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The Effects of Nucleus Accumbens Injections of Receptor-Selective Opiate Agonists on Brain Stimulation Reward

Thomas E.G. West

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ABSTRACT

The effects of nucleus accumbens injections of receptor-selective opiate agonists on brain stimulation reward

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Concordia University, 1991

Opiates are rewarding in their own right and potentiate the rewarding effect of lateral hypothalamic electrical stimulation. One of the sites in the brain where opiates have rewarding action is the nucleus accumbens (NAS), but injections into this site have been reported not to potentiate brain stimulation reward. Few animals have been tested, however, and insensitive methods have been used. The purpose of the present study was to reinvestigate the effects of nucleus accumbens opiates on lateral hypothalamic brain stimulation reward.

The "curve-shift" paradigm was used to assess the effects of morphine, the mu-selective opiate agonist DAGO, the delta-selective opiate agonist DPDPE, and the kappa-selective opiate agonist U50,488H injected into each of four regions of the nucleus accumbens. Morphine, DAGO, and DPDPE, but not U50,488H, potentiated the rewarding effects of lateral hypothalamic stimulation. The effectiveness of morphine and DAGO, but not DPDPE, was dependent on the region of injection. Morphine was effective only when injected into the caudo-medial and caudo-lateral regions of NAS. DAGO was effective when injected into both caudal

regions and the rostro-medial region. Morphine and DAGO were effective only when injected at doses higher than those tested in previous studies. The effects of NAS opiates were blocked by naltrexone, indicating that the opiate effects were receptor-mediated rather than non-specific.

The relative potency of equi-molar doses of DAGO and DPDPE were compared following injections into the caudo-lateral region of the nucleus accumbens. DAGO was observed to be at least 100 times more potent than DPDPE.

Injections of morphine, DAGO, and DPDPE into an area of the caudate dorsal to the caudo-lateral region of NAS did not facilitate brain stimulation reward. Thus, it appears that opiates injected into the nucleus accumbens are acting within this region rather than diffusing up the cannula shaft to act at a distal site.

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Introduction

A defining characteristic of addiction is the development of compulsive drug self-administration habits (Jaffe, 1985). During the last two decades many drug abuse specialists have focused their attention on the reward-related properties of drugs and the habits they establish (Goldberg & Kelleher, 1976; Griffiths, Brady, & Bradford, 1979; Schuster & Johanson, 1981; Schuster & Thompson, 1969; Woods & Schuster, 1971), rather than on the dependence syndromes that are sometimes seen as an underlying cause of such habits (Wise, 1987). Studies of the direct habit-forming properties of drugs have utilized two basic paradigms: the drug self-administration paradigm (Deneau, Yanagita, & Seevers, 1969; Weeks, 1962) and the conditioned place preference paradigm (Beach, 1957; Rossi & Reid, 1976; Schwartz & Marchok, 1974). In addition, opiates (Cador, Kelley, Le Moal, & Stinus, 1986; Gosnell, Morley, & Levine, 1986; Grandison & Guidotti, 1977; Jenck, Quirion, & Wise, 1987; Kelley, Cador, Stinus, & Le Moal, 1989; Leibowitz & Hor, 1982; Majeed, Przewlocka, Wedzony, & Przewlocki, 1986; McLean & Hoebel, 1983; Mucha & Iversen, 1986; Stanley, Lanthier, & Leibowitz, 1989; Tepperman & Hirst, 1982; Tepperman, Hirst, & Gowdey, 1981; Wise, Jenck, & Raptis, 1986; Woods & Leibowitz, 1985) and other habit-forming drugs including amphetamine (Colle & Wise, 1988; Wise, Fotuhi, & Colle, 1989), benzodiazepines (Cooper, 1980; Wise

& Dawson, 1974), cannabanoids (Drewnowski & Grinker, 1978; Glick & Milloy, 1972; Hollister, 1971), barbiturates (Jacobs & Farel, 1971; Watson & Cox, 1976) and ethanol (Britton & Britton, 1981) facilitate feeding and opiates facilitate sexual behavior (Mitchell & Stewart, 1990), suggesting that rewarding drugs can also facilitate the effectiveness of natural rewards. Finally, most addictive drugs facilitate the reinforcing effects of the laboratory reward of direct electrical stimulation of the brain (Kornetsky, Esposito, McLean, & Jacobson, 1979; Levitt, Baltzer, Evers, Stilwell, & Furby, 1977; Wise, 1989; Wise & Bozarth, 1987).

I. Direct rewarding effects of opiates

The direct rewarding effects of drugs can be assessed in terms of either the response habits or the stimulus preferences they establish. Experimental paradigms for each of these two approaches have been established and used tc determine the brain mechanisms involved in the direct rewarding effects of opiates.

A. Methods for assessing drug reward

The two major paradigms for assessing the direct rewarding effects of drugs are the drug self-administration

paradigm and the conditioned place preference paradigms. The two paradigms derive from very different theoretical origins in experimental psychology, and they are generally thought to reflect two very different principles of learning.

1. Drug self-administration

The most widely accepted indication of the direct rewarding effects of drugs is drug self-administration. Drug self-administration habits are established by what Skinner (1938) has termed "operant reinforcement." Operant reinforcement is defined by Skinner as follows: "If the occurrence of an operant is followed by presentation of a reinforcing stimulus, the strength is increased" (Skinner, 1938, p. 21). The defining characteristic of operant reinforcement is the behavioral-contingency; the reinforcer is administered if and only if the animal meets some predetermined behavioral requirement. Thus the animal must <u>earn</u> reinforcement in the operant paradigm. Because of the response contingency, operant reinforcement is thought to reflect <u>response</u> learning; operant reinforcement plays a role in the learning of contingencies between responses and reinforcers. The concept of "operant reinforcement" is the explanatory principle invoked to explain the development and maintenance of drug self-administration habits.

2. Conditioned place preference

The second paradigm that is thought to reflect direct rewarding effects of drugs is the conditioned place preference paradigm. In this paradigm, animals are restricted to one compartment of a two- or three-compartment test box following one or more drug injections (usually four injections are given on separate days) and to a second compartment following vehicle injections (given on the intervening days). A third compartment--often a passageway between the drug-associated and vehicle-associated compartments--is not associated with any injections. After successive pairings of drug with one compartment and vehicle with the other, the animal is given (on a drug-free day) a choice between compartments; if the drug-associated compartment is preferred to the vehicle-associated compartment the drug is considered to have reinforcing actions. This paradigm involves Pavlovian conditioning, and the reinforcement in Pavlovian conditioning has been presumed to be fundamentally different (Mowrer, 1947; Schlosberg, 1937; Skinner, 1938) from that in operant conditioning; it has been distinguished as "respondent reinforcement" by Skinner (1938). Respondent reinforcement is defined by Skinner as follows: "The approximately simultaneous presentation of two stimuli, one of which (the 'reinforcing' stimulus)

belongs to a reflex existing at the moment at some strength, may produce an increase in the strength of a third reflex composed of the response of the reinforcing reflex and the other stimulus" (Skinner, 1938, p. 18). The characteristic of respondent reinforcement that sets it fundamentally apart from operant reinforcement is the response-independence of the administration of the reinforcer; the animal is presented the reinforcing stimulus regardless of the animal's behavior. Because reinforcement is given in contingency with stimuli rather than with responses, respondent reinforcement is a case of stimulus learning. Respondent reinforcement plays a role in the learning of contingencies between sets of stimuli.

B. Mechanisms of opiate reward

Opiates--such as heroin, morphine--have been shown to be reinforcing agents in both the self-administration (Bozarth & Wise, 1981a; van Ree & de Wied, 1980) and conditioned place preference (Phillips & LePiane, 1980; Bozarth & Wise, 1981b) paradigms. Interestingly, the same brain structures--the ventral tegmental area (VTA) and the nucleus accumbens (NAS)--and the same classes of opiate receptors--mu and delta--are implicated by studies involving each of the two pa adigms. Other brain regions such as the lateral hypothalamic area, periaqueductal gray,

and the hippocampus may also be involved in opiate reward although the present evidence is less well developed.

1. Lateral hypothalamic area

Stein and Olds (1977) were the first to observe that rats will self-administer opiates directly into the brain. They found that rats would lever-press for morphine injections into the lateral hypothalamic area, where electrical stimulation is also rewarding. Subsequent reports from the same laboratory have confirmed this finding (Olds, 1979; Olds & Williams, 1980), and Corrigal (1987) has reported the complementary finding that lateral hypothalamic injections of an opiate antagonist attenuate the rewarding effects of intravenous opiates. It is not clear, however, that the lateral hypothalamic injections have a lateral hypothalamic site of action. With a somewhat different injection paradigm--but one that was effective with another injection site--Bozarth and Wise (1982) were unable to confirm lateral hypothalamic self-administration of morphine and Britt and Wise (1981) reported that destruction of the bed nucleus of the lateral hypothalamus by kainic acid injections failed to alter intravenous heroin self-administration. Thus there remains some question as to whether these opiate injections are indeed acting in the lateral hypothalamic area and not at

some distant site.

A critical step in determining whether the action of a drug is localized to a specific site in the brain is to compare the effectiveness of injections between the putative site of action and closely adjacent sites. Since it is well known that drugs injected into the brain can diffuse and reach sites distal to the site of injection (eg., Johnson & Epstein, 1975), any attempt to localize a drug effect to a central site requires the demonstration that injections into adjacent areas are less effective than injections into the putative site of action. In the case of the studies reporting LHA self-administration, cannulae were used that were much larger than those of Bozarth and Wise (1982) and this can facilitate the spread of drug up the cannula shaft and into the ventricles where it can diffuse to a distal site of action (Routtenberg, 1972). Since there are demonstrations that rats will self-administer opiates into the ventricles (Amit, Brown, & Sklar, 1976; Belluzzi & Stein, 1977) it is possible that injections of opiates into the lateral hypothalamic area diffused dorsally and reached the ventricular system. Because there has yet to be a demonstration that opiate injections are more effective in the lateral hypothalamic area than in adjacent regions, it remains unconfirmed whether this region of the brain is involved in opiate reward.

Opiate injections into the LHA have also been shown to establish conditioned place preferences (van der Kooy et al., 1982). However, the test dose used to establish a place preference was 40 times higher than the dose that is effective in the ventral tegmental area (Bozarth, 1987; Phillips & LePiane, 1980). In addition, a demonstration that opiate injections into the lateral hypothalamic area produce stronger place preferences than injections into adjacent regions is still required. Thus, the present evidence for a role of the lateral hypothalamic area in opiate reward is equivocal.

2. Ventral tegmental area

One region of the brain that seems more clearly involved in opiate reward is the ventral tegmental area. Evidence suggesting a role of the VTA in opiate reward is derived partially from the demonstration by different laboratories that animals will learn to lever-press for injections of opiates into this brain region (Bozarth & Wise, 1981a, 1982; van Ree & de Wied, 1980; Welzl, Kuhn, & Huston, 1989). It appears that the opiate actions are receptor-mediated since peripheral administration of the opiate receptor blocker naloxone attenuates VTA morphine self-administration (Bozarth & Wise, 1981a) and direct administration of an opiate receptor blocker into the VTA

attenuates intravenous opiate self-administration (Britt & Wise, 1983; Vaccarino, Bloom, & Koob, 1985).

Conditioned place preference studies suggest the same conclusion as the self-administration studies--opiates have rewarding actions in the ventral tegmental area. Injections of morphine into the VTA (Phillips & LePiane, 1980; Bozarth, 1987) but not into regions dorsal (Phillips & LePiane, 1980), rostral, caudal, or ventral (Bozarth, 1987) to this area produce a conditioned place preference. A conditioned place preference is produced by injections of the opiate peptide (D-ala^2), Met^5-enkephalin (DALA) into the VTA (Phillips & LePiane, 1982; Phillips, LePiane, & Fibiger, 1983) but not by injections into a region dorsal to the VTA (Phillips & LePiane, 1982). The place preference produced by VTA morphine or DALA is blocked by peripheral administration of naloxone (Phillips & LePiane, 1980, 1982), suggesting that the opiate effect is receptor-mediated.

In summary, the demonstration by different laboratories that rats will self-administer opiates into the VTA and the finding that conditioned place preferences are more effective following opiate injections into the VTA rather than into adjacent regions suggests that the ventral tegmental area is an important site for opiate reward. The anatomical controls for drug diffusion appear to confirm the VTA as a site of opiate reward.

3. Nucleus accumbens

Another region that appears likely to be involved in opiate reward is the nucleus accumbens. Evidence linking this brain region to opiate reward, like the evidence linking the ventral tegmental area to opiate reward, comes from both the results of self-administration and conditioned place preference studies. The first demonstration that animals would self-administer opiates into the nucleus accumbens was reported by Olds (1982) and this finding has been confirmed independently by Goeders, Lane, and Smith (1984). The finding that injection of an opiate receptor blocker into the nucleus accumbens attenuates the reinforcing effect of intravenous heroin self-administration further supports the suggestion that the nucleus accumbens plays a role in opiate reward (Corrigal & Vaccarino, 1988; Vaccarino, Bloom, & Koob, 1985). Not all reports are in agreement, however. Bozarth and Wise (1982) reported that rats would not self-administer morphine into the nucleus accumbens. In their study, however, the animals were not tested for morphine self-administration across a range of doses. Thus, it is not clear whether animals would have self-administered morphine at higher doses.

Injections of morphine into the nucleus accumbens can produce a conditioned place preference (van der Kooy,

Mucha, O'Shaughnessy, & Bucenieks, 1982). This reinforcing effect of morphine appears to be receptor-specific since van der Kooy et al (1982) demonstrated that central administration of the active morphine enantiomer (-)-morphine, and not the inactive enantiomer (+)-morphine, was effective.

While animals will work for NAS opiates and while NAS opiates cause conditioned place preferences, it has not been demonstrated that these injections are more effective than dorsal or adjacent injections. Thus, as in the case of the lateral hypothalamic area, the site of rewarding action of opiates has not yet been convincingly localized to the site at which it has been presumed to act.

4. Other regions

The hippocampus and the periaqueductal gray (PAG) may also be involved in opiate reward. Recent demonstrations that rats will learn to lever-press for injections of opiates into the hippocampus (Stevens, Shiotsu, Belluzzi, & Stein, 1988; Stevens, Shiotsu, & Stein, 1991) and that intra-hippocampal opiate injections can produce a conditioned place preference (Corrigal & Linseman, 1988; Iwamoto, 1988) suggest a possible role for the hippocampus in opiate reward. Again, however, anatomical comparisons have not been made. While the hippocampus appears to be a

potential site of opiate reward, there has yet to be a study showing that opiate injections are more effective when injected within the hippocampus than when injected into regions outside of this structure including the adjacent ventricle. The PAG has also been suggested to be involved in opiate reward since injection of morphine into the periaqueductal grey produces a conditioned place preference (van der Kooy et al., 1982), and injection of an opiate antagonist into the PAG attenuates the rewarding effects of intravenous heroin self-administration (Corrigal & Vaccarino, 1988). Again, however, anatomical comparisons have not been made. Thus, there is no confirmation that these opiate injections are acting in the PAG. Moreover, Bozarth and Wise (1982) failed to demonstrate PAG self-administration of morphine. Thus, again, rewarding actions have not been localized to these sites of injection.

In summary, evidence from self-administration and conditioned place preference studies suggest that the rewarding effects of the opiates are mediated, at least in part, by opiate receptor actions in the ventral tegmental area. The nucleus accumbens may also be an important region for opiate action but a demonstration that injections of opiates into neighboring regions are less effective is still required. The lateral hypothalamic area, hippocampus, and PAG are suggested to be involved in opiate reward, but again, the evidence regarding these

regions is not fully developed.

II. Opiate facilitation of brain stimulation reward

Opiates are not only rewarding in their own right; they, like several other drugs of abuse (Wise, 1980; Kornetsky, Esposito, McLean, & Jacobson, 1979; Gardner, 1991), also potentiate the rewarding effects of direct electrical stimulation of the brain. It has been suggested (Esposito & Kornetsky, 1978; Kornetsky et al., 1979; Wise, 1980; Gardner, 1991) that the reward-potentiating effects of opiates are mediated by the same brain mechanisms as are involved in their direct rewarding actions. If this suggestion is valid, the potentiation of brain stimulation reward (BSR) by drugs offers a third paradigm for studying reward-related properties of addictive agents. This third paradigm offers several advantages over drug self-administration and conditioned place preference paradigms, thus it is of considerable interest to determine the mechanism of the reward-potentiating effects of opiates and other habit-forming drugs. However, there is one drug treatment--morphine microinjected into nucleus accumbens--that is rewarding in its own right (Olds, 1982) but has been reported not to potentiate the rewarding effects of electrical stimulation of the brain (Broekkamp, van den Bogaard, Heijnen, Rops, Cools, & van Rossum, 1976). This

treatment is the subject of the present investigation.

A. History of brain stimulation reward

Olds and Milner (1954) discovered that rats would work for electrical stimulation of the medial forebrain bundle and related brain regions. This discovery led Olds (1956) to speculate that the brain must contain specialized anatomical circuits which are involved in reward function. It is now widely believed that the phenomenon of brain stimulation reward involves the activation of endogenous reward circuitry--circuitry that evolved to serve natural rewards such as feeding, drinking, and sexual behavior.

B. Effect of opiates on brain stimulation reward

The opiates were among the first drugs of abuse to be shown to facilitate brain stimulation reward. Moreover, opiate antagonists can attenuate self-stimulation--at least in the case of some stimulation sites (Belluzzi & Stein, 1977; Collier & Routtenberg, 1984; Franklin & Robertson, 1982; Freedman & Panghorn, 1984; Glick, Weaver, & Meibach, 1982; Ichitani, Iwasaki, & Satoh, 1985; Kelsey, Belluzzi, & Stein, 1984; Reymann, Wulcko, Ott, & Matties, 1986; Schaefer, & Michael, 1981, 1985; Stapleton, Merriman, Coogel, Gelbard, & Reid, 1979; Stein, 1985; Trujillo,

Belluzzi, & Stein, 1989; West & Wise, 1988). Thus there is some reason to believe that endogenous opiates may play a role in the mechanism of brain stimulation reward. This, in turn, offers an explanation of why exogenous opiates should facilitate brain stimulation reward.

1. Opiates

Olds and Travis (1960) observed that peripheral administration of morphine caused a decrease in responding for brain stimulation reward. Subsequent investigators have confirmed this observation, but have also reported facilitatory effects. It is now clear that these opposite effects of morphine on responding for BSR are related to the level of opiate in the brain. At moderate to high doses, systemic morphine and heroin produce a biphasic effect which is characterized by an initial depression of responding--during the phase of high drug levels--followed by a period of enhanced responding--as the drug is metabolized to low levels (Adams, Lorens, & Mitchell, 1972; Bermudez-Rattoni, Cruz-Morales, & Reid, 1983; Bush, Bush, Miller, & Reid, 1976; Koob, Spector, & Meyeroff, 1975; Lorens & Mitchell, 1973; Wauquier & Niemegeers, 1976). With low doses of systemic morphine (Glick & Rapaport, 1974) and heroin (Gerber, Bozarth, & Wise, 1981), a facilitatory effect can be seen without any depression of

responding for brain stimulation reward.

Several research groups have investigated the effect of opiates on self-stimulation using reward threshold rather than response rate as a measure of the rewarding impact of electrical stimulation of the brain. Opiates produce a decrease in reward threshold (Kornetsky, Esposito, McLean, & Jacobson, 1979; Nazzaro, Seeger, & Gardner, 1981; van Wolfswinkel & van Ree, 1985), even during the period when high doses cause a suppression of response rate. This suggests that these compounds facilitate the effects of rewarding brain stimulation, even at high doses. Indeed, morphine not only reduces the amount of current necessary to support minimal (threshold) levels of responding: it reduces the current necessary to produce normal responding across the whole working range of the animal's response rate. It also suggests that the suppressive effects of morphine on self-stimulation reflect an attenuation of the performance capacity of the animal, and that suppression of performance capacity can occur despite-- and concurrent with--reward facilitation.

2. Opiate antagonists

Opiate antagonists have been reported to block the reward-facilitating effects of exogenous opiates and attenuate the rewarding effects of stimulation even when no

exogenous opiates have been given (Belluzzi & Stein, 1977). This finding implicates endogenous opiates in the mechanism of brain stimulation reward and it offers an explanation of why exogenous opiates should facilitate the behavior. Subsequent studies involving the effect of opiate receptor blockers on brain stimulation reward have not been consistent, however. In some studies opiate antagonists have been reported to decrease responding or increase thresholds for brain stimulation reward while in other studies these agents have been observed to produce no effect. It is now clear that self-stimulation involving different sites in the brain can differ in sensitivity to opiate antagonists (West & Wise, 1988); however, opiate antagonist attenuation of self-stimulation has been reliably demonstrated (Belluzzi & Stein, 1977; Collier & Routtenberg, 1984; Franklin & Robertson, 1982; Freedman & Panghorn, 1984; Glick, Weaver, & Meibach, 1982; Ichitani, Iwasaki, & Satoh, 1985; Kelsey, Belluzzi, & Stein, 1984; Reymann, Wulcko, Ott, & Matthies, 1986; Schaefer & Michael, 1981, 1985; Stapleton, Merriman, Coogel, Gelbard, & Reid, 1979; Stein, 1985; Trujillo, Belluzzi, & Stein, 1989; West & Wise, 1988). Endogenous opiates appear to play strong or weak roles in self-stimulation, depending on the particular circuitry that is stimulated (Collier & Routtenberg, 1984; West & Wise, 1988).

C. Mechanism of facilitation

It has been suggested that the rewarding action of drugs of abuse and electrical stimulation of the brain involve the activation of a common biological mechanism (Esposito & Kornetsky, 1978; Gardner, 1991; Kornetsky et al., 1979; Wise, 1989; Wise & Bozarth, 1987). One prediction of this suggestion is that all drugs that are rewarding in their own right should potentiate the rewarding effects of BSR. This prediction is borne out in the cases of amphetamine (Colle & Wise, 1988; Esposito, Perry, & Kornetsky, 1980; Liebman & Butcher, 1974; Phillips & Fibiger, 1973; Stein & Ray, 1960), morphine (Adams, Lorens, & Mitchell, 1972; Broekkamp, van den Bogaard, Heijnen, Rops, Cools, & van Rossum, 1976; Jenck, Gratton, & Wise, 1987; Olds & Travis, 1960), heroin (Gerber, Bozarth, & Wise, 1981), cocaine (Crow, 1970; Wauquier & Niemegeers, 1974), ethanol (De Witte & Bada, 1983; Lorens & Sainati, 1978), barbiturates (Reid, Gibson, Gledhill, & Porter, 1964), and benzodiazepines (Olds, 1966; Panksepp, Gandelman, & Trowill, 1970). A further prediction is that drugs should potentiate BSR when injected into the specific brain regions where they have direct rewarding effects. This prediction is borne out in the case of nucleus accumbens amphetamine and VTA opiate injections but not, according to current data, in the case of NAS opiate

injections. If the finding that NAS opiates fail to facilitate BSR were reliable, it would falsify the hypothesis that the mechanism of reward-facilitation and the mechanism of direct rewarding effect of opiates are one and the same, since such injections are rewarding in their own right (Goeders, Lane, & Smith, 1984; Olds, 1982; van der Kooy et al., 1982).

1. Ventral tegmental area

Broekkamp, van den Bogaard, Heijnen, Rops, Cools, & van Rossum (1976) identified the VTA as a site at which morphine facilitates BSR. Morphine (2.5 μg), when injected into the ventral tegmental area, produces a biphasic (peripheral-like) effect on responding for brain stimulation reward. The initial suppression of responding observed with this dose may be due to the spread of morphine into more caudal regions including periaqueductal gray (Broekkamp et al., 1976) and reticular formation (Dunstan, Broekkamp, & Lloyd, 1981) where it is reported to produce pure inhibitory or cataleptic effects, respectively. When lower doses (0.05-1.0 μg) of morphine are injected into the VTA a pure facilitation of responding for BSR is observed (Broekkamp, Phillips, & Cools, 1979). VTA injections of the opiate peptides DALA (Broekkamp, Phillips, & Cools, 1979) and DPDPE (Jenck, Gratton, & Wise,

1987) facilitate the rewarding impact of brain stimulation; the facilitation of self-stimulation following VTA morphine (Broekkamp, Phillips, & Cools, 1979; Jenck, Gratton, & Wise, 1987) and DPDPE (Jenck, Gratton, & Wise, 1987) is blocked by peripheral administration of naloxone suggesting that their effects are receptor-mediated. These results suggest that the ventral tegmental area is one site at which morphine and opiate peptides can interact with the mechanisms involved in brain stimulation reward.

