required for naltrexone to have significant effects in the second test session, a second experiment was performed.

EXPERIMENT 2

METHOD

Subjects

Five animals that displayed stable responding for lateral hypothalamic stimulation in the first experiment were selected for the present experiment.

Procedure

Each animal was tested at a stimulation frequency of 40 Hz (the stimulation intensity for each rat was the same as in Experiment 1). Following a 5 minute warm-up period, animals were injected with a 20.0 mg/kg dose of naltrexone. Over the next 3-hour period, beginning 5 minutes after injection, animals were tested in 2-minute trials every 20 minutes. Response rates were recorded during the last minute of each trial. One week later, animals were tested in the same way except that the test session was delayed to begin 45 minutes after injection. One day prior to each naltrexone test session, animals were tested under saline conditions.

Histology

At the completion of the experiment, animals were

anesthetized with chloral hydrate and intracardially perfused with 30 mL of 0.9 % physiological saline followed by 60 mL of 10 % formalin solution. The brains were then removed and fixed in formalin solution for a period of 7 days. Following fixation, the brains were frozen and sliced in 40-um sections. Every fourth section was mounted and stained with thionin.

Statistical Analyses

One-way analyses of variance were used to determine whether responding under naltrexone conditions was significantly different from responding under baseline conditions when measured at 20-minute intervals over a three-hour period in test sessions beginning five and 45 minutes after drug injection.

Since separate analyses of variance were conducted, a conservative level of significance ($\alpha/2$) was adopted to correct for the experimentwise error rate. Thus, findings were considered significant only at obtained probabilities of less than 0.025 (Kirk, 1982, p. 103).

RESULTS

The effect of naltrexone on responding when measured at 20-minute intervals over a three-hour period beginning five minutes after injection is shown in Figure 13. A one-way analysis of variance revealed that the rate of responding of naltrexone-treated animals was significantly different from the rate of responding under saline conditions ($F(1,9)=64.067$, $p<0.001$). Naltrexone-treated animals responded at near-normal rates at the beginning of the test session followed by a decrease in responding 25 minutes after injection. Maximal attenuation of responding was observed 45, 65 and 85 minutes after injection. A near complete recovery of responding was reached by the end of the test session.

The effect of naltrexone on responding when measured at 20-minute intervals beginning 45 minutes after injection is displayed in Figure 14. A one-way analysis of variance shows that the number of responses animals made under naltrexone conditions differed significantly from the number of responses made under saline conditions ($F(1,7)=22.803$, $p<0.002$). Response rates were immediately suppressed at the beginning of the test session (45 minutes after injection) and remained this way when measured 65 and 85 minutes after injection, the same time intervals at which naltrexone produced the greatest suppression in Figure 13. By the end of the test session, the rate of responding had approached near-normal levels.

Figure 13. Time-course of the effects of naltrexone (20.0 mg/kg) (squares) or saline (circles) on responding for lateral hypothalamic stimulation in animals given immediate access to stimulation. Experiment 2.

