A PHARMACOLOGICAL AND NEUROANATOMICAL INVESTIGATION OF THE CONDITIONED PLACE PREFERENCE PRODUCED BY AMPHETAMINE

by

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ABSTRACT

The present study investigated the neural mechanisms by which environmental stimuli guide conditioned behaviors in the amphetamine conditioned place preference (CPP) paradigm. Systemically injected D1 and D2 dopamine antagonists blocked both acquisition and expression of the CPP the selective D1 antagonist more effectively blocked expression than the D2 antagonists. The site of action of the antagonists on expression was the nucleus accumbens. Systemically injected reserpine, but not intra-accumbens *a* -MPT microinjections, also blocked the expression of the amphetamine CPP. Pre-conditioning and post-conditioning electrolytic or excitotoxic lesions of the lateral amygdaloid nucleus impaired the CPP. It was concluded that the effect of conditioned incentive stimuli is mediated by a neural system which involves the reserpine-sensitive dopamine pool and the D1 dopamine receptor in the nucleus accumbens and the lateral amygdaloid nucleus.

RESUME

Dans la présente these, les mecanismes neurologiques à travers lesquels les stimuli environnementaux guident les comportements conditionnés ont été étudiés dans un paradigme où la preference pour un endroit particulier est conditionnée par l'amphetamine (amphetamine conditioned place preference (CPP) paradigm). L'injection systemique des antagonistes D1 et D2 de la dopamine a bloqué l'acquisition et l'expression de la CPP. De plus, l'antagoniste spécifique D1 a bloque l'expression plus efficacement que l'antagoniste D2. Le site d'action des antagonistes sur l'expression du CPP est situé au niveau du noyau accumbens. L'injection systémique de reserpine, mais non les microinjections intra accumbens a -MPT, a bloqué l'expression du CPP de l'amphetamine. Les lesions électrolytiques ou excitotoxiques du noyau latéral de l'amygdala, soit avant ou après le conditionnement, ont inhibé le CPP. On peut conclure que le système neurologique à l'origine l'effect des stimuli incentifs conditionnes implique le compartement dopamine sensible a la reserpine et le recepteur dopaminergique D1 dans le noyau accumbens et dans le noyau lateral de l'amygdala.

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PREFACE

The presence of incentive stimuli dynamically organize animals behavior. Incentive stimuli not only elicit approach but also induce hyperactivity and establish new responses. When environmental stimuli are associated with incentives, they acquire these properties of incentive stimuli (incentive learning). There is an ample body of evidence that the neurotransmitter dopamine is involved in incentive learning. The capacity of natural incentive stimuli to establish incentive learning is eliminated by dopamine antagonists (Beninger and Phillips, 1980, Spyraki, Fibiger and Phillips, 1982c), and direct pharmacological activation of dopamine systems establishes incentive learning (Beninger and Hahn, 1983, Davis and Smith, 1975, Reicher and Holman, 1977, Sherman, Roberts, Roskam and Holmes, 1980, Spyraki, Fibiger and Phillips, 1982a). However, little is known about the neural bases of the expression of conditioned incentive behavior, which is mediated by the effects of conditioned incentive stimuli. The present investigation is an attempt to elucidate the neuroanatomical and neurochemical mechanisms of conditioned incentive behavior in the amphetamine conditioned place preference (CPP) paradigm.

The first set of experiments examined the roles of dopamine pathways and dopamine receptors in the caudate/putamen and nucleus accumbens in the amphetamine CPP. It was shown that systemic injections of dopamine antagonists with higher affinity for D2 than D1 receptors failed to block expression of the amphetamine CPP at doses which blocked acquisition, whereas systemic injections of a selective D1 dopamine antagonist blocked both acquisition and expression equally. The site of action of dopamine antagonists on expression was found to be the nucleus accumbens and not

the caudate/putamen. Control experiments ruled out the possibility that blockade of expression of the CPP by dopamine antagonists was due to reduced locomotion, an effect produced by dopamine antagonists. These findings revealed for the first time that the mesolimbic dopamine projection to the nucleus accumbens, but not the nigrostriatal dopamine pathway, is involved in the expression of the amphetamine CPP and that activation of the D1 dopamine receptor is probably more critical than that of the D2 dopamine receptor in mediating the effect of conditioned incentive stimuli on behavior.

The second set of experiments examined the involvement of two pharmacologically distinct dopamine pools in the expression of the amphetamine CPP. Expression of the amphetamine CPP was blocked by reserpine, but not by alpha-methyl-DL-para-tyrosine (*a* -MPT). Control experiments showed that the doses of reserpine and *a* -MPT used were sufficient to deplete two pharmacologically distinct dopamine pools. Together with the first set of experiments that showed that selective dopamine receptor antagonists injected into nucleus accumbens blocked expression, it was concluded that the blockade of expression of the amphetamine CPP was due to depletion of the reserpine-sensitive dopamine pool in the nucleus accumbens. This revealed for the first time that conditioned incentive behavior is mediated by dopamine released from the reserpine sensitive dopamine pool.