2. Nucleus accumbens

Broekkamp et al. (1976) observed that morphine (2.5 μg) produces a small but nonsignificant facilitation of LHA BSR. De Witte, Heidbreder, and Roques (1989) also failed to observe any facilitation of BSR following intra-NAS injections of [D-Thr^2, Leu^5]-enkephalin-Thr^6 (DTLET), a partial delta opiate receptor agonist (James & Goldstein, 1984), and [D-ala^2, MePhe^4, Gly-ol^5]-enkephalin (DAGO), a selective mu opiate receptor agonist (Handa, Lane, Lord, Morgan, Rance, & Smith, 1981). If it were true that NAS opiate injections do not facilitate BSR, then the theory that reward-facilitation involves that same mechanism as the direct rewarding effects of opiates must be invalid. There are, however, several possible explanations of failure to demonstrate reward-facilitating effects. The

negative effects observed by Broekkamp et al. (1976) and De Witte et al. (1989) may reflect the testing of the wrong doses or the wrong opiates, the wrong injection sites, or the wrong stimulation parameters.

D. Limitations of the previous studies

1. Range of tested doses

In the Broekkamp et al. (1976) study, only one dose (2.5 µg) of morphine was examined; it is possible that this dose was too low to produce a significant change in behavior. Van der Kooy et al. (1982) used higher doses to produce conditioned place preference, though they did not determine that such doses were necessary. The apparent ineffectiveness of intra-accumbens injections of DAGO and DTLET on responding for brain stimulation reward may, similarly, be the result of the dose of administration. DAGO and DTLET, which failed to facilitate self-stimulation when administered in a dose of 300 picomoles (pmoles), are reported to produce an increase in locomotor activity when administered at higher doses (Dauge, Rossignol, & Roques, 1988, 1989); higher doses may also be necessary for a significant change in self-stimulation.

2. Region of opiate injection within nucleus accumbens.

A second possibility is that opiates are effective in only a sub-region of the nucleus accumbens, and that Broekkamp et al. (1976) and De Witte et al. (1989) injected into the wrong region of this structure. Because the nucleus accumbens is a large structure, measuring approximately 3.0 millimeters in length, 2.5 millimeters in width, and 1.5 to 2.0 millimeters in depth (Pellegrino, Pellegrino, & Cushman, 1979), it is possible that injections of a drug into one region of the nucleus accumbens may produce different effects on responding for rewarding brain stimulation than injections into another region of the nucleus. Indeed, injections of proglumide, a cholecystokinin antagonist, into the rostral region of the nucleus accumbens produces a completely different effect on responding for brain stimulation reward than do injections into the caudal region (Vaccarino & Vaccarino, 1989). Stellar and Corbett (1989) report differences in the effect of the neuroleptic, cis-flupenthixol, on brain stimulation reward depending on the region of injection within the nucleus accumbens. The same may be true for injections of opiates into the nucleus accumbens--injections of opiates into one region may affect responding for brain stimulation reward differently than injections into another region of

this structure.

3. Parameters of rewarding stimulation.

It is also possible that the effects of intra-NAS opiates on BSR depend on the stimulation parameters that are used. With high frequencies or intensities and short durations of stimulation, high baseline response rates are usually seen, whereas with low frequencies or intensities and long durations of stimulation, low response rates can be typically obtained from the same animal. Dews (1958) has argued that baseline rate of responding can be a major determinate of the effects of psychomotor stimulants and the same may be true for the effects of the opiates; thus animals should be tested across a range of stimulation parameters (which produce different rates of response) before a general conclusion concerning the effect of intra-NAS opiates on BSR can be drawn.

Another possibility is that stimulation parameters that produce maximal rates of responding obscure any tendency of opiates to facilitate self-stimulation. If an animal is responding maximally for BSR and an opiate is administered, the opiate may increase the rewarding effect of BSR but not the response rate. Thus, it seems clear that arbitrary decisions concerning the choice of stimulation parameters in the Broekkamp et al. (1976) and

DeWitte et al. (1989) studies could have contributed to the apparent ineffectiveness of intra-NAS opiates on brain stimulation reward in previous studies.

E. Potentiation of brain stimulation reward by rewarding drugs--a hybrid paradigm

1. A hybrid paradigm

The potentiation of brain stimulation reward by rewarding drug injections involves a hybrid paradigm--a combination of both operant and respondent elements. The paradigm is a hybrid paradigm because the animal receives two reinforcers, the response-contingent (operant paradigm) brain stimulation and the non-contingent (Pavlovian paradigm) drug. Inasmuch as the paradigm reflects the interaction of a non-contingent drug reinforcer with a response-contingent brain stimulation reinforcer, it appears to tell us something about the <u>additivity</u> of drug reward and brain stimulation reward. This conclusion is consistent with a "dose-response" analysis of the interaction.

2. The measurement of brain stimulation reward

In the brain stimulation reward paradigm, the

rewarding efficacy of electrical stimulation was historically inferred from simple operant response rate. This measure is no longer considered acceptable as a measure of the reinforcing value of brain stimulation (Valenstein, 1964). For one thing, it is difficult to decide whether a drug- or lesion-induced change in the average rate of responding reflects an alteration in the rewarding qualities of the stimulation or whether it reflects an alteration in the performance capacity of an animal. Another problem with using simple response rate is that different researchers have chosen different stimulation parameters. Since different stimulation parameters yield different rates of response, and since drugs produce differential effects depending on baseline response rate, arbitrary decisions concerning stimulation parameters may contribute to whether a drug effect is found in a given study.

Researchers have responded to the need for a rate-independent measure by designing alternative paradigms including the threshold paradigm and the curve-shift paradigm. In the threshold paradigm (Esposito, Perry, & Kornetsky, 1980; Perry, Esposito, & Kornetsky, 1981; Stein & Ray, 1960), the minimum intensity or frequency of stimulation that will sustain some arbitrary level of responding is defined as threshold. The arbitrary levels of responding that have been used to define threshold

include, among others, minimal responding and half-maximal responding. Threshold measures are assumed to reflect the neural sensitivity to the rewarding impact of the stimulation. Since threshold reflects the level of stimulation for which an animal is willing to sustain 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.

Another paradigm that has been used widely to measure the rewarding effect of brain stimulation is the curve-shift paradigm (Franklin, 1978; Gallistel & Freyd, 1987; Milliaressis, Rompré, Laviolette, Phillipe, & Coulombe, 1986; West & Wise, 1988; Yeomans, Kofman, & McFarlane, 1985). In the curve-shift paradigm, response rate is determined across a wide range of stimulation intensities or frequencies, including some that produce maximal rates of responding, some that produce moderate rates of responding, and some that fail to sustain even a minimum level of responding. Like the threshold measure, this method makes it possible to determine whether the effect of a drug treatment alters the rewarding impact of stimulation and not merely the performance capacity of the animal.

The curve-shift method is analogous, in many ways, to the standard dose-response analysis of pharmacology (Liebman, 1983). Just as it is required that a

pharmacological effect be sampled across a range of effective doses of a drug, the curve-shift paradigm requires that a brain stimulation reward effect be sampled across a range of effective "doses" of rewarding stimulation. In the curve-shift paradigm the dose of rewarding stimulation that an animal receives can be varied by changing the intensity, frequency, or train duration. By changing one of these parameters (usually intensity or frequency) and keeping other parameters fixed, a function relating response strength to reward strength can be determined. A curve that represents the function relating response strength to reward strength is the curve for which the curve-shift paradigm is named. Although varying stimulation intensity is preferable in some brain stimulation reward studies--including mapping studies--stimulation frequency should be varied in drug studies. When the stimulation frequency is varied and other parameters are fixed, different "doses" of stimulation are associated with different numbers of evoked action potentials in a fixed population of directly activated neurons. This is useful for measurement purposes, since for example, halving the stimulation frequency reduces the reward signal by a known percentage (half), whereas halving the stimulation intensity reduces the population of directly activated fibers by an unknown percentage (Gallistel, 1987; Milliaressis et al., 1976; Wise, 1989).

The curve in the curve-shift paradigm is shifted in different directions under different experimental conditions (Edmonds & Gallistel, 1974). First, the curve can be 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 et al., 1986), or by making animals run an incline rather than a level runway (Edmonds & Gallistel, 1974).

The rate-intensity or rate-frequency curve can be shifted laterally, left or right, by changing stimulation intensity or by changing stimulation frequency. Lateral shifts of the rate-intensity or rate-frequency curve appear to reflect changes in the rewarding impact of the stimulation. Lateral shifts seem not to reflect a change in the performance capacity of the animal, since they do not alter the asymptotic levels of performance that are eventually reached when stimulation levels are maximal.

Rightward shifts of the rate-frequency curve are caused by decreases in the stimulation intensity; rightward shifts of the rate-intensity curve are caused by decreases in the stimulation frequency. Rightward shifts thus reflect decreases in the total stimulation charge per reinforcement; decreases in one of the parameters

contributing to the total charge per reinforcement must be offset by increases in another if response rate is to be kept constant (Edmonds & Gallistel, 1974; Gallistel, 1974). Treatments that cause rightward shifts of the curve thus have consequences similar to decreasing the magnitude of reward. Because the stimulation strength is in fact constant, the treatment is assumed to cause some degree of decrease in the ability of the stimulation to activate relevant brain circuitry, or in the ability of the relevant brain circuitry to propagate normal reward signals. A rightward shift of the curve is analogous to the way in which a competitive antagonist shifts a pharmacological dose-response curve.

Leftward shifts of the rate-frequency curve are caused by increases in the stimulation intensity; leftward shifts of the rate-intensity curve are caused by increases in the stimulation frequency. Leftward shifts thus reflect increases in the total stimulation per reinforcement; again increases in one of the parameters contributing to the total charge per reinforcement must be offset by decreases in another if response rate is to be kept constant (Edmonds & Gallistel, 1974; Gallistel, 1974). Treatments that cause leftward shifts of the curve thus have consequences similar to increasing the magnitude of reward. Because the stimulation strength is constant, such treatment is assumed to cause some increase in the ability of the stimulation to

activate relevant brain circuitry, or in the ability of the relevant brain circuitry to propagate normal reward signals.

3. Drug interactions with brain stimulation reward

In the brain stimulation reward paradigm, the most important questions are whether a treatment shifts the curve vertically or horizontally, and by how much. Vertical shifts of the curve occur with drugs that are known to have debilitating effects, such as d-tubocurarine (Edmonds & Gallistel, 1974) or methocarbamol (Edmonds & Gallistel, 1974; Milliaressis et al., 1986). 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.

Some drugs cause shifts of the curve to the right. For example, neuroleptics, at low doses, cause rightward shifts (Franklin, 1978; Gallistel & Karras, 1984; Milliaressis et al., 1986). Naltrexone, a competitive antagonist of opiates, also causes a rightward shift of the curve (West & Wise, 1988). Since these results parallel the effect of decreasing the stimulation current, they suggest that neuroleptics and opiate antagonists decrease

the rewarding impact of the stimulation.

Other drugs can cause leftward shifts of the curve. For example, amphetamine produces leftward shifts of the curve (Gallistel & Karras, 1984). Opiates also shift the curve to the left (Rompré & Wise, 1988). The fact that amphetamine and opiates shift the curve to the left, as does increasing the level of the rewarding impact of the stimulation, suggests that these agents amplify the rewarding effect of the stimulation. The fact that pimozide and naloxone are antagonists of amphetamine and opiates, respectively, lends credence to the inference that amphetamine and opiates on the one hand and neuroleptics and opiate antagonists on the other have opposite effects on the rewarding impact of hypothalamic brain stimulation.

F. Advantages of the study of drug self-stimulation interactions

If the ability of drugs is to potentiate the rewarding impact of medial forebrain bundle electrical stimulation and to reflect drug actions on the mechanisms of the primary rewarding effects of those drugs, the BSR paradigm offers several advantages over other paradigms for studying reward-related drug effects. First, it offers the advantage over self-administration paradigms that the experimenter, rather than the animal, controls the drug

dose and injection volume. These are important advantages in studies involving anatomical comparisons. When different animals self-select different doses and volumes of drug, they subject themselves to different levels of drug spread.

Another advantage of the BSR paradigm is that the animal is not learning anything important about the task during the drug treatment. In the self-administration paradigm, the animal is learning to lever-press for drug. If the animal does not learn to self-administer drug it is not clear whether the problem is with the learning ability of the animal or with the rewarding impact of the drug. In the reward potentiation paradigm, the animal learns to lever-press for brain stimulation reward prior to drug testing and continues to perform the well-practiced task during the drug-testing phase. Thus there is no doubt that the animal has the required knowledge of the task. Since drug effects are reflected in established and ongoing performance, a failure to observe a drug effect suggests that the drug has no reward-potentiating effects.

A final advantage of the reward-potentiation paradigm is that estimates of changes in the magnitude of a drug effect on brain stimulation reward can be quantified on the same logarithmic scale that is used to characterize other psychophysical dimensions (Milliaressis et al., 1986; Gallistel & Freyd, 1987). Since the rewarding value of the

stimulation is related logarithmically to the stimulation frequency, equal changes in the log of the required stimulation frequency are taken as equal changes in the perceived impact of the stimulation. Drug treatments that produce the same logarithmic change in the stimulation intensity or frequency are assumed to produce equal changes in the rewarding impact of the stimulation (Gallistel & Freyd, 1987). In the drug self-administration and conditioned place preference paradigms, the rewarding effects of a drug are not easily quantifiable because the effect of an animal's behavior is not measured directly on a ratio (logarithmic) scale.

EXPERIMENT 1

The first experiment was designed to examine the effect of nucleus accumbens opiate injections on lateral hypothalamic brain stimulation reward. This line of study was undertaken because some theorists have suggested that the reward-potentiating effects of opiates are mediated by the same brain mechanisms as are involved in their direct reward actions (Esposito & Kornetsky, 1978; Gardner, 1991; Kornetsky et al., 1978; Wise, 1980). However, the nucleus accumbens which has been suggested as a site of opiate reward by self-administration and condition place preference studies, has been reported not to be involved in opiate-facilitation of the rewarding effect of hypothalamic stimulation. Broekkamp et al. (1976) and De Witte (1989) reported no effects of NAS injections of opiates on BSR. If opiates do have reward-related effects in NAS, an explanation of why NAS opiates failed to facilitate the rewarding effects of brain stimulation in these studies is required. A number of possible explanations might be suggested: the wrong doses, the wrong region of NAS, or the wrong stimulation parameters may have been used. It is expected that rewarding opiate injections in NAS should potentiate BSR, but it was not established that the injections tested by Broekkamp et al. (1976) or De Witte et al. (1989) were rewarding in their own right. Even if the

injections were appropriate, these studies involved simple rate measures of BSR and such measures are known to be susceptible to ceiling effects; thus facilitation may not have been seen because the animals were already at the limits of their performance before drug was administered. The purpose of the present study was to re-examine the effect of NAS opiates on LHA BSR using a more powerful methodology than was employed in the earlier studies of Broekkamp et al. (1976) and De Witte et al. (1989).

METHOD

Subjects The subjects were male Long-Evans rats weighing 325-500 grams. They were individually housed in stainless steel cages in a colony room that was maintained at 70^{o}F and on a 12-hour light and 12-hour dark cycle. Food and water were available ad lib.

Surgery Sixty-two rats were anaesthetized with sodium pentobarbital (Somnotol, 60 mg/kg, ip) and secured in a stereotaxic instrument for electrode and cannula implantation. They each received 0.15 mL of atropine sulfate (0.009 mg/mL, sc) to reduce respiratory distress. Monopolar, stainless-steel electrodes (0.25 mm in diameter), insulated with varnish except at the cross-section of the tip, were implanted bilaterally into the

lateral hypothalamic area of each rat. With the incisor bar at 5.0 mm above the intra-aural line, the stereotaxic co-ordinates for the lateral hypothalamic area were: 0.8 mm posterior to bregma, 1.6 mm lateral to the midline, and 7.8 mm below the dural surface. Each animal was also implanted with bilateral stainless steel guide cannulae (22-gauge) aimed the rostro-medial, rostro-lateral, caudo-medial or caudo-lateral accumbens nuclei. The co-ordinates for each of the four targets in the accumbens are shown in Table 1.

Five stainless-steel screws threaded into the skull served as anchors for each electrode-cannula assembly. An uninsulated wire was wrapped around two of these screws to serve as a current return for electrical stimulation. The assembly was anchored to the skull screws with acrylic cement. Stainless steel blockers, extending 1.5 mm beyond the tip of each cannula were inserted into the guide cannula after the dental cement hardened.

Apparatus Animals were trained in operant chambers (28 X 28 cm) having three aluminum walls and a clear plastic front wall. A lever, 5 cm above a grid floor, protruded through one of the aluminum walls. Depression of the lever resulted in delivery of a 0.5-sec train of 0.1 msec square wave cathodal pulses. The pulse amplitude was controlled by a constant current generator (Mundl, 1980). An oscilloscope was used to monitor current intensity by

Table 1

The stereotaxic co-ordinates for the four target regions in the nucleus accumbens

NAS region	Angle[1]	Anterior[2]	Lateral[2]	Ventral[2,3]
Rostro-Medial	10	3.4-4.0	2.4	.8
Rostro-Lateral	0	3.4-4.0	2.4	5.7
Caudo-Medial	10	2.2-2.8	2.4	5.1
Caudo-Lateral	0	2.2-2.8	2.8	5.6

1 Cannulae were angled 10^{o} from the vertical when implanted into a medial region of the nucleus accumbens in order to avoid penetration of the ventricular system.

2 The anterior co-ordinates were measured from bregma, lateral from the midline, and ventral from the dural surface. Co-ordinates are expressed in millimeters.

3 Each cannula was implanted with its tip 1.5 mm above the injection target.

reading the voltage drop across a 1 kohm resistor connected in series with the animal. Electrical stimulation was delivered through flexible wires connected through a commutator so that the animal was able to move freely within the chamber.

Procedure

Screening. One week after surgery, each rat was placed into an operant chamber and allowed to lever-press spontaneously for electrical stimulation of the lateral hypothalamic area at a frequency of 60 Hz and an intensity of 100 μA. Rats that did not learn to lever-press spontaneously were given successive approximation training: to approach and press the lever. Successive approximation training involved rewarding an animal with electrical stimulation for closer and closer approximations of the lever-pressing response: first an animal was rewarded for movement towards the lever, then for contact with the lever, and then for depression of the lever. If an animal did not learn to lever-press during the initial successive approximation training, the stimulation intensity was increased in 25-μA increments from 100 μA to a maximum of 700 μA. An animal that did not learn to respond for 700 μA (through at least one electrode) was eliminated from the experiment. Once an animal learned to lever-press, the

stimulation intensity was re-adjusted in 25-μA increments every 50 seconds to establish a stable rate of responding. When a current was found that produced a stable rate of responding (more than 20 presses per 50 seconds) over three consecutive test sessions, rate-frequency curves for that stimulation site were determined.

Animals were tested at several stimulation frequencies, beginning with frequencies that produced high rates of responding and descending in 10% steps every 50 seconds until a stimulation frequency was reached for which the rat would not continue to respond. The initial 10 seconds of testing at each frequency was treated as an adjustment period; response rates were recorded during the last 40 seconds of testing at each stimulation frequency. When a stimulation frequency was found which would not maintain at least 3 responses per 40 seconds, the rate-frequency curve was considered complete. The highest frequency of stimulation that maintained 3 or more responses during the last 40 seconds of a 50 second trial was defined as the frequency threshold. The amount of current was then adjusted so that a frequency threshold of 40 Hz was obtained. When the frequency thresholds associated with four consecutive rate-frequency curves did not differ from 40 Hz by more than 10 percent within a test session and over three consecutive test days, the threshold was considered to be stable. When this criterion was met,

the animals were considered ready for baseline testing.

Baseline testing. With the stimulation intensity fixed at the same value that produced frequency thresholds of 40 Hz in the screening phase, four baseline rate-frequency curves were determined for each animal. If the frequency thresholds of the first four baseline curves did not differ by more than 10 percent, an animal's baseline behavior was considered stable. In cases where an animal's baseline behavior was not stable, additional rate-frequency curves were determined. When the frequency thresholds of four consecutive baseline curves did not differ by more than 10 percent (which usually required four to six curves) animals were removed from the operant chamber and given a drug injection. In all experimental conditions, animals were assessed for baseline responding prior to the administration of drug.

Drugs. Drugs used were morphine sulfate (Department of Health &Welfare, Canada), [D-Ala^2, N-methyl-Phe^4, Gly-ol^5]-enkephalin (DAGO) (Sigma Chemical Co., St. Louis, MO), [D-Pen^2, D-Pen^5]-enkephalin (DPDPE) (IAF Biochem Int. Inc., Laval, Quebec), (trans-3,4-dichloro-N-methyl-N[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide) (U50,488H) (Upjohn Company, Kalamazoo, MI), and naltrexone hydrochloride (Sigma Chemical Co., St. Louis, MO). All

drugs were dissolved in 0.9% saline.

DAGO, DPDPE, and U50,488H were chosen because they have been demonstrated to have high affinity and selectivity for mu (Handa, Lane, Lord, Morgan, Rance, & Smith 1981), delta (Mosberg, Hurst, Hruby, Gee, Yamamura, Galligan, & Burks, 1983), and kappa (Von Voightlander, Lahti, & Ludens, 1983) opiate receptors, respectively. Morphine was chosen to compare the results of the present study against the standard of previous studies.

Drug injection. All drugs were injected into the nucleus accumbens ipsilateral to the working electrode in a volume of 0.5 µL. For injections, the blocker was removed and a 30-gauge stainless-steel injector cannula, connected to a 1 µL Hamilton syringe via polyethylene tubing, was inserted into the guide cannula. The tip of the injector cannula extended beyond the guide cannula by 1.5 mm. Drugs were administered over a 60 second period and the injector was left in the guide cannula for an additional 60 seconds to ensure complete delivery of drug. Immediately following the delivery of drug, the blocker was reinserted into the guide cannula and the animal was placed back into the operant chamber. The injectors and tubing used to administer DPDPE were coated with bovine serum albumin in order to minimize binding of the peptide to these surfaces.

Drug testing. Beginning 10 minutes after drug injection, rate-frequency curves were determined every 15 minutes for a period of two hours. Animals were tested for two hours to assess the time of each opiate to produce peak behavioral effects. All animals were first tested under saline prior to testing under three doses of morphine (0.625 µg, 2.5 µg, 10 µg) and four doses each of DAGO (50 ng, 200 ng, 800 ng, 3200 ng) and DPDPE (50 ng, 200 ng, 800 ng, 3200 ng). Eighteen of these animals were randomly selected to be tested under four doses of U50,488H (50 ng, 200 ng, 800 ng, 3200 ng). The drugs and doses were given in a random order and at least two non-drug days separated the testing days.

To determine whether the effects of nucleus accumbens opiate injections were receptor-mediated, animals were given subsequent tests under naltrexone. Animals that displayed a decrease in frequency threshold of at least 10 percent following injections of morphine (10 µg), DAGO (3200 ng), or DPDPE (3200 ng) were selected to receive injections of naltrexone (1.0 and 2.5 mg/kg). Naltrexone was given intraperitoneally 10 minutes prior to nucleus accumbens drug injections. Finally, 14 animals were randomly selected, independent of region of NAS cannulae implantation, to receive post-test injections of saline. Animals received post-test saline injections in order to determine whether conditioning factors played a role in the

effect of opiate injections on lateral hypothalamic frequency thresholds.

Determination of rewarding effectiveness of stimulation. In all experimental conditions, changes in the rewarding effectiveness of lateral hypothalamic stimulation were inferred from the idealized threshold estimate "theta 0" (Milliaressis et al., 1986). Theta 0 was estimated from each rate-frequency function by extrapolating the stimulation frequency required to sustain 20, 30, 40, 50, and 60 percent of the maximal rate of responding and fitting a linear function to the extrapolated points. The point of intersection of the fitted line with the "X" (frequency) axis was taken as the value of theta-0. This analysis was performed on each of the four rate-frequency curves determined during baseline testing and on each of the eight rate-frequency curves determined after drug administration for each experimental test session. The theta-0 values (reward threshold) associated with each of the four curves in the baseline condition were then averaged. Theta-0 values were also averaged for each pair of rate-frequency curves determined during each half hour of testing under drug. Changes in the rewarding effectiveness of lateral hypothalamic stimulation following each experimental condition were then determined by calculating the percent change in theta-0

between baseline and each of the four time periods under drug. Reward thresholds less than 100 % were presumed to reflect increases in the rewarding effectiveness of lateral hypothalamic stimulation; reward thresholds greater than 100% were presumed to reflect decreases in the rewarding effectiveness of lateral hypothalamic stimulation.

Histology. At the completion of each experiment, the animals were anaesthetized with chloral hydrate and perfused with 30 mL of 0.9% saline followed by 60 mL of 10% formalin solution. The brains were then removed and fixed in formalin solution for seven days. Following fixation, the brains were sliced in 40 micron coronal sections and stained with thionin for the purpose of electrode and cannula localization. The position of the electrodes and cannulae were compared and transposed to diagrams of coronal sections taken from the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979).