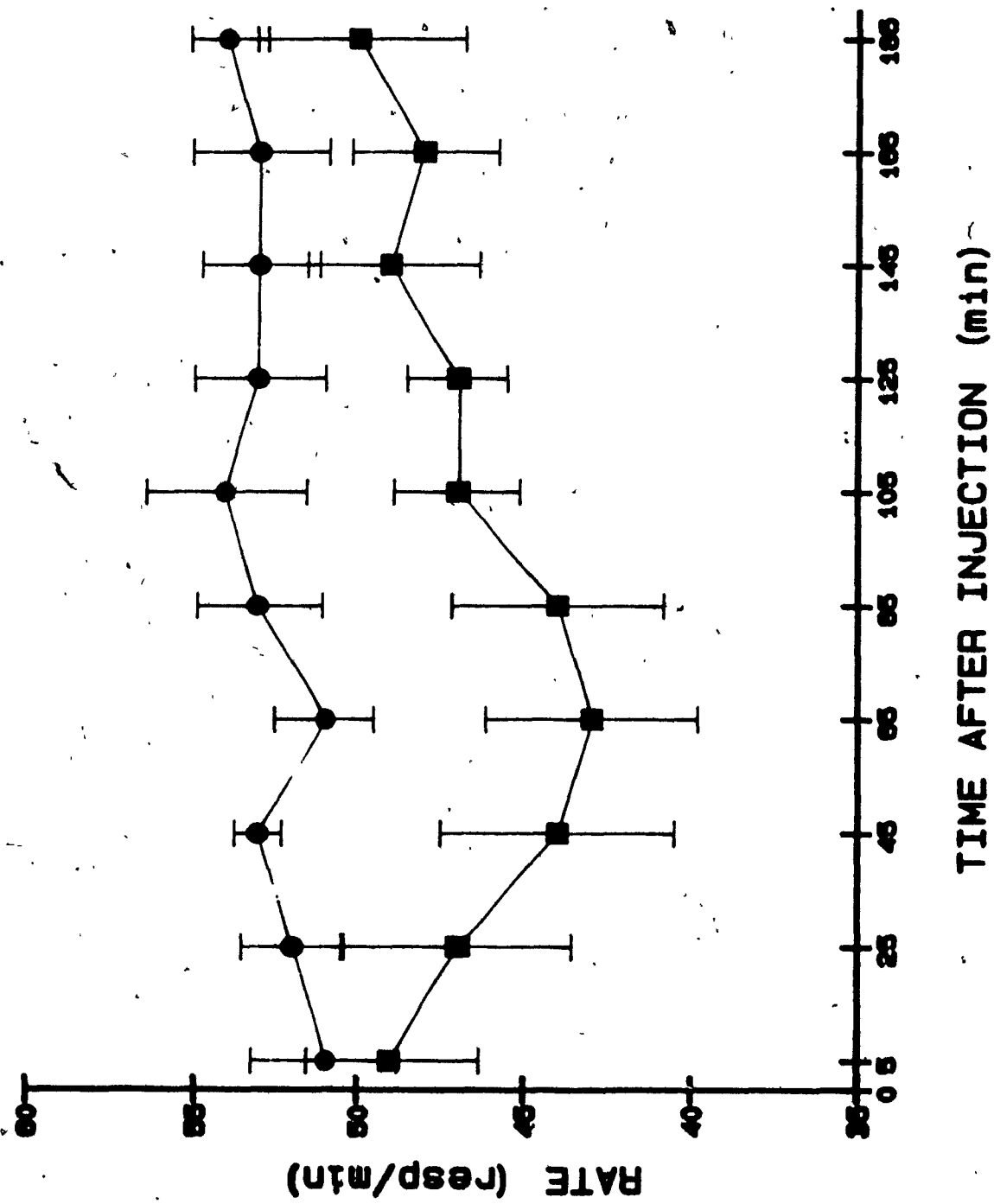