In the last set of experiments, involvement of limbic structures in the amphetamine CPP was examined. Small electrolytic lesions and fiber-sparing excitotoxic (N-methyl-d-aspartic acid (NMDA)) lesions of the lateral amygdaloid nucleus impaired the CPP when they were made before

conditioning. In contrast, the CPP was not impaired by electrolytic lesions of the central or basolateral amygdaloid nucleus, endopyriform nucleus or ventral hippocampus, which were partly damaged by NMDA injected into the lateral amygdaloid nucleus. Radiofrequency lesions of the fornix fimbria had no effect on the CPP. These findings suggest that the lateral amygdaloid nucleus is involved in either acquisition or expression of the amphetamine CPP and that neither acquisition nor expression of the amphetamine CPP involves the central or basolateral amygdaloid nucleus or the hippocampus accumbens system. Subsequent experiments showed that small electrolytic and large excitotoxic lesions of the lateral amygdaloid nucleus impaired expression of the amphetamine CPP when they were made after conditioning but before testing. The strategy of using both types of lesions overcame the limitations of using either of the techniques alone, electrolytic lesions are well confined anatomically, but damage fibers of passage, while excitotoxic lesions spare fibers of passage, but damage a relatively large area because the spread of the injected substance is uncontrollable. By making small electrolytic lesions confined to each subdivision of the NMDA damaged area, the critical role of intrinsic neurons of the lateral nucleus of the amygdala was revealed for the first time.

In summary, the present investigation is the first to elucidate the roles of the mesolimbic and nigrostriatal dopamine systems, the dopamine receptors, the dopamine pools, and the lateral amygdaloid nucleus in conditioned incentive behavior in the CPP paradigm. These new findings lead to the resolution of some hitherto unexplained or conflicting data in the literature and to the elaboration of a general model of incentive learning.

CHAPTER 1

Animals approach food, water and sexual partners. These stimuli are defined as incentive stimuli because animals naturally approach and maintain contact with them (Young, 1959). These behaviors make it possible to maximize contact with biologically essential stimuli (Glickman and Schiff, 1967, Schneirla, 1959). In natural settings, incentive stimuli are often "hidden" in a constellation of environmental stimuli, which vary from one environment to another. If animals could also maximize contact with the environmental stimuli that are most likely to be associated with incentive stimuli, their chances of survival would be greatly enhanced. Thus there may be an evolutionary pressure for this type of adaptive behavioral modification. In fact, most learning in animals occurs as a means of maximizing contact with incentive stimuli. Therefore, studying this type of learning is a step toward understanding motivation and learning.

The role of dopamine pathways in motivational behaviors

The neural basis of motivational behaviors has been extensively studied. Early studies showed that the hypothalamus is an important neural structure for various motivational behaviors. Lesions of the lateral hypothalamic area produce aphagia (Anand and Brobeck, 1951) and adipsia (Montemurro and Stevenson, 1957). Stimulation of the area induces hyperphagia (Delgado and Anand, 1953, Miller, 1960) and hyperdipsia (Greer, 1955, Mogenson and Stevenson, 1966).

Although lesions of the lateral hypothalamus reliably produce aphagia and adipsia, the entire region is not involved in eating and drinking. Lesions of the far lateral hypothalamus produce severe aphagia and adipsia. Lesions

of the most medial part of the lateral hypothalamus induce only mild aphagia and adipsia (Morgane, 1961), and lesions of the medial part of the medial forebrain bundle coursing through the lateral hypothalamus have little effect on food or water intake (Morgane, 1961). Based on these findings, Morgane (1961) suggested that the medial forebrain bundle is not a critical element for controlling feeding and that the severe aphasia and adipsia observed after lesions of the far-lateral hypothalamus are due to interruption of the pallidofugal fibers coursing through the area.

Ungerstedt (1971c) revealed the involvement of dopamine pathways coursing through the far-lateral hypothalamus in eating and drinking. Aphagia and adipsia are produced when damage includes the medial part of the crus cerebri and the lateral part of the medial forebrain bundle (Ungerstedt, 1971c), through which dopamine pathways course (Ungerstedt 1971a). Consistent with this, Oltmans and Harvey (1972) showed that small electrolytic lesions to the nigrostriatal dopamine pathway at the level of the lateral hypothalamus produced more severe aphagia and adipsia than those of the medial forebrain bundle. They suggested that mild aphagia and adipsia induced by the lesions of the medial forebrain bundle are due to partial interruption of the nigrostriatal dopamine pathway.

The neurotoxin 6-hydroxydopamine (6-OHDA), which depletes dopamine in the caudate/putamen, nucleus accumbens, and olfactory tubercle when injected into the lateral hypothalamus or substantia nigra (SN), produces severe aphagia and adipsia (Fibiger, Zis and McGeer, 1973, Marshall, Richardson and Teitelbaum, 1974, Ungerstedt, 1971c). 6-OHDA also depletes norepinephrine. But the aphagia and adipsia do not seem to be due to this effect, because 6-OHDA applied to an area caudal to the SN preferentially

depletes norepinephrine without producing aphagia or adipsia (Ungerstedt, 1971c).

One explanation for aphagia and adipsia comes from the observation that lesions which cause aphagia and adipsia also produce sensory neglect (Marshall and Teitelbaum, 1974, Marshall, Turner and Teitelbaum, 1971). Sensory neglect is produced by lesions of the lateral hypothalamus (Marshall and Teitelbaum, 1974, Marshall et al., 1971) and by treatments affecting the nigrostriatal dopamine pathway, including 6-OHDA injected into the SN (Ljungberg and Ungerstedt, 1976b; Marshall and Gotthelf, 1979; Marshall et al., 1974), into the caudate/putamen (Marshall, Berrois and Sawyer, 1980), near the origin of the nigrostriatal and mesolimbic dopamine pathways (Marshall, 1979, Marshall et al., 1980), and into the middle of the dopamine pathway at the level of the lateral hypothalamus (Schallert et al., 1982, Schallert, Upchurch, Wilcox and Vaughn, 1983). Sensory neglect is ameliorated by a dopamine agonist injected systemically (Marshall and Gotthelf, 1979, Schallert et al., 1983) or into the caudate/putamen (Marshall et al., 1980). These are the same treatments that ameliorate aphagia and adipsia in animals with lesions (Ljungberg and Ungerstedt, 1976a). The sensory deficit is reinstated by dopamine antagonists in recovering animals (Marshall 1979). During recovery, animals with lateral hypothalamic lesions start accepting highly palatable food on the same day that they show orientation to olfactory stimuli and whisker touch (Marshall et al., 1971). Animals with unilateral dopamine depletion in the caudate/putamen do not respond to food and sensory stimuli on the contralateral side of the body (Ljungberg and Ungerstedt, 1976b). The impressive correlation between aphagia/adipsia and sensory neglect led some to suggest that sensory neglect