Statistical analysis

Morphine, DAGO and DPDPE. Individual three-factor analyses of variance (Region x Dose x Time) were used to determine the effects of morphine, DAGO, and DPDPE on LHA frequency thresholds following injections of each opiate into four different regions of the nucleus accumbens. In

order to assess the hypothesis that the four regions of the nucleus accumbens were independent of each other, in each three-way analysis of variance, Region was considered a between subjects factor. The other two factors of Dose and Time were within subjects factors. In the three-way analysis of variance for morphine, there were four levels of Dose (saline, 0.625, 2.5, and 10.0 µg) and four levels of Time (30, 60, 90, and 120 minutes). In the three-way analyses of variance for DAGO and DPDPE, there were five levels of Dose (saline, 50, 200, 800, and 3200 ng) and four levels of Time (30, 60, 90, and 120 minutes).

Three-way analyses of variance were also conducted on the effect of the drugs on asymptotic responding. The maximal response rate was defined as the fastest rate of responding that occurred at any frequency during the determination of a rate-frequency function. Each of the four regions of the nucleus accumbens was considered a level of the between factor of Region. Dose and Time were considered to be repeated factors. Comparisons of interest were analyzed using the Tukey test.

U50,488H. A two-way repeated measures analysis of variance was used to determine the effect of U50,488H on LHA frequency thresholds following injection into the nucleus accumbens. The two repeated factors were Dose and Time. The number of subjects from each of the four

"Regions" of the nucleus accumbens was too low to perform a between factor analysis, consequently, subjects from the four Regions were pooled in this analysis.

A two-way repeated measures analysis of variance was also conducted on the effect U50,488H on asymptotic responding. Dose and Time were considered repeated factors.

Pre-drug saline versus post-drug saline. A two-way repeated measures analysis of variance was performed on the effect of saline prior to and following opiate injections into the nucleus accumbens on LHA frequency thresholds. Dose and Time were considered repeated factors. The number of subjects from each of the four "Regions" was too low to perform as between factor analysis, consequently, the subjects from the four Regions were pooled in this analysis.

A two-way repeated measures analysis of variance was conducted on the effect of pre- and post-drug saline on asymptotic responding. Dose and Time were considered repeated factors.

Naltrexone versus opiates. Individual two-way repeated measures analyses of variance were used to assess the effect of morphine, DAGO, and DPDPE on LHA frequency thresholds following pre-treatment with naltrexone (1.0 and

2.5 mg/kg). There were four levels to each of the repeated factors of Dose and Time.

Individual two-way repeated measures analyses of variance were also used to assess the effects of morphine, DAGO, and DPDPE on asymptotic responding following pre-treatment with naltrexone. The repeated factors were Dose and Time. In each analysis of variance, comparisons of interested were analyzed with the Tukey test.

RESULTS

Injections of morphine, DAGO, and DPDPE, caused leftward shifts of the rate-frequency function when injected into some regions of the nucleus accumbens. Injections of U50,488H into the nucleus accumbens were not effective in shifting the rate-frequency function. Morphine was effective only when injected into the caudal regions of the nucleus accumbens. DAGO produced a leftward shift of the rate-frequency curve when injected into both caudal regions and the rostral-medial region of the nucleus. Injections of DPDPE into each of the four regions of the nucleus accumbens were effective in producing a leftward shift of the rate-frequency curve.

Morphine

Frequency thresholds. Injections of morphine into the caudo-medial [$F(9,45)=3.5$, $p<0.05$] and caudo-lateral [$F(9,90)=2.9$, $p<0.05$] but not rostro-medial or rostro-lateral regions of the nucleus accumbens, caused significant reductions in the self-stimulation thresholds, shifting the rate-frequency functions to the left across the range of tested frequencies. When there were shifts in the rate-frequency functions, the shifts were reasonably parallel (Figure 1). Tukey multiple comparison tests revealed that the high dose of morphine (10 µg) produced a significant decrease in the lateral hypothalamic frequency threshold for the first 30 minutes of testing following injection into the caudo-medial region (Figure 2) and significantly decreased lateral hypothalamic thresholds for the first 60 minutes of testing following injections into the caudo-lateral region of the nucleus accumbens (Figure 3). Injections of morphine into the two rostral regions of the nucleus accumbens did not produce significant effects on lateral hypothalamic frequency thresholds (Figures 4 and 5).

Maximal rate of responding. Injections of morphine into the four different regions of the nucleus accumbens did not significantly alter maximal rate of responding for

Figure 1 (see page 50). Effect of nucleus accumbens morphine (10 µg: circles) and saline (squares) on lateral hypothalamic brain stimulation reward during the first half hour after injection. The data were obtained from a single animal that received injections into the caudo-lateral region of the nucleus accumbens. Experiment 1.

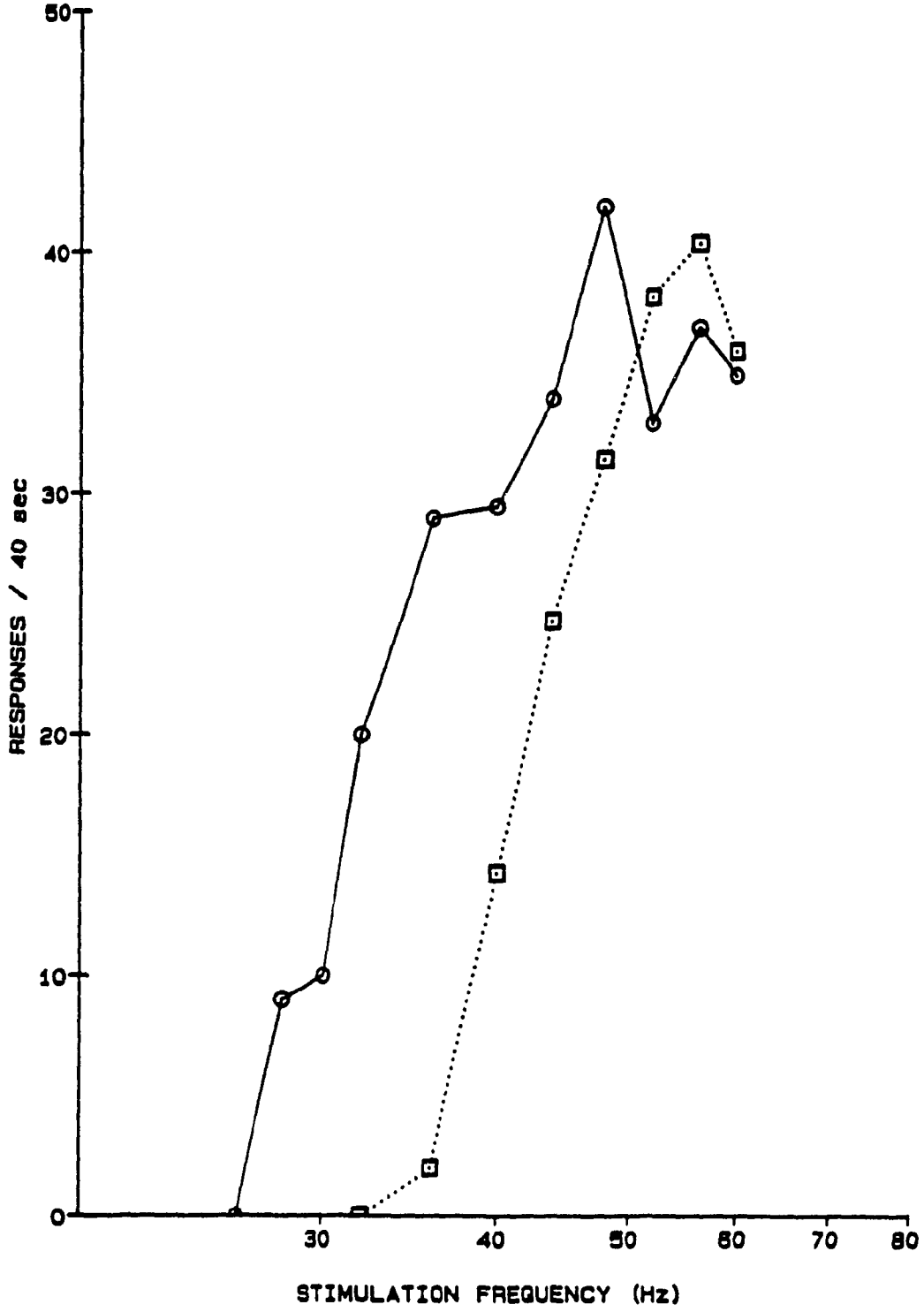

50
40
30
20
10
0
RESPONSES / 40 sec
30
40
50
60
70
80
STIMULATION FREQUENCY (Hz)

Figure 2. (see page 52). Frequency threshold (+/- S.E.M., n=6) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time after microinjections of morphine or saline into the caudo-medial region of the nucleus accumbens. Experiment 1.

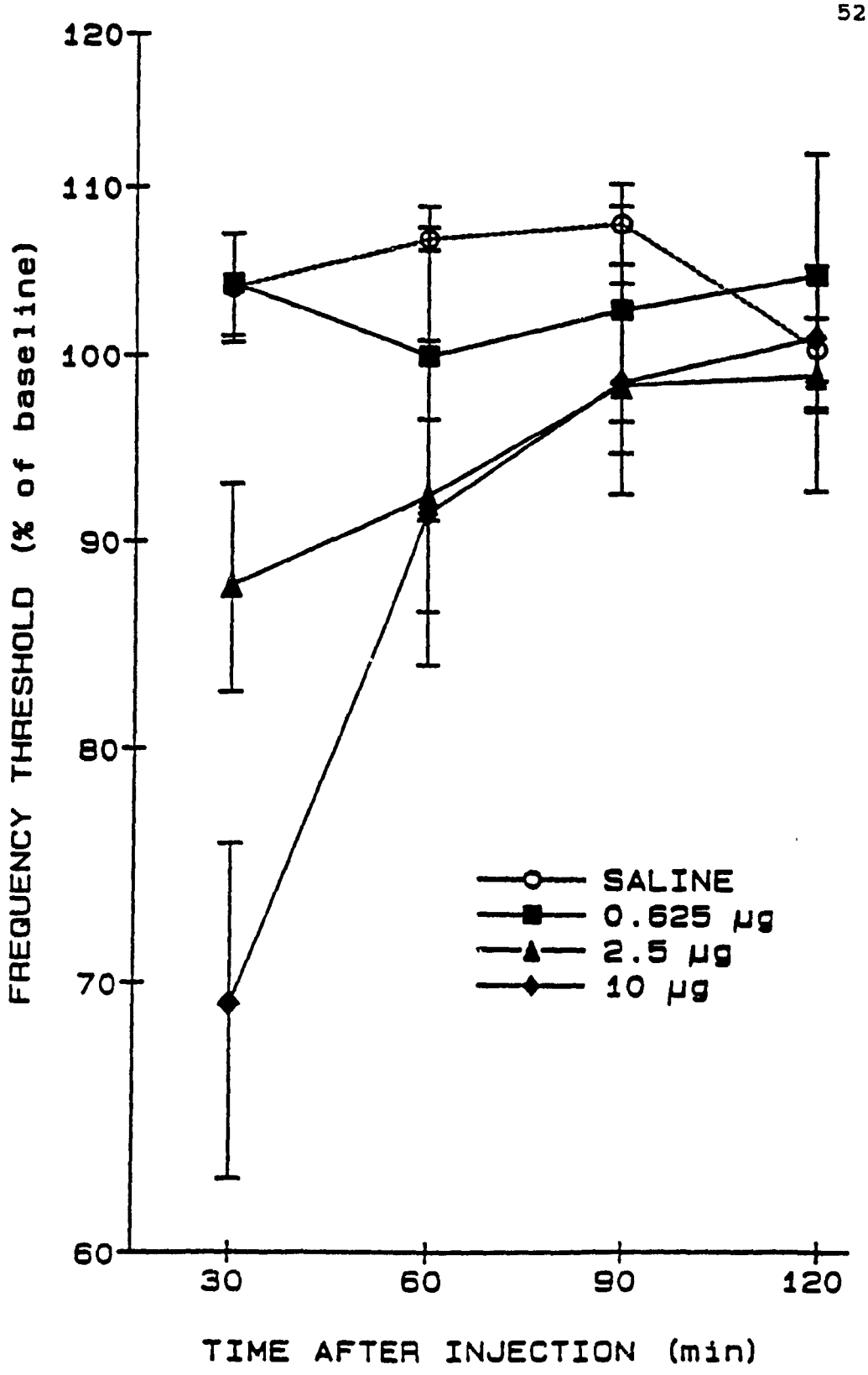

120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
0.625 µg
2.5 µg
10 µg

Figure 3. (see page 54). Frequency threshold (+/- S.E.M., n=11) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time after microinjections of morphine or saline into the caudo-lateral region of the nucleus accumbens. Experiment 1.

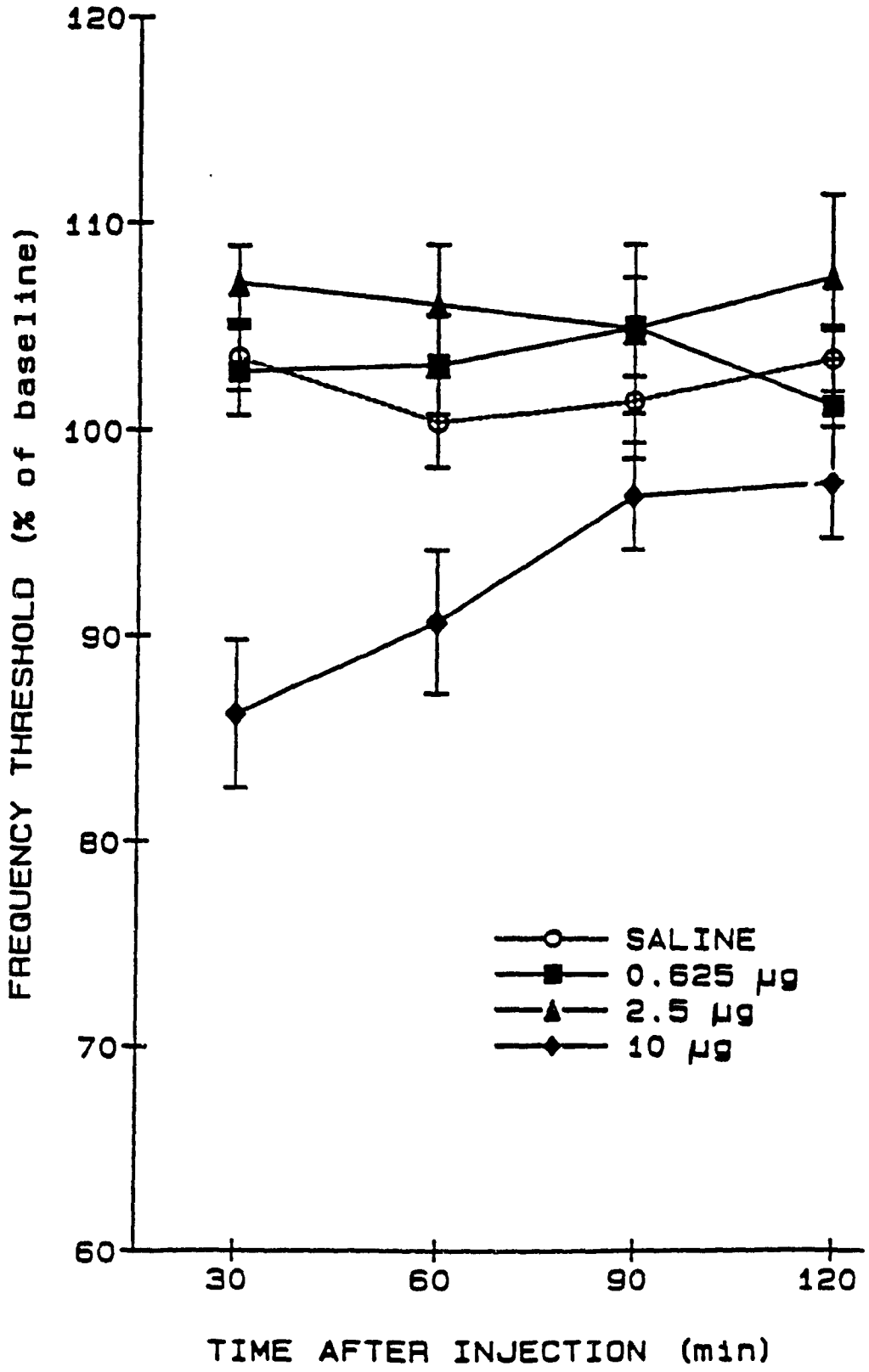

120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
0.625 µg
2.5 µg
10 µg

Figure 4. (see page 56). Frequency threshold (+/- S.E.M., n=5) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time after microinjections of morphine or saline into the rostro-medial region of the nucleus accumbens. Experiment 1.

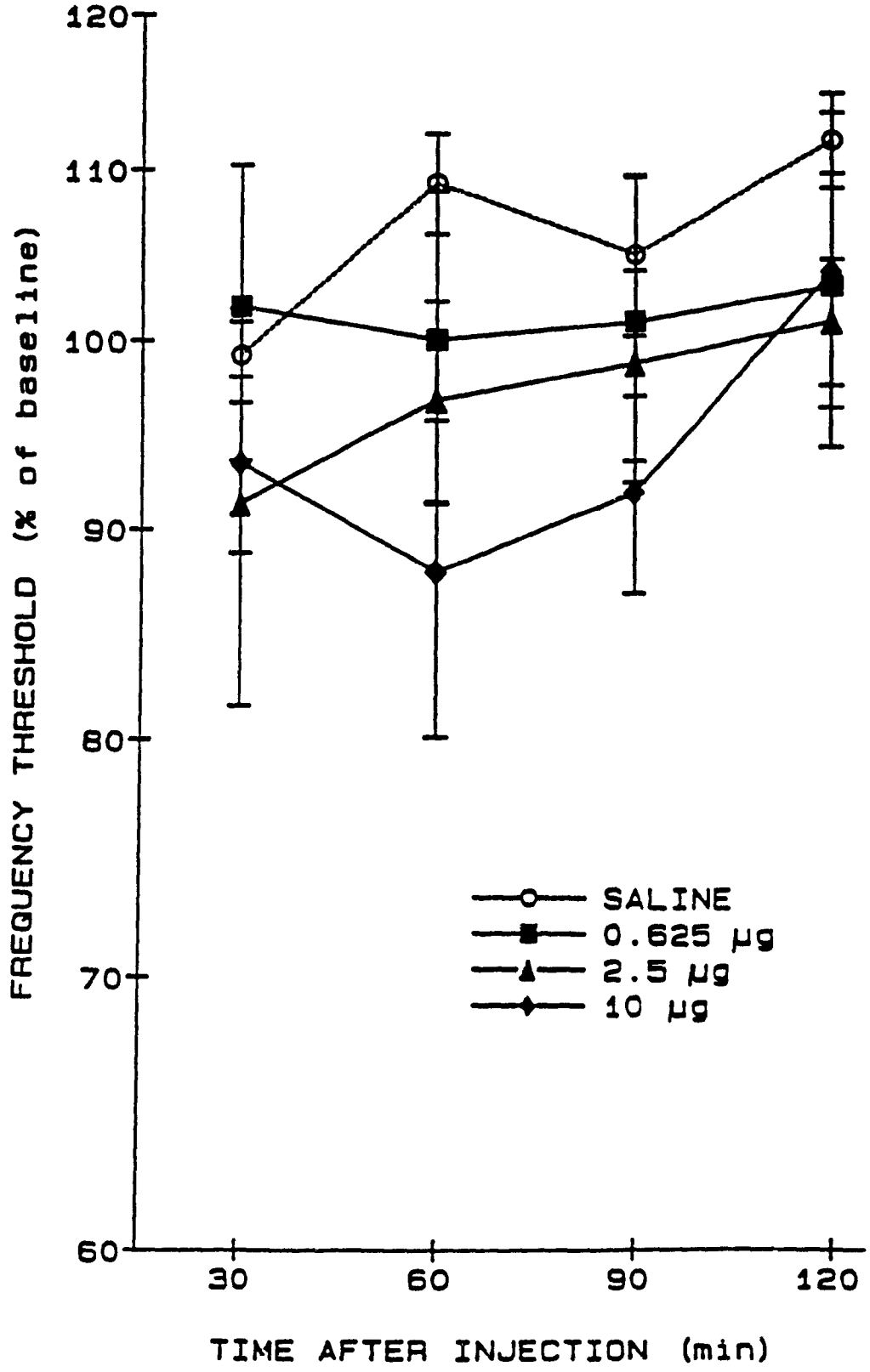
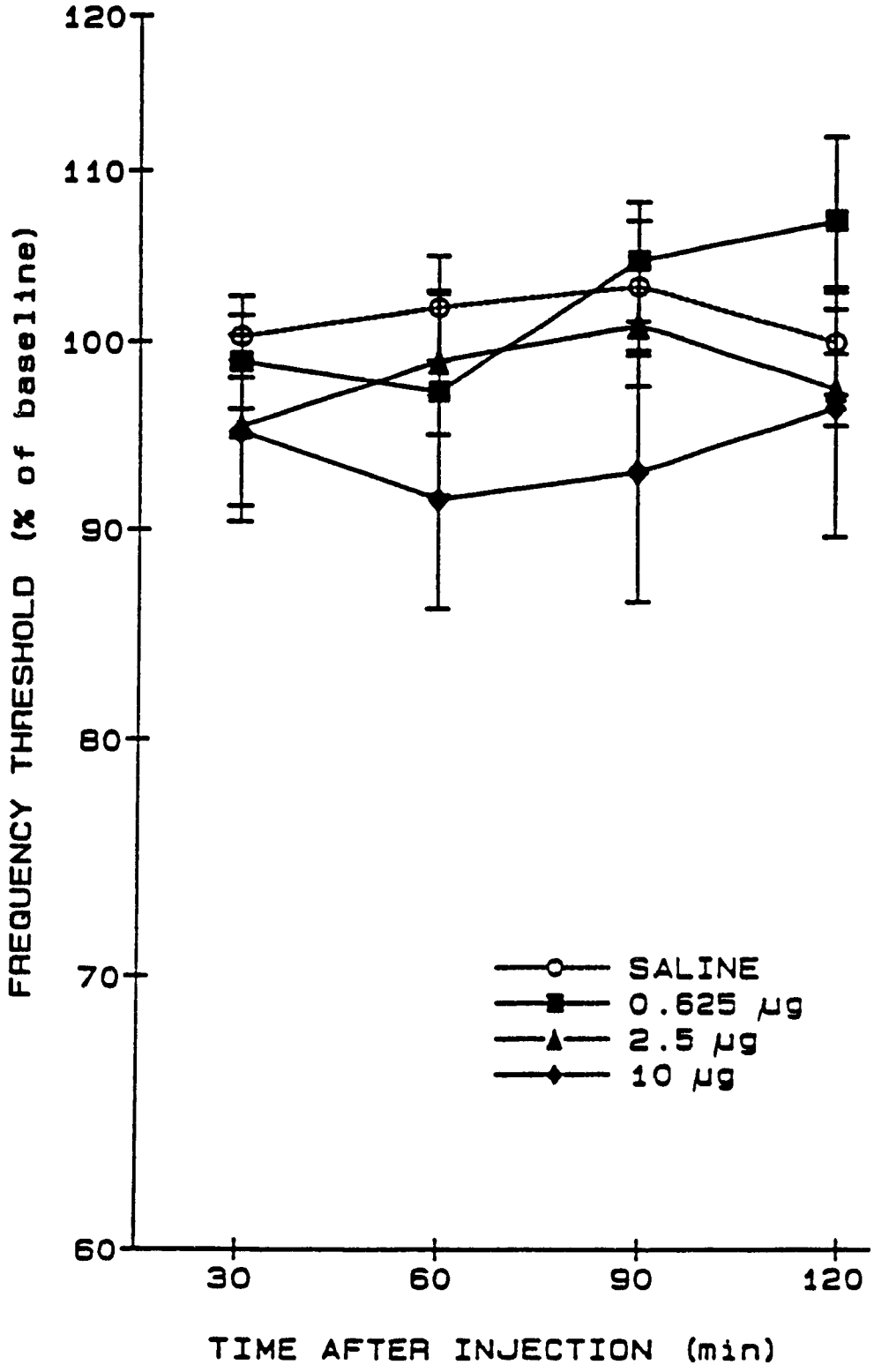

120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
0.625 µg
2.5 µg
10 µg

Figure 5. (see page 58). Frequency threshold (+/- S.E.M., n=6) of lateral hypothalamic frequency brain stimulation reward (expressed as percentage of baseline threshold) as a function of time after microinjections of morphine or saline into the rostro-lateral region of the nucleus accumbens. Experiment 1.


120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
0.625 µg
2.5 µg
10 µg

rewarding lateral hypothalamic stimulation.