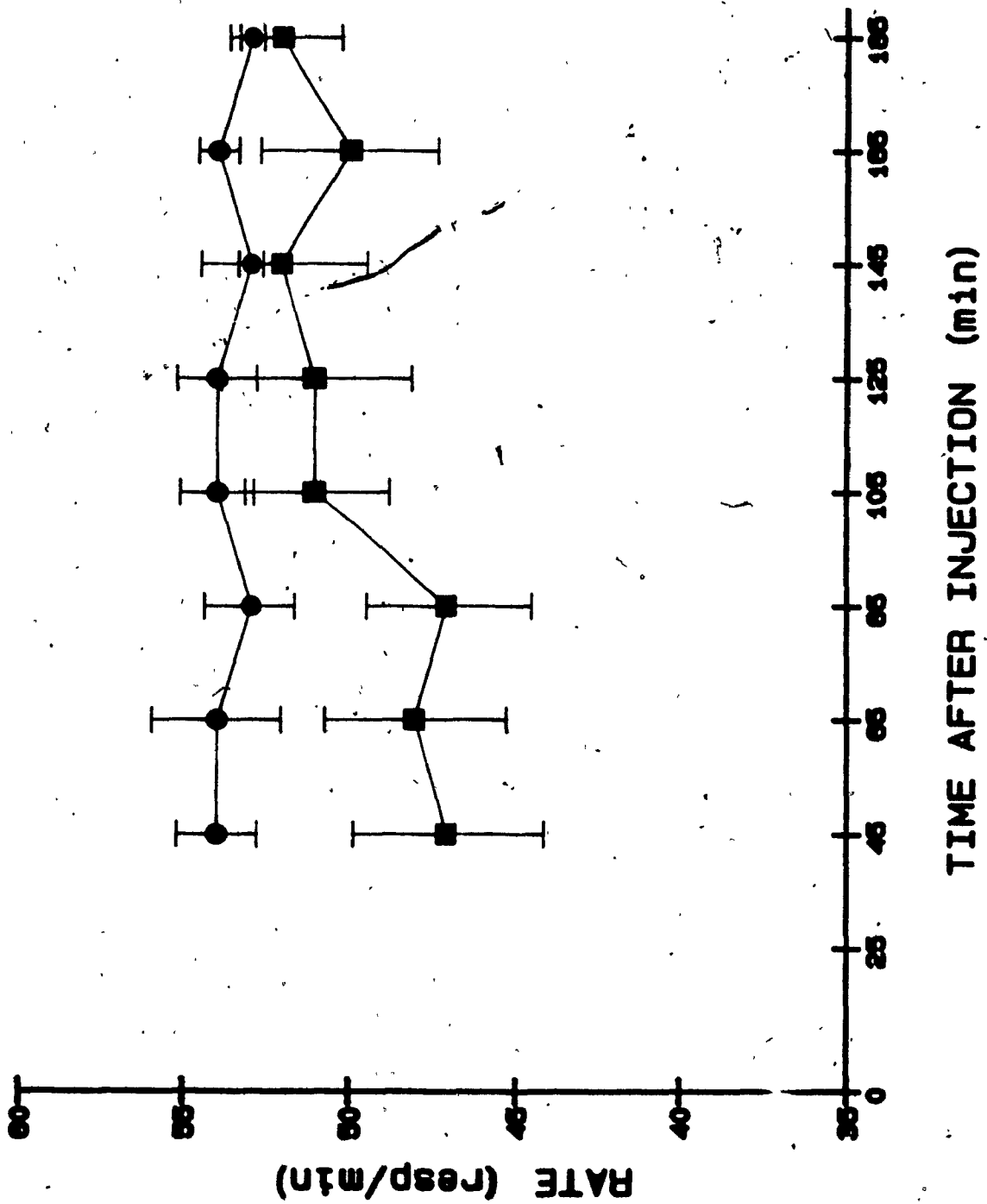