is causally implicated at least in the initial phase of aphagia and adipsia (Marshall and Teitelbaum, 1974, Marshall et al., 1971).

The nucleus accumbens, a terminal area of the mesolimbic dopamine pathway, is not directly involved in basic maintenance of eating and drinking. 6-OHDA lesions affecting dopamine in the nucleus accumbens and olfactory tubercle have little effect on feeding and drinking (Robbins and Koob, 1980; Ungerstedt, 1971c) or cause *hyper*phagia (Koob, Riley, Smith and Robbins, 1978).

Yet subtle impairments are observed in animals with dopamine depletion in the nucleus accumbens. When normal animals are given food pellets periodically, they tend to display excessive drinking immediately following food delivery (Falk, 1971). This food-associated "adjunctive" drinking is severely impaired by dopamine depletion in the nucleus accumbens (Koob et al., 1978, Mittleman, Whishaw, Jones, Koch and Robbins, 1990, Robbins and Koob, 1980). Nucleus accumbens dopamine depletion also suppresses the hyperactivity accompanying food intake (Koob et al, 1978) and hoarding, which precedes eating (Kelley and Stinus, 1985).

These findings are generally interpreted as suggesting that mesolimbic dopamine mediates the general "excitement" that is characteristic of motivational states (Kelley and Stinus, 1985, Robbins and Koob, 1980) It is relatively easy to understand hyperactivity as an expression of this excitement, but it is not an easy matter to explain a highly organized behavior such as hoarding simply in these terms. Rather, these findings might be interpreted as suggesting that the two dopamine systems mediate two distinct phases of motivated behaviors. In the presence of food, animals move forward, approach, sniff, and, if possible, bring the food to a safe place

(appetitive behaviors) and then eat or drink (consummatory behaviors) (Craig, 1918). It seems that lesions of the mesolimbic dopamine system affect a class of behaviors which correspond to appetitive behaviors, whereas those of the nigrostriatal dopamine system affect consummatory behaviors.

Motivational behaviors depend on internal factors such as hunger and thirst and external factors such as incentive stimuli. Dopamine systems seem to be involved in the latter factor. Food deprivation does not activate dopamine systems (Heffner, Hartman and Seiden, 1980). In contrast, when animals approach food and engage in eating, dopamine systems are activated (Blackburn, Phillips, Jakuvobic and Fibiger, 1986, Church, Justice and Neill, 1987, Heffner et al., 1980, Holmes, Smythe and Storlien, 1989, Radhakishun, van Ree and Westernik, 1988).

Other evidence also suggests that both distal and proximal incentive stimuli require normal dopamine function to elicit approach and eating. Both D1 and D2 dopamine antagonists increase the latency of food intake, which reflects approach to food (Koechling, Colle and Wise, 1988; Wise and Cole 1984, Wise and Raptis, 1986). Dopamine antagonists also impair the consummatory phase, which is reflected in the speed of eating after animals make contact with food (Koechling et al., 1988, Wise and Colle, 1984, Wise and Raptis, 1986). This phase seems to be mediated by some process generated by stimulation of peripheral sensory receptors above the stomach since rats take sucrose even if it is completely drained from the stomach through a tube (Geary and Smith, 1985, Schneider, Gibbs and Smith, 1986), and dopamine antagonists decrease this sham feeding (Geary and Smith, 1985, Schneider et al., 1986).

Taken together, these data suggest two points. First, the mesolimbic

and nigrostriatal dopamine systems may be essential for the appetitive and consummatory phases, respectively. Second, dopamine systems seem to be essential for incentive behaviors, which are the aspects of motivational behaviors initiated and maintained by incentive stimuli. This idea is considered further in the next section.

Dissociation of the appetitive and consummatory dopamine systems

Supporting the notion that the mesolimbic and nigrostriatal dopamine systems mediate appetitive and consummatory incentive behaviors, respectively, direct pharmacological activation of these dopamine systems induce behaviors which resemble the respective incentive behaviors. Microinjections of dopamine agonists into the caudate/putamen induce gnawing and licking (Costall, Naylor and Neumeyer, 1975, Costall, Naylor and Olley, 1972). Lesions of the caudate/putamen abolish gnawing and licking induced by systemic injections of dopamine agonists (Fog, Randrup and Pakkenberg, 1970, Fuxe and Ungerstedt, 1970). Direct application of dopamine agonists into the nucleus accumbens induces intense downward sniffing and hyperactivity (Costall and Naylor, 1975, 1976, Jackson, Anden and Dahlstrom, 1975; Pijungberg and van Rossum, 1973). Hyperactivity and downward sniffing induced by systemic injections of amphetamine are blocked by 6-OHDA lesions of the nucleus accumbens (Costall, Marsden, Naylor and Christopher, 1977, Fink and Smith, 1980, Kelly and Iversen, 1976, Kelly, Seviour and Iversen, 1975). Dopamine depletion in the caudate/putamen abolishes stimulant-induced gnawing and licking, and that of the nucleus accumbens abolishes stimulant-induced downward sniffing and hyperactivity (Costall, Naylor and Owen, 1977, Fink and Smith, 1980,

Kelly and Iversen, 1976, Kelly et al., 1975).