Morphine and naltrexone

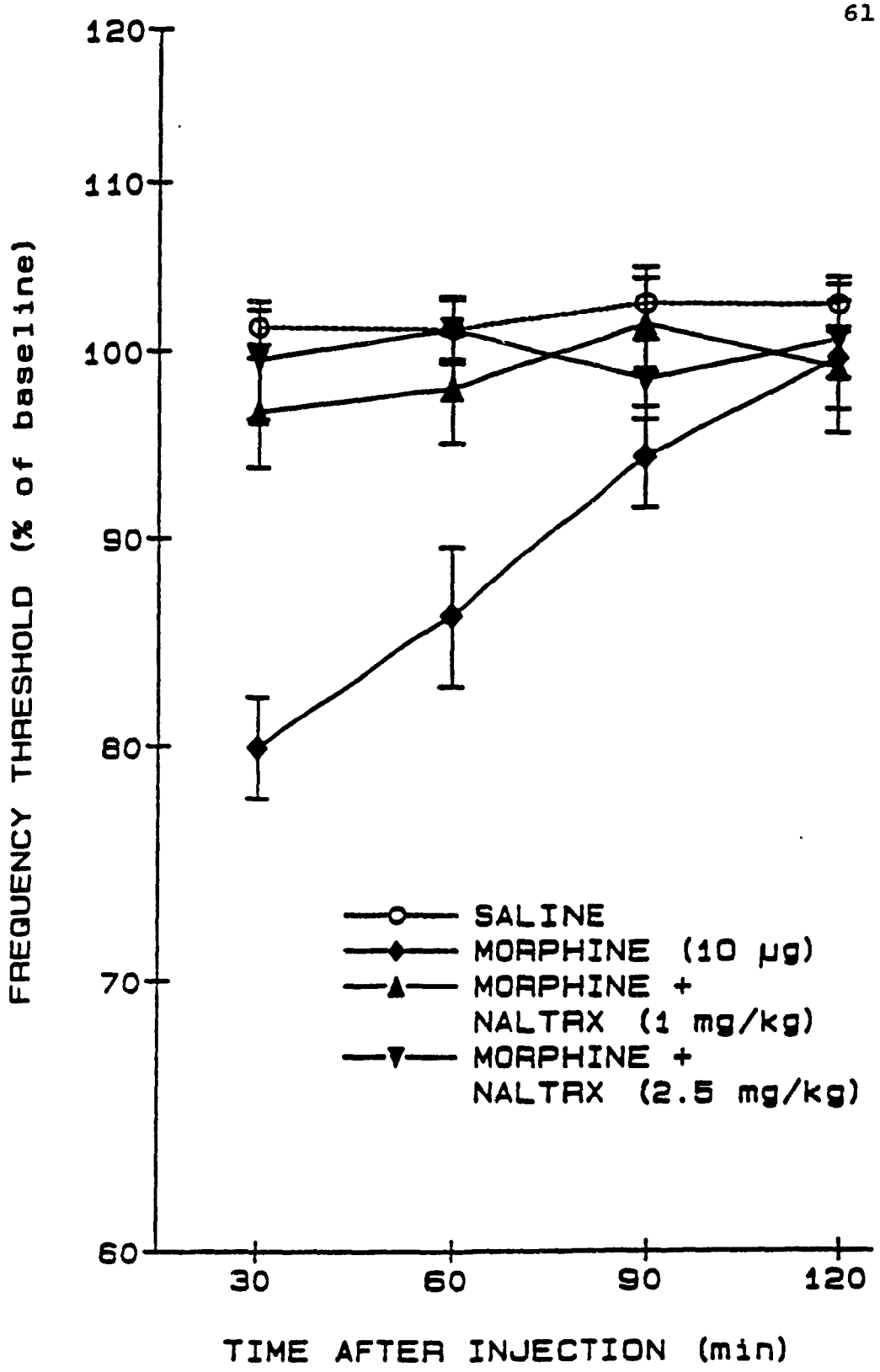
Frequency thresholds. Naltrexone effectively blocked the rewarding effect of intra-NAS morphine (10 µg) on LHA BSR (Figure 6). A two-way analysis of variance revealed a significant Dose by Time interaction [$F(9,108)=3.8$, $p<0.05$]. Post-hoc analysis of the interaction revealed that the decrease in LHA frequency threshold following intra-NAS morphine was significantly attenuated by naltrexone (1 and 2.5 mg/kg) during the first hour of testing ($p<0.05$).

Maximal rate of responding. Analysis of variance revealed that intra-NAS injection of morphine and peripheral administration of naltrexone did not affect maximal rate of responding for lateral hypothalamic brain stimulation reward.

DAGO

Frequency thresholds. Three-factor analysis of variance of the effects of intra-NAS DAGO on LHA frequency thresholds yielded a significant Region by Dose by Time interaction [$F(36,288)=1.74$, $p<0.05$]. The three-way

Figure 6. (see page 61). Frequency threshold (+/- S.E.M., n=13) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time after peripheral injection of naltrexone and microinjection of morphine into the nucleus accumbens. Experiment 1.


120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
MORPHINE (10 µg)
MORPHINE + NALTRX (1 mg/kg)
MORPHINE + NALTRX (2.5 mg/kg)

interaction reflects the fact DAGO produced different effects on LHA frequency thresholds depending on the region of injection within the nucleus accumbens. Separate two-way analyses of variance (Dose by Time) of each region revealed that injections of DAGO into the rostro-medial and both of the caudal regions of the nucleus accumbens caused significant decreases in lateral hypothalamic frequency thresholds, shifting the rate-frequency functions to the left across the range of tested frequencies. When there were shifts in the rate-frequency functions, the shifts were reasonably parallel (Figure 7). Injections of DAGO into the rostro-lateral region were ineffective (Figure 8).

Rostro-medial. Injections of DAGO into the rostro-medial region of the NAS produced a significant decrease in frequency thresholds depending on the dose and time of testing [$F(12,48)=2.3$, $p<0.05$]. The high dose of DAGO (3200 ng) was effective in significantly reducing LHA frequency thresholds, but only during the second and fourth half hours of testing (Figure 9).

Caudo-medial. Injections of DAGO into the caudo-medial region produced different effects depending on dose and time of testing. Post-hoc analysis of the dose by time interaction [$F(12,60)=2.4$, $p<0,05$) indicated that in the first 30 minutes of testing, the 200 ng dose significantly decreased LHA frequency thresholds. During the second half hour of testing, the two high doses (800 and 3200 ng)

Figure 7. (see page 64). Effect of nucleus accumbens DAGO (3200 ng: circles) and saline (squares) on lateral hypothalamic brain stimulation reward during the first half hour after injection. The data were obtained from a single animal that received injections into the caudo-lateral region of the nucleus accumbens. Experiment 1.

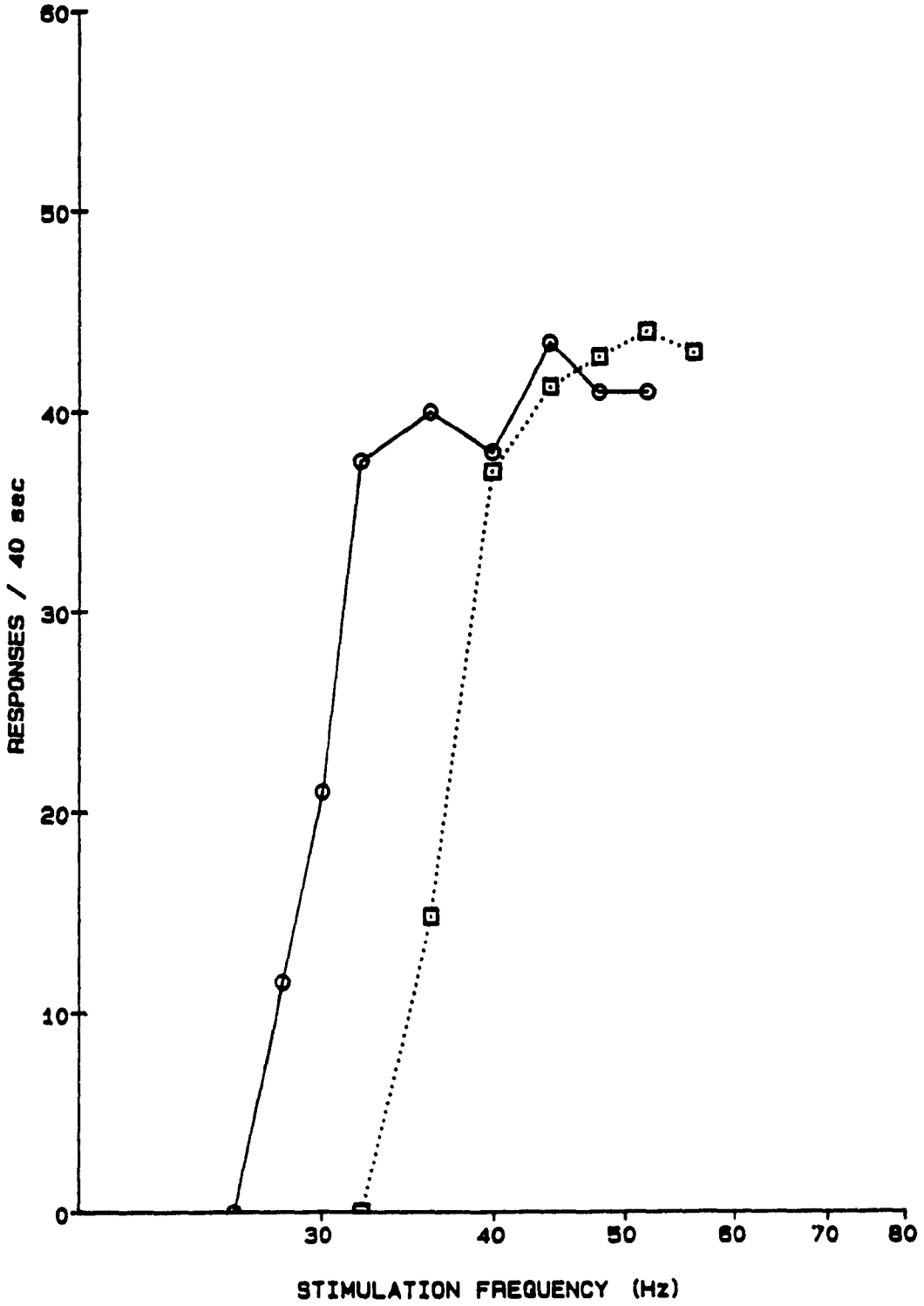

60
50
40
30
20
10
0
RESPONSES / 40 sec
30
40
50
60
70
80
STIMULATION FREQUENCY (Hz)

Figure 8. (see page 66). Frequency threshold (+/- S.E.M., n=6) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time following microinjections of the selective mu receptor agonist, DAGO, into the rostro-lateral region of the nucleus accumbens. Expermiment 1.

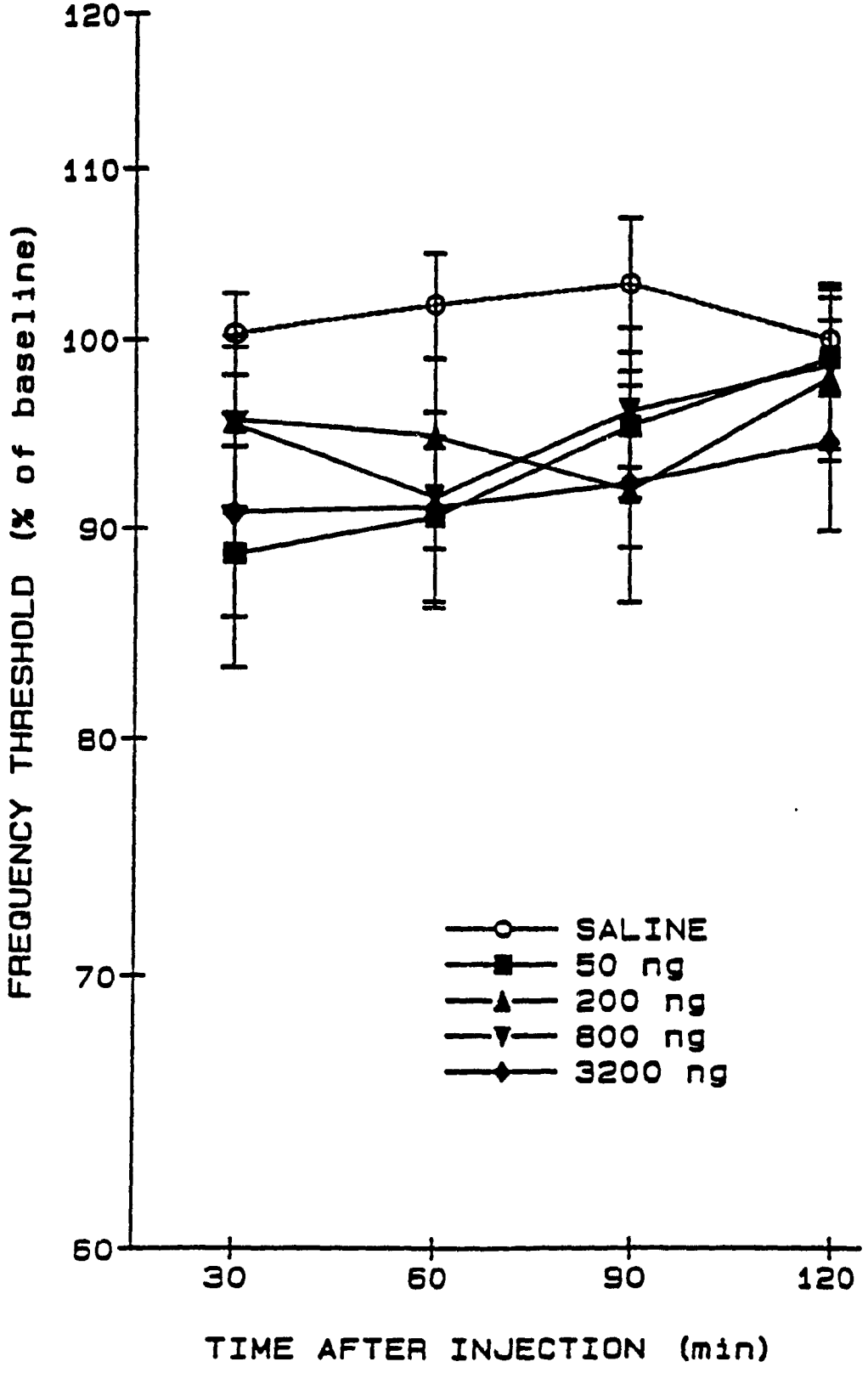
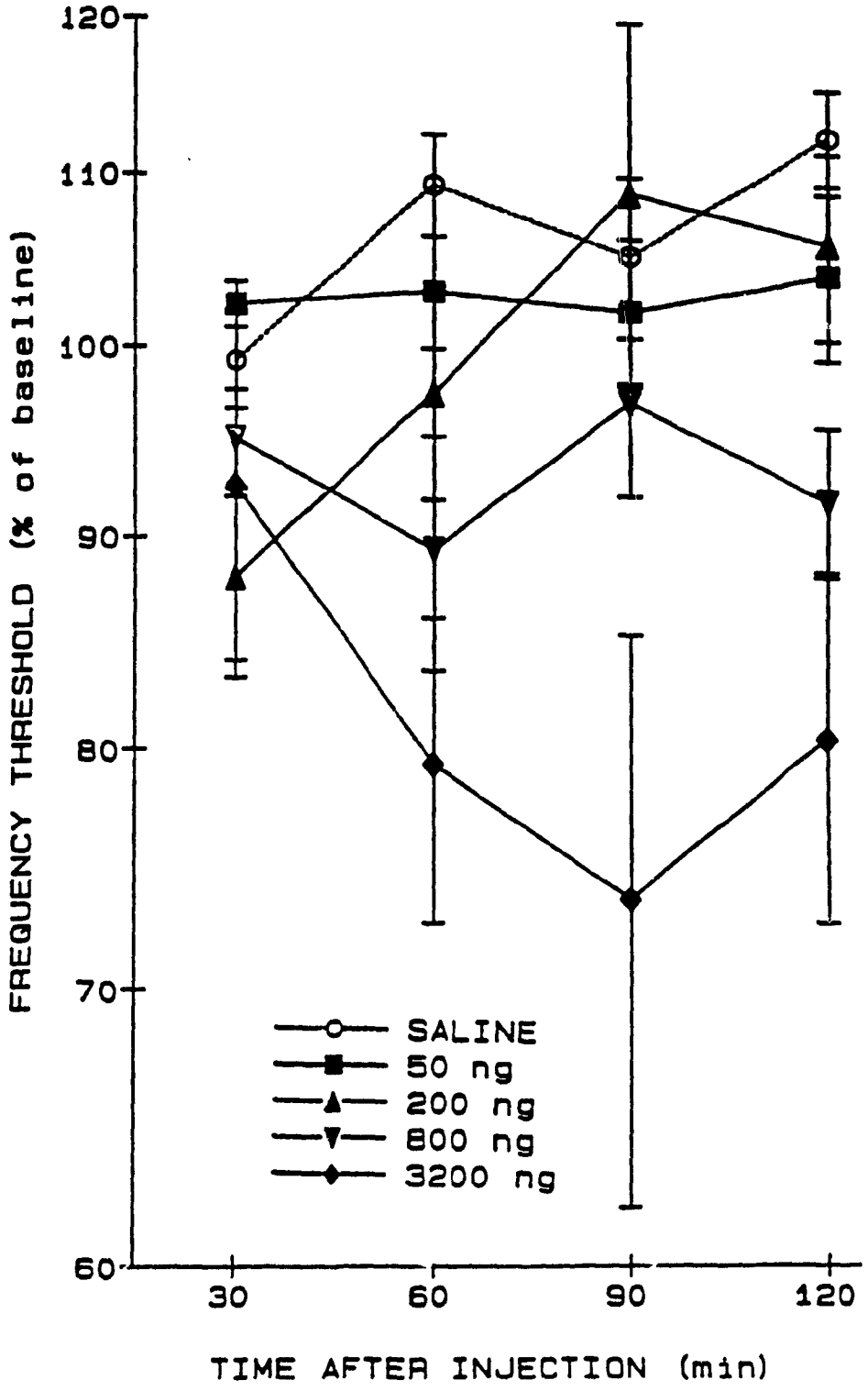

120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
50 ng
200 ng
800 ng
3200 ng

Figure 9. (See page 68). Frequency threshold (+/- S.E.M., n=5) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time following microinjections of the selective mu opiate receptor agonist, DAGO, into the rostro-medial region of the nucleus accumbens. Experiment 1.


120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
50 ng
200 ng
800 ng
3200 ng

significantly decreased LHA frequency thresholds, and during the third half hour of testing only the high dose produced a significant decrease in lateral hypothalamic frequency thresholds (Figure 10).

Caudo-lateral. The administration of DAGO into the caudo-lateral region of the nucleus accumbens significantly decreased LHA frequency threshold depending on dose [$F(4,40)=6.84$, $p<0.05$]. Post hoc analysis revealed that only the high doses were effective and that a dosage of 800 ng produced a larger decrease in frequency threshold than a dosage of 3200 ng (Figure 11).

Maximal rate of responding. Analysis of Variance revealed a significant Region by Dose by Time interaction [$F(36,288)=1.8$, $p<0.05$]. The three-way interaction reflects the fact that DAGO produced different effects on maximal rate of responding depending on the region of injection in the nucleus accumbens. Separate analyses of variance for each region revealed a significant Dose by Time interaction [$F(12,60)=2.66$, $p<0.05$] for injection of DAGO into the rostro-lateral region. Tukey pairwise comparisons of the simple main effects of this interaction revealed that in the second half hour of testing, 800 ng of DAGO significantly decreased maximal responding. During the third half hour of testing, all but the low dose of DAGO produced a small but significant decrease in maximal

Figure 10. (see page 71). Frequency threshold (+/- S.E.M., n=6) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time following microinjection of the selective mu opiate receptor agonist, DAGO, into the caudo-medial region of the nucleus accumbens. Experiment 1.

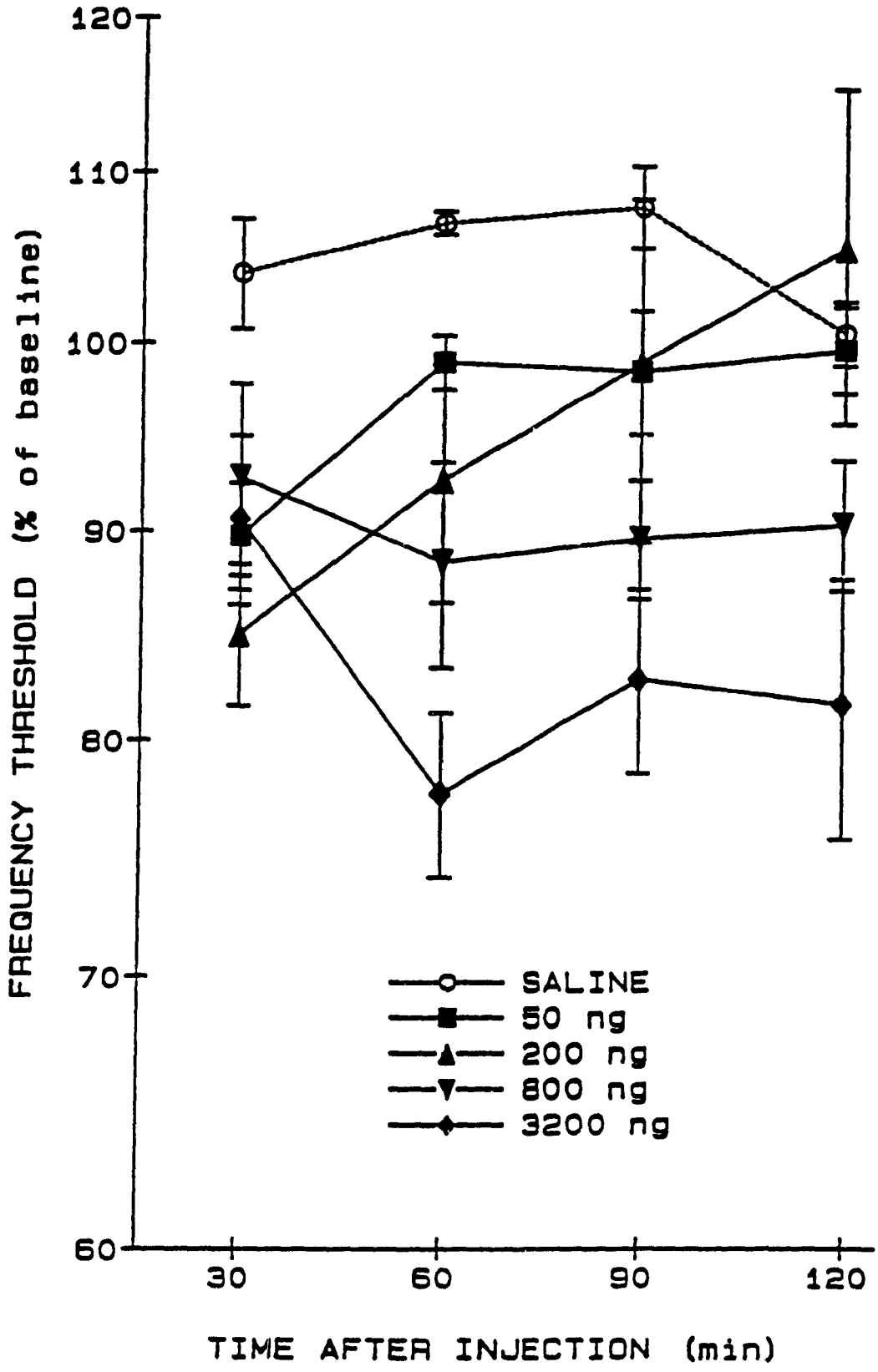
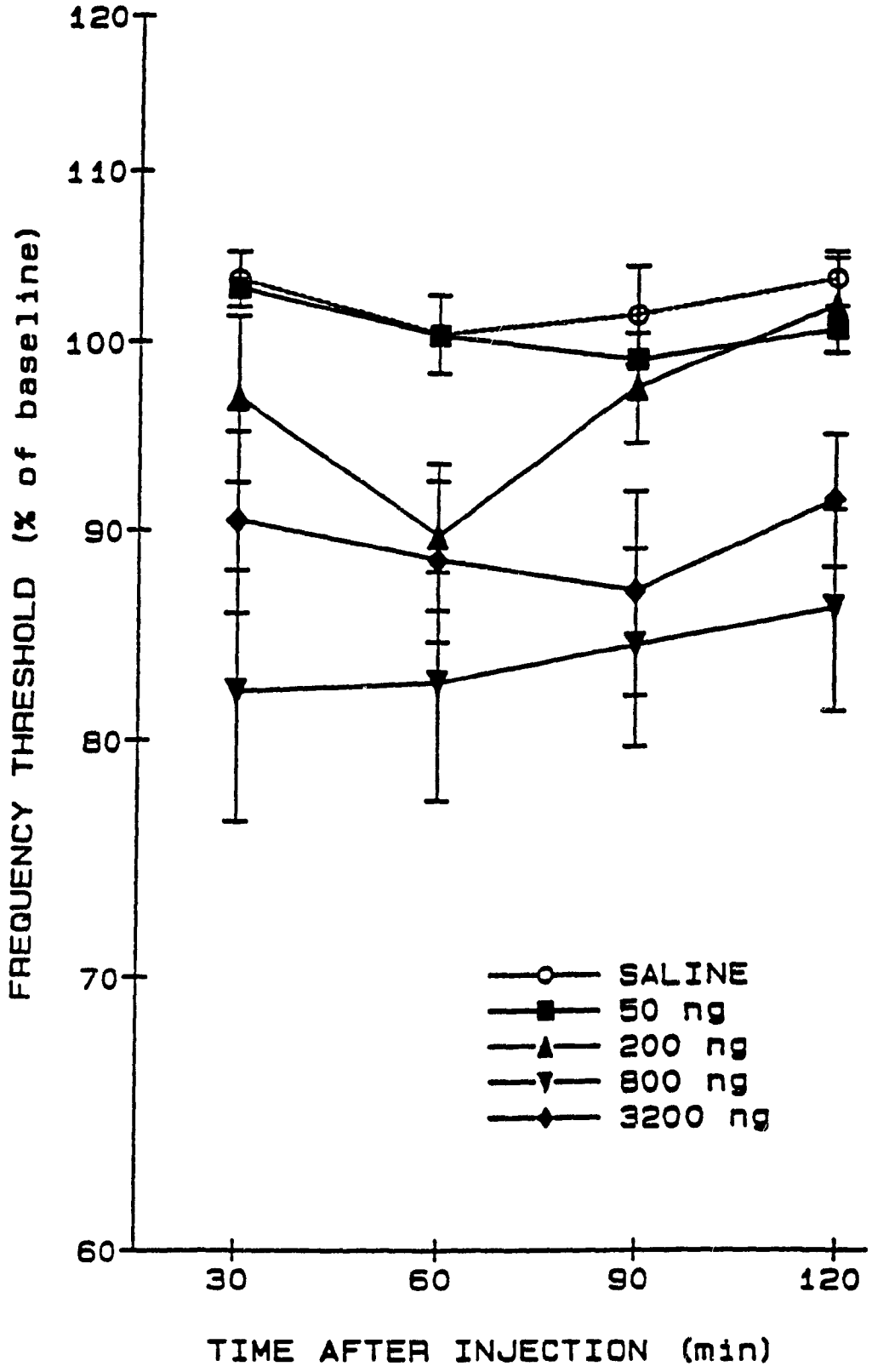

120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
50 ng
200 ng
800 ng
3200 ng

Figure 11. (See page 73). Frequency threshold (+/- S.E.M., n=11) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time following microinjection of the selective mu receptor agonist, DAGO, into the caudo-lateral region of the nucleus accumbens. Experiment 1.


