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The Effects of Lesions of the Medial Frontal Cortex and Habenular Nuclei on the Development of Sensitization to the Activational Effects of Repeatedly-Administered Morphine

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ABSTRACT

The Effects of Lesions of the Medial Frontal Cortex and Habenular Nuclei on the Development of Sensitization to the Activational Effects of Repeatedly-Administered Morphine

Douglas Funk

Moderate to high doses of morphine (MOR) administered systemically in the rat result in an initial depression of locomotor activity followed by a later increase. Repeated, intermittent treatment leads to the progressive enhancement, or sensitization, of these activating effects. MOR applied directly to the cell bodies of the mesolimbic DA system in the ventral tegmental area (VTA) leads only to behavioral activation, which also sensitizes with repeated treatment. Behavioral sensitization is accompanied by increases in the responsiveness of the mesolimbic DA system to the disinhibitory effects of the drug.

A series of experiments was carried out to assess the effects of lesions of the medial frontal cortex (MFC) or of the habenular nuclei, two areas of the brain which inhibit the mesolimbic DA system, on the sensitization of MOR-induced behavioral activation in the rat. Lesions of the MFC or habenular nuclei did not affect the development of a sensitized activational response to repeated, intermittent injections of MOR when administered either systemically or directly into the VTA. Cortical lesions increased baseline activity scores in one experiment. Habenular lesions

enhanced the acute locomotor effect of systemic MOR, and led to an increased incidence of stereotypical behaviors when MOR was injected into the VTA.

These results suggest that the habenular nuclei and the MFC, although participating in the inhibitory control of the mesolimbic DA system, do not regulate the development of the mesolimbic DA system's sensitized response to MOR when it is administered repeatedly.

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TABLE OF CONTENTS

LIST OF FIGURES

Introduction

In the rat, the behavioral activational effects of morphine (MOR) have been shown to sensitize with repeated administration. The acute locomotor action of moderate to high doses of systemically-injected MOR is biphasic in nature, with an initial sedative effect, followed by a later increase in locomotion. Daily systemic administration of MOR results in tolerance to it's sedative action and an enhancement of it's motor stimulant effect (Babbini & Davis, 1972; Buxbaum, Yarbrough & Carter, 1973; Di Chiara & Imperato, 1988; Jorenby, Keesey & Baker, 1988).

Morphine and the Dopamine Systems

The stimulant effects of MOR appear to be mediated, at least in part, by the mesotelencephalic dopamine (DA) systems (for discussions of this point, see Swerdlow, Vaccarino, Amalric & Koob, 1986; Vezina, Kalivas & Stewart, 1987). These originate in the ventral mesencephalon, and ascend via the medial forebrain bundle, to ramify in a number of forebrain areas. In the case of the mesolimbic DA system, the cells of origin are located in the ventral tegmental area (VTA), also known as the A10 region, and project most heavily to the nucleus accumbens (NAcc), and also to the caudate-putamen (CPu). The mesolimbic DA system is implicated in the mediation of locomotor activity as well as motivated behavior. A sub-population of DA cells in this region project to the medial frontal cortex (MFC), cingulate cortex and orbital frontal cortex, and this projection is known as the mesocortical DA system. In the case of the nigrostriatal system, the DA-ergic cell bodies are located more laterally in the substantia nigra (SN), or A9 region, and project mainly to the CPu. This system is thought to play a role in the integration of motor behavior (Dahlstrom & Fuxe, 1964; Moore & Bloom, 1978; Graybiel & Ragsdale, 1983; Fallon & Loughlin, 1987; Mogenson, 1987; Oades & Halliday, 1987).

Mechanism of Morphine-induced Behavioral Activation

MOR exerts stimulant effects on locomotion and exploration by increasing the firing rate of DA neurons in the mesolimbic system. This appears to be accomplished via an inhibitory action on inhibitory non-DA neurons that impinge on A10 DA cells (Nowycky, Walters & Roth, 1978; Ostrowski, Hatfield & Caggiula, 1982; Gysling & Wang, 1983; Matthews & German, 1984). In support of this view is the fact that moderate levels of mu opiate receptor binding have been found in the VTA, and that fibre-sparing lesions, but not selective destruction of DA cells via 6-hydroxydopamine (6-OHDA) infusion into the VTA reduce mu opiate receptor binding in this region (Mansour, Khachaturian, Lewis, Akil & Watson, 1987; Dilts & Kalivas, 1989). Both systemic MOR and intra-VTA opioid peptide injections, furthermore, lead to increases in locomotor activity that are accompanied by enhanced DA-ergic activity in the NAcc (Kalivas, Widerlov, Stanley, Breese, & Prange, 1983; Latimer, Duffy & Kalivas, 1987; Kalivas, 1985; Kalivas & Duffy, 1987; Di Chiara & Imperato, 1988); these behavioral effects of MOR can be attenuated by treatment with either DA receptor antagonists or blockers of DA synthesis (Buxbaum et al., 1973; Iwamoto, 1981). Moreover, the stimulatory effects caused by application of MOR or opioid peptides to the DA cell body region, which leads only to locomotor stimulation (Joyce & Iversen, 1979; Stinus, Koob, Ling, Bloom &

LeMoal, 1980; Vezina & Stewart, 1984), can also be blocked by systemically administered DA receptor blockers or selective destruction of the DA terminals in the NAcc (Sinus et al., 1980; Vezina & Stewart, 1984). Furthermore, the locomotor effects produced by either systemic or intra-VTA injections of MOR can be blocked by systemic or intra-VTA co-administration of opiate receptor blockers (Iwamoto, 1981; Vezina & Stewart, 1984; Kalivas & Duffy, 1987). Taken together, these data strongly support the conclusion that the locomotor effects of MOR are mediated by a disinhibitory action on mesolimbic DA cells, at the cell body region of these neurons.

The DA Systems and the Development of Sensitization to the Stimulant Effects of Morphine

There is considerable evidence that MOR acting at opiate receptors in the region of the A10 DA cell bodies mediates the development and expression of the sensitized effects. Repeated intra-VTA administration of MOR results in a sensitization of it's behavioral activational effects (Joyce & Iversen, 1979; Vezina & Stewart, 1984; Kalivas, Taylor & Miller, 1985; Vezina, Kalivas & Stewart, 1987). Intra-VTA application of an opiate receptor blocker prior to each exposure to systemic MOR, blocks the development of a sensitized locomotor response to a later challenge administration of systemic MOR (Kalivas & Duffy, 1987). Additional support for this hypothesis is derived from the observation that a sensitized locomotor response is noted in rats previously exposed to systemic MOR, when challenged with an intra-VTA infusion of an opiate receptor agonist (Kalivas & Duffy, 1987).

This sensitized response to MOR, in animals that have received prior exposure to the drug, is paralleled by similar increases in DA-ergic activity in the NAcc. There are enhanced rates of DA utilization and synthesis in the NAcc of rats previously exposed to systemic MOR or other opioid peptides in response to a challenge dose of such drugs (Kalivas, 1985; Kalivas [illegible]ffy, 1987). It is thought that this enhancement of DA-ergic activity in the NAcc is responsible for the sensitized locomotor effects.

Stress, Opiates and the Mesolimbic DA System

Another finding which bears directly on the interaction between opiates and the mesolimbic DA system, and on the phenomenon of sensitization, is that exposure to intense peripheral stimuli, such as foot-shock or tail pinch, can produce increases in locomotor activity (Fanselow, 1984; Arnsten, Berridge & Segal, 1985; Leyton & Stewart, 1990, manuscript submitted for publication) and other motivated behaviors, such as eating (Antelman, Szechtman, Chin & Fisher, 1975; Rowland & Antelman, 1976). It has been shown that stress-producing stimuli increase DA-ergic activity in the mesolimbic, as well as mesocortical terminal fields (Fadda, Argiolas, Melis, Tissari & Onali, 1978; Herman, Guillonneau, Dantzer, Scatton, Semerdjian-Rouquier & Le Moal, 1982; Herman, Stinus & Le Moal, 1984; Abercrombie, Keefe, DiFrischia & Zigmond, 1989). This locomotor activation induced by stressors has been shown to sensitize with repeated exposure (Leyton and Stewart, manuscript submitted for publication). Moreover, rats previously exposed to repeated footshock stress show enhanced locomotor activity compared to stress-naive animals when challenged with either systemic or intra-VTA opioid administration (Kalivas, Richardson-Carlson & Van Orden 1986; Leyton & Stewart, manuscript submitted for publication), an effect that is blocked by intra-VTA infusion of an opiate receptor blocker prior to each daily footshock preexposure (Kalivas & Abhold, 1987). These findings show that peripheral sensory input has access to the mesolimbic DA system, and can produce effects similar to those seen following drug treatment. These results also suggest that the mechanisms that underlie the development of behavioral sensitization to the repeated administration of opioid drugs and repeated exposure to stress may be similar.

Connections of the DA systems with Other Brain Regions

The mesolimbic DA system, through it's projections to the NAcc and CPu, can influence neural substrates underlying motor activity and motivated behavior (Mogenson, 1987). It in turn receives input at both cell body and terminal regions from a wide variety of central nervous system (CNS) sites (Phillipson, 1979; Groenewegen, Becker & Lohman, 1980; Fuller, Russchen & Price, 1987; Oades & Halliday, 1987). Stimulation, lesion, or pharmacological manipulation within a number of such areas has been shown, in a variety of studies, to alter the acute behavioral, biochemical and electrophysiological responses of the DA systems.

THE MEDIAL FRONTAL CORTEX

One forebrain site implicated in the regulation of mesolimbic DA function is the medial frontal cortex (MFC). Often described as "limbic cortex" due to its close anatomical and functional association with the limbic system, this area is defined as the projection zone of the mediodorsal thalamic nucleus (Krettek & Price, 1977). This cortical region receives extensive projections from other areas of the cortex and from limbic areas such as the amygdala (McDonald, 1987). The MFC, in addition, receives a moderately dense DA-ergic projection which originates in the VTA (Thierry, Blanc, Sobel, Stinus & Glowinski, 1973; Berger, Thierry, Tassin and Moyne, 1976; Descarries, Lemay, Doucet & Berger, 1987; Fallon & Loughlin, 1987). The cells of this cortical field project, in turn, to other cortical regions, as well as to a number of subcortical structures, such as the NAcc, CPu, VTA, SN, thalamus and amygdala (Beckstead, 1979; Phillipson, 1979; Christie, Bridge, James & Beart, 1985; Cassel & Wright, 1986; Ferino, Thierry, Saffroy & Glowinski, 1987; Fuller, Russchen & Price, 1987; Sesack, Deutch, Roth & Bunney, 1989). The MFC is therefore well-situated to influence the activity of the mesolimbic DA system, through direct projections to the VTA and NAcc as well as through indirect projections to other nuclei which project to the mesolimbic DA system.

The MFC sends a projection to the VTA region, which is thought to utilize an excitatory amino acid (EAA) transmitter. Receptors for both glutamate (GLU) and aspartate are present in this region, and VTA tissue contains relatively high levels of these transmitters. Excitotoxin-induced lesions of the MFC, furthermore, reduce the uptake of radiolabeled EAAs in VTA synaptosomal preparations (Gundlach & Beart, 1982; Halpain, Wieczorek & Rainbow, 1984; Christie et al., 1985; Monaghan & Cotman, 1985).

The projection from the MFC to the NAcc may also use an EAA transmitter. The NAcc contains significant levels of EAA receptors. Injection of radiolabeled EAAs into the NAcc results in retrograde labelling of cells in the MFC, and aspiration lesions of the MFC reduce high-affinity GLU uptake in the NAcc (Halpain et al., 1984; Reibaud, Blanc, Studler, Glowinski & Tassin, 1984; Monaghan & Cotman, 1985; Fuller, Russchen & Price, 1987).

Cortical Influence on NAcc DA Activity

DA-ergic activity in the MFC has been observed to influence DA-ergic activity in the mesolimbic and nigrostriatal DA systems, and this influence has most often been found to be inhibitory. The findings in this area of study are not, however, entirely consistent. Lesion studies have led to conflicting results, which may depend on variables such as the type of lesion, period of recovery and type of bioassay used.

There is evidence that selective destruction of DA terminals in the MFC by intra-cortical 6-OHDA infusion leads to changes in the baseline concentrations of DA and DA metabolites in the NAcc. Increases in DA release, metabolism and synthesis have been observed for up to 30 days following DA-selective lesions of the MFC (Carter & Pycock, 1978b, 1980; Pycock, Carter & Kerwin, 1980; Pycock, Kerwin & Carter, 1980; Martin-Iverson, Szostak & Fibiger 1986). More recently, however, a number of studies utilizing DA-selective lesions of the MFC have failed to find any evidence for changes in DA-ergic activity in the NAcc after both short and long recovery periods (Joyce, Stinus & Iversen, 1983; Goeders & Smith, 1986; Oades, Taghzouti, Rivet, Simon & Le Moal, 1986; Clarke, Jakubovik & Fibiger; 1988; Bubser & Schmidt, 1990). There does not appear to be any systematic variation in experimental procedures which could account for these variable results.

Few studies have assessed the effect of non-selective lesions of the MFC on DA-ergic activity in the NAcc. Reibaud et al., (1984) found no effect of aspiration lesions of the MFC on the DA content of the NAcc. A recent study, however, did observe moderate increases in DA and DA metabolites in the NAcc of rats after lesions of the intrinsic cells of the MFC (Jaskiw, Braun, Karoum, Breslin & Weinberger, 1989). The discrepancy in the findings of these two studies may be explained by the use of different lesion techniques, and the fact that Jaskiw et al., (1989) carried out the biochemical assay 7 days after making the lesions, while Reibaud et al., (1984) used a 6-12 week recovery interval.

Louilot, Le Moal & Simon (1989), using in-vivo voltammetry, found that stimulation of DA release in the MFC via local application of amphetamine (AMPH) led to decreases in the level of DA metabolite detected in the NAcc. Increases in DA metabolites in the NAcc were observed following intra-MFC infusion of a DA receptor blocker. These two observations suggest that enhancement of DA receptor stimulation in the MFC inhibits DA-ergic activity in the NAcc. In addition, the infusion of the nerve impulse blocker tetrodotoxin (TTX) into the MFC, which would block the descending output of the MFC, led to increases in the NAcc DOPAC signal, suggesting that the output from the MFC to the mesolimbic DA system is inhibitory.

There is also evidence that the MFC may be involved in the regulation of DA receptors of the D_1 type in the NAcc. Pycock, Kerwin & Carter (1980) observed increases in DA receptor binding and DA-stimulated activity of adenylate cyclase, the second messenger associated with the D_1 receptor, in the CPu and NAcc of rats with DA-selective lesions of the MFC. Reibaud et al., (1984) found that bilateral aspiration of the MFC led to a moderate enhancement of the DA-stimulated activity of adenylate cyclase in the NAcc. It was also noted that 6-OHDA-induced lesions of the NAcc alone did not lead to alterations in adenylate cyclase activity in the NAcc, but when combined with cortical ablation, dramatically increased the activity of the second messenger. These results suggest that the MFC might exert an inhibitory influence on DA receptor activity in the NAcc.

Cortical Influence on DA Cell Electrophysiology

Manipulations of the MFC have also been observed to result in changes in the firing rate and pattern of DA cells. Electrical stimulation of the MFC and adjacent cortical regions has been shown to result mainly in inhibition of the firing of DA-ergic neurons in both the VTA and SN (Nakamura, Iwatsubo, Tsai & Iwama, 1979; Thierry, Deniau & Feger, 1979; Gariano & Groves, 1988). Another result suggesting that the MFC inhibits the electrical activity of DA cells is the fact that excitotoxin-induced lesions of the MFC enhance the basal firing rates of DA-ergic A10 cells (Ceci & French, 1989).

Some electrophysiological evidence exists suggesting that a MFC-VTA projection might regulate the firing pattern of DA-ergic cells in the A10 region. It has been found that stimulation of this cortical region elicited excitatory responses, including burst firing, in a small population of DA neurons (Gariano and Groves, 1988). Cooling the MFC, which would be expected to block or reduce cortical output, was demonstrated to elicit a "pacemaker"-like firing pattern in DA-ergic A10 neurons in the anesthetized rat, in contrast to the irregular, bursting firing pattern normally seen. The fact that this effect of cortical cooling was blocked by systemically-administered ritanserin, a drug which disinhibits DA-ergic A10 cells in the intact rat, suggests that the inhibition is normally mediated by a serotonergic mechanism. The systemic administration of an EAA antagonist also results in a similar regularization of firing; this finding suggests that EAA receptor stimulation is necessary for burst firing (Grenhoff, Tung & Svensson, 1988; Grenhoff, Ugedo & Svensson, 1988; Svensson & Tung, 1989; Svensson, Tung & Grenhoff, 1989; Ugedo, Grenhoff, & Svensson, 1989). The effect of firing in bursts on DA release in the NAcc is not known. There is some evidence to suggest, however, that dramatic increases in DA release in terminal regions accompany bursts (Gonon, 1988; see also Grace & Bunney, 1984b for an earlier discussion of this material). These results suggest that the GLU-ergic projection from the MFC to the VTA is necessary for the maintenance of burst-firing in these DA neurons.

Lesions of the MFC and Baseline Locomotor Activity

The effect of DA-selective lesions of the MFC on spontaneous locomotor activity has been studied. Carter & Pycock (1980) and Pycock, Kerwin & Carter (1980) found that 6-OHDA lesions of the MFC led to enhanced open field activity. More recently, Bubser &

Schmidt (1990) observed increased levels of exploratory activity in a radial arm maze after DA-selective MFC lesions. Joyce et al., (1983) and Clarke et al., (1988) found no differences between lesioned and sham-operated groups in photocell tests of locomotor activity after 6-OHDA treatment of the MFC. The variation in the results of these studies does not appear to be related to the length of the post-lesion recovery period used in each. One possible explanation may be the fact that Pycock, Kerwin & Carter (1980), Carter & Pycock (1980) and Bubser & Schmidt (1990) tested spontaneous activity only in the first minutes following introduction of the rat into the testing environment, a time when novelty-induced exploration would be at a high level, while Joyce et al., (1983), Oades et al., (1986) and Clarke et al., (1988) used tests of longer duration.

The effect of non-selective lesions of the MFC on locomotor activity has been assessed in relatively few studies. Kolb (1974) found consistent, near-significant increases in running wheel activity in rats with aspiration lesions of the MFC. It is not clear, however, if running wheel scores can be directly compared with other measures of locomotor activity. More recently, Isaac, Nonneman, Neisewander, Landers & Bardo (1989) found no differences in either running wheel or open field activity in rats with similar aspiration lesions of the MFC.

It is not clear whether DA-selective or non-selective lesions of the MFC result in persistent changes in baseline locomotor activity. Factors such as recovery interval and duration of test may play some role in the expression of alterations in baseline activity following such lesions.

The MFC and Behavioral Responses to DA-ergic Drugs

Although lesions of the MFC have inconsistent effects on spontaneous locomotor activity some researchers have reported alterations in the behavioral effects of DA-ergic drugs. Enhancement of AMPH-induced locomotor activation and stereotypical behavior in rats with 6-OHDA-induced lesions of the MFC have been reported (Carter & Pycock, 1978a, 1980; Pycock, Kerwin & Carter, 1980). Joyce et al., (1983) and Clarke et al., (1988), however, found that DA-selective lesions of the MFC did not effect the locomotor activation or stereotypical behavior seen after AMPH administration.

The locomotor and stereotypic effects of the direct-acting DA agonist apomorphine (APO) may also be altered by lesions of the MFC. Carter & Pycock (1978a, 1980) found reductions in the stereotypical behavior seen after the administration of moderate doses of APO in rats with 6-OHDA lesions of the MFC. Oades et al., (1986) observed increased levels of locomotor activity in response to a relatively low dose of APO in similarly lesioned rats. Joyce et al., (1983) and Clarke et al., (1988), however, failed to find any differences in the locomotor effects of a low dose of APO between animals with DA-selective lesions of the MFC and sham-operates.

Tassin, Vezina, Blanc & Glowinski (1988) recently observed that intra-MFC infusions of AMPH attenuated the locomotor-activating effects of AMPH administered into the NAcc, which suggests that DA acting in the MFC can inhibit DA-ergic activity in the NAcc. Intra-MFC AMPH alone had a minimal effect on locomotor activity. On the other hand, it has been demonstrated that unilateral infusions of cocaine or AMPH into the MFC results in contraversive circling, a pattern of behavior suggesting that the drug infusion actually stimulated the activity of the DA systems in the hemisphere it was applied to (Stewart, Morency & Beninger, 1985; Morency, Stewart & Beninger, 1987).

Although at times inconsistent, these results do suggest that the MFC is involved in the regulation of the mesolimbic, and to some extent, the nigrostriatal DA system. Anatomically, the MFC is in a prime position to influence mesolimbic DA function; it projects to both the cell body and terminal regions of this system, as well as to other nuclei that have been shown to project, in turn, to the mesolimbic DA system. In addition, there is evidence that experimentally-induced changes in the MFC can alter the biochemical and electrophysiological activity of this system. Furthermore, the behavioral response to DA-ergic drugs may be altered by lesions or other manipulations of this cortical region.

THE HABENULA

Another brain region which has been implicated in the regulation of the DA systems is the habenular complex, a key component in the proposed dorsal diencephalic conduction system. The dorsal diencephalic pathway is an extensive descending projection system linking forebrain areas with the midbrain (Sutherland, 1982). Electrophysiological, biochemical and behavioral studies support the idea that the habenular complex may be part of an inhibitory projection to the DA-ergic nuclei of the mesencephalon.