These behavioral effects of stimulants and dopamine agonists are brought about by binding of endogenous dopamine or dopamine agonists to dopamine receptors. Naturally, dopamine receptor antagonists block these behavioral effects (Randrup, Munkvad and Udsen, 1963, Anden, Butcher, Corrodi, Fuxe and Ungerstedt, 1970, Costall and Naylor, 1976, Janssen and Van Bever, 1978, Randrup and Munkvad, 1967, Randrup et al., 1963).

It is again noteworthy that sniffing and locomotor activity are parts of appetitive incentive behaviors and gnawing and licking are parts of consummatory incentive behaviors.

Roles of D1 and D2 dopamine receptors

Taking advantage of the recent development of selective D1 and D2 dopamine receptor agonists and antagonists, studies have further revealed the roles of dopamine receptor subtypes in these behaviors. Gnawing and licking are not induced by either selective D1 or D2 agonists alone (Braun and Chase, 1986, Johansson, Levin, Gunne and Ellison, 1987, Molloy and Waddington, 1983, 1984, 1985a). Only when both selective D1 and D2 agonists are given in combination are these behaviors induced (Arnt, Hyttel and Perrgaard, 1987, Dall'Olio, Gandolfi, Vaccheri, Roncada and Montanaro, 1988, White, Bednarz, Wachtel, Hjorth and Brooderson, 1988). Gnawing and licking induced by dopamine receptor agonists or stimulants are blocked by either selective D1 or D2 antagonists (Arnt, 1985; Arnt et al., 1987, Christinsen, Arnt, Hyttel, Larsen and Svedsen, 1984, Mailman, Schultz, Lewis, Staples, Rollema and Dehaven, 1984). These observations suggest some synergistic interaction of D1 and D2 dopamine receptors in eliciting gnawing

and licking (Arnt, 1985; Arnt et al., 1987, Braun and Chase, 1986, Mashurano and Waddington, 1986, Pugh, O'Boyle, Molloy and Waddington, 1985, White et al., 1988).

Hyperactivity and downward sniffing seem to be differentially dependent on dopamine D1 and D2 receptors. Systemic injections of D2 agonists alone produce hyperactivity and downward sniffing (Arnt, Bogeso, Hyttel and Meier, 1988, Christensen et al., 1984, Dall'Olio et al., 1988, Dall'Olio, Roncada, Vaccheri, Gandolfi and Montanaro, 1989, Jackson and Hashizume, 1986, Jenkins and Jackson, 1986, Mashurano and Waddington, 1986, Molloy, O'Boyle, Pugh and Waddington, 1986, Pugh et al., 1985, White et al., 1988). When injected systemically, selective D1 dopamine agonists do not induce hyperactivity or downward sniffing (Arnt et al, 1987, Dall'Olio et al., 1988, Mashurano and Waddington, 1986). The behaviors induced by selective D2 dopamine agonists are blocked by both D1 (Breese and Mueller, 1985, Christensen et al., 1984, Dall'Olio et al., 1989; Jackson and Hashizume, 1986, Molloy et al., 1986, Molloy and Waddington, 1985a,b, Pugh et al., 1985) and D2 dopamine antagonists (Breese and Mueller, 1985, Christensen et al., 1984, Dall'Olio et al., 1989, Jackson and Hashizume, 1987; Molloy et al., 1986, Pugh et al., 1985). These data are consistent with the hypothesis that stimulation of the dopamine D2 receptor is essential to induce downward sniffing and hyperactivity and that tonic stimulation of the D1 dopamine receptor enables the D2 receptor-mediated behaviors (Jackson and Hashizume, 1986, Jackson, Jenkins and Ross, 1988, Mashurano and Waddington, 1986, Molloy and Waddington, 1984, 1985a,b, Waddington, 1986, White et al., 1988).

Although the effects of systemically injected D1 and D2 agonists and antagonists are straightforward, some evidence questions the proposed

enabling role of the D1 dopamine receptor. When injected into the nucleus accumbens, either selective D1 or D2 agonists alone induce hyperactivity (Dreher and Jackson, 1989, Freedman, Wait and Woodruff, 1979). This indicates that stimulation of the D1 dopamine receptor in the nucleus accumbens induces locomotor activity. Interestingly, if endogenous dopamine is removed by dopamine depletion, microinjections of D1 or D2 agonists alone into nucleus accumbens no longer induce the behaviors (Dreher and Jackson, 1989), suggesting that both the D1 and D2 dopamine receptors in the nucleus accumbens possess the enabling as well as the inducing role. The fact that systemically injected D1 agonists are unable to induce the behaviors might be due to their weak ability to penetrate the blood-brain barrier (Dreher and Jackson, 1989).