120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
50 ng
200 ng
800 ng
3200 ng

responding (Figure 12). DAGO did not cause a statistically significant decrease in maximal responding during the first and last half hours of testing.

DAGO and naltrexone

Frequency thresholds. Naltrexone (1 and 2.5 mg/kg) significantly blocked the reward facilitating effects of intra-NAS DAGO on LHA frequency thresholds [$F(3,30)=14.1$, $p<0.05$). As can be seen from Figure 13, both doses of naltrexone were effective in attenuating the rewarding effects of intra-NAS DAGO during the two hour test session.

Maximal rate of responding. Analysis of variance revealed a significant main effect of Dose ($F(3,30)=3.3$, $p<0.05$). Tukey pairwise comparisons revealed that the combination of DAGO (3200 ng) and the high (2.5 mg/kg) dose of naltrexone produced a small but significant decrease in maximal responding in comparison to maximal responding following intra-NAS saline (Figure 14).

DPDPE

Frequency thresholds. Injections of DPDPE into each of the four regions of the nucleus accumbens produced parallel leftward shifts of the rate-frequency function

Figure 12. (see page 76). Maximal rate of responding (+/- S.E.M., n=6) for lateral hypothalamic brain stimulation reward (expressed as percentage of baseline maximal rate of responding) as a function of time following microinjection of the selective mu receptor agonist, DAGO, into the rostro-lateral region of the nucleus accumbens. Experiment 1.

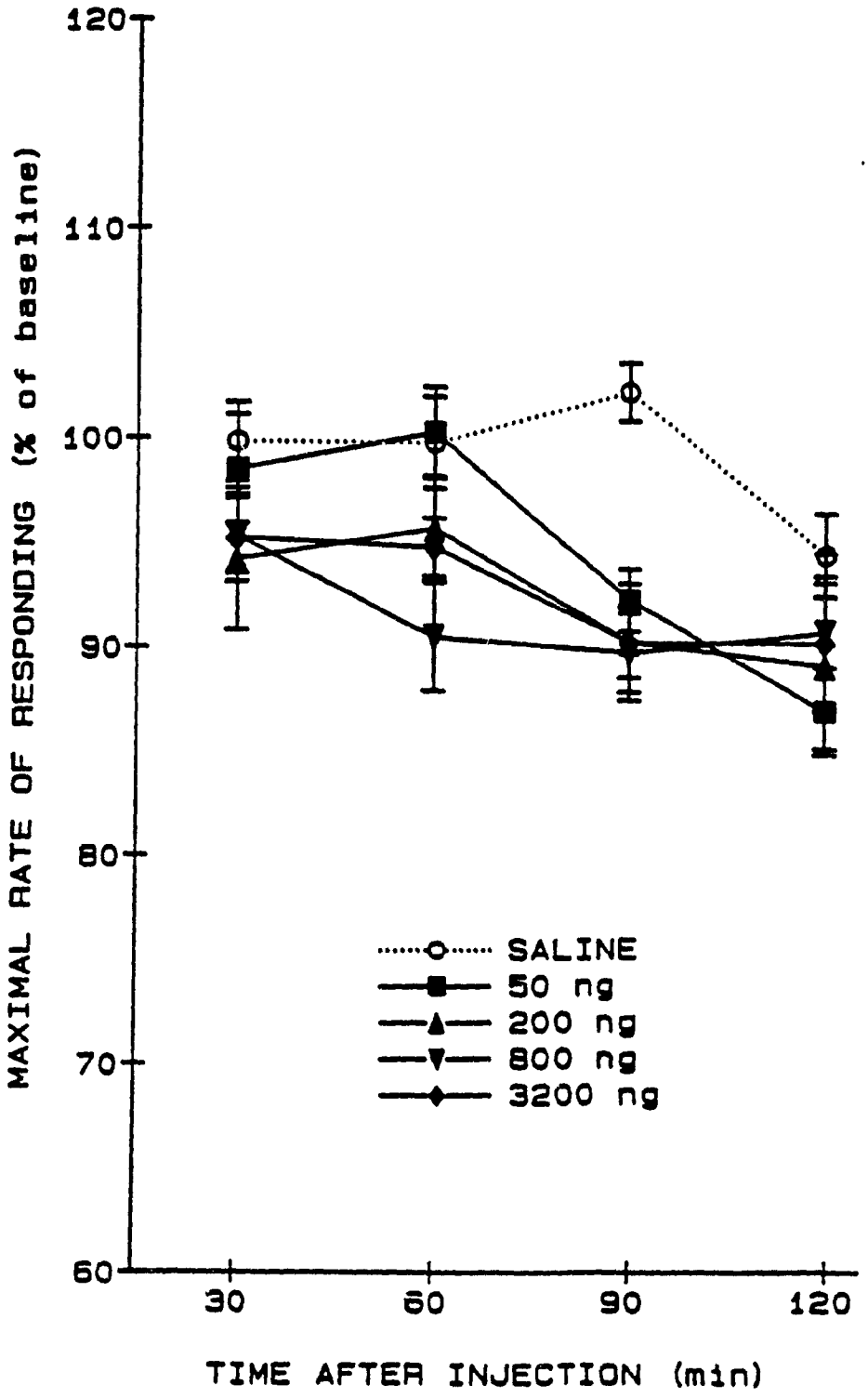

120
110
100
90
80
70
60
MAXIMAL RATE OF RESPONDING (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
50 ng
200 ng
800 ng
3200 ng

Figure 13. (see page 78). Frequency threshold (+/- S.E.M., n=11) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time following peripheral injection of naltrexone and microinjection of the selective mu receptor agonist, DAGO, or saline into the nucleus accumbens. Experiment 1.

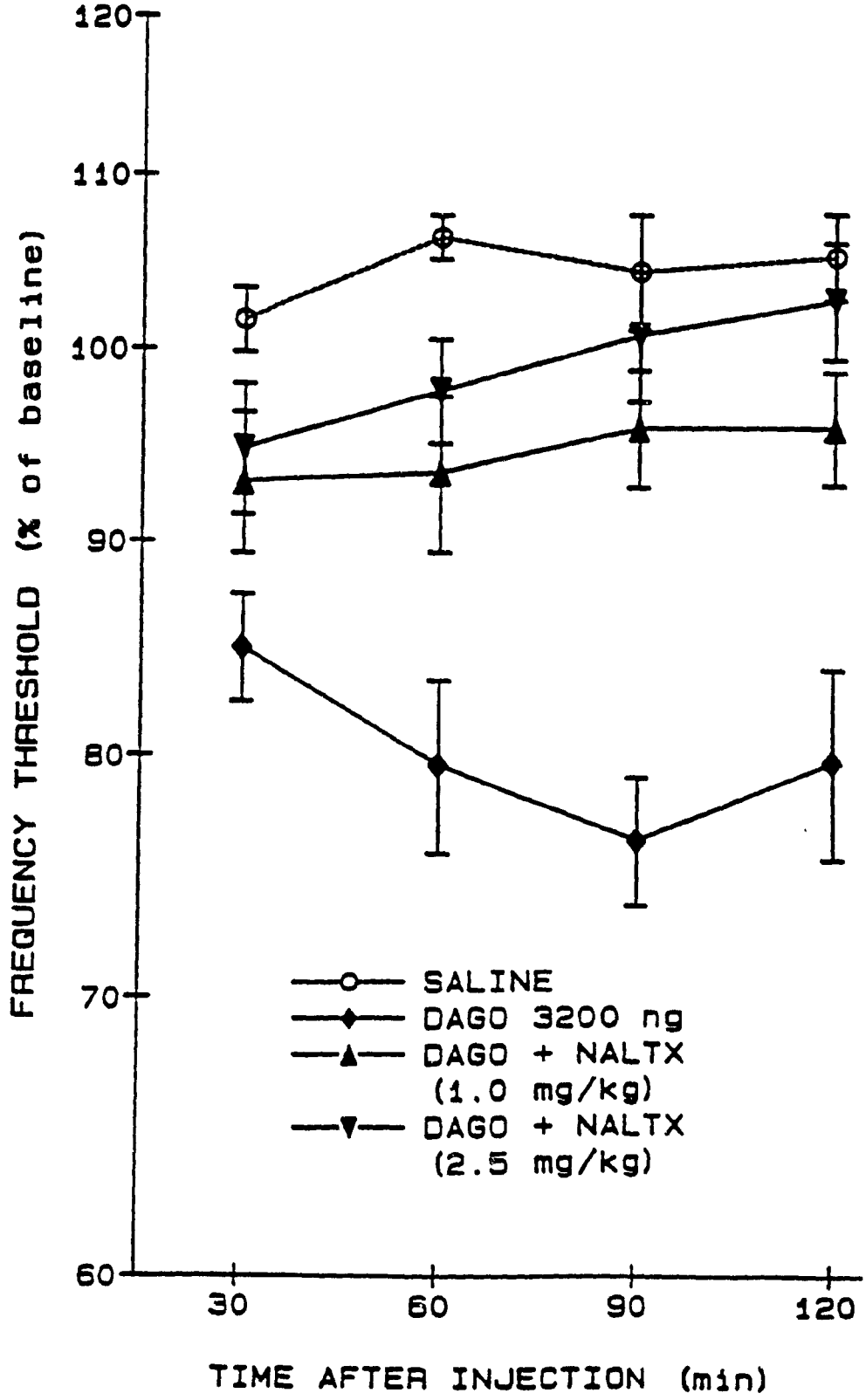
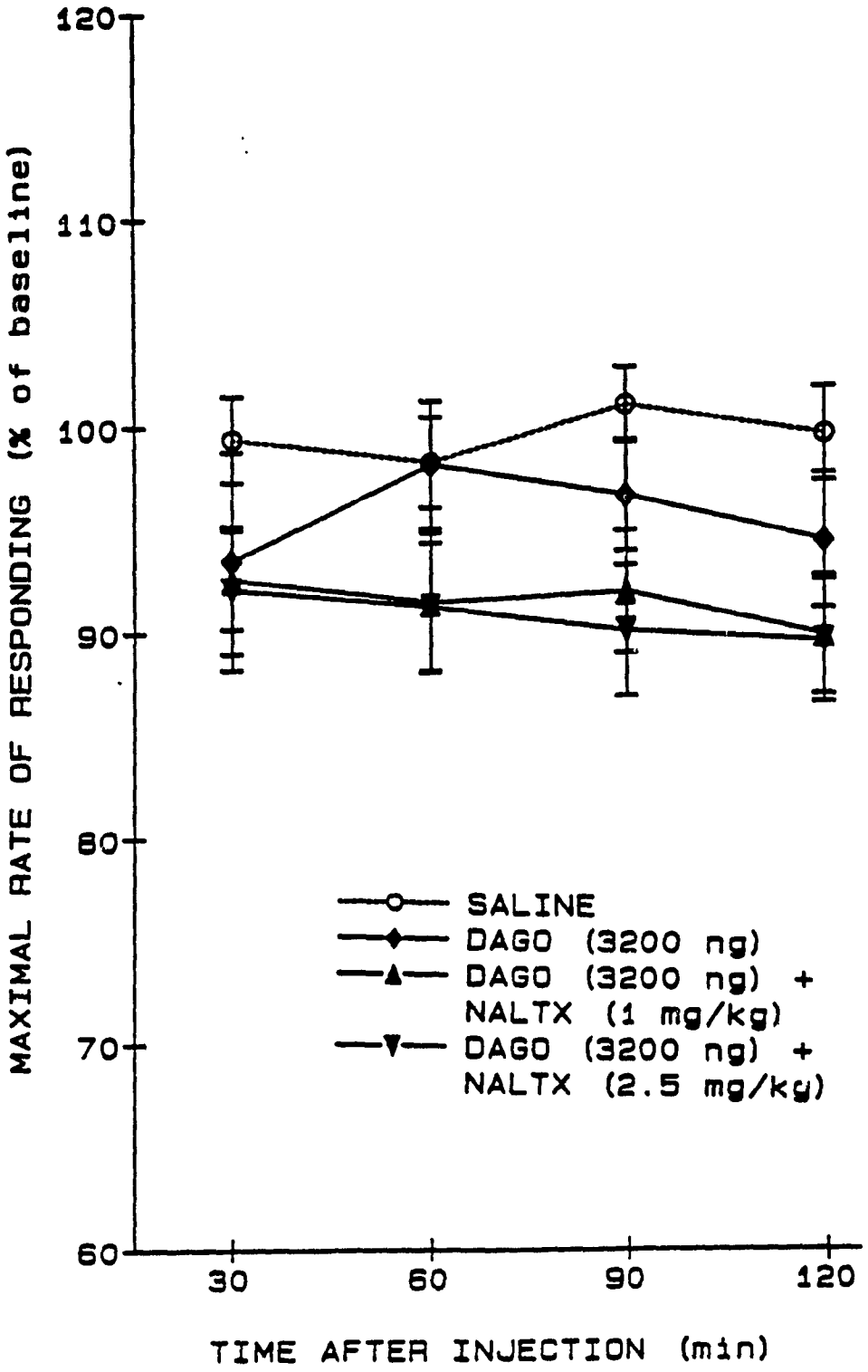

120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
DAGO 3200 ng
DAGO + NALTX (1.0 mg/kg)
DAGO + NALTX (2.5 mg/kg)

Figure 14. (see page 80). Maximal rate of responding (+/- S.E.M., n=11) for lateral hypothalamic brain stimulation reward (expressed as percentage of baseline maximal rate of responding) as a function of time following peripheral injection of naltrexone and microinjection of the selective mu receptor agonist, DAGO, or saline into the nucleus accumbens. Experiment 1.


120
110
100
90
80
70
60
MAXIMAL RATE OF RESPONDING (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
DAGO (3200 ng)
DAGO (3200 ng) + NALTX (1 mg/kg)
DAGO (3200 ng) + NALTX (2.5 mg/kg)

(Figure 15); injections of DPDPE into each region were effective in decreasing lateral hypothalamic frequency thresholds. Analysis of variance revealed that the effectiveness of DPDPE in decreasing lateral hypothalamic frequency thresholds was dependent on the dose and time of testing (Dose by Time interaction [$F(12,288)=1.81$, $p<0.05$). Analysis of the interaction revealed that the two high doses of DPDPE significantly decreased lateral hypothalamic frequency thresholds during the first 60 minutes of testing. The 200 ng dose produced a small but statistically significant decrease in frequency threshold in the second half hour of testing. As can be seen by Figure 16, the lowest dose of DPDPE did not significantly decrease lateral hypothalamic frequency thresholds.

Maximal rate of responding. A three-way (Region, Dose, Time) analysis of variance revealed that intra-NAS injections of DPDPE did not affect maximal rate of responding for lateral hypothalamic stimulation.

DPDPE and naltrexone

Frequency thresholds. A two factor (Dose, Time) analysis of variance revealed a significant main effect of Dose [$F(3,21)=9.9$, $p<0.05$]. Post hoc analysis revealed that both doses of naltrexone (1 and 2.5 mg/kg)

Figure 15. (see page 83). Effect of nucleus accumbens injections of DPDPE (3200 ng: circles) and saline (squares) on lateral hypothalamic brain stimulation reward during the first half hour after injection. The data were obtained from a single animal that received injections into the caudo-medial regions of the nucleus accumbens. Experiment 1.

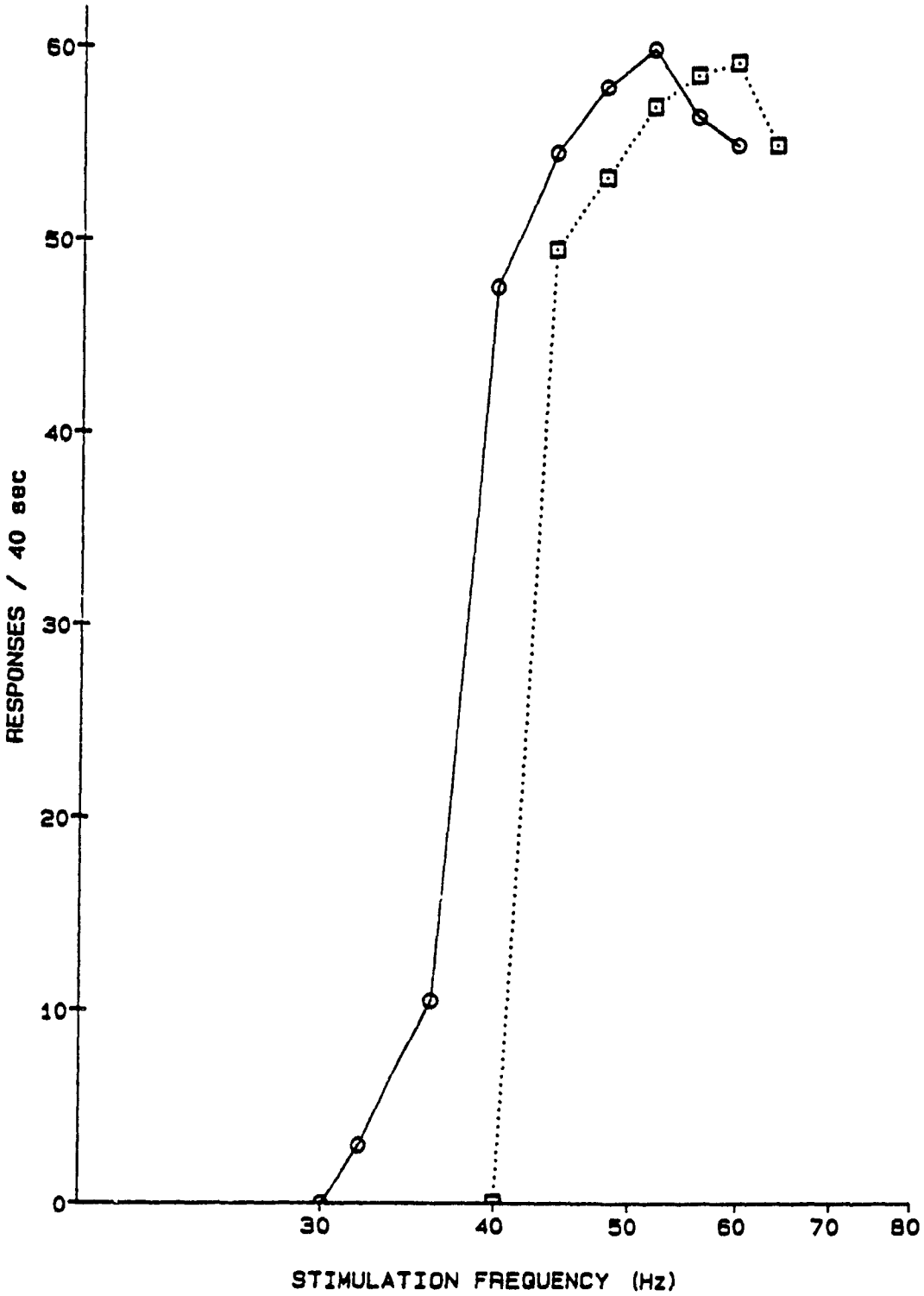
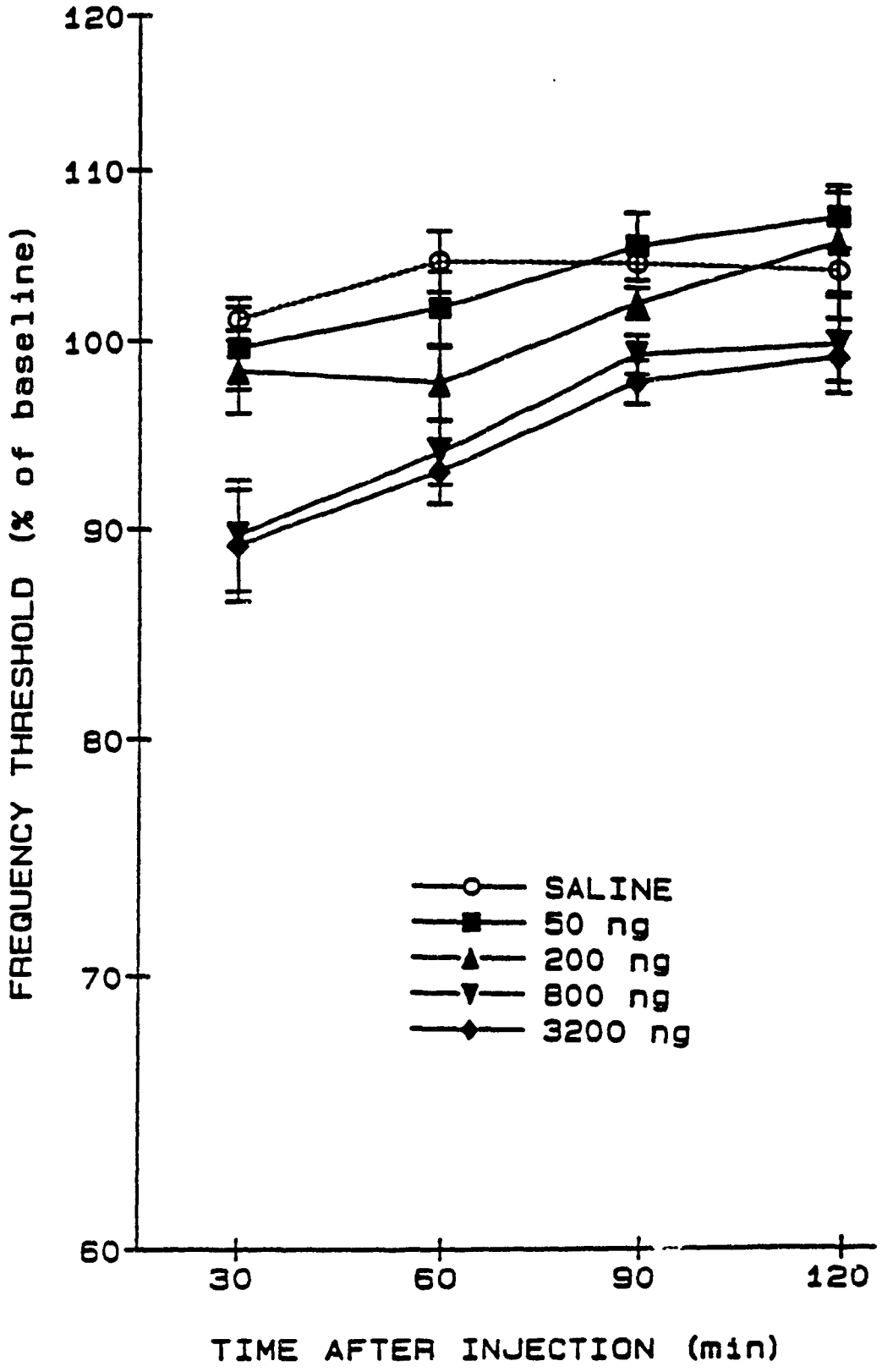

60
50
40
30
20
10
0
RESPONSES / 40 sec
30
40
50
60
70
80
STIMULATION FREQUENCY (Hz)

Figure 16. (see page 85). Frequency threshold (+/- S.E.M., n=28) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time following microinjection of the selective delta opiate receptor agonist, DPDPE, or saline into the nucleus accumbens. Experiment 1.