The habenular complex is composed of two adjacent nuclei, the medial habenula and the lateral habenula (LHb); each of these subdivisions possess separate inputs and outputs. Efferents from forebrain areas are collected together in a fibre bundle, the stria medullaris , which projects to the habenular nuclei. The habenular nuclei, in turn, projects to midbrain structures through the fasciculus retroflexus pathway. The habenular complex may therefore serve to route a descending forebrain influence on the activity of a number of mesencephalic nuclei.

The existing evidence suggests that the LHb plays a much greater role in the regulation of the DA systems than does the medial habenula. Anatomically, the LHb maintains connections with the DA systems and nuclei which project to the DA systems that are far more extensive than those of the medial habenula. Although the almost exclusive efferent target of the medial habenula, the interpeduncular nucleus, may take part in some drug-related phenomenon, such as opiate withdrawal (Contestabile et al., 1987; Cutlip, Lenn & Wooten, 1988), there is little evidence for their influence on the DA systems. Stimulation of the medial habenula, furthermore, has little effect on the activity of the DA systems, in contrast to the effect seen during stimulation of the LHb. This issue is confused, however, by the difficulty encountered in making selective lesions of the LHb. Most studies have used relatively non-selective electrolytic lesion techniques, which often destroy both habenular nuclei, making it impossible to assess their individual contributions to the regulation of the DA systems.

Some of the afferent sources and efferent targets of the LHb are components of the mesotelencephalic DA systems. The LHb, furthermore, is connected with other nuclei implicated in the regulation of the DA systems. The LHb receives an extensive projection from the entopeduncular nucleus, a structure which receives a substantial proportion of striatal efferents, as well as a moderate projection from the ventral pallidal region, the analogous output nucleus of the NAcc. Another major afferent source of the LHb is the lateral hypothalamus. The VTA sends a moderately dense DA-containing projection to the LHb. Other afferents of the LHb include the MFC, lateral preoptic area, median raphe nucleus, nucleus basalis and the central grey region (Herkenham & Nauta, 1977; Parent, Gravel & Boucher, 1981; Greatrex & Phillipson, 1982; Phillipson & Pycock, 1982; Skagerberg, Lindvall & Bjorklund, 1984).

The LHb, in turn, projects to the lateral hypothalamus, VTA, SN and the raphe nuclei, among others. The LHb is therefore anatomically well-positioned to participate in the regulation of the DA systems (Herkenham & Nauta, 1979; Araki, McGeer & Kimura, 1988).

Habenular Influence on the Electrophysiology of DA cells

Christoph, Leonzio & Wilcox (1986) found that stimulation of the LHb led to inhibition of DA-ergic neurons in the VTA and SN, while stimulation of the medial habenula had little effect. The most common pattern of inhibition was a suppression of ongoing DA cell activity followed by rebound excitation, while a smaller proportion of DA neurons showed pure inhibition. This effect was blocked by either kainate-induced lesion of the LHb or electrolytic lesions of the fasciculus retroflexus. These results suggest that the influence of the LHb on the activity of DA cells is inhibitory, and that a projection originating in the LHb mediates the effect.

Lesions of the habenular nuclei have also been shown to reduce the inhibition of the DA systems caused by DA-ergic autoreceptor stimulation. Sasaki et al., (1988) observed that electrolytic lesions of the habenular complex led to an attenuation of the methamphetamine-induced inhibition of DA cells in the SN, again suggesting that the habenular influence on the activity of the DA cells is inhibitory.

Habenular influence on DA Release

Changes in DA and DA metabolite levels in the terminal fields of the mesotelencephalic DA systems have been observed following manipulations of the habenula. Lisoprawski, Herve, Blanc, Glowinski & Tassin (1980) found increases in the DOPAC/DA ratio in the MFC of rats, 6 days after destroying the habenula complex. Nishikawa, Fage, & Scatton (1986) blocked impulse transmission in the fasciculus retroflexus or stria medullaris with TTX, a treatment which would block the descending output of the habenula complex, and observed increased levels of DA and DA metabolites in the MFC, NAcc, and CPu. Similar increases in DA utilization and synthesis were noted in these areas in this study. These observations further support the notion that the habenula participates in a pathway which inhibits the activity of the DA systems.

Other biochemical changes in the DA systems have been noted following lesions of the habenula complex which are perhaps secondary to the lesion-induced increases in the activity of the DA systems. Levels of a-neoendorphin-like immunoreactivity were shown to be increased in both the VTA and SN following electrolytic lesions of the habenula (Ichikawa, Nishikawa, Mitsushio & Takashima, 1988). This finding also suggests that the habenula inhibits the DA systems, since habenular lesions mimicked the enhancing effect of DA agonist administration on levels of dynorphinergic peptide immunoreactivity in the SN (Li, Sivam & Hong, 1986; Nylander and Terenius, 1987; Hanson, Merchant, Letter, Bush & Gibb, 1988).

Taken together, the results of these studies suggest that the habenula complex normally exerts an inhibitory influence on the activity of the DA systems.

The Habenula and Metabolism in the CNS

Metabolic activity in the LHb, measured by the uptake of radiolabelled 2-DG (2-deoxyglucose), can be altered by the administration of DA-ergic drugs. Furthermore, lesions of this nucleus can affect the metabolic activity of a number of brain areas associated with the DA systems.

McCulloch, Savaki & Sokoloff (1980) observed decreases in 2-DG uptake in the LHb after systemic administration of APO, while haloperidol led to the opposite effect. These data suggest that the LHb may participate in the response to DA-ergic drugs, and are especially interesting in view of the DA-ergic projection to the LHb from the VTA. Bilateral electrolytic lesions of the LHb have been shown to result in decreases in 2-DG uptake in the VTA (Ito, Kadekaro & Sokoloff, 1985). However, a later study failed to replicate this effect (Motohashi, MacKenzie & Scatton, 1986). Very long-lasting decreases in metabolism in the raphe nuclei, which are known to project to the mesolimbic DA system, have also been observed after lesions of the habenular nuclei (Motohashi, Nishikawa, Scatton & MacKenzie, 1986).

The results of the 2-DG uptake studies are difficult to interpret, since it is not clear which neural elements are responsible for the changes in 2-DG uptake caused by a given treatment. These results, however, do suggest that the activity of the LHb is altered by the administration of DA-ergic drugs and therefore be important in mediating the behavioral response to such drugs. Furthermore, lesions of the habenula can have long term effects on metabolism in components of the DA systems, as well as in structures that provide input to the DA systems.

Habenular Lesions and Baseline Locomotor Activity

Lesions of the habenula have been shown to result in increases in baseline locomotor activity, which are thought to be mediated by disinhibition of the mesolimbic DA system. The literature dealing with the effects of habenular lesions on baseline locomotor activity is confused by the presence of methodological differences between studies, most notably the variation in the duration of the activity tests. A number of studies have shown that habenular lesions lead to increases in basal locomotor activity (Nielson & McIver, 1966; Lee and Huang, 1988; Nguyen, Jackson & Caldecott-Hazard, 1989; Thornton Bradbury, Evans & Wickens, 1989). Thornton & Evans

(1982) and Thornton, Evans & Barrow (1983), however, found no effect of habenular lesions on locomotor activity. The animals in these studies, however, were tested for only 6 and 10 min, respectively, whereas those studies finding significant effects of lesions utilized substantially longer test durations.

Rats with lesions of the habenula, furthermore, show more spontaneous gnawing behavior than intact animals (Cooper & Van Hoesen, 1972). This latter finding is also consistent with the idea that habenula lesions disinhibit the DA systems, since activation of the nigrostriatal or mesolimbic DA systems lead to, respectively, stereotyped oral behaviors and eating (Staton & Solomon, 1984; Evans & Vaccarino, 1986; Sharp, Zetterstrom, Ljungberg & Ungerstedt, 1987; Wise, Fotuhi & Colle, 1988). In keeping with the notion that lesions of the habenula disinhibits the activity of the DA systems, Lee & Huang (1989) found that relatively selective electrolytic destruction of the LHb reduced the inhibition of locomotor activity seen immediately after exposure to footshock stress.

Although there are some inconsistencies, possibly arising due to methodological differences among studies, these results support the proposal that the projection from the habenula to the DA-ergic cell bodies in the mesencephalon inhibits the activity of the DA systems. Destruction of the habenula, especially the LHb, disinhibits the DA systems, leading to increases in DA-mediated behaviors.

Habenular Lesions and Behavioral Responses to DA-ergic Drugs

Carvey, Kao & Klawans (1987) assessed the effects of fiber-sparing lesions of the LHb on the stereotypic responses of rats to AMPH or APO administration. Lesioned animals showed increased stereotypical behavior in response to intermediate doses of APO, but not to higher doses or autoreceptor-specific low doses. A similar lesion-induced enhancement of stereotypical behavior was observed following administration of a relatively high dose of AMPH. The stereotypic response of the animals with lesions was described as being more intense than those seen in intact animals, including more chewing, biting, and gnawing, and less locomotor activity. Similar results were obtained by Nguyen et al., (1989), who found that rats with lesions of the habenula showed more locomotor activity as well as stereotypy after 7 days of continuous exposure to AMPH, while still under the influence of the drug. In another recent study, however, Thornton et al., (1989) found no evidence that electrolytic lesions of the habenula affected the locomotor or stereotypic responses of rats to moderate to high doses of APO. Considerable inter-animal variability, however, was noted in this study.

These results suggest that activity in the habenula-midbrain pathway plays a role in the regulation of DA neurotransmission in the forebrain, and that this influence is primarily inhibitory. Stimulation of the LHb results in decreases in the firing of DA-containing cells in the midbrain. Abolition of habenular output, via either acute blockade or lesion, results in increased DA-ergic activity in the forebrain which in some studies, has been shown to be accompanied by increases in baseline locomotor activity.

Furthermore, habenular lesions have been shown to potentiate the behavioral activation seen after the administration of DA-ergic drugs.

The Present Experiments

The following experiments were carried out to see if destruction of two brain areas projecting to the mesolimbic DA system, the MFC and the habenular nuclei, would alter the course of the development the sensitized locomotor response which accompanies the repeated administration of MOR. In Experiments 1 and 2, rats with aspiration lesions of the medial frontal cortex were repeatedly administered MOR, either systemically or directly to the VTA, and their locomotor activity in response to these repeated treatments recorded. Experiments 3 and 4, utilizing the same overall approach as the first two experiments, assessed the effect of electrolytic lesions of the habenular complex on the locomotor response to repeated treatment with MOR, administered either systemically or directly into the VTA.

EXPERIMENT 1

Although a number of studies have demonstrated alterations in the behavioral effects of acute DA-ergic drug administration following lesions of the MFC, none have assessed the effects of lesions on the development of the increases in activation noted during repeated drug treatment. Experiment 1 was carried out to see if lesions of the MFC would alter the development of the sensitization of locomotor activity that accompany the repeated, systemic administration of MOR.

Methods

Subjects

Thirty-seven male Wistar rats (Charles River, St. Constant, P.Q.), weighing 275-300g at the beginning of the experiment, were housed singly in a reverse cycle animal room (light phase 2000 to 0800 h) maintained at 22 degrees C, for at least one week prior to surgery. Standard lab chow and tap water was available ad libitum. All testing of locomotor activity was carried out between 0800h and 1400h.

Surgery

Rats were anesthetized with sodium pentobarbital (Somnotol, M.T.C Pharmaceuticals, Toronto), administered intraperitoneally (i.p.) at a dose of 65.0 mg/kg, which was supplemented with the inhalation anesthetic, methoxyflurane (Metofane, M.T.C. Pharmaceuticals, Toronto) as required. Atropine methyl sulphate (Glaxo Laboratories, Montreal) was also administered to reduce salivation. Under clean conditions, the scalp was retracted, and a dental burr was used to remove the skull overlying the MFC bilaterally. The dura was then retracted, and the MFC was aspirated bilaterally with a glass pipette (tip diameter= 1.0 mm) connected to a suction pump (Fisher Scientific, Montreal). Care was taken to avoid damaging the mid-sagittal sinus. Following aspiration of the MFC, expanded gelatin foam (Gelfoam, Upjohn, Don Mills) soaked in sterile saline was placed in the wound cavity to aid in hemostasis. Once this occurred, the scalp incision was closed with suture clips, and the rats were injected with 30 000 units of intramuscular (i.m.) penicillin G (Ayercillin, Ayerst, Montreal). They were then warmed gently under an incandescent lamp until recovered from anesthesia, and were returned to their home cages. The rats in the sham-operated groups were treated identically to those in the lesioned groups, except that the cortex was not disturbed.

During the 14 day post-surgical recovery period, all rats remained in their home cages, and were habituated to handling for 2 min, once every three days. For the first 2 days following surgery, all rats were administered 30,000 units of penicillin G, i.m., to prevent infection.

Apparatus

In this and all succeeding experiments, the locomotor activity of animals in response to drug treatment was carried out in a bank of twelve individual photocell boxes, each measuring 41 cm by 20 cm by 35 cm. The rear and two side panels of the boxes were made of white-painted plywood, while the roof was made of 5.0 mm wire mesh. The front panel of the boxes consisted of clear Plexiglas, hinged in the middle to provide an opening, and was equipped with a magnetic catch at the top to facilitate secure closure. The floors were comprised of rows of 3 mm stainless steel rods separated by 1 cm, running the length of the box. Each box was equipped with two photocells located 3.5 cm from the box floor spaced evenly along the long axis of the box, to record horizontal activity. Two similarly spaced photocells located 16.5 cm from the box floor on the side panels recorded rearing activity. Photocell beam interruptions were tabulated separately for both horizontal locomotion and rearing activity by an Apple microcomputer located in an adjoining room. The room containing the activity boxes was dimly lit with red light, and a white noise generator maintained an ambient sound level of 75 decibels during the activity testing sessions.

Design and Procedure

The design and procedure of this and all following experiments consisted of two phases, the drug preexposure phase, when animals were repeatedly exposed to either MOR or saline in the activity boxes, and the test for sensitization of the locomotor effects of MOR, when all animals, irrespective of preexposure drug group were administered a challenge injection of MOR.

Following surgery, the MFC lesioned animals were randomly assigned to one of two drug preexposure groups. The animals in the lesioned group MFMOR were administered MOR repeatedly in the activity boxes, while those in the lesioned group MFSAL received saline. The sham-operated animals were similarly assigned to the two corresponding MOR or saline-treated groups, SHAMOR and SHASAL, respectively.

Morphine Preexposure: Fourteen days after surgery the drug preexposure sessions were initiated. Once every three days, on 6 separate occasions, animals in groups MFMOR and SHAMOR were administered i.p. injections of morphine sulphate (10 mg/kg) (BDH Chemicals, Toronto), while those in groups MFSAL and SHASAL were administered 0.9% saline vehicle (1 ml/kg, i.p.). Rats were placed in the activity boxes for 2 h immediately after injection. Rats were left undisturbed in their home cages in between preexposure days.
Test for Sensitization: A test for sensitization of the locomotor response to repeated MOR treatment, administered systemically was carried out on the third day following preexposure day 6. Animals in all four groups were administered one-half the preexposure dose of systemic MOR (5 mg/kg i.p.) before being placed in the activity boxes for 2 h.

Histology

At the end of this and all succeeding experiments, rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with 0.9% saline followed by 10% formaldehyde solution. The brains were then removed from the skull and stored in 10% formaldehyde solution for at least 5 days before slicing. At this time, the brains were frozen, cut in 30 um sections, stained with thionin, and the assessment of lesions was carried out with the aid of a slide viewer and stereotaxic atlas (Pellegrino, Pellegrino and Cushman, 1979).

Statistics

For this and all following experiments, analyses of variance (ANOVA), including repeated measures and simple main effects, were done using BMDP statistical software on a VAX mainframe computer.

Results

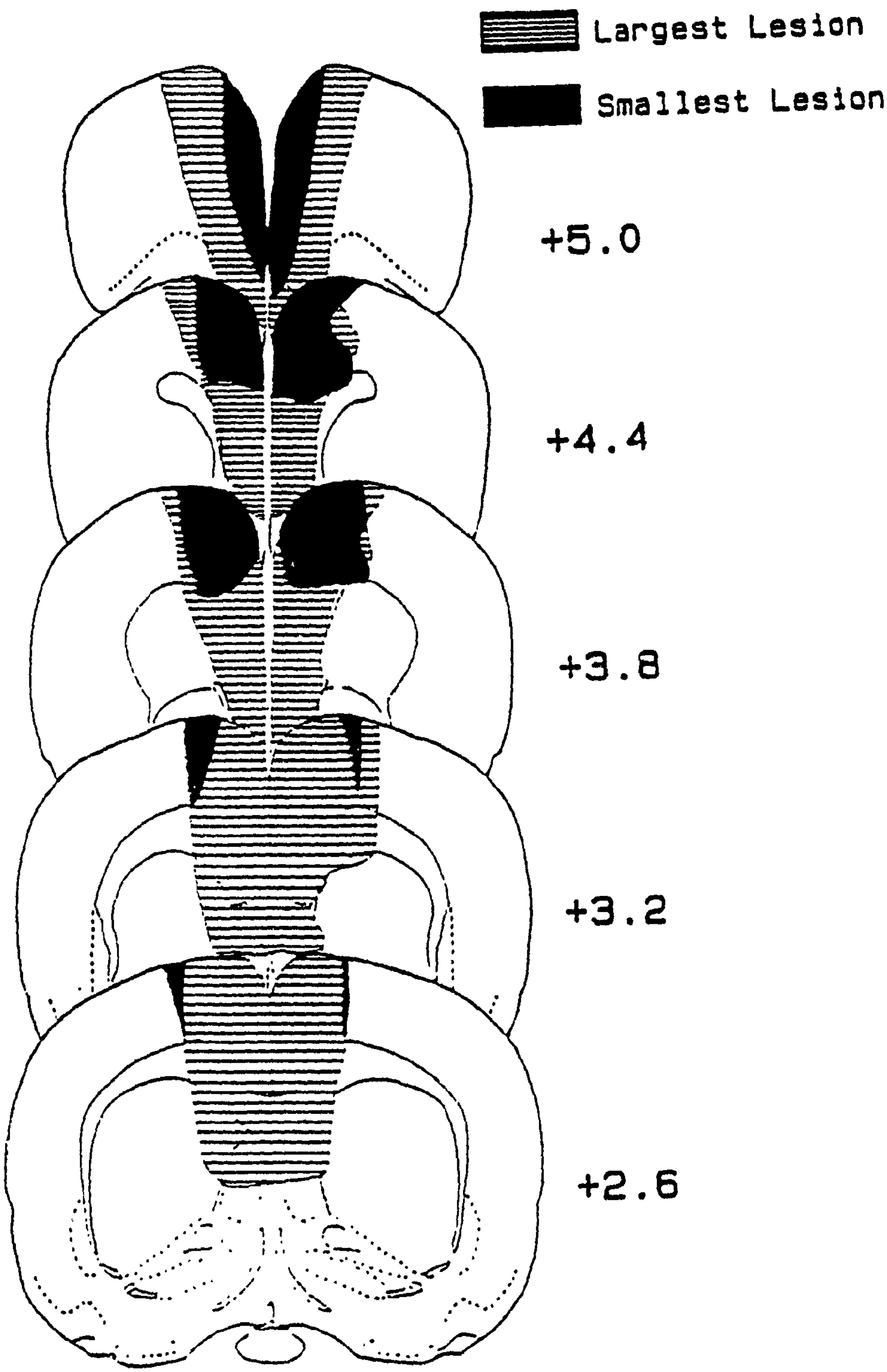
Extent of Lesions

Figure 1 depicts the largest and smallest cortical lesions mapped onto a series of brain atlas plates, through the extent of the MFC (Pellegrino et al., 1979). Other areas typically damaged by the lesions included the cingulate cortex, septal nuclei and corpus callosum. In two animals, the MFC was almost completely spared, and in one case, unilateral destruction of the anterior NAcc was observed; the data from these animals were excluded from analysis. The final number of subjects per group was as follows: group MFMOR, n=7; group MFSAL, n=10; group SHAMOR, n=8; group SHASAL, n=9.

Morphine Preexposure

The effects of lesion of the MFC on the locomotor activity scores seen in response to repeated systemic injections of MOR were assessed by analysis of variance (ANOVA) for lesion x preexposure drug with the two repeated factors of hour following injection, and preexposure day. In this and all succeeding experiments the combined activity scores (horizontal + rearing photocell counts), totaled separately for the first and second hours following drug administration, were used as the dependent measure.

Figure 1 Extent of lesions of the MFC in Experiment 1. Striped areas represent the largest lesion and shaded areas the smallest.


Largest Lesion
Smallest Lesion
+5.0
+4.4
+3.8
+3.2
+2.6

The mean combined activity scores for each group on the 6 preexposure days are depicted for the first and second hours separately in Figure 2.

As expected, MOR initially depressed activity in both the lesioned and sham-operated groups during the first hour (Figure 2a). In the second hour, however, MOR-treated animals showed higher levels of activity than did those treated with saline (Figure 2b). This biphasic effect of MOR is reflec i in a significant preexposure drug x hour interaction [$F(1,30)=49.39$, $p < .0001$].