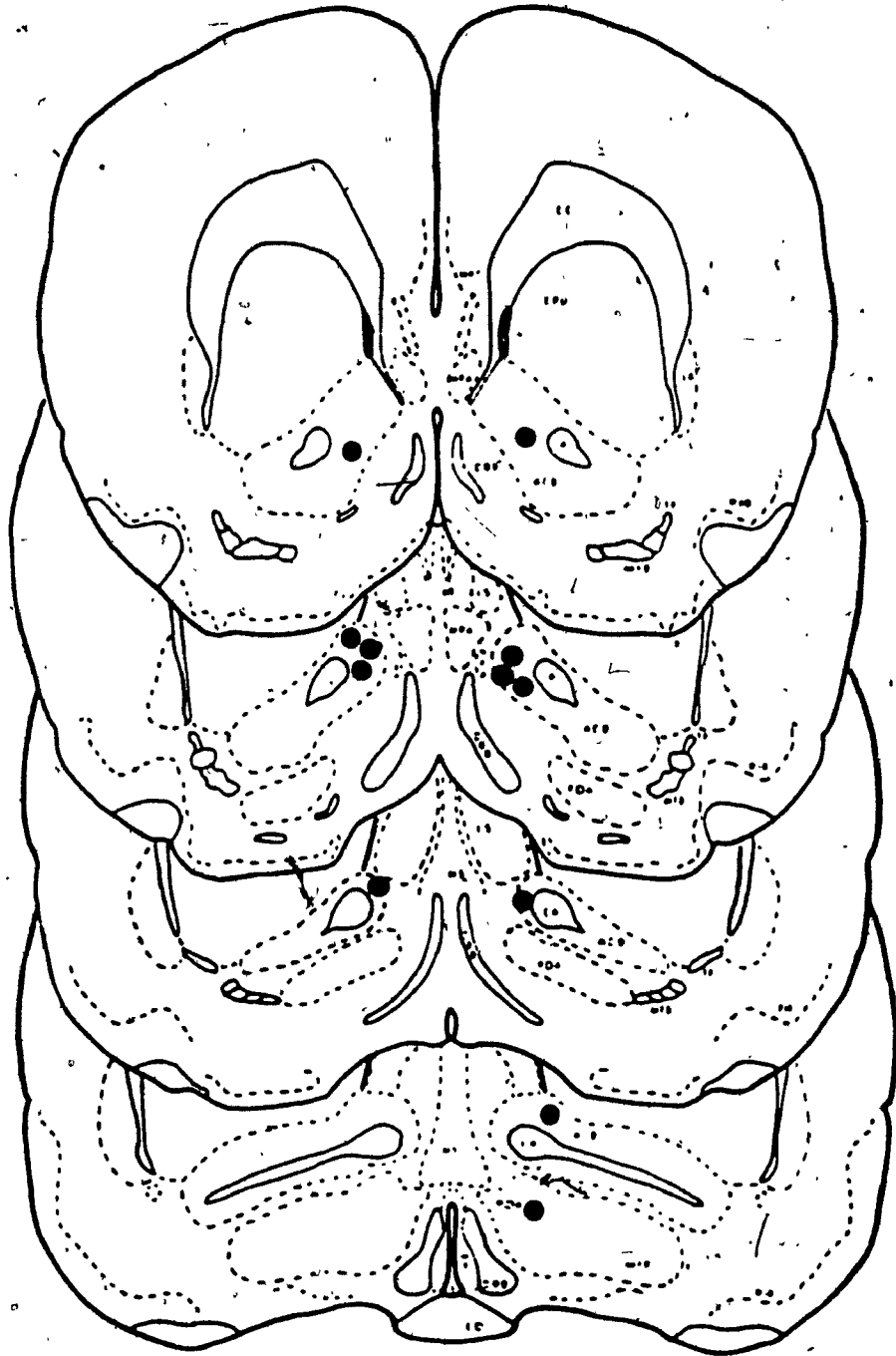
Figure 14. Time-course of the effects of naltrexone (20.0 mg/kg) (squares) or saline (circles) on responding for lateral hypothalamic stimulation in animals given delayed access to stimulation. Experiment 2.