120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
50 ng
200 ng
800 ng
3200 ng

significantly blocked the rewarding effects of intra-NAS DPDPE (3200 ng) (Figure 17).

Maximal rate of responding. A two factor (Dose, Time) analysis of variance revealed that DPDPE did not alter maximal rate of responding for lateral hypothalamic brain stimulation reward.

U50,488H

Frequency thresholds. Injections of U50,488H into the nucleus accumbens did not significantly decrease lateral hypothalamic frequency thresholds. A two factor (dose, time) analysis of variance revealed a significant main effect of Time [$F(3,48)=11.2$, $p<0.05$]. Further analysis revealed that during the two hour test session, U50,488H produced a small but significant increase in frequency threshold during the last 90 minutes of testing in comparison to the first 30 minutes of testing (Figure 18).

Maximal rate of responding. A two factor analysis of variance yielded a significant main effect of Time [$F(4,64)=6.0$, $p<0.05$]. Post-hoc analysis revealed that during the two hour test session, U50,488H produced a small but significant decrease in maximal rate of responding during the last 30 minutes of testing in comparison to the

Figure 17. (see page 88). Frequency threshold (+/- S.E.M., n=8) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time following peripheral injection of naltrexone and microinjection of the selective delta receptor agonist, DPDPE, or saline into the nucleus accumbens. Experiment 1.

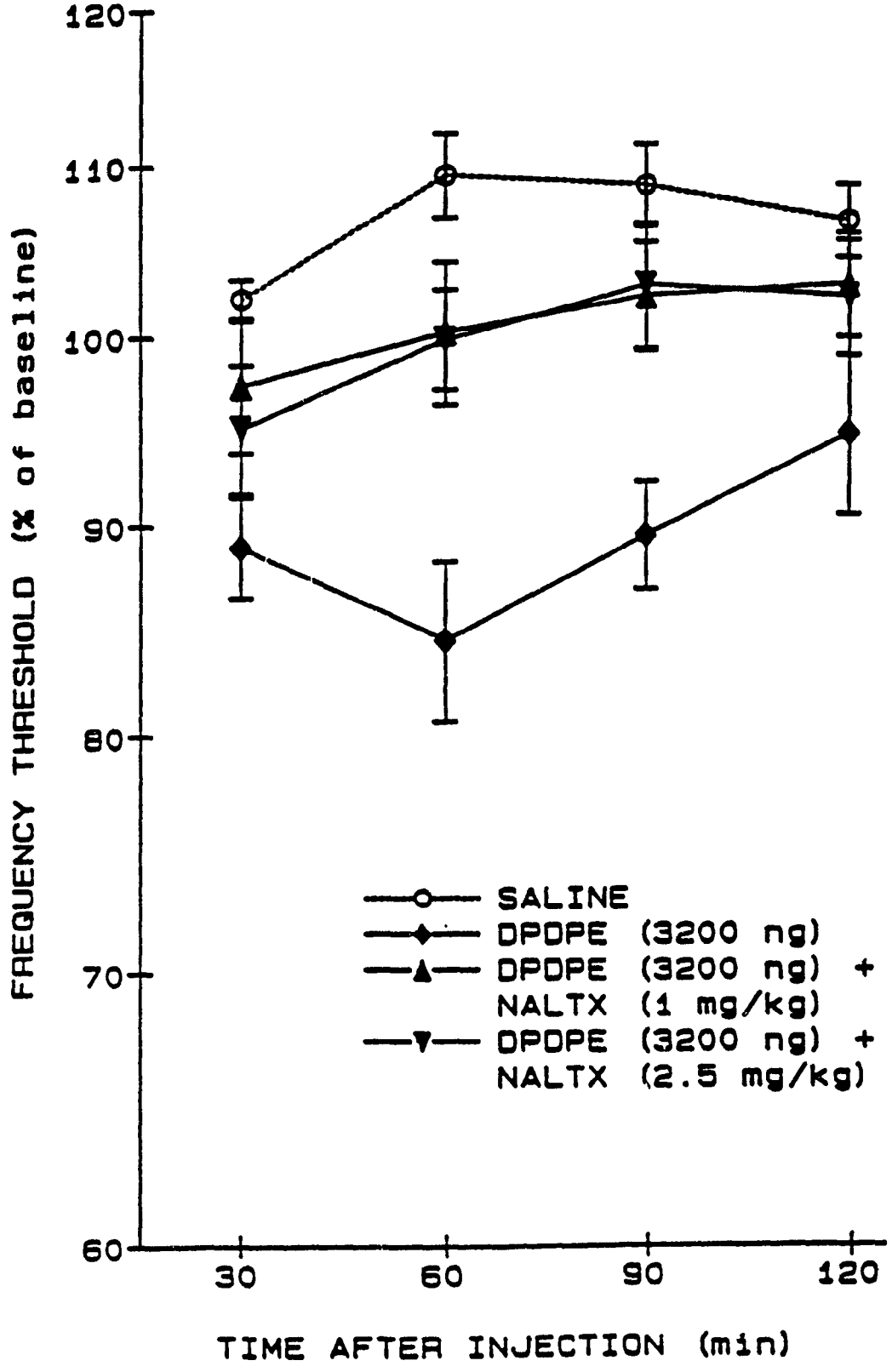
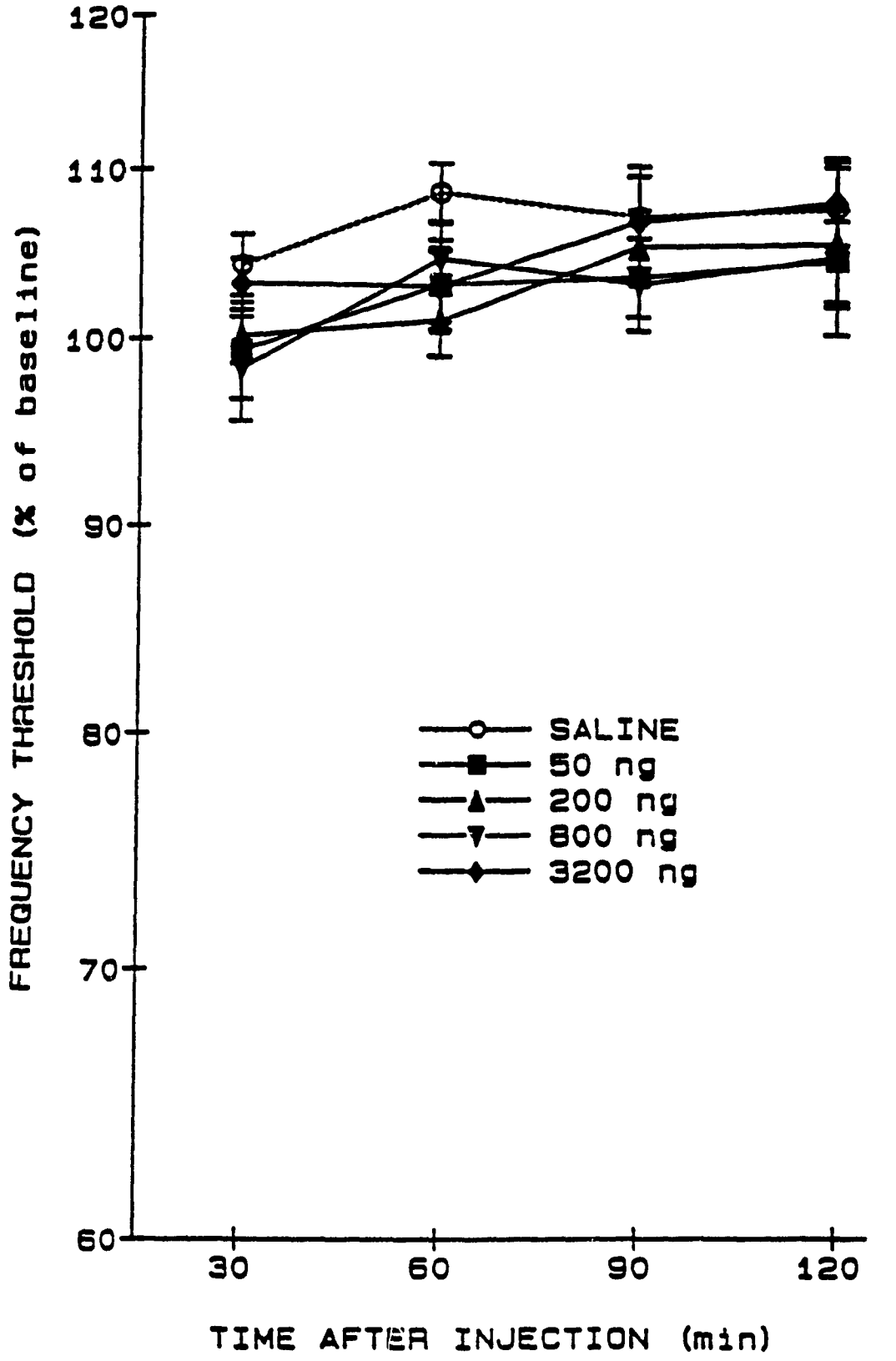

120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
DPDPE (3200 ng)
DPDPE (3200 ng) + NALTX (1 mg/kg)
DPDPE (3200 ng) + NALTX (2.5 mg/kg)

Figure 18. (see page 90). Frequency threshold (+/- S.E.M., n=17) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time following microinjection of the selective kappa receptor agonist, U50,488H, or saline into the nucleus accumbens. Experiment 1.


120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE
50 ng
200 ng
800 ng
3200 ng

first 30 minutes of testing (Figure 19).

Pre-drug saline versus post-drug saline

Frequency thresholds. As can be seen by Figure 20, there is no significant difference between the effects of intra-NAS saline prior to and following injections of morphine, DAGO, DPDPE, and U50,488H on lateral hypothalamic frequency thresholds.

Maximal rate of responding. A two factor analysis of variance revealed that intra-NAS injection of saline prior to and following injections of morphine, DAGO, DPDPE, and U50,488H did not affect maximal rate of responding for lateral hypothalamic electrical stimulation.

Confirmation of cannula and electrode placements

The locations of injection sites in the rostro-medial or rostro-lateral region of the nucleus accumbens are displayed in Figure 21. Figure 22 displays the locations of injection sites in the caudo-medial or caudo-lateral region of the nucleus accumbens. The locations of electrode tips in the area of lateral hypothalamus are shown in Figure 23.

Figure 19. (see page 93). Maximal rate of responding (+/- S.E.M., n=17) for lateral hypothalamic brain stimulation reward (expressed as percentage of baseline maximal rate of responding) as a function of time following microinjection of the selective kappa receptor agonist, U50,488H, into the nucleus accumbens. Experiment 1.

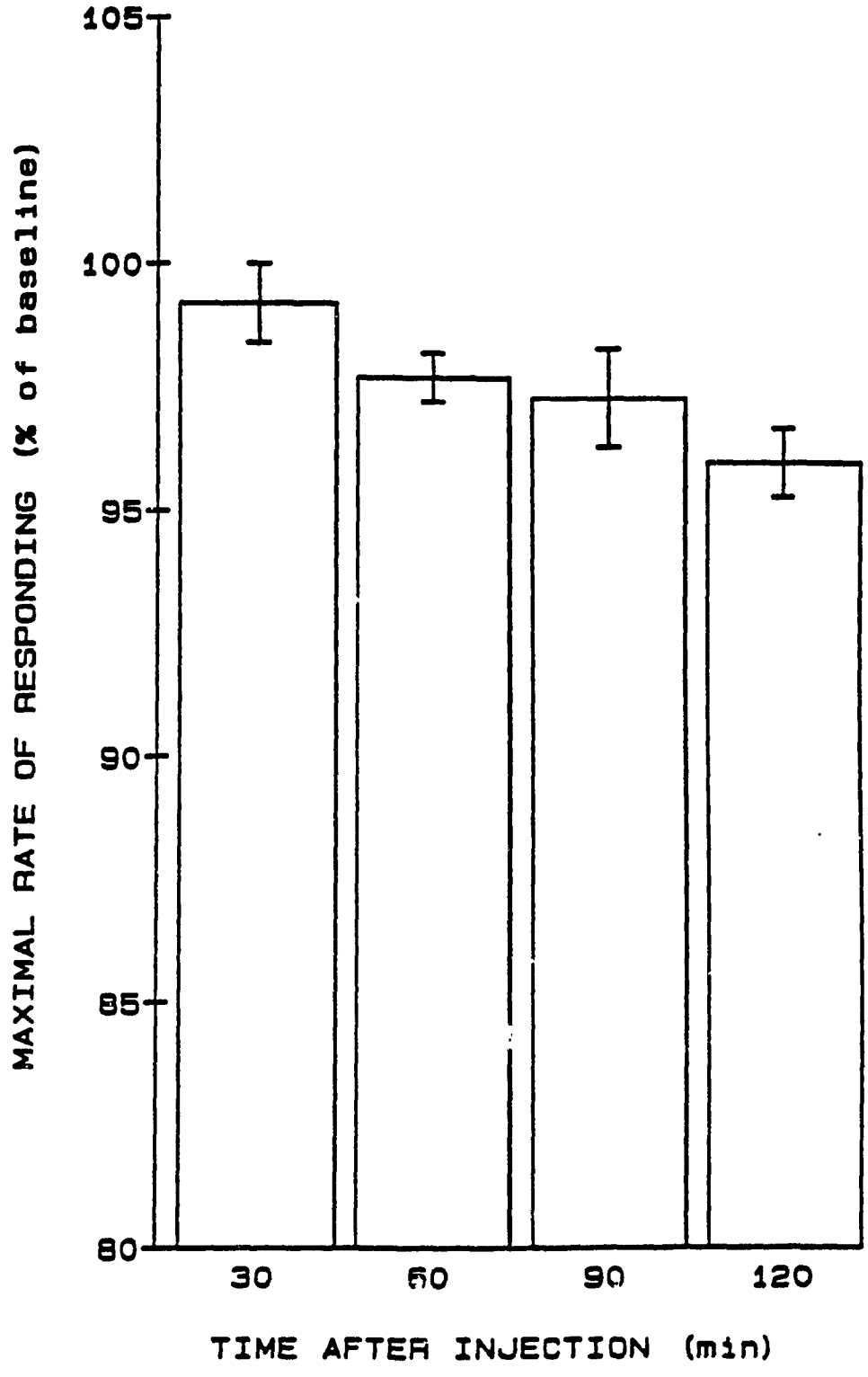

105
100
95
90
85
80
MAXIMAL RATE OF RESPONDING (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)

Figure 20. (see page 95). Frequency threshold (+/- S.E.M., n=13) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time after microinjection of saline prior to and following opiate administration into the nucleus accumbens. Experiment 1.

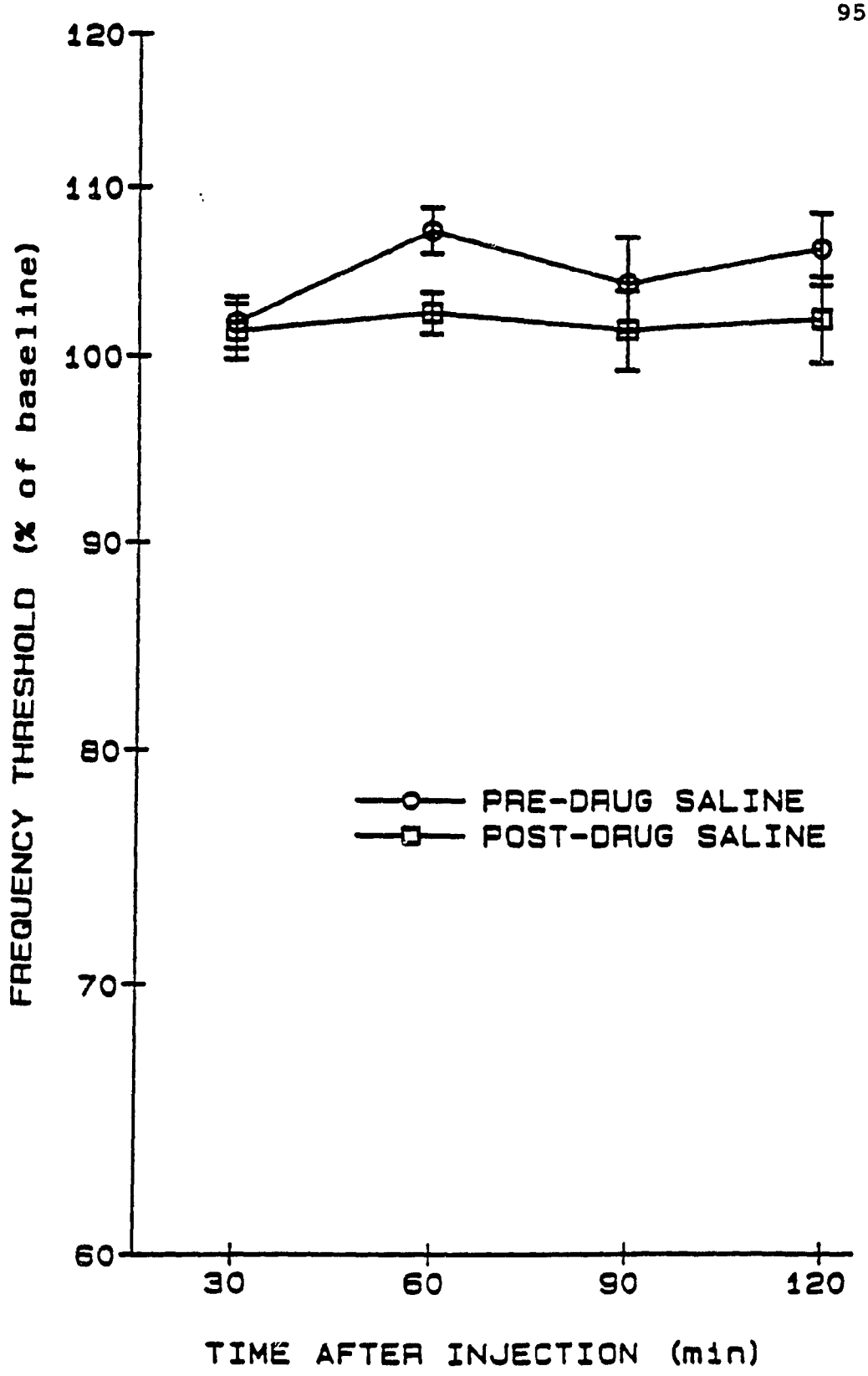

120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
PRE-DRUG SALINE
POST-DRUG SALINE
30
60
90
120
TIME AFTER INJECTION (min)

Figure 21. (see page 97). The locations of injection sites in the rostro-medial (triangles) or rostro-lateral (circles) region of 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 (in millimeters) anterior to bregma. Experiment 1.

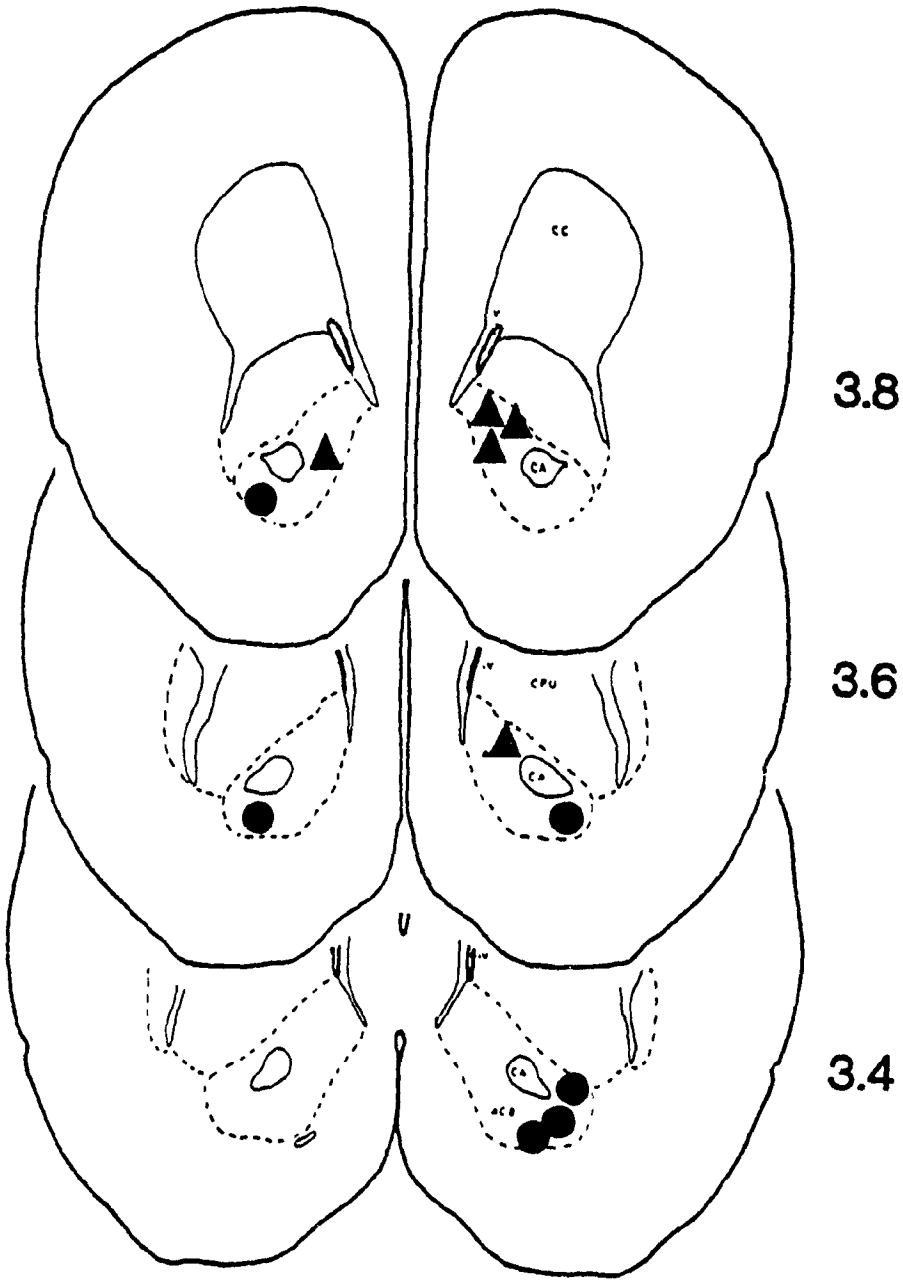

CC
CA
CPU
V
3.8
3.6
3.4

Figure 22. (see page 99). The locations of injection sites in the caudo-medial (triangles) or caudo-lateral (circles) region of 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 (in millimeters) anterior to bregma. Experiment 1.