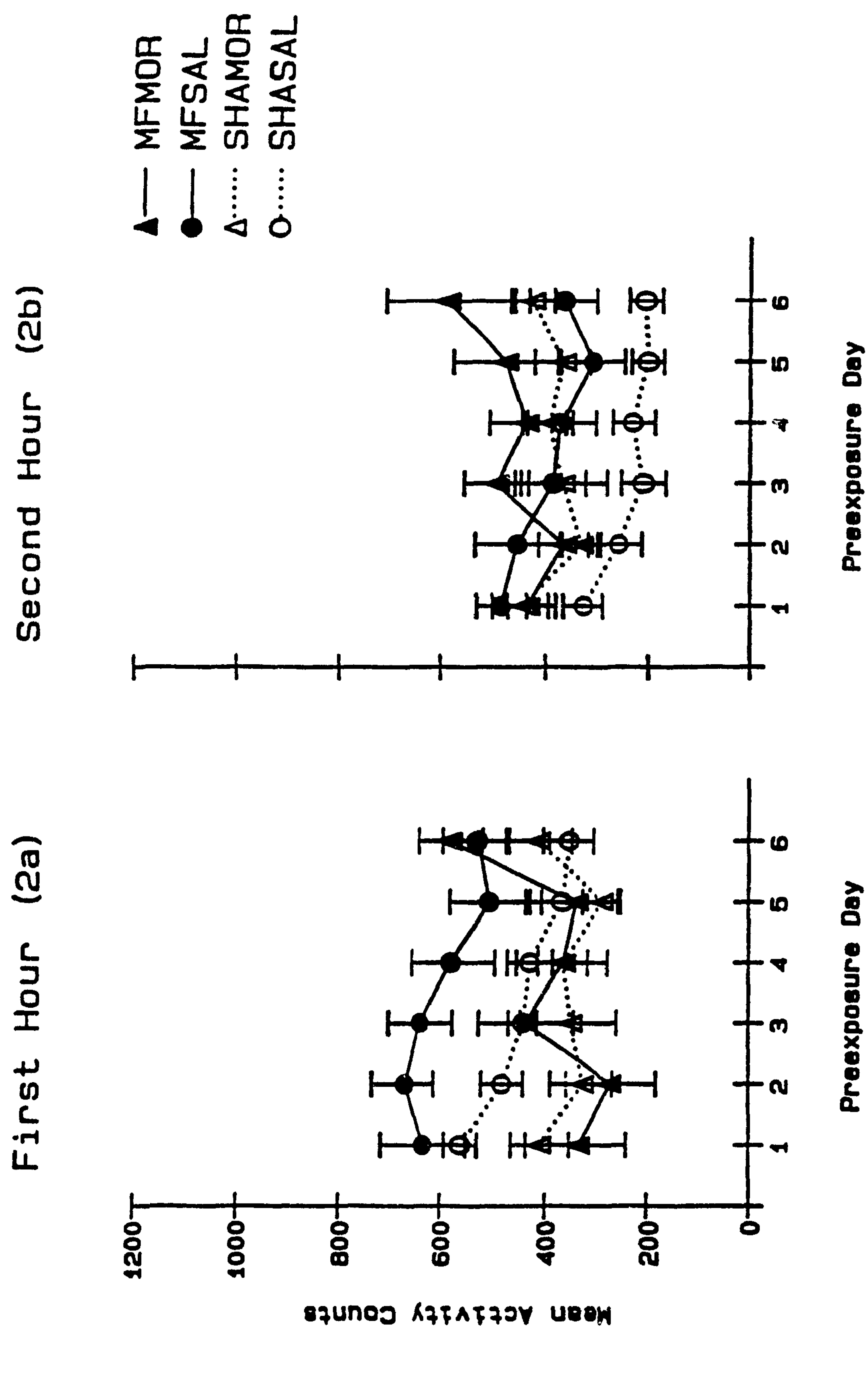
There was also a significant preexposure drug x day interaction [$F(5,150)=9.33$, $p < .0001$], reflecting the fact that the activity of MOR-treated animals tended to increase over days, while the activity of those treated with saline tended to decrease.

There was a significant main effect of lesion [$F(1,30)=4.60$, $p < .05$]. Animals with lesions of the MFC tended to be more active than sham-operated animals, irrespective of drug treatment. This effect is most clearly evident in the second hour of the preexposure sessions. Lesions of the MFC, interestingly, had no effect on the early-appearing depression of locomotor activity seen following the administration of this dose of MOR. In the first hour, lesioned and sham-operated animals showed a similar degree of depression of locomotor activity in response to MOR.

Test for Sensitization

The combined activity scores from the test for sensitization, when animals in all four groups were administered MOR systemically at one-half the preexposure dose (5.0 mg/kg, i.p.), were subjected to an ANOVA for lesion x preexposure drug x hour of test. The mean

Figure 2 Group mean activity counts (+/- 1 S.E.M.) for the 6 days of MOR preexposure in Experiment 1 plotted separately for the first (2a) and second hours (2b) following MOR or saline administration. Animals in groups MFMOR and SHAMOR received MOR (10 mg\kg, i.p.) while those in groups MFSAL and SHASAL received saline (1 ml/kg, i.p.).



First Hour (2a)
Second Hour (2b)
MFMOR
MFSAL
SHAMOR
SHASAL
Mean Activity Counts
1200
1000
800
600
400
200
0
1
2
3
4
5
6
Preexposure Day

scores in the first and second hours for each group are shown in Figure 3.

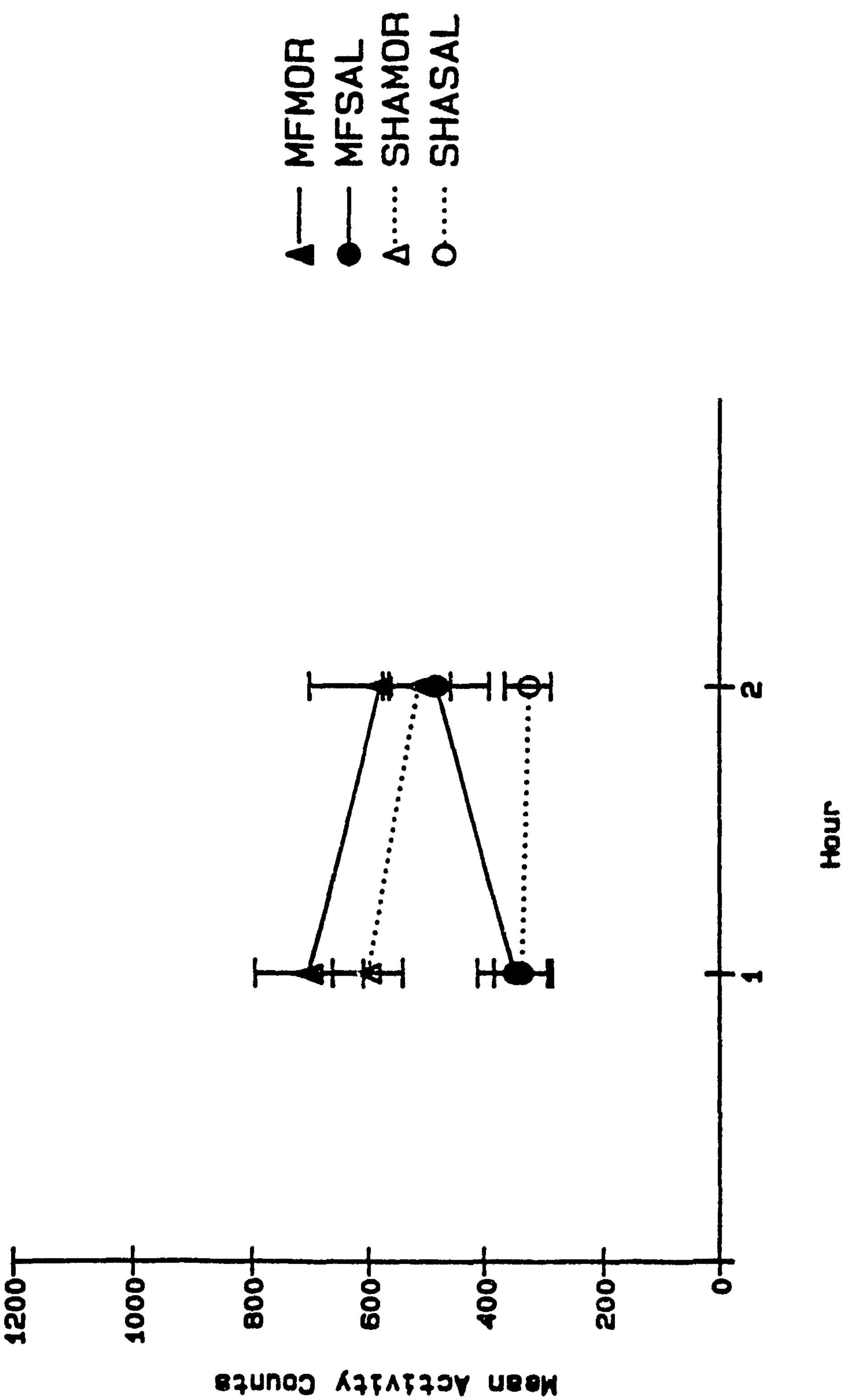
There was a significant effect of preexposure drug [$F(1,30)=11.10$, $p < .005$]. As expected, rats previously exposed to repeated injections of MOR showed higher levels of activity in response to MOR challenge in the test for sensitization than did the control animals receiving MOR for the first time.

Lesioned rats previously exposed to saline showed an enhanced response to challenge with MOR relative to sham operated animals preexposed to saline, during the second hour of the test for sensitization. Analysis of simple main effects revealed that the effect of hour was significant only in this group [$F(1,30)=6.37$, $p < .05$].

Discussion

This experiment was carried out to test the hypothesis that lesions of the MFC might, by releasing mesolimbic DA neurons from inhibitory control, facilitate the development of sensitization to the repeated administration of MOR. No evidence for this was found. A major finding of this experiment, however, was that the type of cortical ablation used in this experiment led to enhanced levels of spontaneous activity which persisted over the days of repeated testing (Figure 2). Rats with such lesions differed little from sham-operated animals on the first day of exposure to the testing environment, but, unlike that of intact animals, their locomotor

Figure 3 Group mean activity counts (+/- 1 S.E.M.) during the test for sensitization in Experiment 1 for the first and second hours following MOR administration. Animals in all four groups were administered MOR (5.0 mg/kg, i.p.).


MFMOR
MFSAL
SHAMOR
SHASAL
Mean Activity Counts
0
200
400
600
800
1000
1200
Hour
1
2

activity stayed at high levels across testing sessions. These findings may serve to explain the lack of effect of non-selective lesions of the MFC on locomotor activity seen in a number of previous studies (e.g. Isaac et al., 1989). In those studies, in which no effects on activity were found, lesion-induced differences in locomotor activity were assessed in a single test which was often of short duration.

In the present study, it was found that during the drug preexposure phase the activating effects of ablation of the MFC summated with the late-appearing enhancement of activity following the systemic administration of MOR. As discussed in the introduction, the locomotor-activating effect of MOR is correlated with enhanced DA-ergic activity in the NAcc (Kalivas & Duffy, 1987; Latimer et al., 1987; Di Chiara & Imperato, 1988). The present findings are consistent, therefore, with those of earlier studies suggesting that cortical lesions potentiate the locomotor responses to DA agonists through their releasing effects on the activity of the mesolimbic DA system (Carter & Pycock, 1978b, 1980; Pycock, Kerwin & Carter, 1980; Oades et al., 1986; Jaskiw et al., 1989). The lesions, however, did not appear to affect the development of sensitization to MOR per se. On the test for sensitization, when all animals were challenged with injections of MOR, the magnitude of the differences in locomotor activity between the lesioned and sham-operated groups preexposed to MOR, and the lesioned and sham-operated groups preexposed to saline were similar.

Finally, the lack of an effect of lesions on the early-appearing depression of locomotor activity, seen after the systemic administration of MOR, suggests that these depressant effects arise from actions of MOR in areas of the brain not directly regulated by the MFC.

EXPERIMENT 2

The preceding experiment demonstrated that lesions of the MFC did not affect the development of the sensitization of locomotor activity seen with the repeated, systemic administration of MOR, although they did enhance baseline locomotor activity. In order to extend these findings, it was decided to carry out a second experiment, this time repeatedly applying MOR directly into the VTA, the brain area where MOR acts to produce increased locomotion, via a disinhibitory effect on the mesolimbic DA system.

Methods

Subjects

Twenty-one male Wistar rats (Charles River, St. Constant, P.Q.) were housed singly in flat-bottomed cages containing wood shavings as bedding in an animal room maintained at 22 degrees C (light phase 0800 to 2000 h), for at least one week prior to surgery. Standard lab chow and tap water was available ad libitum. All testing of locomotor activity was carried out between 0800h and 1400h.

Surgery

Animals were prepared for surgery as in Experiment 1. All rats were implanted bilaterally with 22 gauge stainless steel cannulae (Plastics One Inc., Roanoke, VA) aimed at the VTA (coordinates relative to bregma: A-P= -3.6 mm; LAT= 0.6 mm; D-V= -7.4 mm; toothbar set at +5.0 mm) using standard stereotaxic equipment (David Kopf Instruments, Tujunga, CA). Cannulae were implanted at an angle 16 degrees from the vertical, and were held in place with a cap of dental acrylic anchored to the skull with jeweller's screws. After surgery, stainless steel obturators which protruded 1 mm from the cannula tips were inserted into each cannula to keep them free from dirt; these were removed only during infusions of drug or vehicle solution.

In addition to cannulation of the VTA, the rats in the lesioned groups received bilateral aspiration lesions of the MFC, using the same procedure as described in Experiment 1, immediately prior to implantation of the VTA cannulae. The rats that received sham lesions of the MFC were treated identically to those in the lesion groups, except that the cortex was left undisturbed. During the post-operative period, the rats were treated identically to those in Experiment 1.

Apparatus

The locomotor activity of the animals in response to the repeated, intra-VTA application of MOR was measured in the same activity boxes used in Experiment 1.

Measurement of Stereotypical Behavior

It was observed that lesioned animals administered saline during the preexposure sessions showed stereotypical behaviors compared to animals in the other groups in response to the challenge injection of intra-VTA MOR during the test for sensitization. In order to quantify this response, videotapes were made of the animals in all four groups in the activity boxes during the test for sensitization, from which stereotypy scores for each rat were later derived using the following scoring procedure. Each rat was observed for 2-10 s intervals separated by 50 s every 10 min, beginning 10 min after drug administration, throughout the duration of the 2 h session. For each interval, the most prominent behavior engaged in by each rat was recorded. In order to simplify the scoring of the stereotypy data, and to provide a more conservative estimate of this behavior, it was decided to express the stereotypy of each animal as the percentage of observation intervals where marked stereotypical behavior was observed out of the total number of observations. A morphine-treated rat was deemed to be showing marked stereotypical behavior if it demonstrated repetitive licking, gnawing or head-bobbing behavior for at least half of a particular 10 sec observation period.

In order to verify that this stereotypical response appeared only in lesioned animals preexposed to saline, and only in response to a challenge application of MOR to the VTA, the behavior of both lesioned and sham-operated rats administered MOR during the preexposure sessions was similarly quantified during preexposure days 2, 3, 4, 5 and 6.

Design and Procedure

The experiment consisted of two phases, the MOR preexposure phase, and the testing phase. Following surgery, the lesioned animals were randomly assigned to one of two preexposure drug groups. Lesioned animals in group MFMOR were administered MOR to the VTA in the activity boxes, while the lesioned rats in group MFSAL received saline. The sham-operated animals were similarly assigned to the two corresponding MOR or saline-treated groups SHAMOR and SHASAL.

Intracranial Drug Infusions: Following recovery from surgery, rats were brought to the activity testing room in groups of 8, and received bilateral intra-VTA infusions of either MOR or physiological saline immediately preceding their placement into the activity boxes. Twenty-eight gauge stainless steel injection cannulae (Plastics One, Roanoke, VA) connected to 1 ul microsyringes (Hamilton, Reno, Nevada) via PE-20 polyethylene tubing (Clay Adams, Parsippany, New Jersey)) were inserted in the guide cannulae, and protruded 1 mm from the guide cannulae tips. The intracranial infusions were made bilaterally in a volume of 0.5 ul/side over a period of 45 s and were followed by a further 45 s diffusion period, after which the injection cannulae were removed, the blockers replaced, and the rat placed in the activity box. The integrity of the intracranial infusion equipment was assessed before each individual injection. Two bilateral infusion setups were used, which allowed two animals to be injected simultaneously. The average time taken to administer drug or vehicle to the 8 animals in each session was 20 min, so the order in which individual animals were injected was randomized across sessions.

MOR for intracranial infusion was dissolved in physiological saline and administered at a dose of 5.0 ug/0.5 ul/side. Rats administered saline received bilateral infusions of the same volume of physiological saline.

Drug Preexposure: Animals in groups MFMOR and SHAMOR received intra-VTA infusions of MOR in the activity boxes once every three days, on 6 separate occasions, while animals in groups MFSAL and SHASAL were administered saline. Animals received the intracranial injections immediately prior to their placement in the activity boxes for 2 h. Immediately following termination of each preexposure session, animals in all four groups were administered gentamicin sulphate (Schering, Pointe Claire, P.Q.) in a dose of 1 mg/kg, i.m., to guard against infection. Rats were left undisturbed in their home cages on the two days between preexposure sessions. Test for Sensitization: On the test day for the development of sensitization to repeated intra-VTA MOR, which was carried out the third day following preexposure day 6, the animals in all four groups received intra-VTA infusions of MOR (5.0 ug/side) immediately prior to being placed in the activity boxes for 2 h.

Results

Assessment of Lesions and Placement of VTA Cannulae

Figure 4 depicts the largest and smallest lesions of the MFC. The cortical lesions tended to be somewhat smaller than those made in the previous study. All animals, however, were observed to have sustained damage to the MFC.

Figure 5 depicts the location of the cannulae aimed at the VTA. All cannulae were observed to be within this region. The data from one lesioned animal that became ill with a pulmonary infection during the preexposure phase was not included in the analysis. The final number of subjects in each group was as follows: group MFMOR, n=6; group MFSAL, n=5; group SHAMOR, n=5; group SHASAL, n=4.

Morphine Preexposure

To assess the effects of lesions of the MFC on the locomotor response to MOR applied repeatedly to the VTA, an ANOVA for lesion x preexposure drug with the repeated factors of hour following administration and preexposure day was carried out on the combined activity scores for the 6 days of drug preexposure.

As expected, there was a significant effect of preexposure drug [$F(1,16)=12.00$, $p < .005$] due to the fact that rats administered MOR to the VTA showed higher levels of activity than those administered saline (Figure 6). The drug x day interaction was also significant [$F(5,80)=11.63$, $p < .0001$], reflecting the fact that the locomotor activity of rats administered MOR increased as a function of repeated exposure, while the activity of rats administered saline decreased over days.

There was no significant effect of lesion on the locomotor activation induced by the repeated intra-VTA application of MOR. There was also no evidence for a lesion-induced increase in baseline locomotor activity. It was noted, however, that the mean locomotor scores of the sham-operated group administered saline to the VTA were somewhat higher than those of the lesioned group administered saline (Figure 6). This arose due to the fact that two of the four animals in the sham-operated group administered saline showed abnormally high levels of activity. The locomotor activity of these two rats, furthermore, did not decrease with repeated exposure to the activity boxes as would normally be expected. No explanation for the anomalous behavior of these two animals could be found.

Figure 4 Extent of lesions of the MFC in Experiment 2. Striped areas represent the largest lesion and shaded areas the smallest.