Histological reconstructions

Electrode placements of animals that participated in Experiment 1 and Experiment 2 are shown in Figures 15, 16, and 17. Figure 15 shows the locations of electrode tips in the region of the nucleus accumbens. All electrode tips were located within this region with the exception of one. Although the electrode tip of this animal was located within the preoptic area, the results of this animal were averaged with the rest of the group because of the similarity of its results to the others. Figure 16 displays the locations of electrode tips in the area of the lateral hypothalamus. The tips of electrodes aimed at the lateral hypothalamus were all located within this area. Figure 17 shows the locations of electrode tips in the area of the ventral tegmentum. The tips of electrodes aimed at the ventral tegmentum were all located within this area.

Figure 15. Histological localization of electrode tips aimed at the nucleus accumbens. Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The number beside each brain slice represents the distance anterior to bregma.



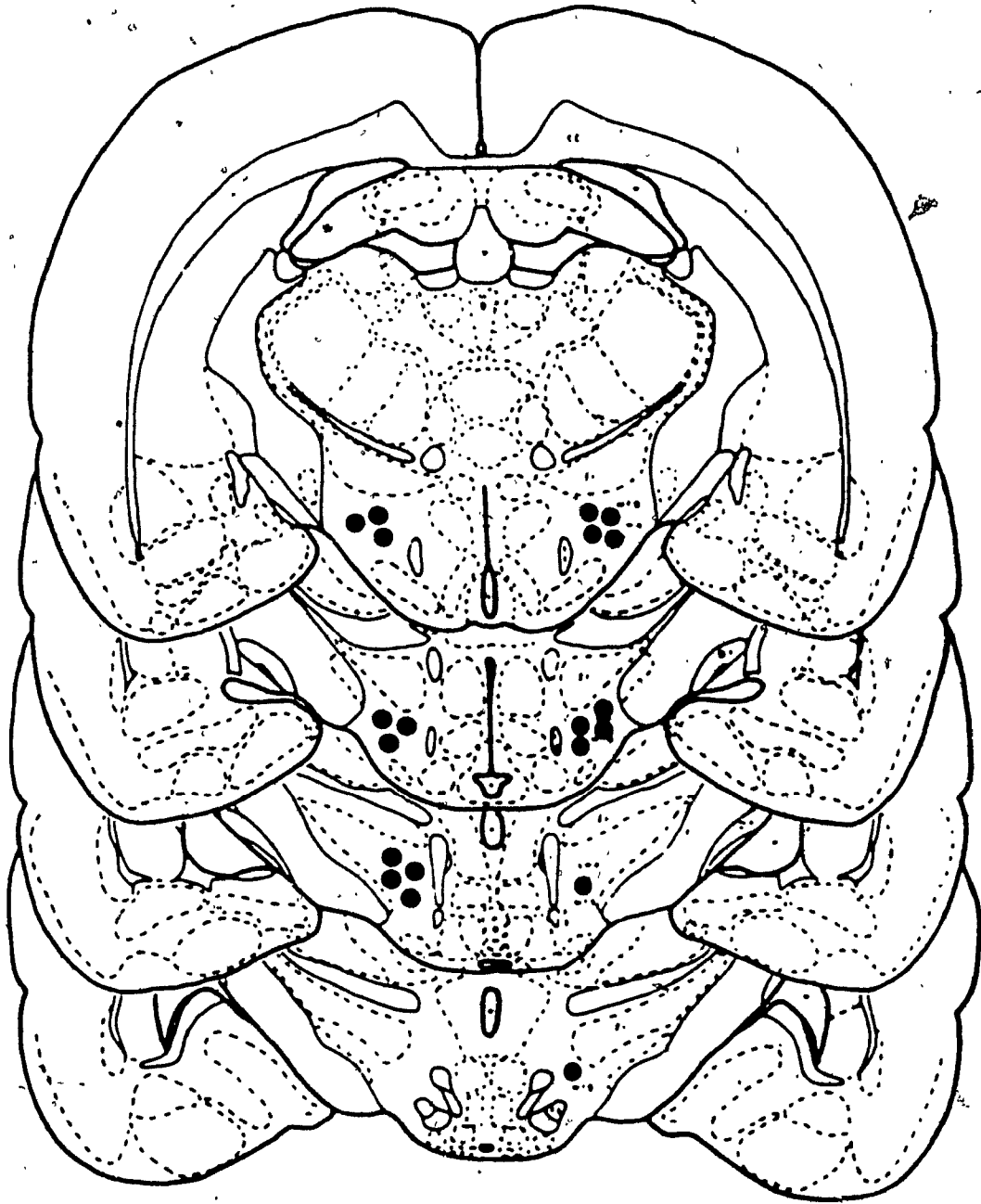
3.4

3.0

2.6

2.2

Figure 16. Histological localization of electrode tips aimed at the lateral hypothalamic area. Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The number beside each brain slice represents the distance posterior to bregma.