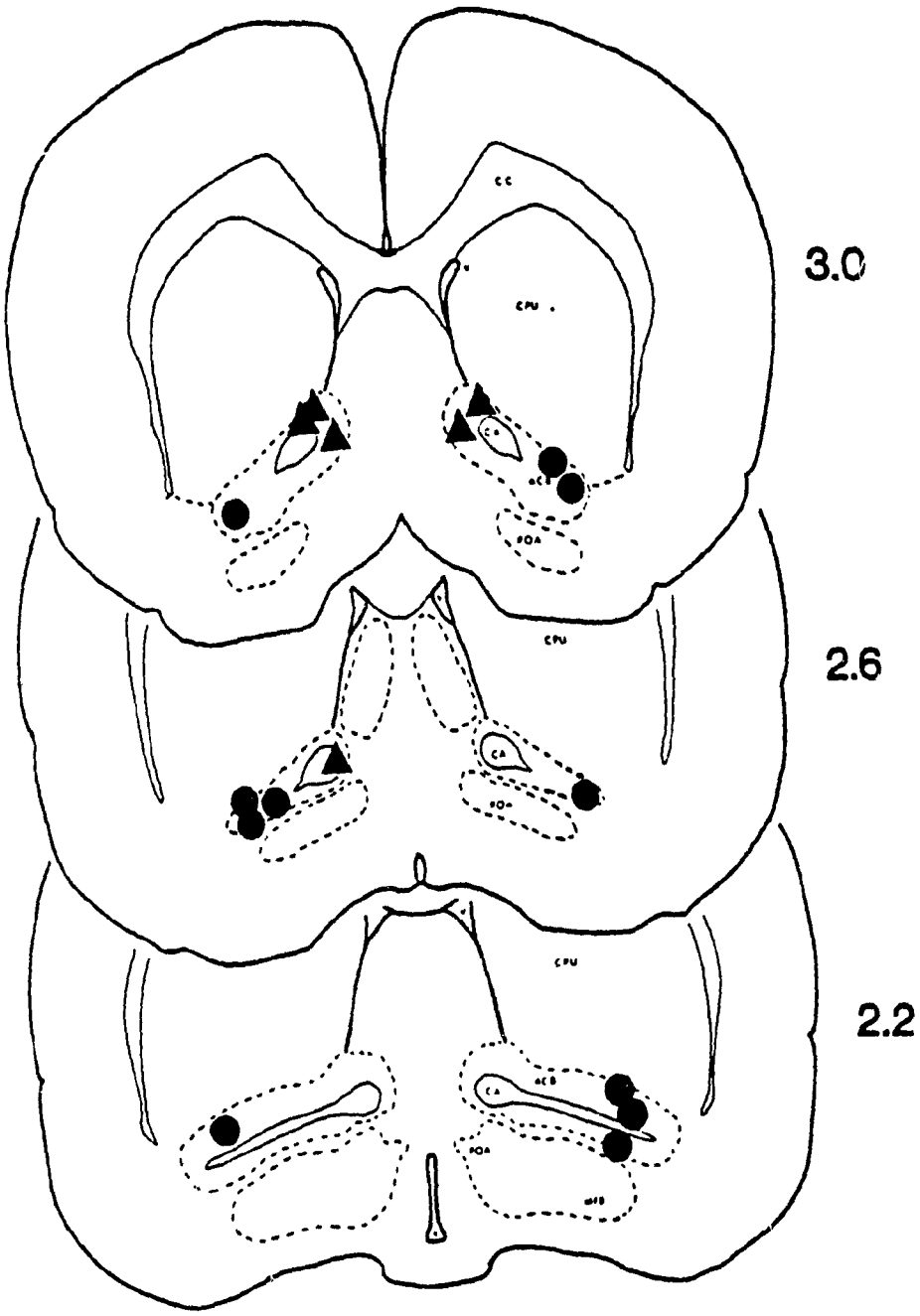
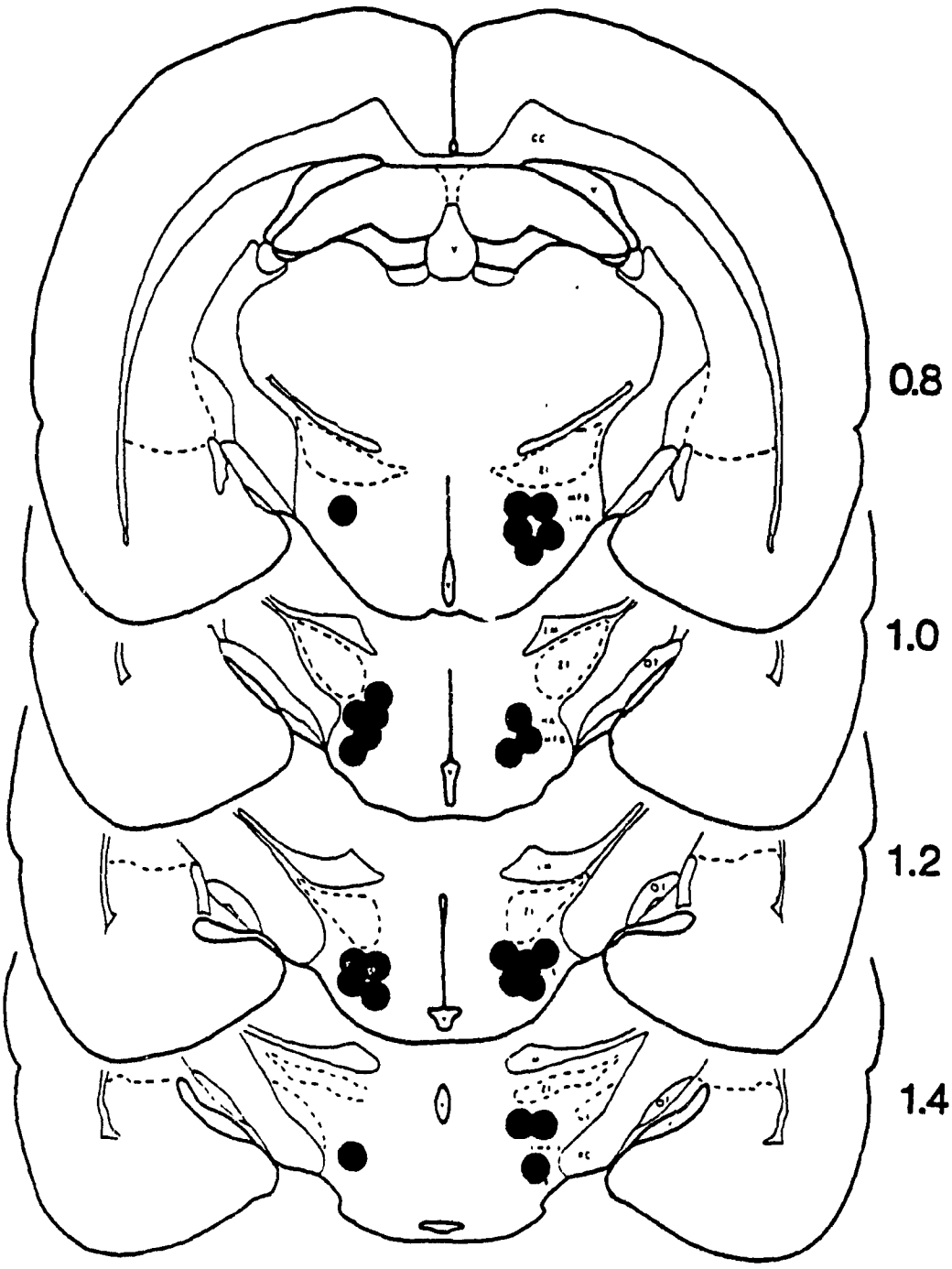

3.0
2.6
2.2

Figure 23. (see page 101). The locations of electrode tips in the area of the lateral hypothalamus. Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The number beside each brain slice represents the distance (in millimeters) posterior to bregma. Experiment 1.


0.8
1.0
1.2
1.4

DISCUSSION

Nucleus accumbens injections of morphine, DAGO, and DPDPE reliably facilitated lateral hypothalamic brain stimulation reward. Each agent caused a parallel leftward shift of the rate-frequency function, decreasing the level of stimulation required to produce normal levels of response. The effects on maximal rate of responding were rare and minor. The effects of central opiates were blocked by naltrexone, indicating that they were due to receptor-mediated pharmacological effects of the drugs and not to non-specific local effects of the injections (eg., changes in osmolarity, pH, or hydraulic pressure). The kappa agonist U50,488H was ineffective when injected into any of the four regions of the nucleus accumbens.

These findings are not in obvious agreement with two earlier reports. Broekkamp et al. (1976) reported that morphine failed to produce an increase in responding for brain stimulation reward. De Witte, Heidbreder, and Roques (1989) also failed to observe a significant facilitation of responding for brain stimulation reward following injections of either the delta agonist, DTLET, or the mu agonist, DAGO. Because of the number of differences between the present study and the studies of Broekkamp et al. (1976) and De Witte et al. (1989) it is not clear what accounts for the differences in findings; differences in

dose, differences in parameters of stimulation, and differences in injection site are all potentially important.

I. Dose of administration

The failure of Broekkamp et al. (1976) and De Witte et al. (1989) to observe a facilitation of brain stimulation reward with nucleus accumbens injections of opiates would appear to be due largely to the use of inadequate doses. In the present experiment, intra-accumbens morphine was effective only when administered at the relatively high dose of 10 µg. At doses of 2.5 µg or lower, morphine produced a small but non-significant facilitation of brain stimulation reward, a finding that appears to be in agreement with that reported by Broekkamp et al. (1976). Even though Broekkamp et al. (1976) reported that nucleus accumbens morphine failed to increase responding for self-stimulation, there was a tendency of morphine to produce some degree of facilitation in the few animals that were tested. The reason that higher doses were not explored by Broekkamp et al. (1976) appears to have been that lower doses were sufficient when injected into other brain regions. Broekkamp (1976) found that doses of 1 µg facilitated responding when injected into the ventral tegmental area and posterior hypothalamus. Thus in their

hands a dose more than double the effective dose in the ventral tegmental area and posterior hypothalamus was ineffective when injected into the nucleus accumbens.

The effectiveness of DAGO and DPDPE is also dependent on the dose of administration. In the present study, DAGO facilitated lateral hypothalamic brain stimulation reward when administered at doses of 200 ng, 800 ng, and 3200 ng. De Witte et al. (1989) failed to observe a facilitation of responding following nucleus accumbens DAGO, but they used doses that were lower than the lowest effective dose in the present study; De Witte et al. (1989) used 150 ng (300 pmol) as their highest dose. In the present experiment, nucleus accumbens DPDPE facilitated brain stimulation reward when administered in a dosage of either 800 ng (1.2 nmol) or 3200 ng (5.0 nmol). Although the effects of NAS DPDPE have not been examined by other researchers, De Witte et al. (1989) reported that nucleus accumbens injections of the delta agonist DTLET, at a dose of 1 nmol, produced a small but non-significant facilitation of brain stimulation reward. Although De Witte et al. (1989) failed to determine whether higher doses of DTLET would be effective, the results of the present study clearly indicate that nucleus accumbens injections of the delta agonist DPDPE facilitate brain stimulation reward. Indeed, in the present study DPDPE was the only opiate to facilitate brain stimulation reward regardless of what sub-region of NAS

received the injection (see below).

U50,488H, not tested by other investigators, was ineffective in facilitating BSR at a dose of 3.2 µg. It does not seem likely that higher doses of U50,488H would have facilitated brain stimulation reward since U50,488H has been reported to produce aversive effects in conditioned place preference following injections into the lateral ventricle (Bals-Kubik, Herz, & Shippenberg, 1989). Thus, the finding s of the present experiment suggest that NAS kappa receptors do not play a role in lateral hypothalamic brain stimulation reward.

II. Parameters of stimulation

In the present experiment, the effectiveness of nucleus accumbens opiate injections was dependent on an animal's baseline rate of responding. When animals were tested at stimulation frequencies that produced low to moderate baseline rates, nucleus accumbens opiates facilitated brain stimulation reward. When animals were tested at stimulation frequencies that produced asymptotic baseline rates of responding, nucleus accumbens opiates were not effective in elevating response rate to a higher asymptote.

The rate-dependency of the present findings appears to represent some form of ceiling effect; opiates failed to

increase responding only when response rates were already maximal. It is possible that the stimulation parameters of Broekkamp et al. (1976) were such as to produce near maximal response rates and that this contributed to their failure to observe statistically reliable facilitation of responding with NAS morphine injections. De Witte et al. (1989) also reported that nucleus accumbens opiates failed to significantly increase responding for brain stimulation reward. In this case, however, it is unlikely that the choice of stimulation parameters was a major determinate of the effects of nucleus accumbens opiates since the investigators intentionally chose stimulation parameters that produced moderate rates of responding.

III. Region of opiate injection within nucleus accumbens

The effectiveness of morphine and DAGO was dependent, to some extent, on the region of injection within the nucleus accumbens. Morphine or DAGO injected into the caudal regions and DAGO injected into the rostro-medial region of the NAS facilitated brain stimulation reward; injections of morphine and DAGO into the rostro-lateral region were ineffective. These results do not seem consistent with the anatomical distribution of mu receptors within the NAS; mu receptors are reported to be densely distributed in the medial region of the nucleus (Tempel &

Zukin, 1987) with a more diffuse pattern laterally (Mansour, Khachaturian, Lewis, Akil, & Watson, 1987).

The results of the present experiment suggest that mu receptors in the caudal region of the nucleus accumbens play an important role in brain stimulation reward. Injections of morphine and DAGO into the caudo-medial and caudo-lateral regions each produced a significant facilitation of brain stimulation reward and these effects were observed in the first half hour of testing. It seems unlikely that mu receptors in the rostral region play an important role in the rewarding effects of brain stimulation reward because morphine and DAGO were ineffective in the rostro-lateral region and while DAGO was effective in the rostro-medial region, it was only effective starting in the second half hour of testing. Since injections of DAGO into the rostro-medial region were not effective in the first half-hour of testing, it is likely that this agent spread to the caudo-medial region to produce its effect. The region of injection within the nucleus accumbens may have contributed to differences between the effectiveness of the highest doses of opiates in the Broekkamp and De Witte studies and the lowest effective dose in the present study. Since these authors did not report the exact regions of injection for their animals, it is possible that they injected in a less sensitive region of the nucleus accumbens than the region

where these opiates were effective in the present study.

Injections of DPDPE into each of the four regions of the NAS were effective in facilitating BSR. These results appear consistent with the anatomical distribution of delta receptors in the NAS in that delta receptors are distributed in a diffuse pattern throughout the entire nucleus (Mansour et al., 1987). Thus it appears that DPDPE is effective in facilitating BSR regardless of the region of injection within the nucleus accumbens.

The activation of kappa opiate receptors by U50,488H failed to facilitate the rewarding effects of brain stimulation regardless of the region of injection within the nucleus accumbens.

IV. Potency of DAGO and DPDPE

The present experiment suggests that the activation of nucleus accumbens mu receptors by morphine or DAGO, and nucleus accumbens delta receptors by DPDPE, facilitates brain stimulation reward. It is not clear, however, whether DAGO and DPDPE play similar roles in facilitating brain stimulation reward. Inferences on this matter require a careful comparison of effective doses. In order to compare the potency of nucleus accumbens DAGO and DPDPE on brain stimulation reward, a second experiment was performed.

EXPERIMENT 2

The present experiment was conducted to determine the relative potency of equi-molar doses of DAGO and DPDPE on rewarding lateral hypothalamic stimulation. The drugs were injected into the caudo-lateral region of the nucleus accumbens because in Experiment 1 injections of each opiate were found to be significantly effective here.

METHOD

Subjects Nine male Long-Evans rats weighing 300-400 grams at the beginning of surgery served as subjects. They were housed individually in stainless-steel cages in the same colony room as described in Experiment 1.

Surgery Each rat was implanted bilaterally with electrodes aimed at the lateral hypothalamic area and cannulae aimed at the caudo-lateral region of the nucleus accumbens. The co-ordinates were the same as described as Experiment 1.

Apparatus The same apparatus as in Experiment 1 was used.

Procedure The procedure was the same as in Experiment 1, except for the following changes. All animals were tested

under saline prior to and following drug testing. Under drug testing, each animal received equi-molar doses of DAGO (0.1, 1.0, 10.0 nmol) and DPDPE (0.1, 1.0, 10.0 nmol). The doses of each drug were administered randomly and at least two non-drug days separated each test day.

Statistical Analysis Separate two-way repeated measures analyses of variance were used to assess the effects of DAGO and DPDPE on lateral hypothalamic frequency thresholds and on maximal rate of responding. In both analyses, the two repeated factors were Dose and Time.

RESULTS

Injections of DAGO and DPDPE into the caudo-lateral region of the nucleus accumbens each significantly decreased lateral hypothalamic frequency thresholds without altering maximal response rates. The effect of DPDPE, but not DAGO, was dependent on the dose of injection [$F(7,42)=6.6$, $p<0.05$]; DAGO was effective across the range of tested doses (0.1, 1.0, and 10.0 nmol). DPDPE produced a significant decrease in lateral hypothalamic frequency thresholds only after administration of the highest dose (10 nmol). Post hoc multiple comparison tests revealed that DAGO and DPDPE were equally effective at the high

dose, but DAGO was significantly more effective than DPDPE at a dose of 1 nmol. Comparison tests also revealed that DAGO, at doses of 10 and 1 nmol, was significantly more effective than the low dose of DPDPE (Figure 24).

The effectiveness of DAGO and DPDPE was also dependent on the time of testing [$F(3,18)=9.1$, $p<0.05$]. Each opiate significantly decreased frequency thresholds during the first two half hours of testing but not in the last half hour of testing (not shown).

Confirmation of cannula and electrode placements The locations of injections in the caudo-lateral region of the nucleus accumbens are shown in Figure 25. Figure 26 displays the locations of electrode tips in the area of the lateral hypothalamus.

DISCUSSION

DAGO was more effective than DPDPE in facilitating brain stimulation reward following equi-molar dose injections into the caudo-lateral region of the nucleus accumbens. It appears that DAGO is at least 100 times more potent than DPDPE since the lowest effective dose for DAGO was 0.1 nmol and the lowest effective dose for DPDPE was 10 nmol. It is possible that the potency of DAGO may be even

Figure 24. (see page 113). Threshold change (+/- S.E.M., n=7) in lateral hypothalamic brain stimulation reward (expressed as a percentage difference of saline) as a function of dose following equi-molar microinjections of DAGO, DPDPE, or saline into the caudo-lateral region of the nucleus accumbens. Experiment 2.

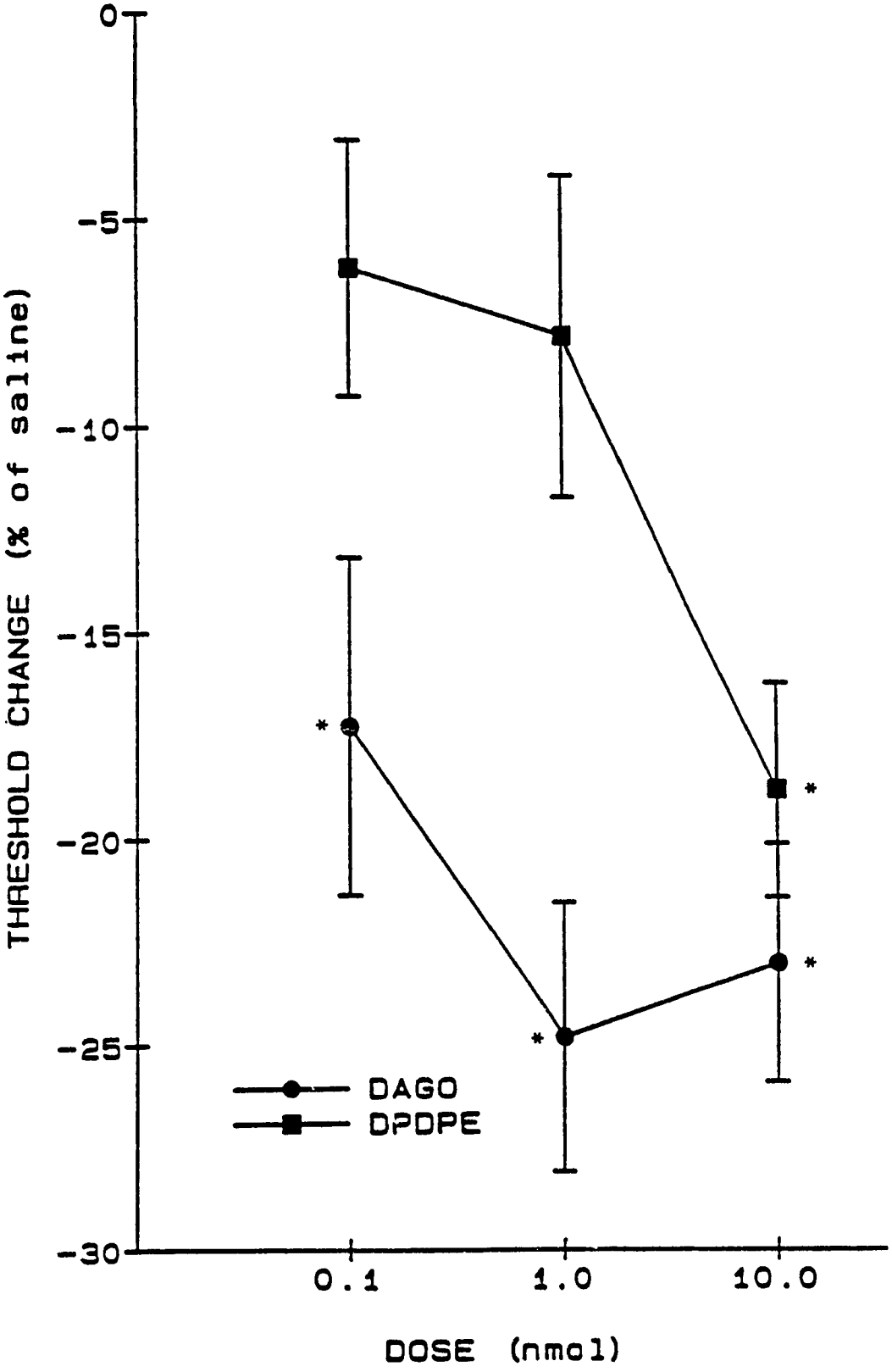

0
-5
-10
-15
-20
-25
-30
THRESHOLD CHANGE (% of saline)
0.1
1.0
10.0
DOSE (nmol)
DAGO
DPDPE
*
*
*
*

Figure 25. (see page 115). The locations of injection sites in the caudo-lateral region of 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 (in millimeters) anterior to bregma. Experiment 2.

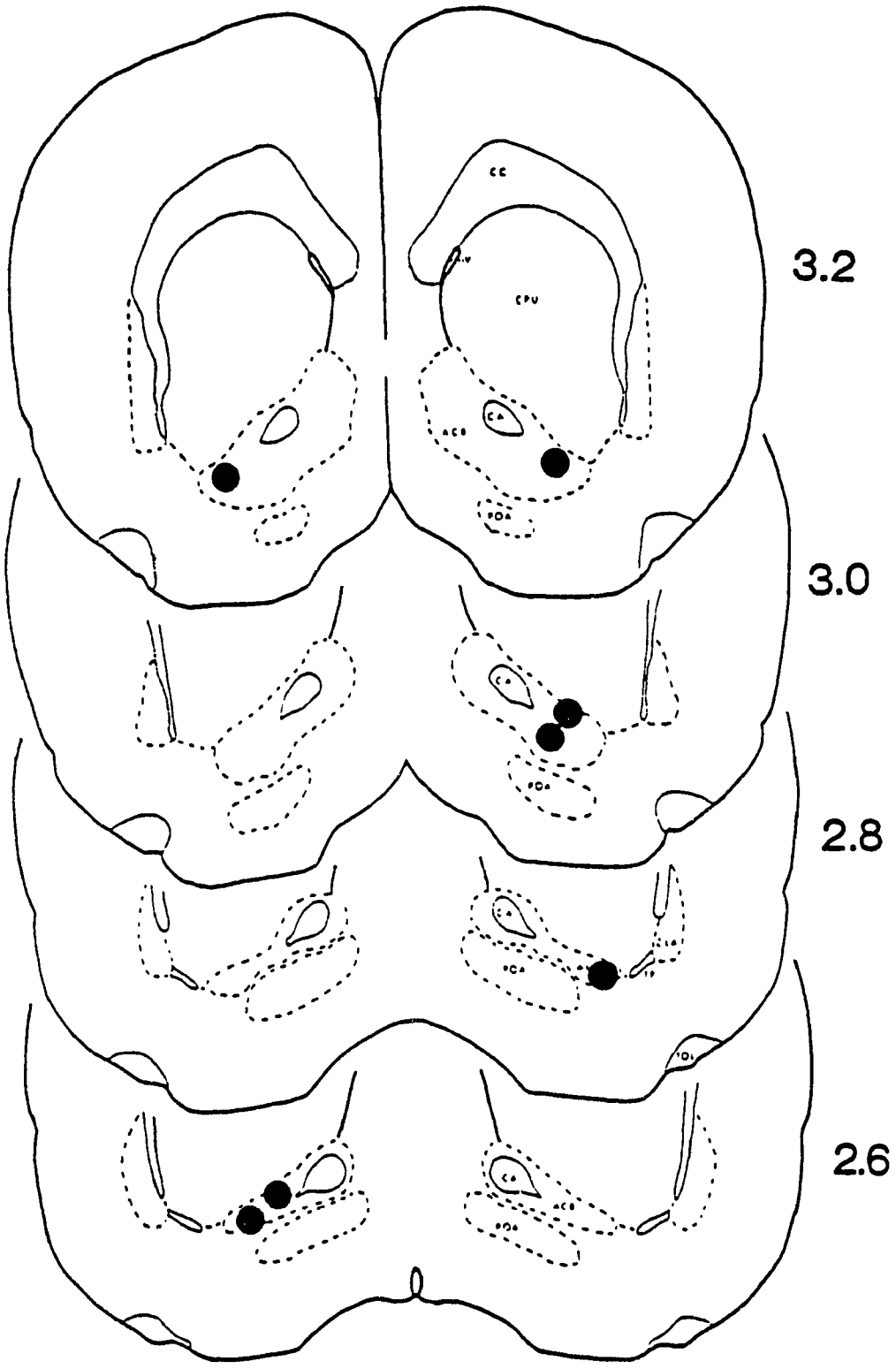
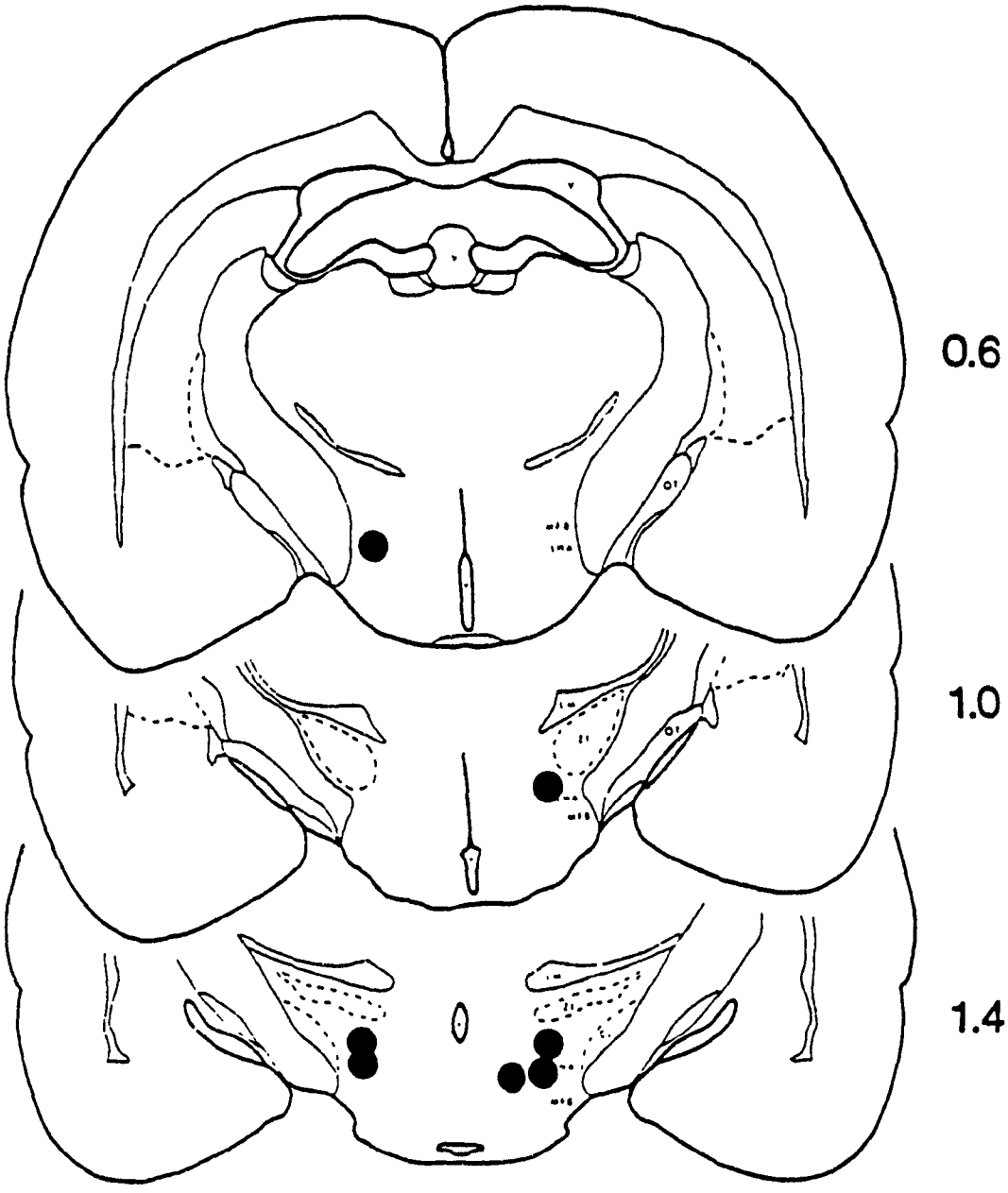

cc
CPU
3.2
3.0
2.8
2.6

Figure 26. (see page 117). The locations of electrode tips in the area of 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 (in millimeters) posterior to bregma. Experiment 2.