The interpretation of behaviors mediated by dopaminergic activation

There has been much speculation about why dopaminergic activation causes these behaviors. One hypothesis is that, since stimulation of the extrapyramidal motor system induces these behaviors, they are purely motor responses (Anden et al., 1970, Fuxe and Ungerstedt, 1970). This explanation is probably not true for several reasons. One is that amphetamine-induced stereotyped head movements are dramatically decreased by blindfolding (Stevens, Livermore and Cronan, 1977). If stereotypies were purely motor responses they would be expressed independently of sensory inputs. The importance of sensory inputs as a determinant of stereotyped behaviors was further substantiated by the finding that, when treated with apomorphine, rats which show clockwise rotation along the outeredge of a donut-shaped table exhibit *counter*clockwise rotation along the inner edge of the table (Pisa

and Szechtman, 1986). Again, these animals are not simply exhibiting a peculiar response, but are responding to particular sensory stimuli. Given that sensory inputs are a critical determinant of stereotyped behaviors, it seems to be difficult to conceptualize stereotyped behaviors as pure motor responses.

Another hypothesis is that stereotypies are induced by accidental conditioning. It has been noted that any given behavior in which animals are engaged just before amphetamine effects take place tends to be repeated and that that behavior develops into a stereotypy (Ellinwood, 1971, Ellinwood and Kilbey, 1975). According to this hypothesis the topography is accidentally selected from species-specific responses, and its frequency increases due to the reinforcing effect of amphetamine. A number of investigators have taken this position (Ellinwood, 1971, Ellinwood and Kilbey, 1975, Robbins, 1976).

What is difficult to explain by this hypothesis, however, is the dose dependency of the topography of stereotypies. Rats, for example, display gnawing and licking whenever they are given high doses of dopamine agonists (Randrup and Munkvad, 1967, Randrup et al., 1963) But because the frequency of occurrence of spontaneous gnawing and licking is extremely low in experimental settings, it is highly unlikely that whenever animals are to be given high doses of dopamine agonists they are engaged in spontaneous gnawing or licking. Furthermore, there is clear evidence that an ongoing behavior can be disrupted, rather than strengthened, by a dopamine agonist (Szechtman, 1986).

The topography might be better understood if one considers the neuroanatomical basis of stereotypies. Stereotypies are not a singular event from a neuroanatomical point of view. Stereotypies mediated by the

nigrostriatal dopamine system resemble consummatory incentive behaviors. Those mediated by the mesolimbic dopamine system resemble appetitive incentive behaviors. Together with the findings that low level dopaminergic activation exaggerates feeding in the presence of food (Dobrzanski and Doggett, 1976, Evans and Vaccarino, 1986, 1987, 1990, Holtzman, 1974, Winn, Williams and Herberg, 1982) and that lesions of the two dopamine systems affect the two distinct aspects of incentive behaviors (Kelley and Stinus, 1985, Ungerstedt, 1971c), it might be suggested that drug-induced stereotypies reflect two distinct types of exaggerated incentive behaviors: exaggerated appetitive incentive behaviors such as downward sniffing and hyperactivity and exaggerated consummatory incentive behaviors such as licking and gnawing.

The role of dopamine systems in the acquisition of incentive learning

Animals exhibit a variety of behavioral changes which are brought about by pairing neutral sensory stimuli with incentive stimuli, and these have been demonstrated in several different experimental paradigms. Neutral stimuli whose presence is correlated with that of incentive stimuli acquire the property of incentive stimuli to establish and maintain a response (conditioned reinforcement paradigm). The property of incentive stimuli to induce behavioral activation is acquired by neutral stimuli (conditioned locomotor activation (CLA) paradigm). The property of incentive stimuli to induce approach and maintained contact is shown in the autoshaping and conditioned place preference (CPP) paradigms. These paradigms all follow the principle of classical conditioning neutral stimuli are paired with incentive stimuli independently of responses. Some property of the incentive

stimuli is acquired by originally neutral sensory stimuli, which then become conditioned incentive stimuli (Bindra, 1969). These types of classical conditioning have been called incentive learning (Beninger, Hoffman and Mazurski, 1989).

Natural incentive stimuli such as food and water establish conditioned reinforcers (Beninger and Phillips, 1980; Hill, 1970, Robbins, 1978), conditioned locomotor activity (Bindra and Palfai, 1967), autoshaping (Brown and Jenkins, 1968; Leslie, Boakes, Linaza and Ridgers, 1979, Peterson, Ackil, Frommer and Hearst, 1972) and CPPs (Papp, 1988, Spyraki, Fibiger and Phillips, 1982c; Tombaugh, Grandmaison and Zito, 1982). Given that central dopamine systems play an important role in incentive behaviors, one might suspect that dopamine is also involved in incentive learning with natural incentive stimuli. This conjecture is supported by the fact that dopamine antagonists block establishment by food of a conditioned reinforcer (Beninger and Phillips, 1980) and a CPP (Spyraki et al., 1982c).

Direct pharmacological activation of dopamine systems also establishes incentive learning. Conditioned reinforcers are established by apomorphine (Davis and Smith, 1977), piribedil (Davis and Smith, 1977), and amphetamine (Davis and Smith, 1975). CLAs are established by amphetamine (Beninger and Hahn, 1983, Gold et al., 1988, Pickens and Crowder, 1967, Schiff, 1982, Schiff, Bridger, Sharpless and King, 1980, Tilson and Rech, 1973) and cocaine (Barr, Sharpless, Cooper, Schiff, Peredes and Bridger, 1983). CPPs are established by amphetamine (Reicher and Holman, 1977; Sherman, Roberts, Roskam and Holman, 1980), cocaine (Mucha, van der Kooy, O'Shaughnessy and Bucenieks, 1982, Spyraki et al., 1982c), methylphenidate (Martin-Iverson, Ortmann and Fibiger, 1985, Mithani, Martin

Iverson, Phillips and Fibiger, 1986), apomorphine (Spyraki et al., 1982a), bromocriptine (Hoffman, Dickson and Beninger, 1988), and nomifensine (Martin-Iverson et al., 1985). Dopamine antagonists block establishment of conditioned reinforcers by apomorphine and piribedil (Davis and Smith, 1977), establishment of amphetamine CLA (Beninger and Hahn, 1983; Poncelet, Dangoumau, Soubrie and Simon, 1987), and establishment of amphetamine CPPs (Hoffman and Beninger, 1989, Mithani et al., 1986; Spyraki et al., 1982a).