0.8

1.2

1.6

2.0


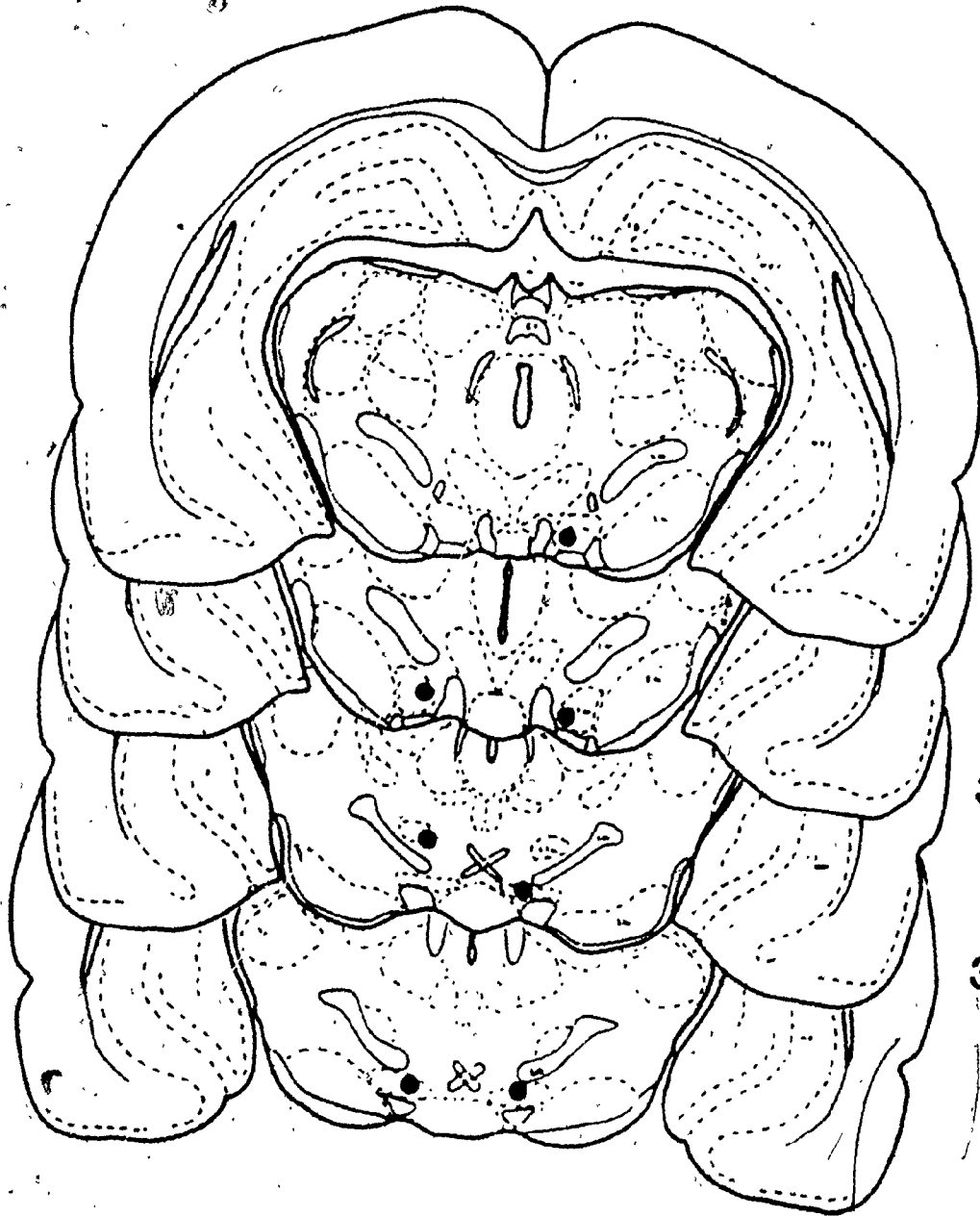


Figure 17. Histological localization of electrode tips aimed at the ventral tegmental area. Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The number beside each brain slice represents the distance posterior to bregma.



3.0

3.2

3.4

3.6

DISCUSSION

In the present experiment, the greatest decrease in self-stimulation was observed 45, 65 and 85 minutes after naltrexone injection, regardless of whether animals merely absorbed drug or actively lever-pressed for stimulation while absorbing drug in the first 30 minutes after injection. Since naltrexone reaches peak brain levels within 30 minutes of injection (Misra, Block, Vardy, Mule, & Verebely, 1976), these results suggest that the greatest suppression of brain stimulation reward behaviour occurs some time after peak concentrations reach the brain. The delay between peak whole-brain drug concentration and peak behavioural effect may be a result of the time it takes naltrexone to reach and occupy the critical receptors.