0.6
1.0
1.4

more than 100 times that of DPDPE, since DAGO may have been effective at even lower doses, had they been tested.

The greater potency of DAGO suggests that nucleus accumbens mu receptors play a greater role than delta receptors in NAS opiate facilitation of brain stimulation reward. Since DAGO has a high affinity and selectivity for mu opiate receptors (Handa, Lane, Lord, Morgan, Rance, & Smith, 1981) and was effective at a low dose, it is clear that it is mu receptors that mediate the DAGO effect.

It is also possible that mu receptors mediate the effects of DPDPE. Although DPDPE has a high affinity and selectivity for the delta opiate receptor (Mosberg, Hurst, Hruby, Gee, Yamamura, Galligan, & Burks, 1983), a high dose of DPDPE was required to have effects equal to those of a low dose of DAGO. At a high dose, it is possible that DPDPE may cross-react with mu receptors to produce its effect.

A high dose of DPDPE is also reported to be necessary in other DAGO-sensitive behaviors. Latimer, Duffy, and Kalivas (1987) found that ventral tegmental injections of DAGO and DPDPE each facilitated locomotor activity but that DAGO was 100 to 1000 time more potent. Austin and Kalivas (1990) and Hoffman, West, and Wise (1991) found that DAGO and DPDPE each cause locomotion when injected into the ventral pallidum but that DAGO was again more potent. Although in these studies DPDPE was required to be

administered at a higher dose than DAGO to be equally effective, it is unlikely that mu receptors were responsible for mediating the DPDPE effect. It was the case in Experiment 1 that DPDPE was effective when injected into regions of the nucleus accumbens where DAGO was ineffective. Thus, it appears that nucleus accumbens delta receptors may play a role, albeit a minor one relative to nucleus accumbens mu receptors, in brain stimulation reward.

While the results of the present experiment demonstrate that injections of opiates into the nucleus accumbens facilitate brain stimulation reward, this region appears to be less sensitive to the effects of opiates than another brain region where opiates have this effect: the ventral tegmental area. The same can be said of the locomotor-stimulating effects of opiates injected into these two areas (Joyce & Iversen, 1979; Kalivas, Widerlov, Stanley, Breese, & Prange, 1983; Stinus, Winnock, & Kelley, 1985).

EXPERIMENT 3

Experiment 3 was designed to determine whether any of the reward-facilitating effects of NAS opiates might be due to diffusion of the drugs up the cannula shaft into the overlying tissue of the caudate nucleus. To assess this possibility, morphine, DAGO, and DPDPE were injected into an area of the caudate nucleus, dorsal to the caudo-lateral region of NAS, where injections were made in the dose response comparison of Experiment 2.

METHOD

Subjects Eight male and naive Long-Evans rats weighing 300-400 grams at the time of surgery served as subjects. Each animal was housed individually in stainless-steel cages in the same colony room as described previously.

Surgery Each animal was implanted bilaterally with electrodes aimed at the lateral hypothalamic area and cannulae aimed at an area of the caudate dorsal to the caudo-lateral region of the nucleus accumbens. The co-ordinates for the lateral hypothalamic area were the same as in the previous two experiments, and the co-ordinates for the cannulae were: 3.0 mm anterior to bregma, 2.8 mm lateral to the midline, and 4.2 mm below the dural surface.

Procedure The procedure was the same as in Experiment 1 except for the following changes. Each animal received injections of saline prior to and following drug testing. Under drug testing, each animal was injected with morphine (10 µg), DAGO (10 nmol), and DPDPE (10 nmol). Each opiate was administered randomly and at least two non-drug days separated each test day.

RESULTS

Injections of a high dose of morphine, DAGO, or DPDPE into an area dorsal to the caudo-lateral region of the nucleus accumbens failed to facilitate the effects of lateral hypothalamic brain stimulation reward and did not alter maximal rate of responding.

Frequency thresholds A two-way repeated measures (Dose, Time) analysis of variance revealed that the effects of morphine (10 µg), DAGO (10 nmol), and DPDPE (10 nmol) were not statistically different from the effects of saline (pre- and post-drug) on lateral hypothalamic frequency thresholds (Figure 27).

Maximal rate of responding A two-way repeated measures (Dose, Time) analysis of variance revealed no significant difference between the effects of saline (pre- and post-

Figure 27. (see page 123). Frequency threshold (+/- S.E.M., n=6) of lateral hypothalamic brain stimulation reward (expressed as percentage of baseline threshold) as a function of time following microinjection of morphine, DAGO, DPDPE, or saline into the region dorsal to the caudo-lateral region of the nucleus accumbens. Experiment 3.

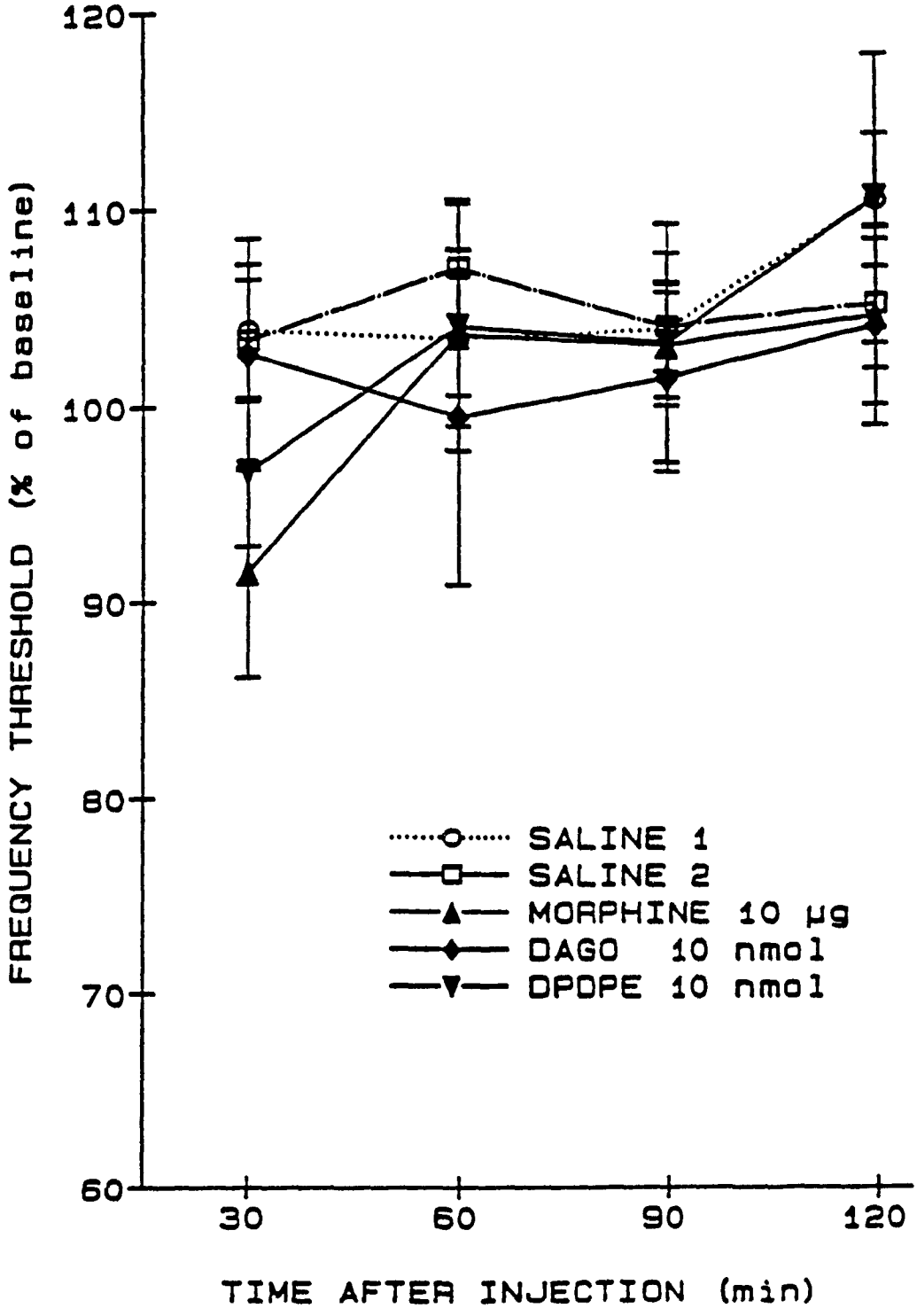

120
110
100
90
80
70
60
FREQUENCY THRESHOLD (% of baseline)
30
60
90
120
TIME AFTER INJECTION (min)
SALINE 1
SALINE 2
MORPHINE 10 µg
DAGO 10 nmol
DPDPE 10 nmol

Figure 28. (see page 125). The locations of injection sites in the striatum. Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The number beside each brain slice represents the distance (in millimeters) anterior to bregma. Experiment 3.

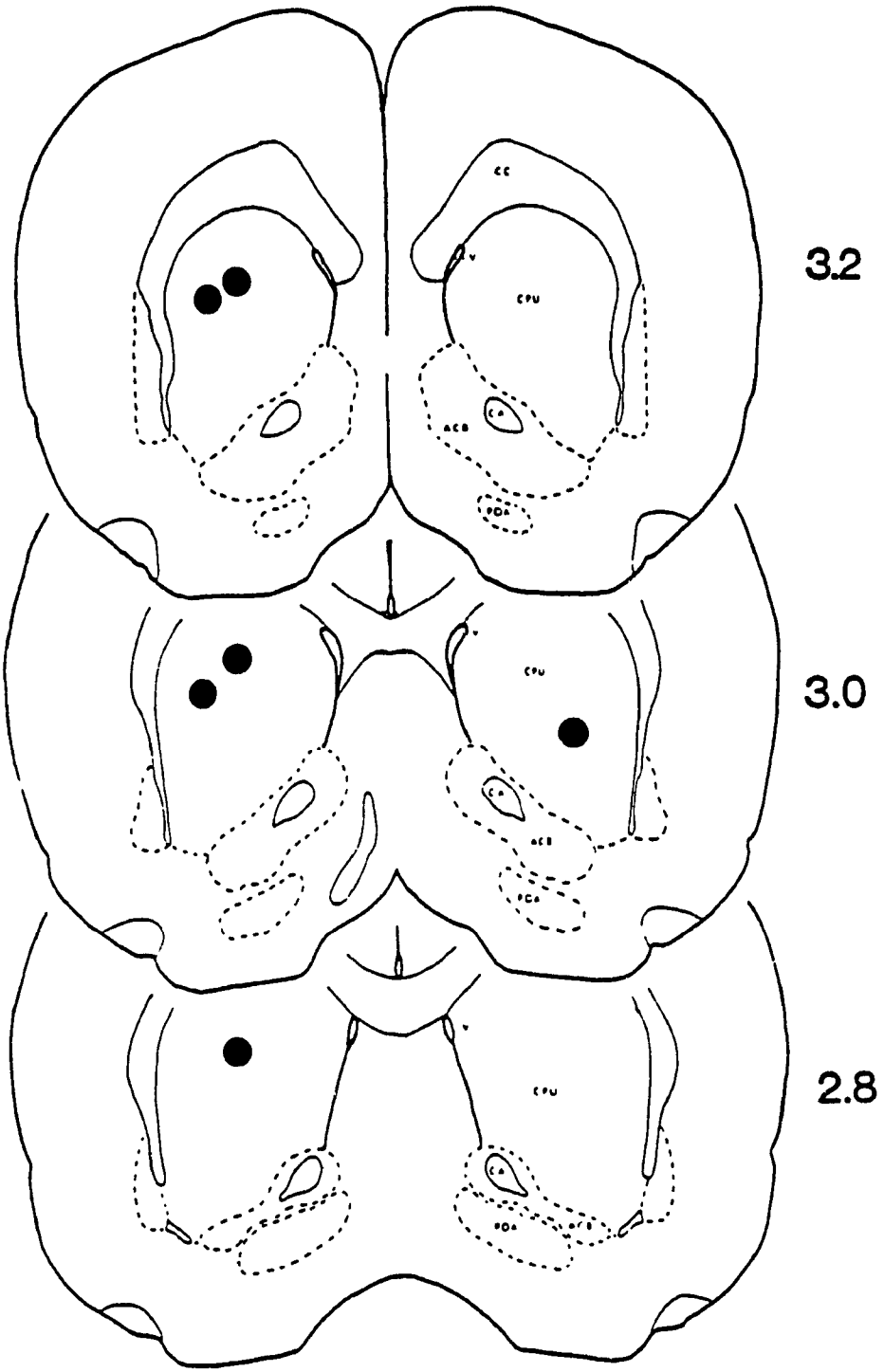
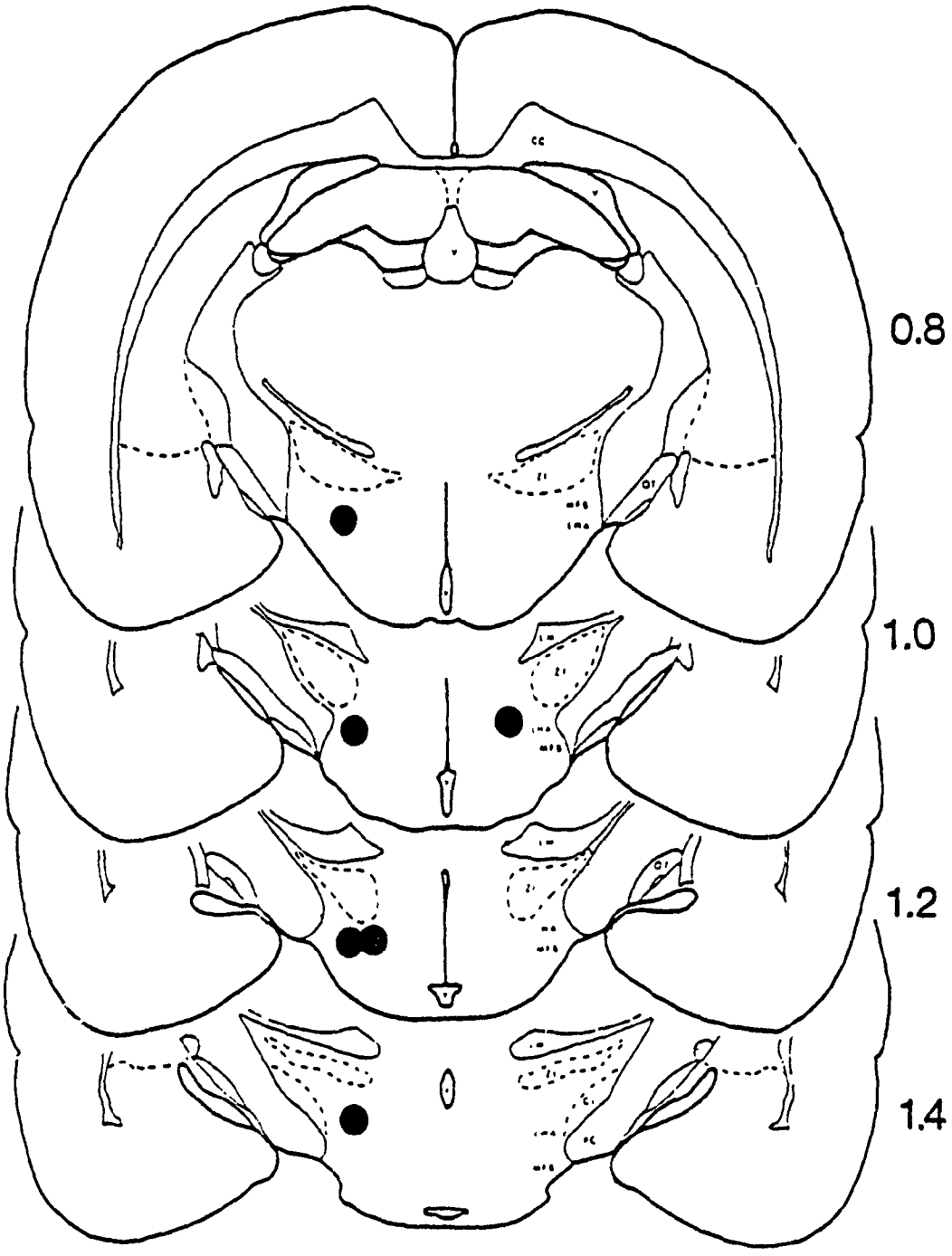

3.2
3.0
2.8

Figure 29. (see page 127). The locations of electrode tips in the area of the lateral hypothalamus. Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The number beside each brain slice represents the distance (in millimeters) posterior to bregma. Experiment 3.


0.8
1.0
1.2
1.4

drug) and morphine (10 µg), DAGO (10 nmol) or DPDPE (10 nmol) on maximal rate of responding for lateral hypothalamic stimulation.

Confirmation of cannula and electrode placements The locations of injections in the area of the caudate nucleus dorsal to the caudo-lateral region of the nucleus accumbens are shown in Figure 28. Figure 29 displays the locations of electrode tips in the area of the lateral hypothalamus.

DISCUSSION

Injections of opiates into the caudate nucleus did not facilitate the rewarding effects of brain stimulation reward. Thus diffusion from nucleus accumbens opiate injections in Experiments 1 and 2 is unlikely to have made any significant contribution to the facilitation of brain stimulation reward observed in those experiments.

There are two ways that diffusion might have played a role in the rewarding effects of brain stimulation reward. First, a drug may diffuse up the cannula shaft to the overlying tissue of the caudate nucleus. This is the most likely route of drug migration from the injection site (Johnson & Epstein, 1975; Simpson & Routtenberg, 1972). Since the opiates were not effective when injected directly

into the caudate nucleus, whatever drug diffused up the cannula shaft to this overlying structure must have had insignificant effects.

Second, the drug may have diffused through the interstitial spaces to act at sites adjacent to the injection site. The ineffectiveness of injections in the caudate nucleus indicates that these injections are not spreading more than the 2 to 3 mm between the ineffective caudate sites and the effective NAS sites directly below. This, in turn, suggests that the NAS injections were acting within approximately 2 to 3 mm of the injection site, confirming the role of the nucleus accumbens in this behavior.

SUMMARY AND CONCLUSIONS

In the first experiment, injections of morphine, DAGO, and DPDPE into the nucleus accumbens decreased lateral hypothalamic frequency thresholds; this is presumed to reflect an increase in the rewarding impact of the stimulation. Injections of the kappa agonist U50,488H did not affect lateral hypothalamic frequency thresholds, suggesting that this agent does not alter the rewarding impact of the stimulation by an action in this brain region. The effectiveness of morphine and DAGO, but not DPDPE, was dependent on the region of injection within the nucleus accumbens. Morphine was effective only when injected into the caudal region, and DAGO produced strongest effects when injected into the same region. These data raise the possibility that mu opiate receptors in the caudal region and delta opiate receptors throughout the nucleus accumbens play a role in potentiating brain stimulation reward. The effectiveness of morphine, DAGO, and DPDPE was dependent on the dose of administration. Morphine and DAGO were effective at doses higher than those explored in previous studies, suggesting that the failure of previous researchers to observe a significant facilitation of brain stimulation reward was due the use of insufficient doses. The effectiveness of nucleus accumbens opiates was also dependent on an animal's baseline rate of

responding. Nucleus accumbens opiates were effective only when animals were tested at stimulation frequencies that produced low to moderates rates of responding and not at stimulation frequencies that produced high rates of response. These data suggest that the choice of dose, stimulation parameters, and region of injection within the nucleus accumbens, are important considerations for observing an opiate-potentiation of brain stimulation reward.

Experiment 2 demonstrated that DAGO was more effective than DPDPE in facilitating brain stimulation reward following equi-molar dose injections into the caudo-lateral region of the nucleus accumbens. DAGO seems to be at least 100 times more potent than DPDPE and was effective at a low dose, suggesting that mu receptors play an important role in mediating the reward-facilitating effects of opiates. With respect to delta receptors, two possibilities must be considered. First, it may be that delta receptors contribute to the reward-facilitating effects of opiates but play a minor role relative to mu receptors. Alternatively, it may be that at the high concentrations of DPDPE that were required to facilitate the rewarding effects of stimulation, DPDPE cross-reacted with mu receptors. Further work is needed to clarify this matter.

Experiment 3 revealed that injections of opiates into the caudate nucleus did not facilitate the rewarding

effects of brain stimulation reward. These data suggests that opiate diffusion from injections in the nucleus accumbens is unlikely to make any significant contribution to the facilitation of brain stimulation reward observed in Experiments 1 and 2.

The present data resolve an apparent contradiction in the early literature on the direct rewarding and reward-potentiating effects of nucleus accumbens morphine. It is now clear that opiates have reward-potentiating as well as direct rewarding actions in the nucleus accumbens as well as in the ventral tegmental area. It is possible that there remain additional sites of opiate rewarding action; for example, rewarding effects of hippocampal opiates have recently been reported (Stevens, Shiotsu, & Stein, 1991).

Rewarding opiate actions in the ventral tegmental area appear to involve synaptic inputs to ventral tegmental dopamine neurons. Rewarding opiate actions in the nucleus accumbens appear to involve intrinsic neurons of the nucleus accumbens, presumably the targets of ventral tegmental dopamine terminals. Rewarding opiate actions of the hippocampus may involve related circuitry since a major neuronal output of the hippocampus synapses with cells of the nucleus accumbens. While the effects of hippocampal opiates on hypothalamic brain stimulation reward are not known, the available data involving other sites are consistent with the assumption that the reward-potentiating

and direct rewarding actions of opiates involve a common brain substrate.

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