Site of action. The neural bases of incentive learning have been studied in the CPP paradigm, and the site of action of amphetamine in this paradigm has been extensively investigated. Dopamine depletion in the nucleus accumbens blocks the establishment of CPPs by systemically administered amphetamine (Spyraki et al., 1982a). Amphetamine establishes CPPs when injected into the nucleus accumbens, but not into the amygdala, caudate/putamen, or medial frontal cortex (Carr and White, 1983, 1986). The effect of intra-accumbens amphetamine is attenuated by simultaneous intra-accumbens administration of a dopamine receptor antagonist (Aulisi and Hoebel, 1983). These data clearly suggest that amphetamine interacts with the mesolimbic dopamine pathway in the nucleus accumbens to establish CPPs.

Cocaine injected into the nucleus accumbens establishes a CPP, and this CPP is antagonized by co-administration of a dopamine antagonist into the nucleus accumbens (Aulisi and Hoebel, 1983). Yet systemically injected dopamine antagonists do not block CPPs induced by systemic cocaine injections (MacKey and van der Kooy, 1985, Spyraki et al., 1982b). Dopamine depletion in the nucleus accumbens by 77 percent has no effect on cocaine

CPPs (Spyraki et al., 1982b). On the other hand, animals with suction lesions of the medial prefrontal cortex, another target of the mesolimbic dopamine pathway, develop cocaine conditioned place aversion at the same dose that produces CPPs in normal animals, and animals with lesions of the orbital and precentral cortex do not develop any place conditioning with cocaine (Issac, Nonneman, Niesewander, Landers and Bardo, 1989). These studies, however, pose procedural as well as interpretative problems. First, cocaine injected into nucleus accumbens might spread to adjacent areas. This cannot be ruled out unless it is demonstrated that microinjections of cocaine into adjacent areas do not establish CPPs. Second, regarding the lack of an effect of dopamine depletion in the nucleus accumbens on cocaine CPPs, it should be noted that dopamine release remains normal until depletion is more than 90 % (Robbinson and Whishaw, 1988, Zhang et al, 1988). Third, the effects of lesions of the frontal cortex on cocaine CPPs should also be interpreted with caution, since suction lesions are not specific to dopamine terminals in the area. The lesions might affect fibers of passage or projections to the nucleus accumbens, thereby secondarily interfering with the establishment of cocaine CPPs. In fact, lesions of the prefrontal cortex produce sensory neglect (Welch and Stutterville, 1958), which might prevent CPPs from developing. Thus, the site of action of cocaine remains obscure.

Methylphenidate establishes a CPP (Martin-Iverson et al, 1985, Mithani et al., 1986). This CPP is unaffected by the dopamine receptor antagonist haloperidol (Mithani et al., 1986) or is blocked only by a high dose of haloperidol (Martin-Iverson et al., 1986). 6-OHDA lesions of the nucleus accumbens have no effect on this CPP (Martin Iverson et al, 1985). The site of action of methylphenidate remains undetermined.

Receptor subtypes. While the site of action of stimulants remains elusive except for amphetamine, some progress has been made in understanding how the two dopamine receptor subtypes are involved in the establishment of incentive learning. Systemically administered D2 agonists establish conditioned reinforcers (Davis and Smith, 1977). Systemically administered D2, but not D1 agonists, establish CPPs (Hoffman and Beninger, 1988, 1989). Both D1 and D2 dopamine antagonists block the establishment of amphetamine CPPs (Hoffman and Beninger, 1989, Leone and DiChiara, 1987, Martin-Iverson et al., 1985, Mithani et al., 1986, Spyraki et al., 1982a) and the establishment of CPPs by D2 dopamine agonists (Hoffman and Beninger, 1989). These findings led some investigators to conclude that stimulation of the D2 receptor is essential for the establishment of the conditioning and that tonic activation of the D1 receptor is necessary for the effect of D2 dopamine receptor stimulation (Hoffman and Beninger, 1989). This, however, must be taken with caution because both D1 and D2 dopamine agonists establish CPPs when injected into the nucleus accumbens (White, Packard and Hiroi, in press).

The role of dopamine systems in the expression of incentive learning

Incentive learning also has a phase which occurs in the absence of unconditioned incentive stimuli or drugs animals learn a new response, exhibit CIA, and exhibit a CPP, if only conditioned incentive stimuli are present. This aspect of incentive learning has not been the subject of much study, and the role of dopamine in it is far from clear.

One line of evidence strongly implicates dopamine release in the nucleus accumbens in the expression of incentive learning, which is initiated

and guided by conditioned incentive stimuli. When animals are engaged in conditioned amphetamine locomotor activity in the absence of the stimulant, dopamine metabolism is elevated in the nucleus accumbens (Schiff, 1982, Schiff et al., 1980). When animals approach and sniff conditioned incentive stimuli, an increase in dopamine metabolism is observed in the nucleus accumbens (Blackburn, Phillips, Jakuvobic and Fibiger, 1989). Complementing these findings, it was shown that the expression of an amphetamine CLA was abolished by 6-OHDA lesions of the nucleus accumbens (Gold et al., 1988). Yet another line of evidence suggests an entirely opposite conclusion. Beninger and Hahn (1983) reported that pimozide had no effect on the expression of amphetamine CLA at a dose which completely blocked unconditioned amphetamine-induced locomotor activity. Based on this finding, Beninger (1983) suggested that dopamine is an essential neurotransmitter to establish learning in general, but that once established, learned behaviors are expressed independently of dopamine function. The reason for this discrepancy is addressed in the present thesis.