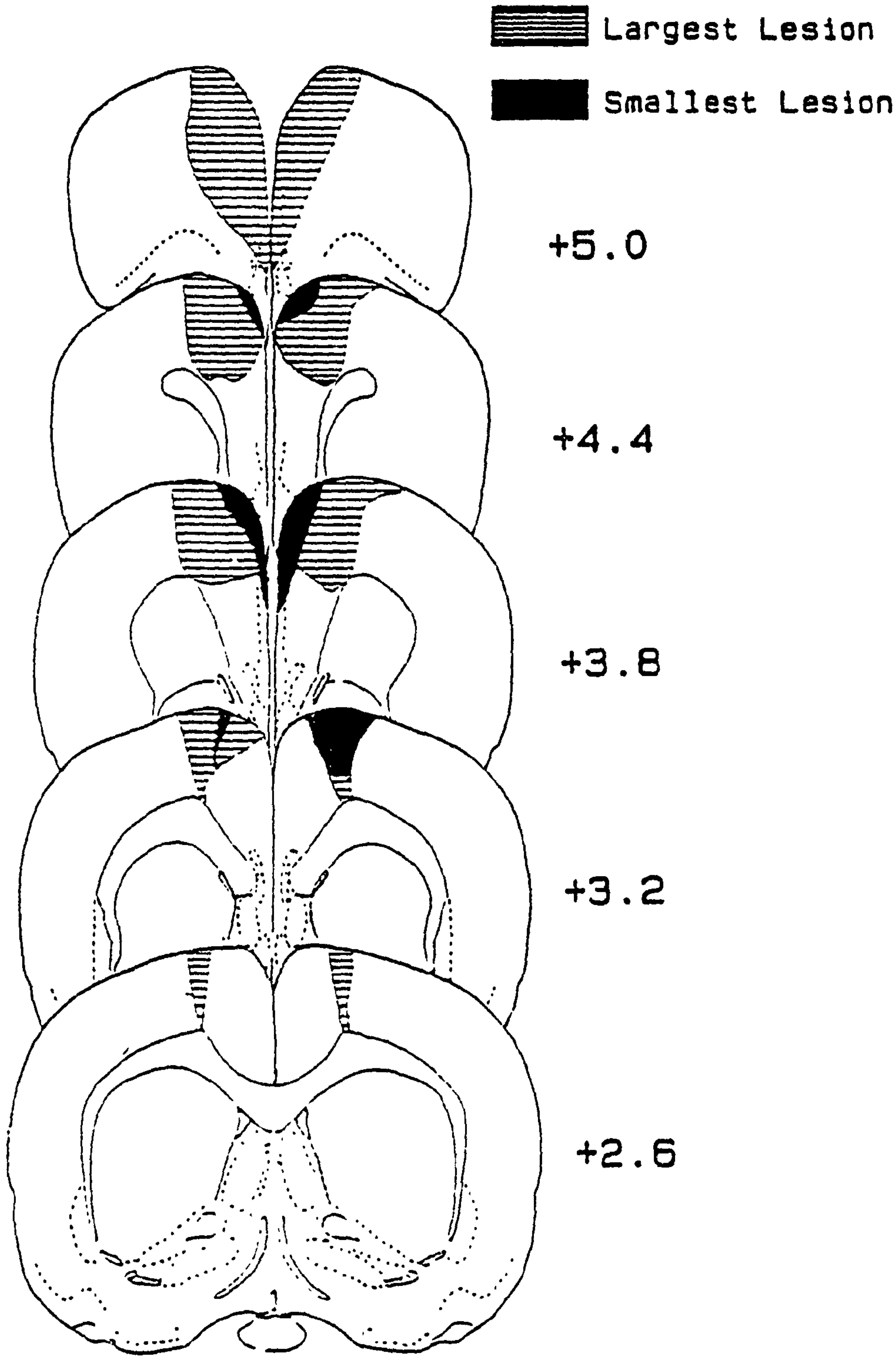
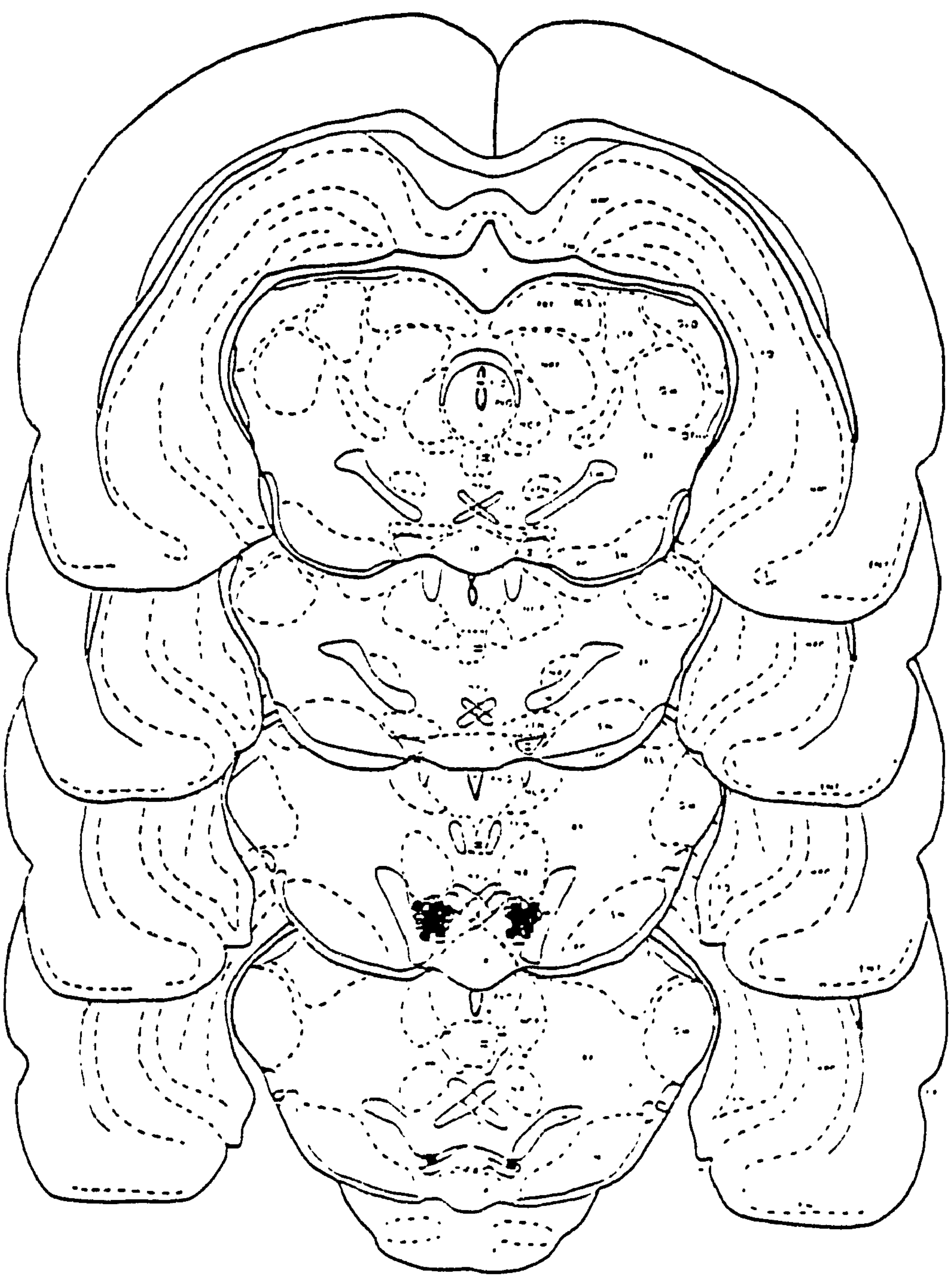

Largest Lesion
Smallest Lesion
+5.0
+4.4
+3.8
+3.2
+2.6

Figure 5 Location of the tips of guide cannulae aimed at the VTA in Experiment 2.



-3.4

-3.6

-3.8

-4.0

Test for Sensitization

To assess the effects of lesions on the development of the sensitized locomotor response to MOR administered repeatedly to the VTA, an ANOVA for lesion x preexposure drug, with the repeated factor of hour of test, was carried out on the combined activity scores following a challenge dose of intra-VTA MOR administered to all four groups. The mean scores of the four groups for each hour are shown in Figure 7.

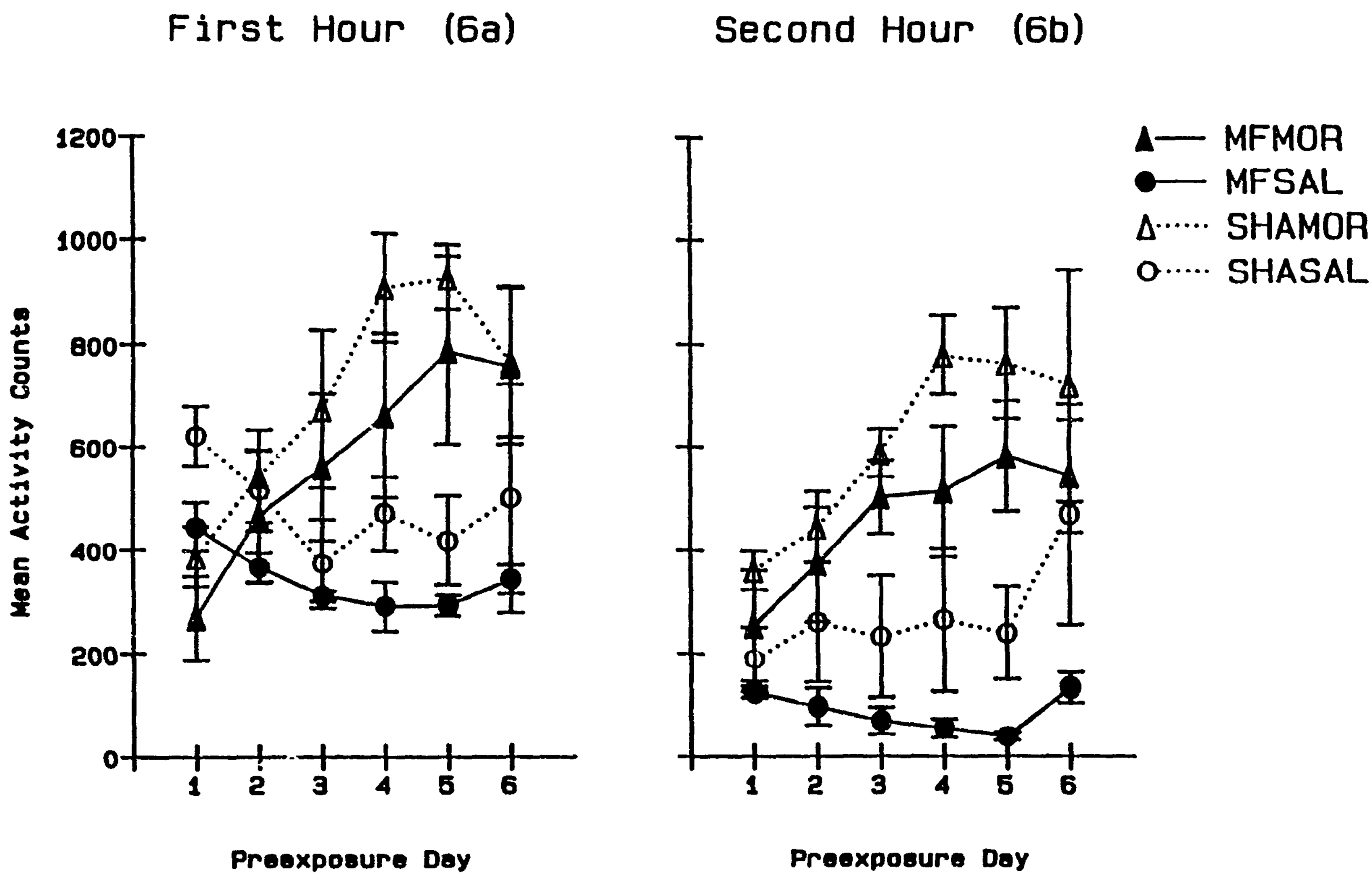
There was a significant effect of preexposure drug [$F(1,16)=14.59$, $p < .005$]; as expected, rats previously administered MOR were more active than those that had previously received saline.

There was also a significant effect of lesion [$F(1,16)=5.05$, $p < .05$]. This arose from the fact that rats with lesions of the MFC that had been previously treated with saline, showed a depression of activity relative to the other groups in response to MOR challenge. Observation of these rats during the test for sensitization revealed that they were engaging in stereotyped behaviors, including prolonged bouts of gnawing, licking and repetitive head movements in one location. It was therefore desirable to provide some index of the degree of stereotypy in these rats relative to that of the other groups.

Stereotypical Behavior in Lesioned Rats Induced by Challenge With Intra-VTA Morphine

Figure 8 illustrates the relationship between locomotor activity and stereotypical behavior during the test for sensitization for groups MFSAL and SHASAL by plotting the mean

Figure 6 Group mean activity counts (+/- 1 S.E.M.) for the 6 days of MOR preexposure in Experiment 2 plotted separately for the first (6a) and second hours (6b) following MOR or saline administration. Animals in groups MFMOR and SHAMOR were administered MOR (5.0 ug/0.5 ul saline/side) bilaterally to the VTA, while those in groups MFSAL and SHASAL received equal volumes of saline into the VTA.



First Hour (6a)
Second Hour (6b)
Mean Activity Counts
1200
1000
800
600
400
200
0
1
2
3
4
5
6
Preexposure Day
MFMOR
MFSAL
SHAMOR
SHASAL

percentage of observations with stereotypical behavior (Figure 8a), beside the mean combined activity scores (Figure 8b) of each group, for each hour of the test for sensitization. It can be seen that group MFSAL shows more stereotypical behavior relative to group SHASAL in response to MOR challenge. A two way ANOVA for lesion x preexposure drug with the repeated factor of hour of test was carried out on the percentage stereotypy scores of the animals in all four groups during the test for sensitization. In response to MOR challenge, lesioned animals that had been preexposed to saline showed significantly more stereotypical behavior than did the other groups. This is reflected in a significant effect of lesion [$F(1,16)=10.79$, $p < .005$)].

Interestingly, lesioned animals administered MOR during the preexposure sessions did not evidence any stereotypical behavior in response to the drug at any time. Figure 9 illustrates the relationship between locomotor activity and stereotypical behavior by plotting the mean combined activity scores (Figure 9a) alongside the mean % stereotypical acts observed (Figure 9b) of groups MFMOR and SHAMOR in the second hour of preexposure days 2, 3, 4, 5 and 6. It can be seen that group MFMOR did not differ from group SHAMOR in the level of stereotypical behavior displayed during the MOR preexposure phase.

Discussion

Although the repeated injection of MOR into the VTA resulted in the expected development of sensitization in this experiment,

Figure 7 Group mean activity counts (+/- 1 S.E.M.) during the test for sensitization in Experiment 2 for the first and second hours following MOR administration. Animals in all four groups were administered MOR (5.0 ug/0.5 ul saline/side) bilaterally to the VTA.

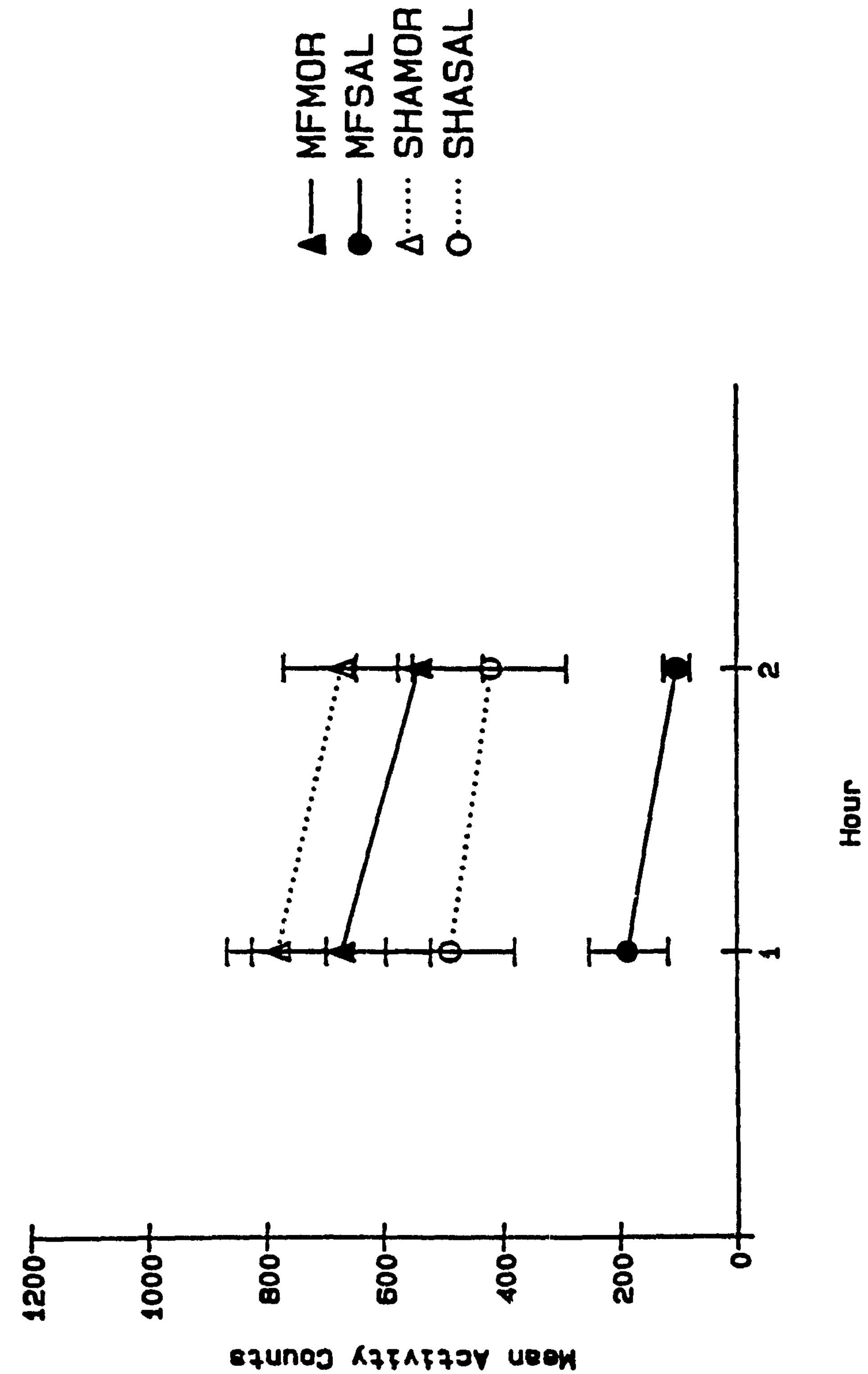

MFMOR
MFSAL
SHAMOR
SHASAL
Mean Activity Counts
0
200
400
600
800
1000
1200
1
2
Hour

Figure 8 Group mean % of observations with stereotypical behavior (+/- 1 S.E.M.) (8a) in groups MFSAL and SHASAL in comparison to locomotor activity (+/- 1 S.E.M.) (8b) in the two hours following intra-VTA MOR administration during the test for sensitization in Experiment 2.

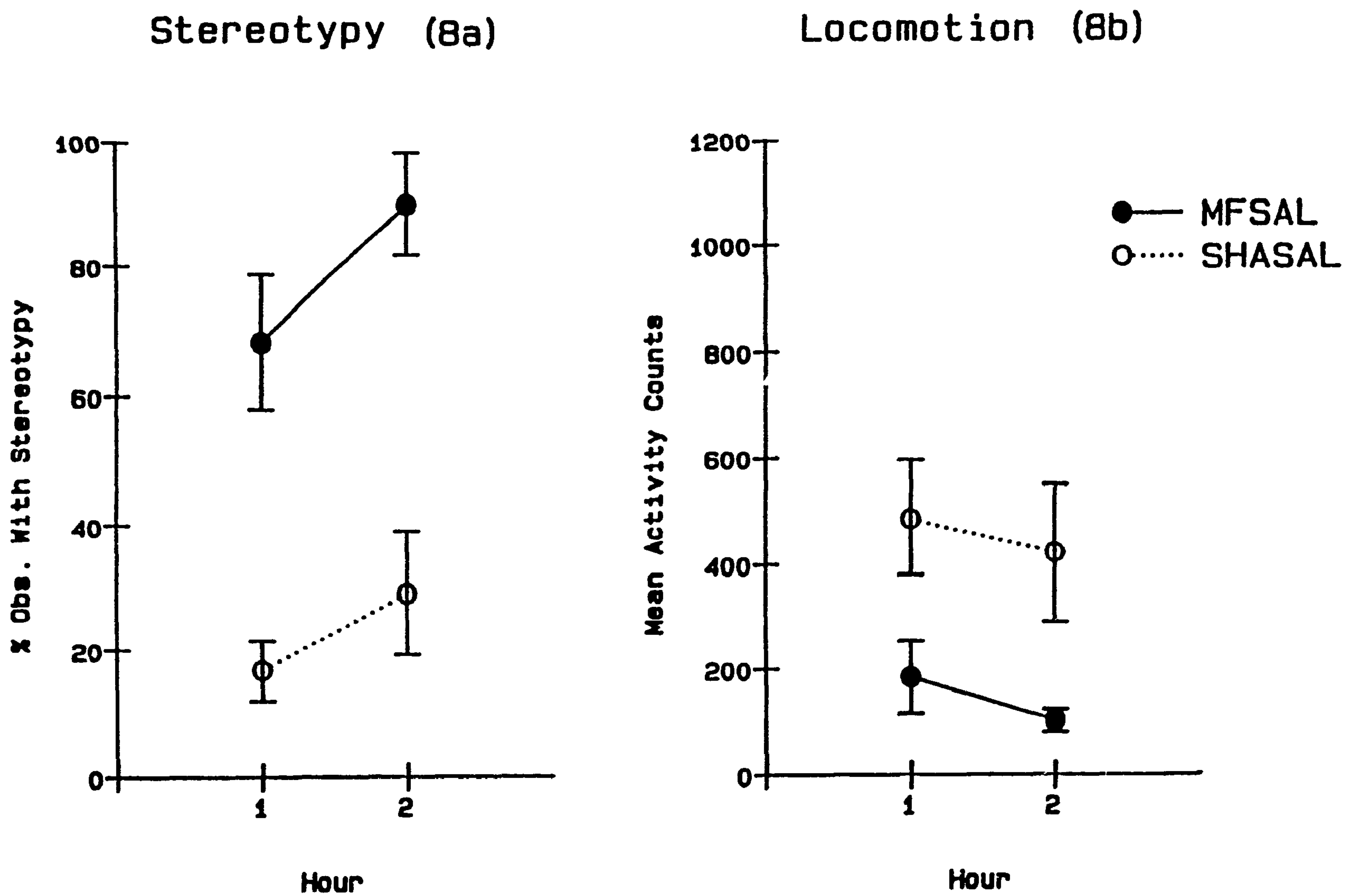
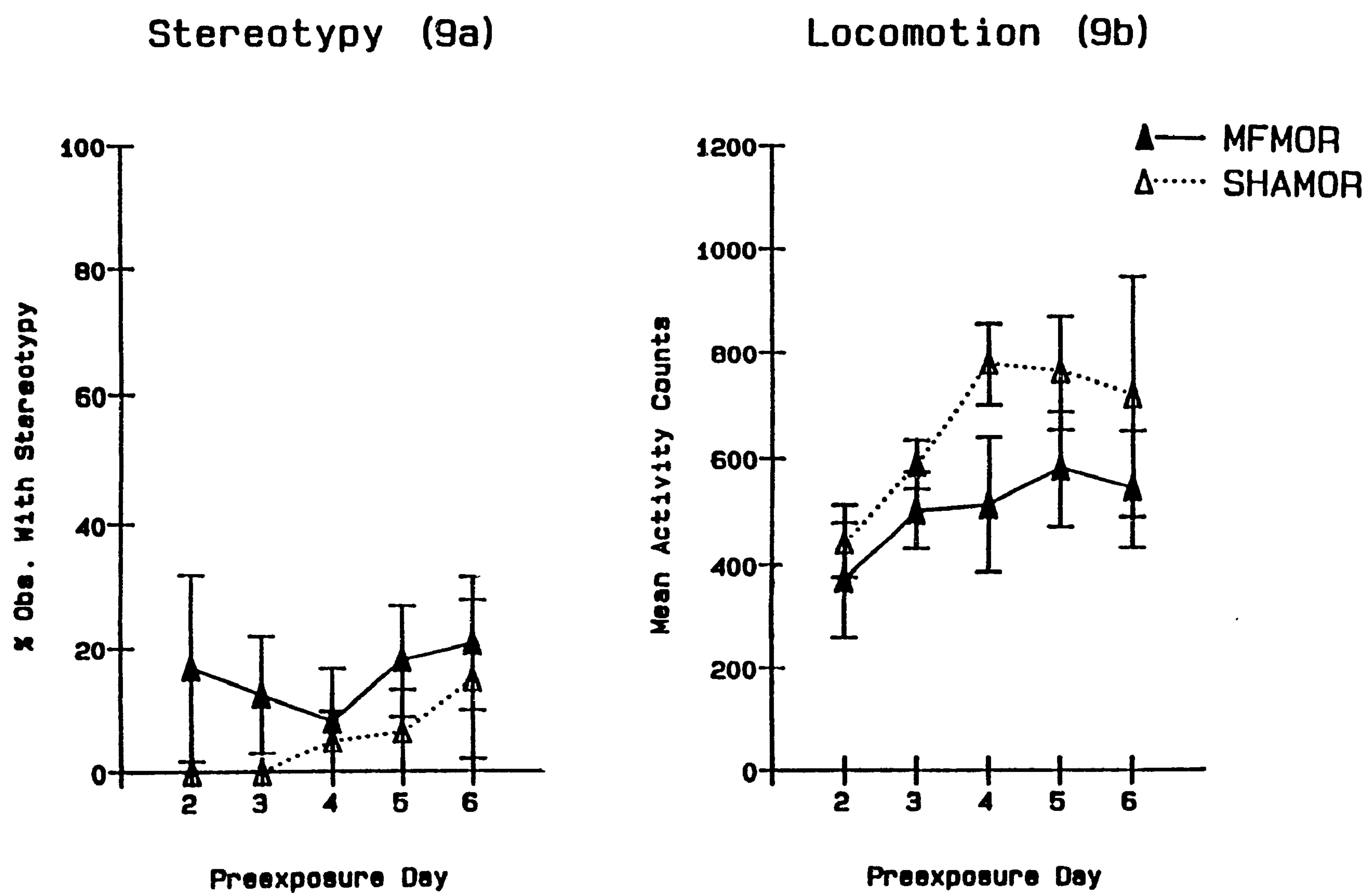