It has been suggested that a period of active responding under naltrexone is required before an animal adjusts its behaviour to reflect an alteration by the drug of the reinforcing impact of stimulation (Trujillo et al., 1984). It is clear from the present experiment, however, that an animal does not require an extended period of active responding under naltrexone in order for a drug-induced change in responding to occur. Naltrexone was effective in decreasing response rates in the first one-minute test (preceded by a one-minute warm-up) of the 45-minute delay condition. Since responding was effectively suppressed in the first one-minute test of the 45 minute delay condition, it would appear to be slow passive

diffusion of the drug to its mechanism of action, and not the need for extended experience with stimulation under drug, that accounts for the delayed suppression of responding in animals that are tested shortly after injection.

SUMMARY AND CONCLUSIONS

In the first experiment, naltrexone shifted the rate-frequency curve to the right, which is presumed to reflect a decrease in the rewarding impact of the stimulation. Stronger naltrexone effects were seen during the second test session, beginning 45 minutes after injection; only minimal effects were seen during the first test session, beginning 15 minutes after injection. Naltrexone had the strongest effect on responding for nucleus accumbens stimulation; it had a weaker, but still reliable, effect on responding for lateral hypothalamic stimulation; it had no effect on responding for ventral tegmental stimulation. The effectiveness of naltrexone was not dependent on an animal's rate of responding; there was no difference between high rate of responding and low rate of responding.

These data confirm earlier reports that naltrexone attenuates brain stimulation reward and that rewarding stimulation associated with some sites is more affected than that associated with other sites. There is no evidence to suggest that differences in stimulation parameters, causing differences in baseline response rate, contribute to the varied effectiveness of naltrexone which is seen in different studies. The fact that naltrexone is more

effective 45 minutes after injection explains some of the inconsistencies in the literature and probably reflects time needed for passive diffusion of naltrexone to its site of action.

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Appendix I

Effects of four doses of naltrexone on nucleus accumbens (NAS), lateral hypothalamic area (LHA), or ventral tegmental area (VTA) reward thresholds. S.E. indicates standard error of the mean. Saline tests and Test 1 began 15 minutes after injection; Test 2 began 45 minutes after injection.

	Dose (mg/kg)							
	2.5		5.0		10.0		20.0	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
NAS								
Saline	35.73	1.04	37.44	1.02	36.06	1.02	37.22	1.04
Test 1	39.90	1.03	39.90	1.03	38.82	1.05	40.83	1.04
Test 2	44.57	1.04	47.95	1.04	46.55	1.04	43.90	1.04
LHA								
Saline	36.98	1.02	36.94	1.01	36.18	1.01	36.94	1.01
Test 1	38.15	1.03	37.41	1.04	38.61	1.02	37.34	1.03
Test 2	40.71	1.03	39.90	1.04	40.55	1.03	42.02	1.03
VTA								
Saline	35.97	1.04	36.31	1.02	35.54	1.02	35.89	1.01
Test 1	39.67	1.04	37.33	1.03	36.89	1.04	35.97	1.05
Test 2	37.15	1.03	38.52	1.03	39.90	1.02	38.64	1.03

Appendix II

Effects of four doses of naltrexone on maximal rate of responding for stimulation of the nucleus accumbens (NAS), lateral hypothalamic area (LHA), or ventral tegmental area (VTA). S.E. indicates standard error of the mean. Saline tests and Test 1 began 15 minutes after injection; Test 2 began 45 minutes after injection.

	Dose (mg/kg)							
	2.5		5.0		10.0		20.0	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
NAS								
Saline	44	1.69	48	2.61	48	3.46	47	2.98
Test 1	47	1.94	47	2.29	46	2.83	46	1.79
Test 2	43	2.44	43	2.19	41	2.63	42	2.17
LHA								
Saline	57	2.62	57	2.26	57	2.60	54	2.77
Test 1	58	2.14	57	2.43	56	2.59	54	3.01
Test 2	57	2.69	57	2.40	57	2.57	54	3.12
VTA								
Saline	56	7.19	50	6.68	56	7.09	56	7.29
Test 1	53	6.82	50	7.07	53	8.10	55	7.67
Test 2	54	7.00	49	6.72	53	8.07	53	7.82