Using the conditioned reinforcement paradigm, Hill (1970) found that responding sustained by a conditioned reinforcer alone was markedly potentiated by the stimulant pipradrol. In the absence of a conditioned reinforcer, the stimulant reduced responding. Thus, the drug seemed to interact with the conditioned reinforcer rather than with general motor responding. Extending these findings, Robbins and his colleagues showed that whereas pipradrol and methylphenidate reliably potentiated responding acquired by a conditioned reinforcer, other stimulants such as amphetamine, cocaine, and nomifensine did not (Robbins, 1978, Robbins, Watson, Gaskin and Ennis, 1983).

These findings raise two questions. First, it is not clear if dopamine release normally mediates the effect of conditioned reinforcers since stimulant-induced dopamine release might artificially augment the effect of conditioned reinforcers. Alternatively, stimulants might exaggerate endogenous dopamine release which normally mediates the effect of conditioned reinforcers. The former possibility was supported by the finding that 6-OHDA lesions of the nucleus accumbens had no effect on the acquisition of lever-pressing by a conditioned reinforcer while the lesions blocked the potentiating effect of a stimulant (Taylor and Robbins, 1986). According to this, endogenous dopamine does not normally mediate the ability of conditioned reinforcers to establish a new response, but if released by stimulants, it potentiates the effect of conditioned reinforcers.

The second question is concerned with the differential effects of several stimulants. Interestingly, the effective stimulants seem to interact with the reserpine-sensitive dopamine pool while the ineffective stimulants interact with the *a* -MPT-sensitive dopamine pool (Glowinski, 1973, Scheel-Kruger, 1971). Taylor and Robbins (1984, 1986), however, showed that microinjections of amphetamine into the nucleus accumbens potentiated the acquisition of responding by a conditioned reinforcer. This suggests that the weak effect of systemic amphetamine might be due to some aversive peripheral effects (Taylor and Robbins, 1984). Yet their data actually show that the amplitude of the potentiation by intra-accumbens amphetamine is far less than that obtained by systemic pipradrol (Robbins et al., 1983) and is in fact comparable to that obtained by systemic amphetamine (Robbins et al., 1983, Taylor and Robbins, 1984, 1986). It is, therefore, undeniable that amphetamine and pipradrol exert differential effects due to some unknown

factor(s) other than peripheral effects. It is unclear why the two types of stimulants exert the different effects. Certainly these studies provide more questions than conclusions. These questions are addressed in the present thesis.

Neuroanatomy of basal ganglia

The neuroanatomical structures in question, the caudate/putamen and the nucleus accumbens, have much in common. One could regard these two telencephalic structures as two parallel systems. We have seen how they differ from behavioral points of view in the previous sections. I shall now review the neuroanatomical literature to illustrate how they differ neuroanatomically. The parallel nature of the caudate/putamen and the nucleus accumbens is best illustrated by their connections with other brain structures.

Both the caudate/putamen and the nucleus accumbens receive dopaminergic innervation from the midbrain. The projection pattern is topographically organized (Fallon, 1988): ventral SN and ventrolateral ventral tegmental area (VTA) send dopaminergic fibers to the caudate/putamen (Fallon, Riley and Moore, 1978), and dorsomedial SN and dorsal VTA send dopaminergic axons to the nucleus accumbens (Fallon and Moore, 1978, Simon, LeMoal and Calas, 1979).

In principle, cortical projections to the caudate/putamen and the nucleus accumbens maintain a topographical order so that cortical areas have connections with immediately adjacent areas in the caudate/putamen and the nucleus accumbens.

Neocortex. Sensorimotor, visual, and auditory cortices project to the

dorsolateral, dorsomedial and posteroventral caudate/putamen, respectively (McGeorge and Faull, 1989; Webster, 1961).

Mesocortex. The cingulate complex of the medial mesocortex innervates the nucleus accumbens and medial caudate/putamen, and another structure of the medial mesocortex, the medial frontal cortex, gives rise to axons to the nucleus accumbens and the olfactory tubercle (Beckstead, 1979; Domesick, 1969, Leonard, 1969, McGeorge and Faull, 1989; Phillipson and Griffiths, 1985). The lateral mesocortex, such as the sulcal, agranular insular, and perirhinal areas, projects to the medial caudate/putamen, the nucleus accumbens, and the olfactory tubercle (Beckstead, 1979, Leonard, 1969; McGeorge and Faull, 1989, Phillipson and Griffiths, 1985).

Paleocortex. The paleocortex gives rise to axons to the caudate/putamen and the nucleus accumbens in a medially skewed fashion: the entorhinal cortex to the ventromedial caudate/putamen and the nucleus accumbens (Haberly and Price, 1978; Kelley and Domesick, 1982; Krayniak, Meibach and Siegel, 1981, Luskin and Price, 1983; McGeorge and Faull, 1989; Phillipson and Griffiths, 1985), the pyriform cortex to the medial caudate/putamen, the nucleus accumbens, and the olfactory tubercle (Haberly and Price, 1978; Lushkin and Price, 1983, McGeorge and Faull, 1989, Price, 1973), and the amygdala to the nucleus accumbens and the entire caudate/putamen except the dorsolateral sector (DeOlmos, 1972, DeOlmos and Ingram, 1972, Kelley, Domesick and Nauta, 1982; Lushkin and Price, 1983; Krettek and Price, 1978, McGeorge and Faull, 1989; Phillipson and Griffiths, 1985, Veening, Cornelissen and Lieven, 1980).