Stereotypy (8a)
% Obs. With Stereotypy
100
80
60
40
20
0
1
2
Hour
Locomotion (8b)
Mean Activity Counts
1200
1000
800
600
400
200
0
1
2
Hour
MFSAL
SHASAL

Figure 9 Group mean % of observations with stereotypical behavior (+/- 1 S.E.M.) (9a) in groups MFMOR and SHAMOR in comparison to locomotor activity (+/- 1 S.E.M.) (9b) in the second hour following intra-VTA MOR administration on MOR preexposure days 2, 3, 4, 5 and 6 in Experiment 2.



Stereotypy (9a)
% Obs. With Stereotypy
100
80
60
40
20
0
2
3
4
5
6
Preexposure Day
Locomotion (9b)
MFMOR
SHAMOR
Mean Activity Counts
1200
1000
800
600
400
200
0
2
3
4
5
6
Preexposure Day

lesions of the MFC did not appear to potentiate the activity-enhancing effects over the days of repeated MOR administration to the VTA. Cortical lesions, furthermore, did not lead to an enhancement of the acute locomotor response to MOR applied to the VTA, a result to be contrasted with that of Experiment 1 where it was found that such lesions did enhance the locomotor response to systemically-administered MOR. It is possible that the lesion-induced enhancement of the activating effects of MOR is expressed only when the drug is administered systemically, and not when it is applied directly to the cell bodies of the mesolimbic DA cells. MOR has been shown to induce locomotor activation independent of the activity of the DA systems when applied directly to the NAcc, an area which receives input from the MFC. It is possible, therefore, that the lesion-induced potentiation of the locomotor response to systemically-administered MOR seen in Experiment 1 was caused by a facilitation of the locomotor effects of MOR acting in the NAcc due to the destruction of MFC afferents to this nucleus. Indirect support for this notion comes from the observation that destruction of the frontal cortex reduces the level of mRNA coding for the precursor of enkephalin in the CPu, suggesting that cortical afferents are necessary for the maintenance of normal levels of this endogenous opiate in the CPu (Uhl, Navia & Douglas, 1988). The chronic reduction in enkephalin levels in striatal regions following cortical ablation may have resulted in the supersensitivity of opiate receptors in the NAcc, which would enhance the behavioral response to MOR when administered systemically, but not when applied to the VTA. An alternative, and possibly more parsimonious hypothesis explaining the lack of a lesion effect on VTA MOR-induced hyperactivity is the fact that the cortical lesions in the present experiment were smaller than those made in Experiment 1. It appears, however that the lesions, although smaller, did alter the behavioral effect of MOR when applied to the VTA, in that lesioned rats previously exposed to saline showed more stereotypical behavior than those in the other groups in response to a challenge administration of intra-VTA MOR.

Neither did the lesions in the present experiment affect baseline locomotor activity. Saline-treated lesioned animals were no more active than sham-operated animals administered saline. This finding is to be contrasted with those of Experiment 1, where lesions of the MFC led to significant increases in spontaneous locomotor activity. There are a number of possible explanations for this disparity. As stated previously, the lesions of the MFC in this experiment tended to be somewhat smaller than those made in the first experiment. Although it is not usually found to be the case, the brain trauma induced by the implantation of guide cannulae may itself have had effects on spontaneous activity in this study, thereby masking any effects induced by cortical lesion. The sham-operated rats preexposed to saline showed unexpectedly high levels of activity. Another possible reason is that the animals in the previous experiment were housed under a reversed light cycle, while those in the present one were housed under a regular light cycle. The former were tested in their normally active phase, while those in the present one were tested during their inactive phase. Kolb (1974) consistently found near-significant increases in running wheel activity in rats with aspiration lesions of the MFC only when animals were tested during the dark phase of their diurnal cycle. Further support for this notion comes from the results of Hepler & Lerer (1986) who found that lesions of the basal forebrain in rats enhanced baseline locomotor activity only when animals were tested during the active phase of their diurnal cycle. On the other hand, Oades et al., (1986), however, found no differences in basal activity between animals with DA-selective lesions of the MFC and sham-operated animals, when tested during either the active or inactive phase of their diurnal cycle.

It is difficult to explain the stereotypical behavior shown by the lesioned animals preexposed to saline when challenged with MOR, especially since no stereotypical behavior was noted in lesioned animals receiving chronic MOR treatment. Since the MFC sends projections to the cell body and terminal regions of the nigrostriatal DA system in addition to the mesolimbic DA system, it is possible that the disinhibitory effects of lesions on the activity of the nigrostriatal DA-ergic projection formed the basis for the increased performance of stereotyped activity in response to MOR challenge (Carter, 1980, 1982; Gerfen, 1984; Donoghue & Herkenham, 1986). This explanation, however, does not address the absence of stereotyped behavior in lesioned animals administered MOR repeatedly during the preexposure phase of the experiment. A possible explanation for this is that the greater length of the drug-free period following cortical lesion in the saline-treated group formed the basis for their stereotypical response to VTA MOR challenge. The effect may also be explained by the fact that the lesioned rats treated repeatedly with saline were well-habituated to the activity boxes at the time of their first exposure to MOR, while lesioned rats preexposed to MOR were administered the drug upon their first exposure to the boxes. There is little knowledge, however, of the effect of arousal state on the behavioral response to DA-ergic drug administration. It was noted, furthermore, that during the first hour of the first MOR preexposure session, the locomotor activity of both lesioned and sham-operated animals receiving MOR was depressed relative to animals receiving saline (Figure 6a). Previous studies have reported that the intra-VTA infusion of MOR leads to immediate locomotor excitation without depression. This raises the possibility that the cannula placements in this study were in regions of the VTA which project relatively more to striatal regions, thereby enhancing the possibility of stereotypical behavior in response to the application of the drug to the VTA. The injection sites in this experiment tended to be located slightly dorsal within the VTA compared with those used in earlier studies. It is not clear, however, whether this region of the VTA sends a relatively higher proportion of DA-ergic projections to the striatum compared to areas located more ventrally in the VTA.

Although no evidence for enhanced locomotor activity in either the baseline condition or in response to MOR was noted, the fact that lesions of the MFC did increase the incidence of stereotypy in response to a challenge dose of MOR in one group of animals is consistent with the notion that the MFC normally inhibits the activity of the DA systems.

EXPERIMENT 3

Although a number of studies have demonstrated that lesions of the habenula, in particular the LHb, may influence the acute behavioral effects of DA-ergic drug administration, no studies have assessed the effects of such lesions on the behavioral responses seen with the repeated administration of DA-ergic drugs. The purpose of the following experiment was to determine whether bilateral lesions of the habenula nuclei would alter the development of sensitization of the locomotor activation seen with the repeated, systemic administration of MOR.

Methods

Subjects

Twenty-nine male Wistar rats (Charles River, St. Constant, P.Q.), weighing 275-300g at the beginning of the experiment, were housed singly in a reverse cycle animal room (light phase 2000 to 0800 h) maintained at 22 degrees C, for at least one week prior to surgery. Standard lab chow and tap water was available ad libitum. All testing of locomotor activity was carried out between 0800 and 1400 h.

Surgery

Animals were prepared for surgery as in Experiment 1. The rats in the lesion groups received bilateral electrolytic lesions of the habenula complex. The skull overlying the habenula was removed bilaterally with a fine dental burr and the dura punctured with a 26 gauge needle, prior to the introduction of the electrode; care was taken to avoid damaging the mid-sagittal sinus. The electrode was lowered bilaterally to the following coordinates relative to bregma: A-P= -2.2 mm; LAT= +/- 0.70 mm; D-V= -4.8 mm from the dural surface; toothbar set at +5.0 mm). A 1 mA current was applied for 11 s at each site with a D.C lesion generator (Grass Medical Instruments, Quincy, MA); the cathode was connected to the lesion electrode, and a rectal anode completed the circuit. The rats in the sham-operated groups were treated identically to those in the lesion groups, except that no electrode was introduced into the brain. Following the lesion or sham surgical procedure, the scalp incision was closed with suture clips. During the post-surgical recovery period, animals were treated identically to those in Experiment 1.

Apparatus

Measurement of the locomotor activity of the rats in response to drug treatment was carried out in the same activity boxes as in the preceding experiments.

Design and Procedure

Following surgery, the habenular lesioned animals were randomly assigned to one of two drug preexposure groups. The animals in the lesioned group HBMOR were administered MOR repeatedly in the activity boxes, while those in the lesioned group HBSAL received saline. The sham-operated animals were similarly assigned to the two corresponding drug groups, namely, groups SHAMOR and SHASAL.

The experiment consisted of two phases, the MOR preexposure phase, and the testing phase, which immediately followed the preexposure phase.

Morphine Preexposure: After the animals had recovered from surgery, the drug preexposure sessions commenced. Animals in groups HBMOR and SHAMOR were administered morphine sulphate (10 mg/kg, i.p.) (BDH Chemicals, Toronto) or 0.9% saline vehicle in 1 ml/kg volume on six separate occasions, once every three days, while those in groups MFSAL and SHASAL received saline immediately prior to being placed in the activity boxes for 2 hours. On the days between preexposure sessions, animals were left undisturbed in their home cages.

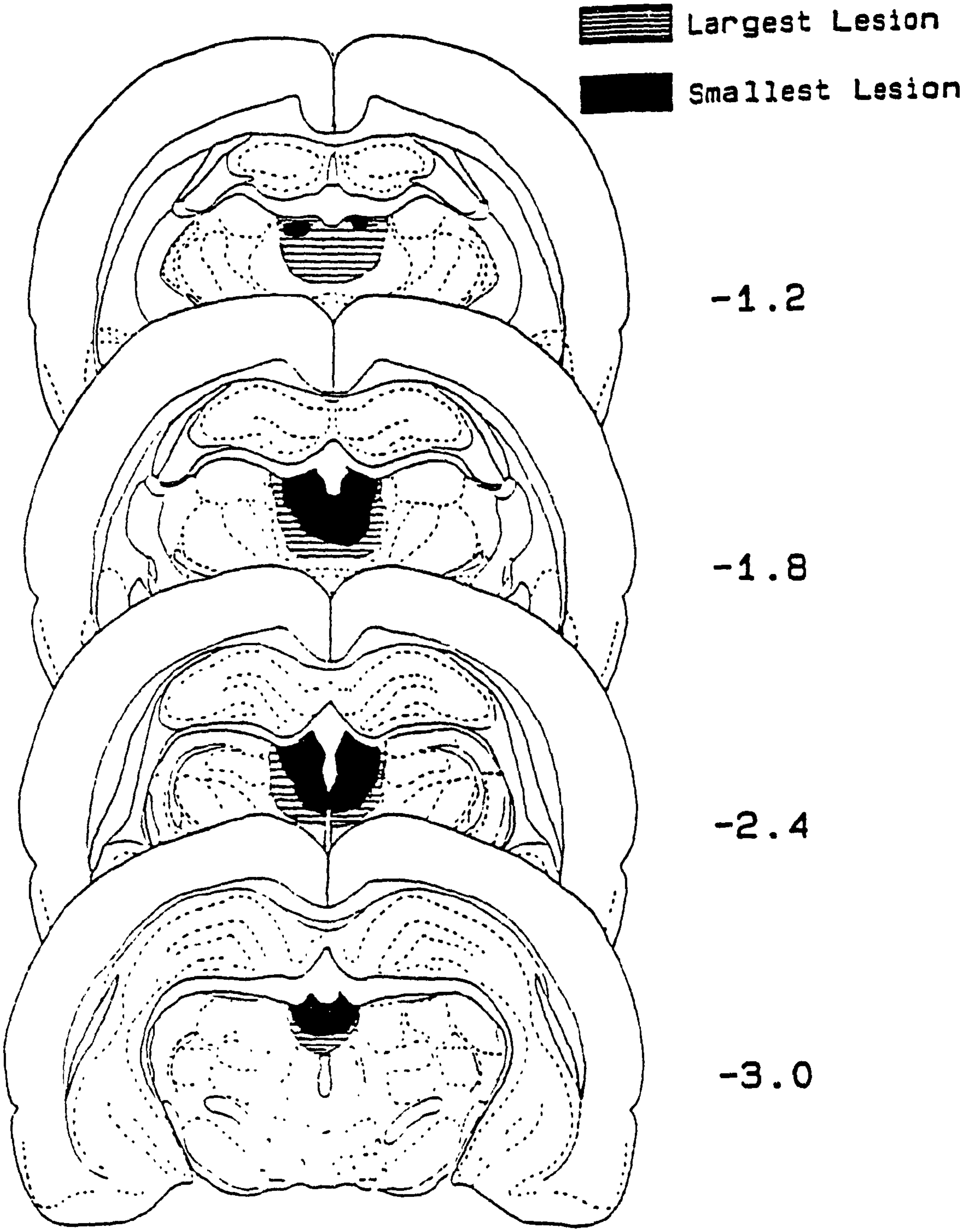
Test for Sensitization: A test for sensitization of the locomotor response to systemic MOR, administered repeatedly, was carried out on the third day following preexposure day 6. Animals in all four groups were administered one-half the preexposure dose of systemic MOR (5 mg/kg i.p.) before being placed in the activity boxes for 2 hours.

Results

Extent of Habenular Lesions

Figure 10 illustrates the largest and smallest habenular lesions. Typically, lesions included bilateral destruction of both medial and lateral habenula, a variable degree of damage to the subjacent midline nuclei of the thalamus and slight damage to the dorsal hippocampus overlying the habenula. In 2 lesioned animals, minimal damage of the habenula complex was observed and the data from these animals were not included in the analyses. The final

Figure 10 Extent of habenular lesions in Experiment 3. Striped areas represent the largest lesion and shaded areas the smallest.


Largest Lesion
Smallest Lesion
-1.2
-1.8
-2.4
-3.0

number of subjects in each group was therefore as follows: group HBMOR, n=6; group HBSAL, n=4; group SHAMOR, n=8; group SHASAL, n=9.

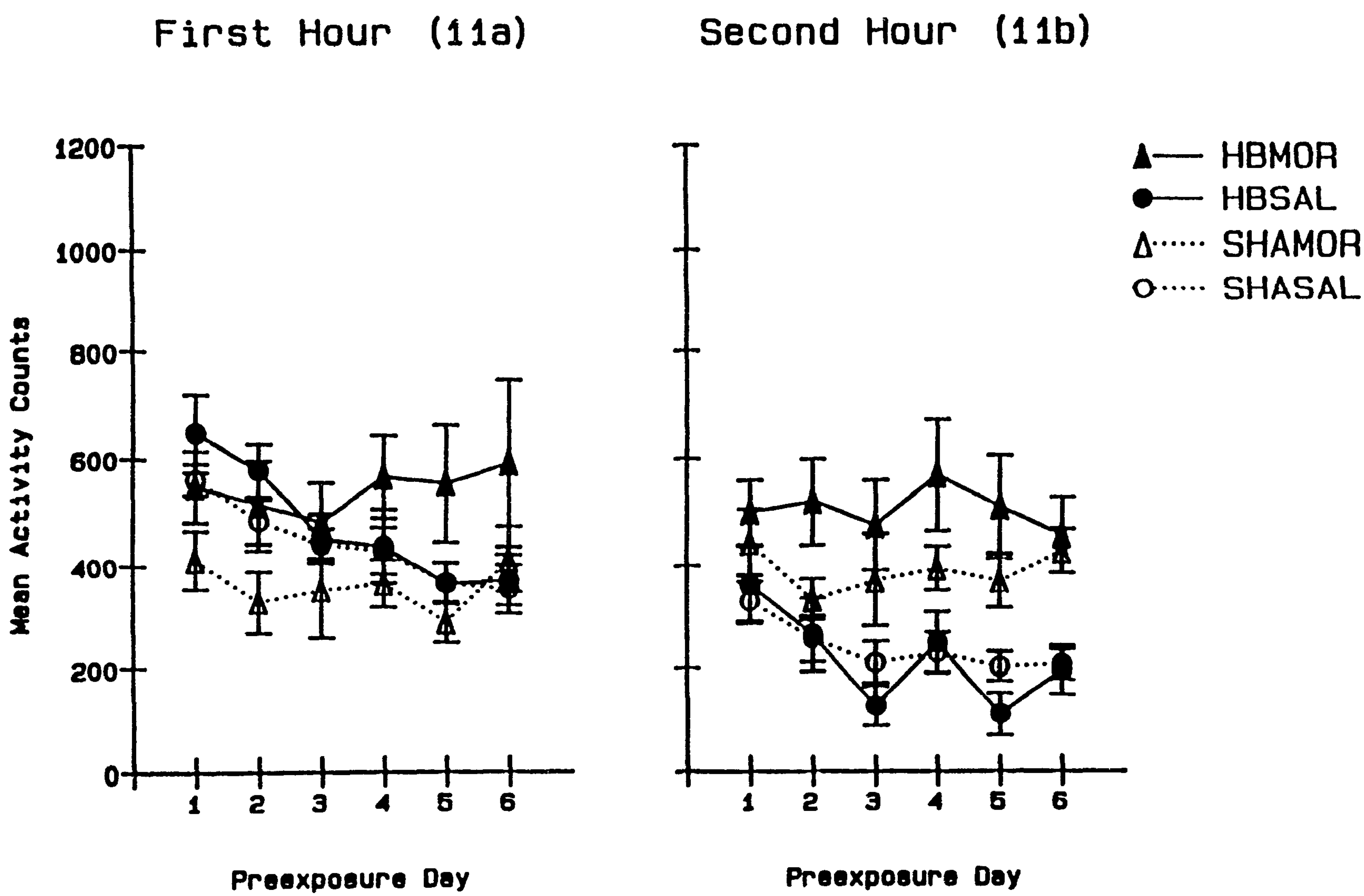
Morphine Preexposure

To assess the effect of habenular lesion on the development of sensitization of the locomotor response to the repeated administration of MOR, administered systemically, an ANOVA for lesion x preexposure drug with the two repeated factors of hour following administration and preexposure day was carried out. The mean combined activity scores of the four groups on the 6 preexposure days are depicted for the first and second hours separately in Figure 11.

As expected, rats administered MOR systemically showed higher levels of locomotor activity than did saline-treated rats [$F(1,23)=4.46$, $p < .05$]. There was also a significant preexposure drug x day interaction [$F(5,115)=4.84$, $p < .001$], reflecting the fact that the locomotor activity of MOR-treated animals increased over repeated treatments, while that of rats administered saline tended to decrease.

Although there was no significant main effect of lesion during the preexposure sessions, there was a significant lesion x hour interaction [$F(1,23)=5.77$, $p < .05$]. This was due to the fact that lesioned animals administered MOR did not show the depression of locomotor activity in the first hour that was seen in sham-operated animals receiving the drug; rather, the activity of lesioned rats treated with MOR tended to increase in the first hour across preexposure sessions, while that of sham-operated rats

Figure 11 Group mean activity counts (+/- 1 S.E.M.) for the 6 days of MOR preexposure in Experiment 3 plotted separately for the first (11a) and second hours (11b) following MOR or saline administration. Animals in groups HBMOR and SHAMOR received MOR (10 mg\kg, i.p.) while those in groups HBSAL and SHASAL received saline (1 ml/kg, i.p.).


First Hour (11a)
Second Hour (11b)
HBMOR
HBSAL
SHAMOR
SHASAL
Mean Activity Counts
1200
1000
800
600
400
200
0
1
2
3
4
5
6
Preexposure Day
Preexposure Day

administered MOR remained depressed. Lesioned animals that received saline, however, showed levels of activity that were similar to those of sham-operated rats administered saline.

Test for Sensitization

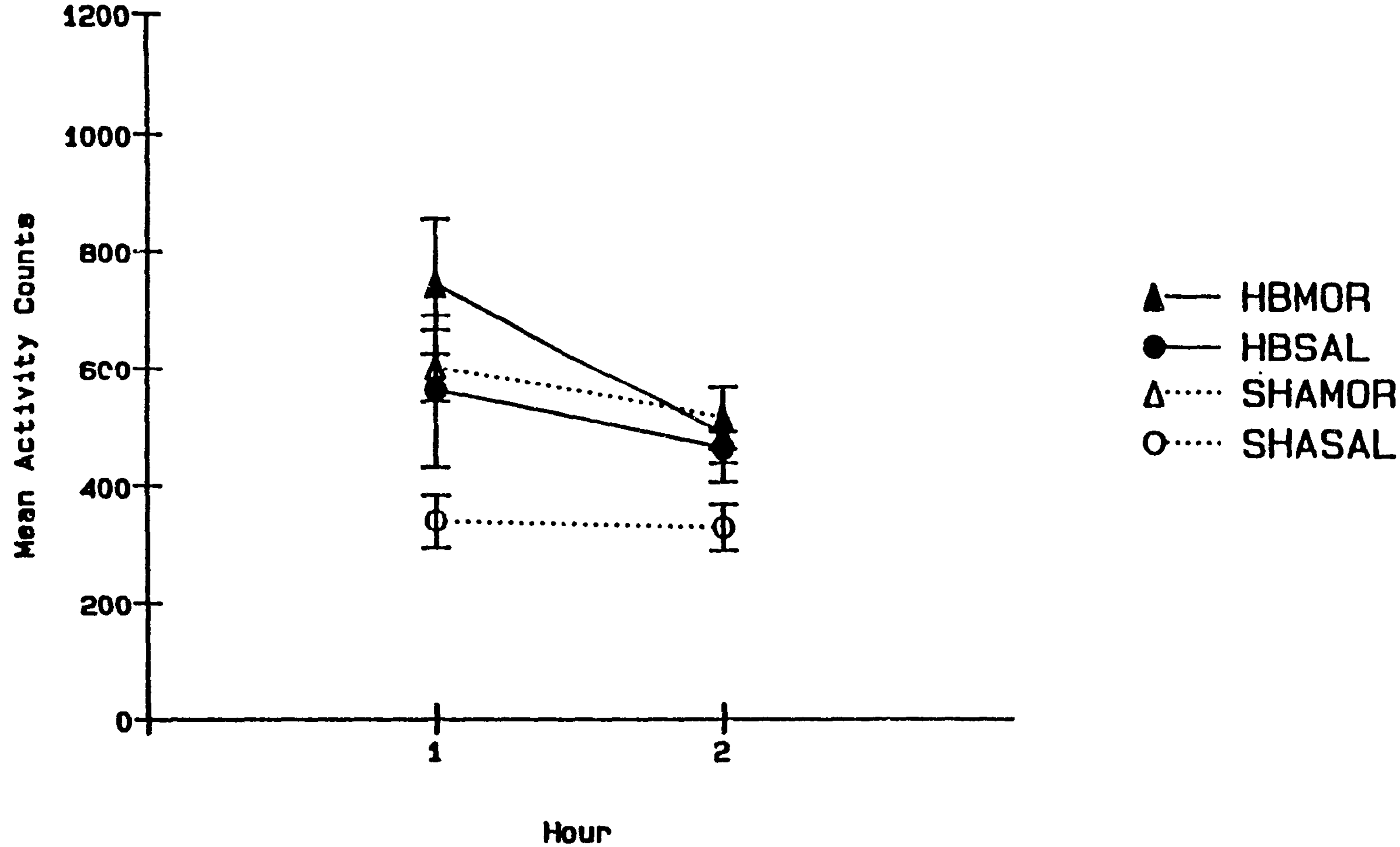
The locomotor activity scores during the test for sensitization, when rats in all four groups were administered one-half the preexposure dose of MOR (5.0 mg/kg, i.p.), were subjected to an ANOVA for lesion x preexposure drug with the repeated factor of hour of test. As shown in Figure 12, animals previously exposed to MOR showed significantly higher levels of activity in response to MOR challenge than did those that were previously exposed to saline [$F(1,23)=5.88$, $p < .05$].

A significant lesion x hour interaction was also found [$F(1,23)=7.84$, $p < .05$]. As illustrated in Figure 12, the locomotor activity of lesioned animals was higher than that of sham-operated animals in response to MOR challenge, but this was significant only in the first hour [($F(1,23)=4.78$, $p < .05$)]. Analysis of simple main effects revealed that the effect of hour was significant within the lesioned groups [$F(1,23)=23.90$, $p < .0001$], but not in the sham-operated groups.

Discussion

Lesions of the habenula enhanced the locomotor response to systemically-administered MOR, without affecting baseline activity. These lesions both attenuated the early-appearing depression and enhanced the later-appearing increases in activity seen after the systemic administration of MOR. Both of these effects of habenular

Figure 12 Group mean activity counts (+/- 1 S.E.M.) during the test for sensitization in Experiment 3 for the first and second hours following MOR administration. Animals in all four groups were administered MOR (5.0 mg/kg, i.p.).



Mean Activity Counts
1200
1000
800
600
400
200
0
1
2
Hour
HBMOR
HBSAL
SHAMOR
SHASAL

lesions persisted across drug preexposure sessions. Such lesions did not potentiate the development of a sensitized locomotor response to repeated injections of systemically-administered MOR.

Inasmuch as the activity-enhancing effects of MOR are mediated in part by activity in the DA systems, these observations are consistent with the results of studies which have shown that lesions of the habenula augment some of the behaviors induced by the administration of DA agonists (Carvey et al., 1987; Nguyen et al., 1989).

Lesions of the habenula did not, however, potentiate the development of the sensitized response to MOR during the test for sensitization, when all rats were challenged with a lower dose of the drug. Both lesioned and sham-operated rats previously exposed to MOR showed high levels of activity, although lesioned animals did tend to be somewhat more active in the first hour after administration.

The absence of an effect of habenular lesions on baseline locomotor activity in this experiment is at odds with the results of a number of studies, discussed in the introduction, where such lesions were observed to enhance baseline activity. The reason for this difference is not clear. It is possible, however, that destruction of midline thalamic nuclei, which was observed in many lesioned animals in the present study, masked the effect of habenular lesions since these thalamic regions have been implicated in the regulation of the DA systems. In support of this notion, Vives, Morales & Mogenson (1986) found that electrolytic lesions of the mediodorsal thalamus resulted in a transient, non-significant decrease in locomotor activity which recovered by the tenth day after surgery. On the other hand, Swerdlow and Koob (1987) found that electrolytically-produced thalamic lesions did not affect baseline locomotor activity 7 days after placement of the lesions. Evidence also exists that the midline thalamic nuclei influence DA-ergic activity in the NAcc, since chemical or electrical stimulation of these nuclei increase the utilization of DA in both the NAcc and MFC; lesions of these nuclei, however, do not alter the utilization of DA in the NAcc or MFC (Jones, Kilpatrick & Phillipson, 1987, 1989). It is possible, therefore, that the destruction of the midline thalamus seen in some animals in the present study masked the activity-enhancing effects of the habenular lesions. This issue is further confused by the fact that the thalamic lesions in both the Vives et al., (1986) and Swerdlow & Koob (1987) studies often included destruction of the habenular nuclei, making a dissociation of the locomotor effects of destruction of each of the two structures impossible. In the present experiment, informal comparisons made among animals did not reveal any consistent relationship between the extent of lesions of the thalamus and levels of baseline or drug-induced activity.

Taken together, the results of the present experiment demonstrate that habenular lesions potentiate the behavioral response to MOR applied directly to the VTA. This finding is consistent with the notion that the habenular nuclei, especially the LHb, participate in the inhibitory regulation of the DA systems. Lesions of the habenular complex had no effect on the development of a sensitized locomotor response to the repeated administration of

MOR . This suggests that the habenular nuclei do not take part in the changes underlying the development of the sensitized response of the mesolimbic DA system to the repeated, systemic administration of MOR.

EXPERIMENT 4

This experiment sought to further clarify the effect of lesions of the habenula complex on the development of the sensitization of locomotor activity seen with the repeated administration of MOR. In this study, MOR was applied directly to the VTA, the CNS region on which MOR acts to produce its effects on locomotor activity.

Methods

Subjects

Twenty male Wistar rats (Charles River, St. Constant, P.Q.) were housed singly in flat-bottomed cages containing wood shavings as bedding in an animal room (light phase 0800-2000 h) maintained at 22 degrees C, for at least one week prior to surgery. Standard lab chow and tap water was available ad libitum. All testing of locomotor activity was carried out between 0800h and 1400h.

Surgery

Rats were prepared for surgery as described in Experiment 1. All animals were implanted bilaterally with indwelling cannulae aimed at the VTA, according to the technique described in Experiment 2. In addition to the implantation of cannulae, the rats in the lesion groups received bilateral electrolytic lesions of the habenula complex as described in the Experiment 2. The rats in the sham-operated groups were treated identically to those that received lesions, except that no electrode was introduced into the brain. During the post-surgical recovery period, animals were treated identically to those in the previous experiments.

Apparatus

The locomotor activity of animals in response to lesion and drug treatment was measured in the same activity boxes used in the previous experiments.

Assessment of Stereotypical Responses to Intracranial Morphine

It was observed that some of the animals in group HBMOR showed stereotypical behaviors in response to repeated intra-VTA morphine treatment. In order to quantify this response, the rats were videotaped during the activity box sessions on preexposure days 2, 3, 4, 5 and 6, as well as during the test for sensitization, and their behavior was later scored for stereotypy from these tapes using the same rating procedure described in Experiment 2. During the drug preexposure sessions, only the animals administered MOR to the VTA were scored for stereotypy, since no stereotypical behavior was observed in animals treated with saline. During the test for sensitization, when all animals were administered MOR to the VTA, the behavior of each animal in all four groups was scored for stereotypy.

Design and Procedure

Following surgery, the habenular lesioned animals were randomly assigned to one of two drug exposure groups. The animals in the lesioned group HBMOR were administered MOR repeatedly in the activity boxes, while those in the lesioned group HBSAL received saline. The sham-operated animals were similarly assigned to the two corresponding drug groups, namely, groups SHAMOR and SHASAL.

Intracranial Drug Infusions: The administration of MOR or saline to the VTA was carried out using the same procedure described in Experiment 2.

Drug Preexposure: Following the recovery period, animals in groups HBMOR and SHAMOR received intra-VTA infusions of MOR once every three days, on 6 separate occasions, while those in groups HBSAL and SHASAL received saline immediately prior to their placement in the activity boxes. Immediately following each preexposure session, animals in all four groups were administered gentamicin sulphate (1 mg/kg, i.m.) to guard against infection. Rats were left undisturbed in their home cages on the two days between preexposure sessions.

Test for Sensitization: On the test day for sensitization to repeated intra-VTA morphine, which was carried out on the third day following preexposure day 6, rats in all four groups received intra-VTA infusions of MOR prior to a 2 hour activity box session.

Results

Assessment of Lesions and Placement of VTA Cannulae

Figure 13 depicts the largest and smallest habenular lesions. Figure 14 depicts the location of the cannulae aimed at the VTA. All cannulae were observed to be within the region of the VTA. Two lesioned animals became sick due to pulmonary infection during the drug preexposure phase of the experiment; their data was not included in the analysis. The final number of animals in each group was as follows: group HBMOR, n=5; group HBSAL, n=4; group SHAMOR, n=5; group SHASAL, n=4.

Figure 13 Extent of habenular lesions in Experiment 4. Striped areas represent the largest lesion and shaded areas the smallest.

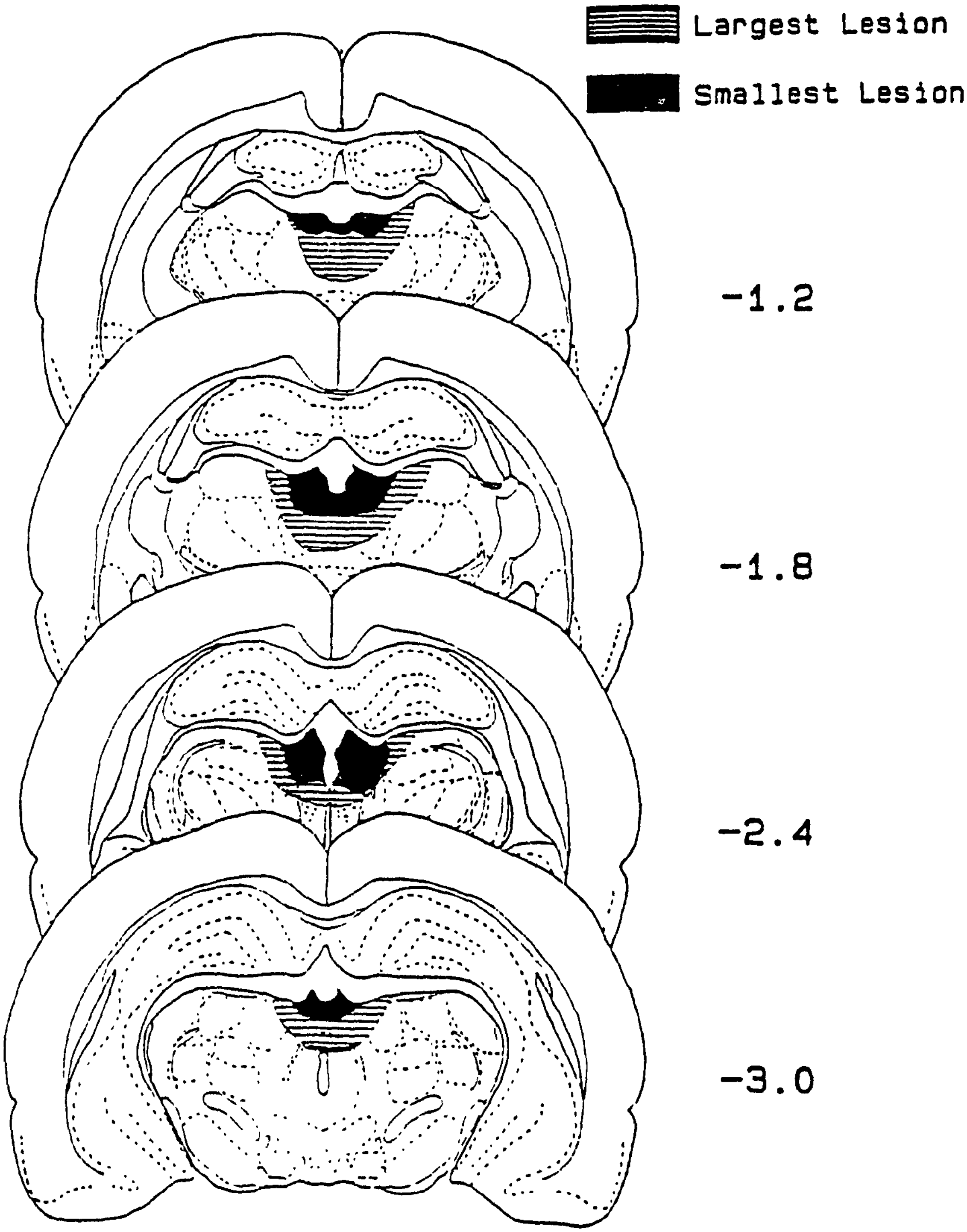

Largest Lesion
Smallest Lesion
-1.2
-1.8
-2.4
-3.0

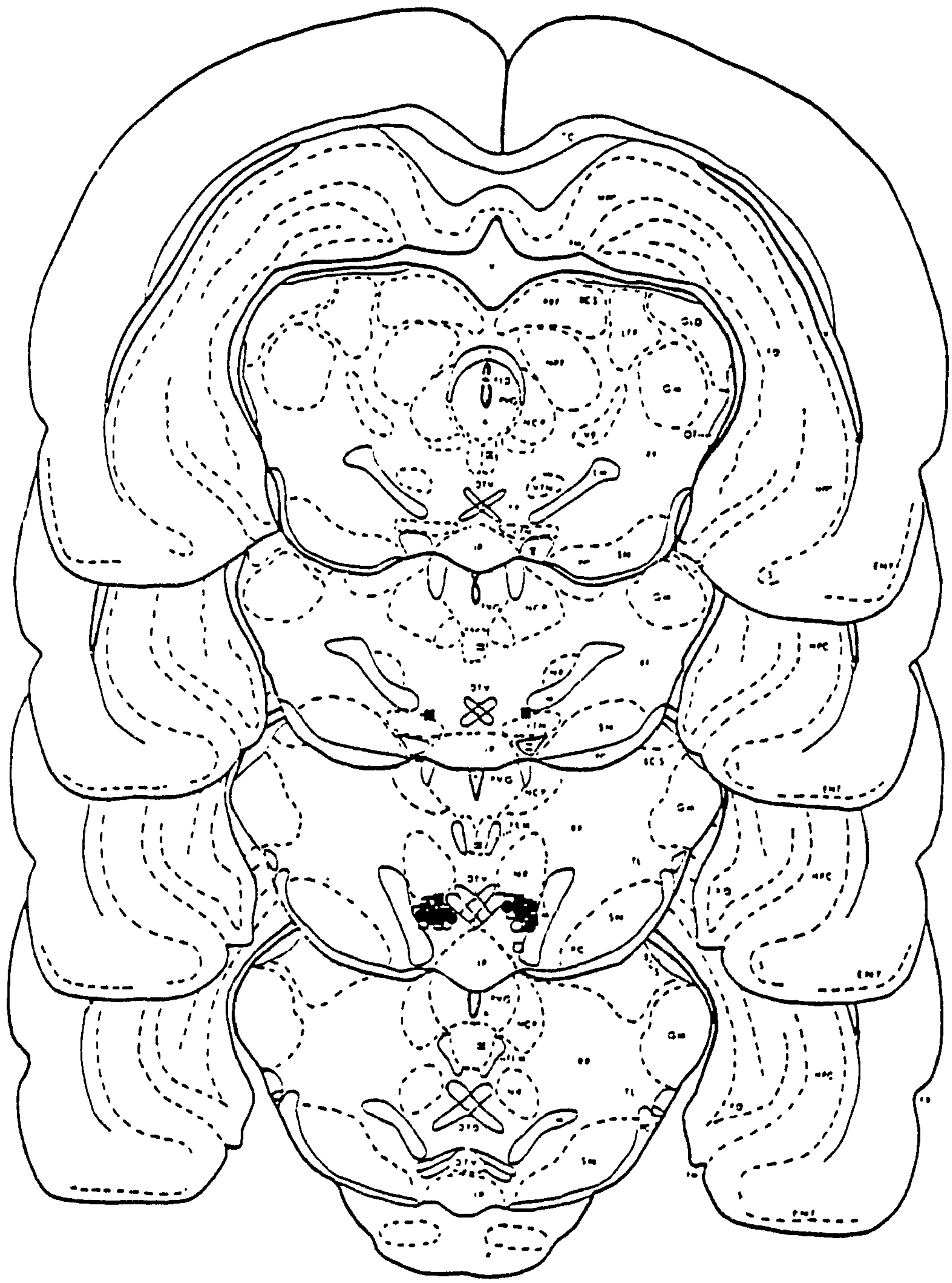
Figure 14 Location of the tips of guide cannulae aimed at the VTA in Experiment 4.

-3.4

-3.6

-3.8

-4.0



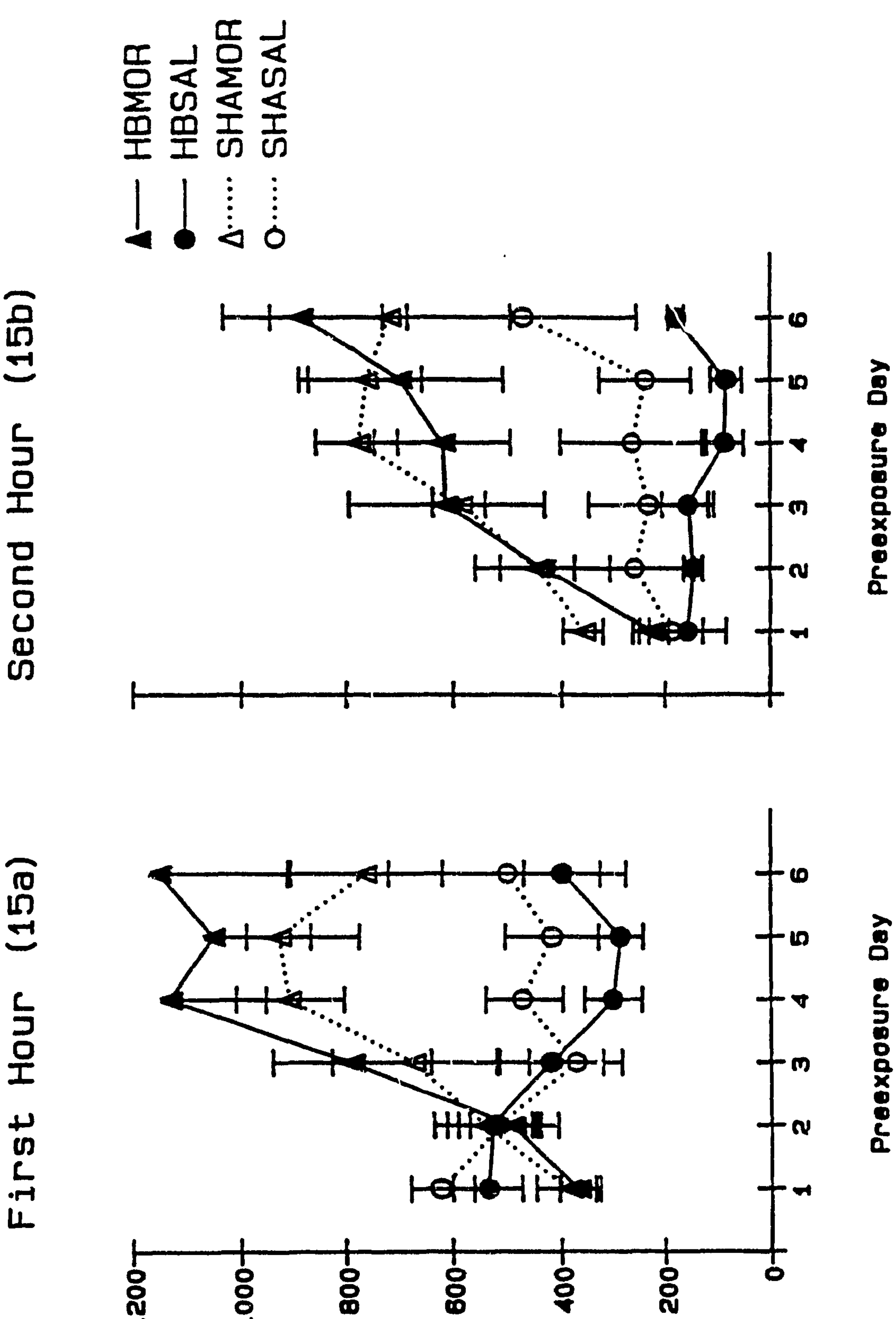
Morphine Preexposure

To assess the effects of lesions of the habenula on the development of the sensitization of locomotor activity seen with the repeated application of MOR to the VTA, an ANOVA for lesion x preexposure drug with the repeated factors of hour following administration and preexposure day was carried out on the combined activity scores on the 6 days of drug preexposure. The mean combined activity scores for each group during the 6 preexposure sessions are depicted in Figure 15 for each hour separately.

There was a significant effect of preexposure drug [$F(1,14)=15.67$, $p < .005$], since, as expected, animals administered MOR to the VTA showed higher levels of activity. The drug x day interaction was also significant [$F(5,70)=10.86$, $p < .0001$], reflecting the fact that the activity of rats administered MOR increased as a function of repeated administration, while the activity of rats treated with saline decreased over days.

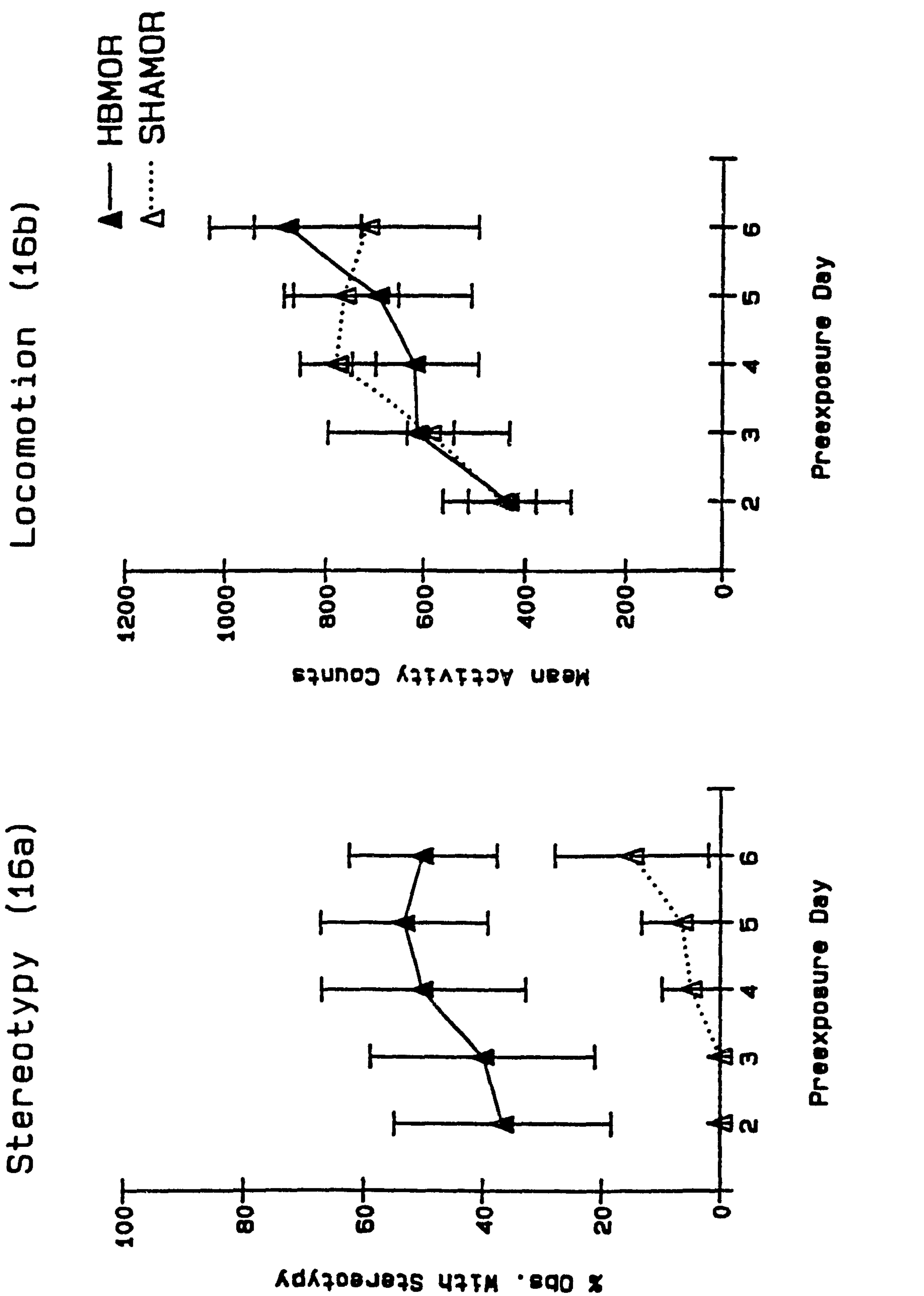
There were no significant effects of lesion on the locomotor activation induced by the repeated application of MOR to the VTA. It was observed, however, that lesioned animals administered MOR during the preexposure sessions engaged in stereotypical behaviors, including persistent gnawing, licking and head bobbing in response to the drug, an indication that the lesions did enhance the response of the animals to the drug. Figure 16 illustrates the relationship between the degree of stereotypical behavior and locomotor activity in the lesioned and sham-operated animals administered MOR during the preexposure phase. In this figure, the mean percentages

Figure 15 Group mean activity counts (+/- 1 S.E.M.) for the 6 days of MOR preexposure in Experiment 4 plotted separately for the first (15a) and second hours (15b) following MOR or saline administration. Animals in groups HBMOR and SHAMOR were administered MOR (5.0 ug/0.5 ul saline/side) bilaterally into the VTA, while those in groups HBSAL and SHASAL received equal volumes of saline into the VTA.



First Hour (15a)
Second Hour (15b)
HBMOR
HBSAL
SHAMOR
SHASAL
Mean Activity Counts
1200
1000
800
600
400
200
0
Preexposure Day
1
2
3
4
5
6

Figure 16 Group mean % of observations with stereotypical behavior (+/- 1 S.E.M.) (16a) in groups HBMOR and SHAMOR in comparison to locomotor activity (+/- 1 S.E.M.) (16b) in the second hour following intra-VTA MOR administration on MOR preexposure days 2, 3, 4, 5 and 6 in Experiment 4.


Stereotypy (16a)
Locomotion (16b)
HBMOR
SHAMOR
% Obs. With Stereotypy
Mean Activity Counts
Preexposure Day
Preexposure Day
0
20
40
60
80
100
0
200
400
600
800
1000
1200
2
3
4
5
6
2
3
4
5
6

of stereotypical acts observed (Figure 16a) are plotted alongside the mean combined activity scores (Figure 16b) of groups HBMOR and SHAMOR in the second hour of preexposure days 2, 3, 4, 5 and 6. It can be seen that the stereotypical response to MOR applied to the VTA is greater in animals with habenular lesions than in sham-operated animals. An ANOVA for lesion group with the repeated factor of day was carried out on these data, revealing a significant effect of lesion [$F(1,8)=8.63$, $p < .05$].

Test for Sensitization

To assess the effects of habenular lesion on locomotor activity during the test for sensitization, when all four groups were administered a challenge dose of MOR to the VTA, an ANOVA for lesion x preexposure drug, with the repeated factor of hour of test was carried out on the combined activity scores. The mean scores of the four groups for the two hours are shown in Figure 17.

There was a significant effect of preexposure drug [$F(1,14)=4.78$, $p < .05$]; as expected, rats preexposed to MOR were more active than those that had previously received saline.

There was no significant effect of lesion on the animals locomotor response to MOR challenge. Observation of the animals in the lesioned groups during the test for sensitization revealed that the animals in both of these groups were engaging in stereotypical behavior. Figure 18 shows the mean percentage of observation intervals where stereotypical behavior was observed to occur (Figure 18a) plotted alongside the mean activity scores (Figure 18b) of animals in groups HBSAL and SHASAL during each hour of the test for sensitization. It is seen that lesioned animals receiving MOR

Figure 17 Group mean activity counts (+/- 1 S.E.M.) during the test for sensitization in Experiment 4 for the first and second hours following MOR administration. Animals in all four groups were administered MOR (5.0 ug/0.5 ul saline/side) bilaterally to the VTA.

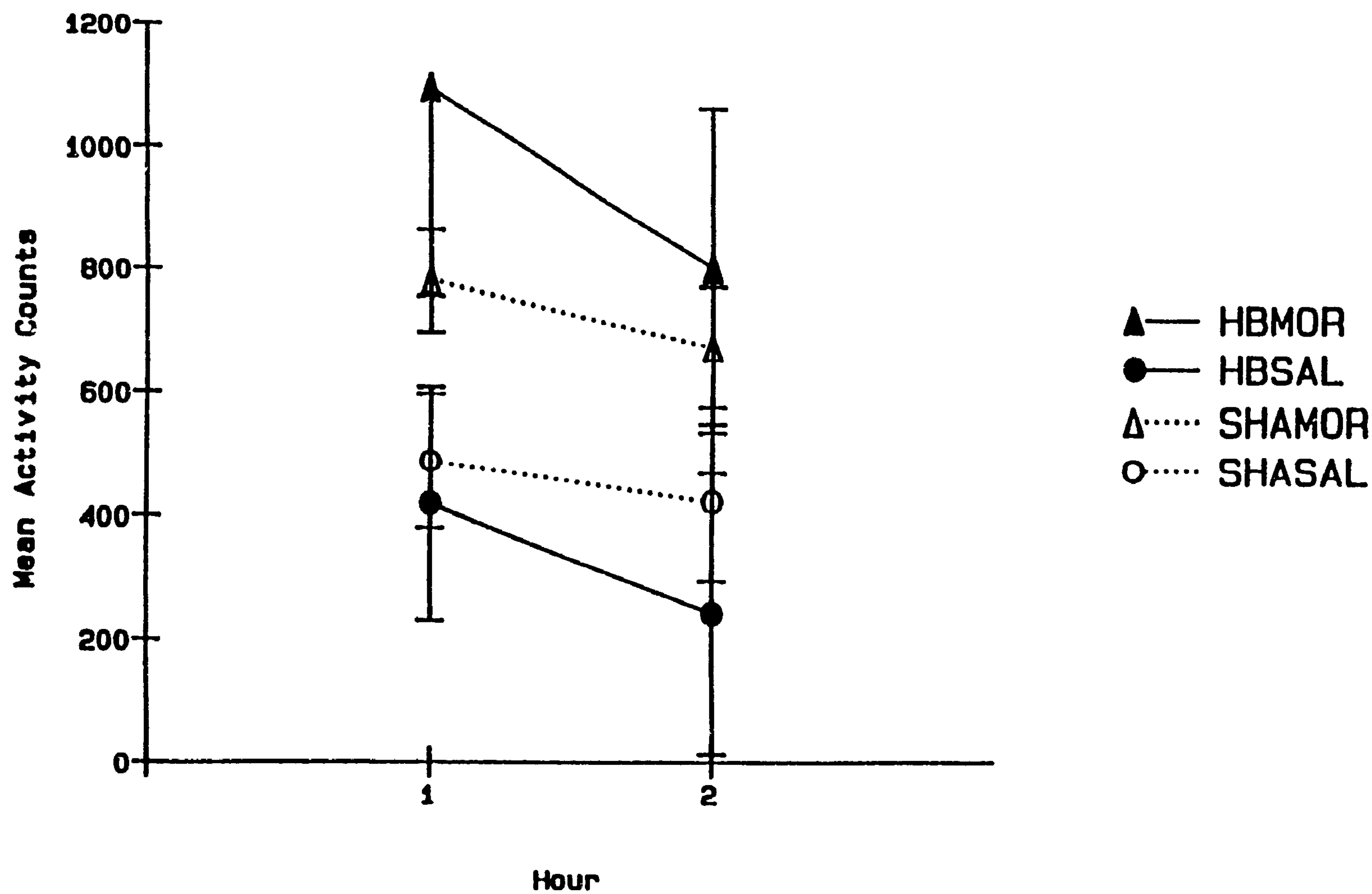
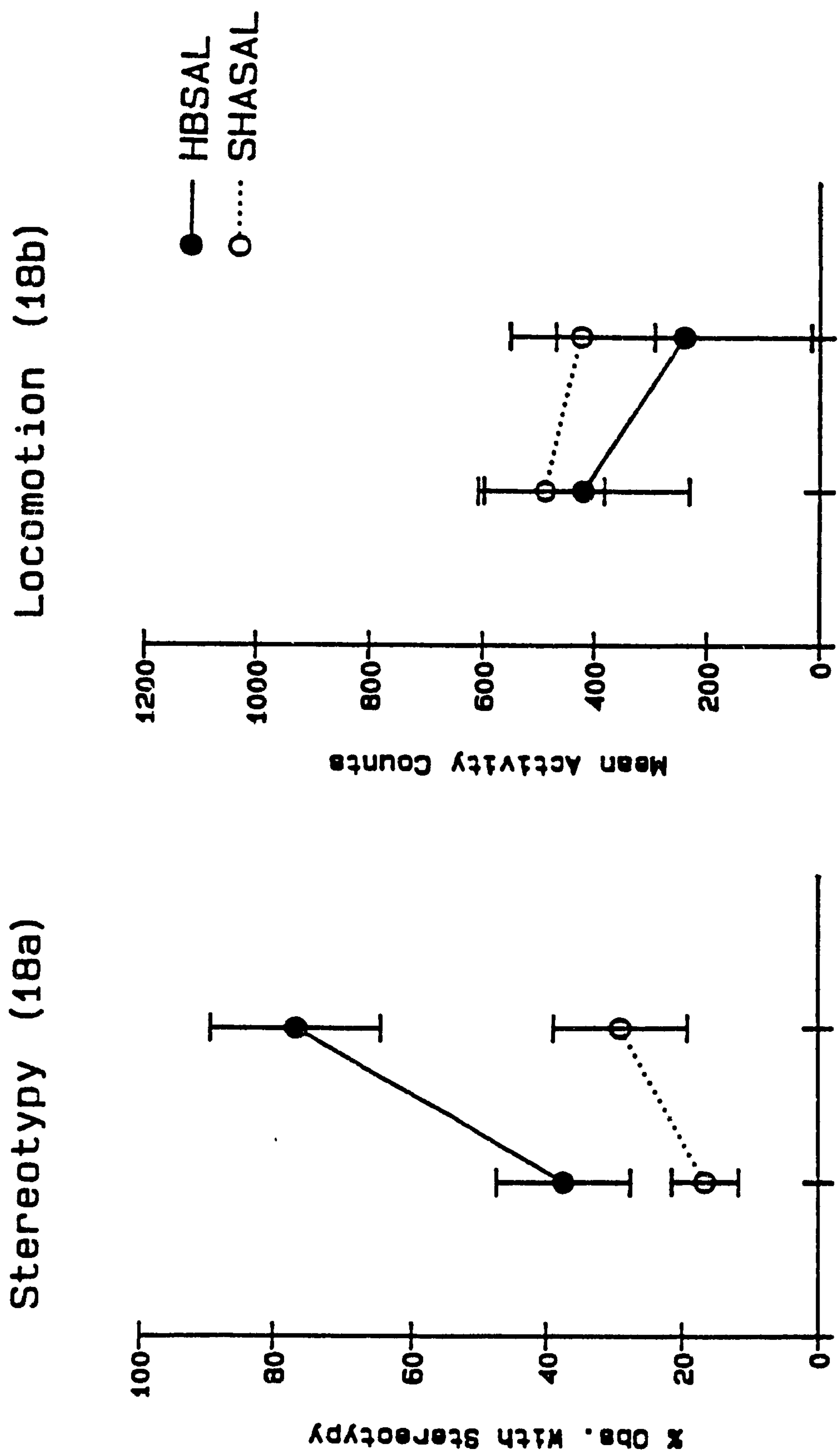

Mean Activity Counts
0
200
400
600
800
1000
1200
Hour
1
2
HBMOR
HBSAL
SHAMOR
SHASAL

Figure 18 Group mean % of observations with stereotypical behavior (+/- 1 S.E.M.) (18a) in groups HBSAL and SHASAL in comparison to locomotor activity (+/- 1 S.E.M.) (18b) in the two hours following intra-VTA MOR administration during the test for sensitization in Experiment 4.


Stereotypy (18a)
% Obs. With Stereotypy
100
80
60
40
20
0
1
2
Hour
Locomotion (18b)
Mean Activity Counts
1200
1000
800
600
400
200
0
1
2
Hour
HBSAL
SHASAL

for the first time demonstrate more stereotypical behavior than do drug-naive intact animals. This was reflected in a significant effect of lesion in a two way ANOVA for lesion x preexposure drug with the repeated factor of hour of test carried out on the stereotypy scores [$F(1,13)=9.32$, $p < .01$].

Discussion

This experiment was carried out to see if lesions of the habenula complex would alter the development of the sensitization to the locomotor-activating effects of MOR when applied repeatedly to the VTA. Lesioned animals receiving MOR showed no more locomotor activity than intact animals administered the drug. Lesioned animals, however demonstrated significantly more stereotypical behavior in response to VTA MOR than did sham-operated animals. It is difficult to interpret the effect the lesions had upon the development of sensitization to repeated MOR administration, since locomotor activity and stereotyped behavior are, to a certain extent, mutually exclusive categories. The presence of stereotypy in the lesioned animals administered MOR does, however, suggest that destruction of the habenula enhanced the response of the DA systems to the drug.

It is thought that activation of the mesolimbic DA system results in locomotor activity, while activation of the nigrostriatal system results in stereotypical behavior (Staton & Solomon, 1984; Sharp et al., 1987). It is possible that the habenular lesions potentiated the stereotypy-inducing effects of MOR by disinhibiting the nigrostriatal DA system. Although the DA cells located in the

VTA project mainly to the NAcc, they also project to the CPu. The LHb receives a massive projection from the entopeduncular nucleus, a pallidal structure which receives a large proportion of the efferent projections from the striatum. The habenula, in turn, projects to the VTA and SN and transmission in this projection has been shown to inhibit the activity of DA cells. It is possible that destruction of the habenula disrupts this inhibitory feedback pathway originating in the CPu, thereby decreasing the threshold level of stimulation required to enhance DA turnover in the CPu to a degree that would result in stereotypy. The enhanced performance of stereotypical behaviors seen in lesioned animals in response to intra-VTA MOR in the present experiment is consistent with those of other studies demonstrating that such lesions do potentiate the stereotypical behavior seen following the administration of DA-ergic drugs (Carvey et al., 1987; Nguyen et al., 1989).

No effects of lesions of the habenula were observed on baseline locomotor activity in this experiment, a result consistent with the findings of Experiment 3, as well as those of previous research (Thornton & Evans, 1982; Thornton et al., 1983). These present results are not in agreement, however, with the findings of a number of previous studies, where lesions of the habenula were observed to lead to increases in baseline locomotor activity (Nielson & McIver, 1966; Lee & Huang, 1988; Nguyen et al., 1989; Thornton et al., 1989). In the introduction of the present work, it was hypothesized that the lack of effect of habenular lesions on baseline locomotor activity observed by Thornton & Evans (1982) and Thornton et al., (1983) was due to the fact that these researchers assessed baseline activity in tests of very short duration compared to those used in the studies finding lesion-induced increases in baseline activity. The results of the present experiment and those of Experiment 3 do not support this hypothesis, since no activational effects of lesion were observed even though the spontaneous activity of animals was repeatedly assessed in test sessions of long duration.

General Discussion

The results of these studies demonstrate that the increase in locomotor activity seen after repeated treatment with the indirect DA agonist MOR can be altered by lesions of brain areas known to participate in the regulation of the DA systems. Destruction of these CNS areas, however, did not affect the development of sensitization of locomotor activity seen with the repeated administration of this drug. The present findings, with the exception of those of Experiment 2, where cortical lesions affected neither baseline nor MOR-induced activity, are in agreement with previous research showing that lesions of the MFC or the habenula enhance the behavioral activation seen after the acute administration of indirect-acting DA agonists. The present results extend these findings to the case of chronic administration of MOR. The implications these results have on the understanding of the substrates which underlie stimulant-induced locomotor activation will be discussed.

The Medial Frontal Cortex

In the first experiment, it was found that lesions of the MFC led to enhanced baseline locomotor activity which did not affect the sensitization of locomotor activity seen with the repeated, systemic administration of MOR. These findings are in keeping with the results of previous studies which show that lesions of the MFC enhance both baseline activity and the response to the administration of DA agonists.

These findings are consistent with the notion that cortical lesions enhance the behavioral response to DA agonists by disinhibiting the mesolimbic DA system via the removal of an inhibitory input. Electrophysiological and biochemical studies suggest that the MFC has an inhibitory influence on this DA system, which is exerted through projections to both the cell body and terminal regions. Lesions of the MFC, however, had no effect on the development of a sensitized locomotor response with the repeated administration of MOR. This suggests that the MFC does not directly control the alterations in the MOR-induced response of the DA systems which accompany repeated exposures to the drug.

There exists evidence to suggest that the inhibitory influence of the MFC on DA transmission is mediated by facilitation of the release of DA from the dendrites of DA neurons. Increases in the release of DA in this region inhibits the firing of the DA cells by acting on DA autoreceptors (Yim & Mogenson, 1980; Freeman, Meltzer & Bunney, 1985; White & Wang, 1984; Freeman & Bunney, 1987), while depletion of DA from dendritic regions results in the enhanced spontaneous firing of DA cells (Kapoor, Webb & Greenfield, 1989). Activation of cortical cells has been shown to evoke the release of dendritic DA (Nieoullon, Cheramy & Glowinski, 1978; Romo, Cheramy, Godeheu & Glowinski, 1986b) and to inhibit the firing of DA-containing neurons in the midbrain (Nakamura et al., 1979; Thierry, Deniau & Feger, 1979; Romo et al., 1986a, 1986b; Gariano & Groves, 1988). Further support for this mechanism comes from the observation that EAAs stimulate the release of DA from slices of SN, an effect which is mediated either directly or via an excitatory action on an interneuron (Marien, Brien & Jhamandas, 1983; Araneda and Bustos, 1989). Taken together, these results suggest that the GLU-ergic cortico-mesencephalic pathway normally inhibits the activity of DA cells by enhancing the release of dendritic DA. Destruction of the GLU-ergic input to the dendritic region of the DA cells would be expected to result in less facilitation of dendritic DA release, leading to the enhanced firing of the DA cells, and increased locomotor activation. The increased baseline locomotor activity seen following lesions of the MFC in Experiment 1 is consistent with this proposed circuitry.

One result which does not support this proposed mechanism is the fact that the local application of EAAs to the region of the DA-ergic cell bodies reliably increases the firing of DA cells, turnover of DA in terminal regions and locomotor activity (Grace & Bunney, 1984b; Kalivas, Duffy & Barrow, 1989), effects which would not be predicted if EAA-containing afferents to the DA cell body region, such as from the MFC, stimulated only the release of dendritic DA. It is possible, however, that EAAs exert a biphasic effect on the activity of the DA cells, inhibiting cell firing by selectively enhancing the release of dendritic DA at low concentrations, an effect that is overshadowed by the excitatory effect of the EAA when applied at higher concentrations. There is, however, little evidence to support this speculation. Another possible explanation is that the electrophysiological and behavioral effects of the local application of EAAs to the DA cell body region depends on whether the EAA is specifically stimulating receptors on the dendrites or on the cell bodies themselves. Iontophoretic or unilateral pressure injection of EAAs to the pars compacta of the SN results in, respectively, the increased firing of DA cells and contralateral circling, suggestive of increases in the activity of the nigrostriatal DA system, while infusion of EAAs into the SN pars reticulata, the region innervated by tne dendrites of the DA cells, leads to behavioral sedation and catalepsy (Pycock & Dawbarn, 1980; Arnt, 1981), which suggests that the application of EAAs to this region inhibits the DA system. Other indirect support for this hypcthesis comes from the observation that serotonin applied to the dendritic regions distal to the DA neurons in the SN pars reticulata can evoke changes in the membrane properties of the cells which are associated with the release of dendritic DA, while application of serotonin to dendritic regions more proximal to the DA cell bodies has no effect (Nedergaard, Bolam & Greenfield, 1988; Nedergaard, Webb & Greenfield, 1989). In the VTA, the origin of the mesolimbic DA projection, these topographical distinctions cannot be made; the cell bodies and dendrites of the DA cells are not compartmentalized as in the case of the nigrostriatal DA system. If the hypothesis outlined above is correct, it would be expected that the local infusion of EAAs to the VTA would result only in increases in the activity of the mesolimbic DA system.

The MFC-midbrain pathway has also been implicated in the control of the firing pattern of DA cells. Stimulation of the MFC has been shown to evoke burst firing in a small population of DA neurons in both the VTA and medial SN. Microiontophoresis of GLU onto the DA cell bodies in the SN also enhances burst activity (Grace & Bunney, 1984b). Cooling the MFC or systemic administration of an

EAA antagonist, furthermore, results in a "pacemaker"-like regularization of the normally burst-oriented firing pattern of DA cells in the VTA (Grenhoff et al., 1988; Svensson & Tung, 1989), while DA cells in slice preparations show a similar regularization of firing activity which is suggested to occur due to removal of regulatory afferents to the DA cells (Sanghera, Trulson & German, 1984; Trulson, Trulson & Arasteh, 1987). Taken together, these results suggest that afferent input to the VTA cells is necessary for the propagation of burst firing, and that this influence may be mediated by an EAA-containing pathway from the MFC to the VTA. Destruction of the MFC, therefore, might also result in decreases in the burst firing activity of DA cells. Burst firing in DA neurons has been suggested to be a mechanism through which large quantities of transmitter can be released from the terminals (Gonon, 1988), so it is possible that a treatment which reduces bursting, such as a lesion, would result in proportionally less DA release and hence, less DA-mediated locomotor activity, a line of reasoning not supported by the results of Experiment 1. It has been reported, however, that although burst activity is reduced in DA cells deprived of afferent input, baseline rates of firing are enhanced (Sanghera et al., 1984; Trulson et al., 1987), and in one study lesions of the MFC were observed to lead to similar increases in the firing of DA cells in the mesolimbic system (Ceci and French, 1988); it may therefore be the case that cortical lesions result in the increased release of DA from the terminals due to their releasing effects on DA cell firing. It is not clear, furthermore, what role the burst firing of DA neurons has in the regulation of DA release in the behaving animal.

It may be that lesions can result in increases in DA-dependent behaviors in the absence of increases in burst firing. It is also possible that the proposed attenuation of burst firing caused by the destruction of the MFC, by decreasing the overall release of DA, actually increases the stores of releasable DA available for liberation in response to environmental stimuli, or to the administration of DA-releasing drugs.

The MFC also sends an EAA-containing projection to the NAcc and CPu which has been implicated in the control of DA release from the terminals. DA-ergic activity in the MFC itself, furthermore, has been demonstrated to regulate the influence of this pathway. Locally applied EAAs evoke the release of DA in the CPu, an effect shown to be mediated by a direct effect on the terminals of the DA neurons (Marien et al., 1983; Cheramy, Romo, Godeheu, Baruch & Glowinski, 1986; Romo et al., 1986b). Activation of cortical cells, furthermore, leads to increases in DA turnover in the CPu, an effect thought to be mediated by enhanced neurotransmission in the cortico-striatal GLU-ergic pathway (Nieoullon et al., 1978; Romo et al., 1986b). This effect of cortical stimulation is not related to the firing rate of the DA cells since, as discussed previously, cortical stimulation also activates EAA-containing pathways to the cell body region of the DA cells, resulting in increases in the release of DA in dendritic regions and decreases in cell firing (Romo et al., 1986a,1986b). The fact that disruption of EAA transmission in the NAcc, furthermore, via intra-NAcc infusion of an EAA blocker results in decreases in AMPH and cocaine-stimulated locomotor activity further supports the proposal that a GLU-ergic projection to the NAcc facilitates the release of DA (Pulvirenti, Swerdlow & Koob, 1988).

The stimulation of DA receptors in the MFC may serve to modulate this facilitatory EAA-containing pathway. Electrical stimulation of the VTA or DA agonist administration evokes primarily inhibitory responses in the post-synaptic cells in the MFC (Mantz, Milla, Glowinski & Thierry, 1988; Shvaloff, Tesolin & Sebban, 1988; Peterson, Olsta & Matthews, 1990), although excitatory responses to DA agonists have also been reported in cortical slice preparations (Penit-Soria, Audinat & Crepel, 1987). If the GLU-containing cells in the MFC projecting to the DA-ergic terminals in the NAcc are inhibited by DA, less facilitation of NAcc DA release would be expected if DA release was enhanced in the MFC. Recently it has been demonstrated that infusion of AMPH into the MFC results in decreased DA metabolism in the NAcc (Louilot et al., 1989). Intra-MFC AMPH infusion, furthermore, attenuates the increase in locomotor activity seen with the co-application of AMPH into the NAcc (Tassin et al., 1988). These results suggest that the EAA-containing projection from the MFC to the NAcc exerts a facilitatory effect on DA release in the NAcc that is normally inhibited by DA-ergic activity in the MFC. This circuit may be used to explain the results of previous research showing that destruction of DA terminals in the NAcc, which would be expected to result in the increased activity of the MFC-NAcc pathway due to release from inhibition, leads to increased baseline locomotor activity and enhanced responsiveness to DA agonists.

On the other hand, if a glutamatergic projection from the MFC serves to facilitate the release of DA from terminals in the NAcc, it might be expected that the non-selective destruction of this pathway, such as via cortical aspiration, would result in a disfacilitation of DA release in the NAcc ultimately leading to decreases in locomotor activity. The results of Experiment 1 are not consistent with this point of view, since such lesions actually resulted in increases in baseline locomotor activity. It is possible, however, that the long-term disfacilitation of the release of DA from the terminals in the NAcc would lead to increased levels of DA stored in the terminals. Proportionally more DA, therefore, would be available for release in response to either environmental stimulation or to the administration of a DA-releasing drug, such as MOR.

Another mechanism by which the MFC may exert its inhibitory effects on the DA systems is through an inhibitory influence on the post-synaptic D_1 receptors in the NAcc (Pycock, Kerwin & Carter, 1980; Reibaud et al., 1984). Reibaud et al., (1984) observed moderate increases in DA-stimulated adenylate cyclase activity in the NAcc following aspiration of the MFC. It is possible that the increases in baseline and MOR-induced activity seen in Experiment 1 resulted from a lesion-induced increase in sensitivity of the D_1 receptors in the NAcc. The increased post-synaptic effect of DA in the NAcc may have resulted in increased activity in the descending pathways mediating locomotor activity. These results suggest a role for the MFC-NAcc projection in the modulation of the sensitivity of DA receptors in the NAcc.

In summary, the results of Experiments 1 and 2 provide additional support for the notion that projections from the MFC inhibit the activity of the DA systems. Lesions of the MFC were shown to increase baseline locomotor activity as well as to augment the behavioral responses induced by the systemic and intra-VTA administration of MOR. The present findings suggest, however, that this cortical region does not control the changes in the substrate that underlies the development of sensitization of the activational effects of MOR seen with repeated administration.

The Habenular Nuclei

Lesions of the habenular nuclei enhanced the locomotor response to systemically-administered MOR, but had no effect on the development of the sensitization of this response. Such lesions also blocked the early-appearing depressant effects of systemic MOR. MOR application to the VTA of animals with habenular lesions resulted in the performance of stereotypical behavior, which appeared at least as early as the second MOR exposure and persisted with repeated applications of the drug. In both Experiments 3 and 4, habenular lesions did not increase baseline locomotor activity.

The enhanced locomotor response to systemic MOR seen in lesioned animals is consistent with earlier research finding increases in both locomotor activity and stereotypical behavior in lesioned animals following the administration of DA agonists. The increased performance of stereotypical behaviors in lesioned rats administered MOR directly to the VTA is also congruent with previous results showing that such lesions lower the threshold dose of DA agonist needed to elicit stereotypical behavior (Carvey et al., 1987; Nguyen et al., 1989). The present results offer further support for the notion that the habenular nuclei, especially the lateral habenula, exert an inhibitory influence on the activity of the DA systems. Lesions of the habenula therefore serve to disinhibit the DA systems.

In the present studies, no evidence was found for lesion-induced increases in baseline locomotor activity, a result in agreement with those of a number of studies (Thornton & Evans, 1982; Thornton et al., 1983) but at variance with others (Nielson & McIver, 1966; Lee & Huang, 1988; Nguyen et al., 1989; Thornton et al., 1989). There appears to be no systematic variation in experimental procedures underlying these discrepant results. As previously suggested in the introduction, the studies that observed increases in baseline locomotor activity following habenular lesions utilized tests of longer duration than those reporting no effect. The present experiments found no evidence for an effect of habenular lesion on baseline locomotor activity in any time interval after introduction of the animals into the activity boxes. Although the effect of habenular lesions on baseline activity is equivocal, the fact that such lesions increased the behavioral responsivity to DA-ergic drug administration can be taken as evidence that they do disinhibit the DA systems.

The habenular complex may be thought of as a relay center in a feedback loop channelling information from the afferent targets of midbrain nuclei back to the cell bodies of origin of these projections. The habenula receives efferents both directly and indirectly from the NAcc, CPu and MFC, and relays this input to the VTA and SN, as well as to non-DA-ergic midbrain nuclei thought to participate in the regulation of the DA systems. As discussed previously, it has been established that neurotransmission in the habenulo-midbrain pathway inhibits the activity of the DA systems. The habenula may exert this influence through a variety of different mechanisms.

A direct inhibitory projection to the VTA and SN from the habenula has been proposed, since the stimulation or interruption of transmission in this pathway inhibits or activates, respectively, the DA systems. Substance P (SP), Substance K (SK) and acetylcholine (ACh) are known to be the major transmitters contained in the habenular projections to the midbrain. The SP and SK-ergic afferents to the VTA region arise from cell bodies located mainly in the medial habenula (Halliday & Tork, 1988; Burgunder & Young, 1989). The ultimate source of the cholinergic projection is still not clear, but it has been suggested that there may be ACh-containing cell bodies and cholinergic fibers, of septal and basal forebrain origin in the medial habenula (Emson, Cuello, Paxinos, Jessel & Iversen, 1977; Cuello, Emson, Paxinos & Jessel, 1978; Vincent, Staines, McGeer & Fibiger, 1980; Murray, Saffroy, Torrens, Beaujouan & Glowinski, 1988). The transmitters contained in the pathway originating in, or passing through the LHb, which has been shown to exert more of an influence on the activity of the DA systems than the medial habenular projection, is less well known. The LHb has recently been shown to contain a few SP-containing cell bodies (Burgunder & Young, 1989) and is well innervated with both SP and cholinergic fibers passing through this nucleus. Since SP, stable SP analogs and SK have been shown to increase DA metabolism and locomotor activity in forebrain regions following infusion into the VTA (Stark Eison, Eison & Iversen, 1982; Deutch et al., 1985; Elliott, Alpert, Bannon & Iversen, 1986; Cador, Rivet, Kelley, LeMoal & Stinus, 1989), and stimulation of the medial habenula, the main source of the habenular SP projection to the VTA, has minimal effects on the firing of DA neurons (Christoph et al., 1986), it is unlikely that the LHb stimulation-induced decrease in the firing of DA cells is caused by the release of SP in this region. It is possible, however, that activity in the SP-ergic LHb-VTA pathway inhibits DA cells in the A10 region through actions on inhibitory interneurons, but this hypothesis has not as yet been addressed in any detail.

There is some evidence for the role of ACh in the habenular influence on DA neurons. Destruction or acute blockade of the fasciculus retroflexus results in decreased ACh activity in the interpeduncular nucleus region adjacent to the VTA (Emson et al., 1977). Stimulation of either lateral or medial habenula causes an atropine-reversible increase in the firing of cells in the interpeduncular nucleus (Sastry, 1977). More recently, Nishikawa et al., (1986) found that the increases in DA release in the NAcc caused by fasciculus retroflexus blockade were reversed by the application of a muscarinic agonist into the interpeduncular nucleus. Intra-interpeduncular nucleus infusion of a muscarinic blocker resulted in increases in DA-ergic activity in the MFC and NAcc. Nishikawa et al., (1986) speculated that the observed disinhibition of the DA systems following the blockade of impulse transmission in the fasciculus retroflexus was mediated by an interruption of the cholinergic habenulo-midbrain pathway or alternatively, via disruption of cholinergic fibers passing through this nucleus, resulting in disfacilitation of inhibitory GABA-ergic interneurons impinging on the DA cells. Additional support for this mechanism was suggested to arise from the observation that lesions of the habenula cause long-term increases in GABA-ergic activity in the interpeduncular nucleus, which might reflect the buildup of GABA in the interneurons of the interpeduncular nucleus due to habenular lesion-induced disfacilitation (Mata, Schrier & Moore, 1977).

The LHb may also participate in the regulation of the DA systems indirectly through a projection to the raphe nuclei. Serotonergic neurotransmission has been shown to have an inhibitory influence on the DA systems (Costall, Naylor, Marsden & Pycock, 1976; Herve et al., 1979; Herve et al., 1981; Beart & McDonald, 1982; Bendotti, Berettera, Invernizzi & Samanin, 1986; Ugedo, Grenhoff & Svensson, 1989) through projections to both cell body and terminal regions of the DA systems (Herve, Pickel, Joh & Beaudet, 1987; Soghomonian, Descarries & Watkins, 1989). Stimulation of the LHb has been shown to result in decreases in the firing of cells in the raphe nuclei and decreases in the release of serotonin in the CPu, suggesting that the habenulo-raphe projection is inhibitory, and is possibly mediated by GABA-ergic interneuron in the raphe nuclei (Wang & Aghajanian, 1977; Stern, Johnson, Bronzino & Morgane, 1979; Reisine, Soubrie, Artaud & Glowinski, 1982; Park, 1987). More recently, however, stimulation of the LHb at higher frequencies was demonstrated to dramatically enhance the release of serotonin in the striatum, an area receiving a projection from the dorsal raphe nucleus, suggesting that the influence of the LHb on the serotonin systems is excitatory. This is consistent with the finding of an EAA-containing projection to the raphe nuclei from the LHb (Kalen, Karlson & Wiklund; Kalen, Pritzel, Nieoullon & Wiklund, 1986). The enhancing effect of LHb stimulation on striatal serotonin release was blocked by the intra-raphe infusion of an EAA antagonist, and potentiated by the intra-raphe infusion of a GABA blocker (Kalen, Strecker, Rosengren & Bjorklund, 1989). These results suggest that an EAA-containing pathway originating in the LHb exerts a facilitatory effect on the activity of the serotonin systems, and that this excitatory effect is modulated by GABA at the level of the raphe nuclei. It is tempting to speculate that the inhibitory effect of LHb stimulation on the firing of DA cells is mediated indirectly via the excitation of an excitatory projection to the raphe nuclei. Increases in DA-ergic activity following lesions of the habenular nuclei may occur as a result of an attenuation of the inhibitory influence that the serotonin-containing projections have on the DA systems.

These results suggest that the habenula, via either a direct or indirect mechanism, exerts an inhibitory influence on the activity of the DA systems. Destruction of the habenula, therefore, leads to disinhibition of the DA systems and increases in the behavioral response to DA-ergic drugs as was found in Experiments 3 and 4. The results of the present experiments show, furthermore, that destruction of this input did not affect the development of sensitization of the loc otor effects of repeated MOR administration.

Conclusions

Taken together, the results of the present experiments demonstrate that lesions of the MFC or habenular nuclei, structures demonstrated to provide inhibitory input to the mesolimbic DA system, facilitate the locomotor activational effects of acutely applied MOR, but do not alter the development of sensitization of the activational effects of MOR seen with repeated administration. These findings further suggest that the mechanisms underlying the sensitized response of the mesolimbic DA system to the repeated administration of MOR or other DA agonists are not subject to control by the MFC or the habenular nuclei.

The actual locus of the changes underlying the enhanced response of the mesolimbic DA system to the repeated administration of DA agonists is not clear. Recently, Stewart & Vezina (1989) presented data demonstrating that D_1 receptor stimulation at the level of the cell bodies of the DA systems is necessary for both the development and the expression of the sensitized locomotor response to the repeated administration of AMPH. The intra-VTA administration of a D_1 receptor antagonist prior to each preexposure injection of systemically-administered AMPH, in addition to blocking the acute locomotor effect of the drug, disrupted the sensitized locomotor response to a later challenge injection of systemic AMPH. In the SN, the cell body region of the nigrostriatal DA system, D_1 receptors are localized primarily on the terminals of a feedback pathway originating in the CPu, and a similar feedback arrangement for the mesolimbic DA system has been proposed. These results suggest that changes in transmission in these feedback pathways might well underlie the sensitized response of the mesolimbic DA system to repeated stimulant administration. Further experiments addressing this hypothesis might assess the effects that selective lesions of components of this feedback pathway have on the development and expression of the sensitized locomotor response which accompany the repeated administration of DA agonists.

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