Archicortex. The archicortex, comprising the subiculum and the hippocampus, innervates the nucleus accumbens and the rostromedial

caudate/putamen (Heimer and Wilson, 1975, Kelley and Domesick, 1982, Kretteck and Price, 1978; Raisman, Cowan and Powell, 1966, Siegel, Edinger and Ohgami, 1974; Swanson and Cowan, 1975, 1977).

The data clearly show slightly overlapping projection patterns of the neocortex and limbic systems to basal ganglia. While the neocortex projects to the caudate/putamen, limbic systems give rise to axons to the anteromedial caudate/putamen, nucleus accumbens and olfactory tubercle. In fact, the very parallel nature led some to suggest the anteromedial caudate/putamen, nucleus accumbens, and olfactory tubercle be called ventral striatum and the rest of the caudate/putamen dorsal striatum (Heimer, Switzer and Van Hoesen, 1982).

Functional interaction of basal ganglia and limbic systems

Behavioral studies have shown functional connections between the basal ganglia and limbic systems. Evidence exists that the basal ganglia are functionally linked to two limbic structures, the hippocampus and the amygdala.

Hippocampus. Mogenson and Nielsen (1984) showed that hyperactivity induced by injections of a cholinergic agonist into the hippocampus was blocked by microinjections of a glutaminergic antagonist into the ipsilateral nucleus accumbens, microinjections of the antagonists into the contralateral nucleus accumbens were without effect. Since a major component of the hippocampal-accumbens pathway is glutaminergic (Fonnum, Karlsen, Matthe-Sorenssen, Skrede and Walaas, 1979, Walaas, 1981), this finding might suggest a functional role of the hippocampal-accumbens pathway in appetitive incentive behaviors. However, it is not clear if the

hyperactivity they produce has any relevance to the locomotor activity involved in incentive behaviors.

Much clearer behavioral evidence for a hippocampal/accumbens interaction is the report that food-associated adjunctive drinking behavior was reduced not only by 6-OHDA lesions of the nucleus accumbens but also by aspirative lesions of the hippocampus (Mittleman et al., 1990). This suggests that the hippocampus and the nucleus accumbens jointly participate in expressing this experimentally-induced behavior.

Evidence also suggests that the hippocampal/accumbens system is involved in learning. Animals with electrolytic lesions of the nucleus accumbens or the fornix/fimbria show impaired acquisition in a Morris water maze task (Sutherland and Rodriguez, 1989), which is sensitive to hippocampal lesions (Morris, Garrud, Rawlins and O'Keefe, 1982). Ibotenic acid lesions of the nucleus accumbens produce deficits in a Morris water maze and spatial T-maze tasks (Annett, McGregor and Robbins, 1989). These results suggest that the hippocampal-accumbens pathway plays an important role in acquisition of learning which is mediated by the hippocampus.

There is little information on how the hippocampal/accumbens system is involved in incentive learning. This issue is dealt with in the present thesis.

Amygdala. The literature shows that the basal ganglia and the amygdala have overlapping functions. Similar to the effects of pharmacological activation of dopamine systems in the basal ganglia, electrical stimulation of the lateral or basolateral nucleus of the amygdala induces sniffing, licking, and chewing (Magnus and Lammers, 1956; Shealy and Peele, 1957, Ursin and Kaada, 1960). Just as dopaminergic activation of

unilateral caudate/putamen causes contralateral turning (Ungerstedt, 1971b), so does stimulation of unilateral amygdala (Magnus and Lammers, 1956, Shearly and Peele, 1957; Ursin and Kaada, 1960). Similar to unilateral dopamine depletion in the caudate/putamen, unilateral lesions of the amygdala produce somatosensory neglect on the contralateral side of the body (Turner, 1973).

Motivational behaviors such as eating and drinking, which are abolished by dopamine depletion of the caudate/putamen (Fibiger et al., 1973, Marshall et al., 1974; Ungerstedt, 1971c), are disrupted by lesions of the amygdala in a more subtle way. Kluver and Bucy (1937, 1939) reported that monkeys with lesions of the temporal lobe showed inadequate incentive behaviors. The monkeys tried to eat any object at hand and approached objects which they normally avoided. Subsequent studies revealed that lesions of the amygdala alone produced these symptoms (Schreiner and Kling, 1953, 1956). Based on these findings, Gloor (1972) suggested that the amygdala is a part of the neural system through which originally neutral stimuli acquire motivational significance.

Overlapping functions are also evident in learning. Turner (1973) reported a study which clearly illustrates a functional link between the caudate/putamen and the amygdala. Animals with unilateral amygdala lesions learn to turn their heads ipsilaterally in order to turn off shock regardless of which side of the body shock is applied to. The animals also learn to turn their heads to the contralateral side when shock is applied to the ipsilateral side of the body. However, the animals cannot learn contralateral head turning in response to shock applied to the contralateral side. This is not a motor deficit, because the animals are capable of turning

their heads in both directions. Neither is the deficit sensory in nature, since the animals respond to shock applied to either side of the body. This raises the possibility that the amygdaloid complex in each hemisphere is involved in sensorimotor learning within the same hemisphere. What is remarkable in this study is the demonstration that a sensorimotor function which is mediated by the caudate/putamen (Marshall and Teitelbaum, 1974; Marshall et al., 1971) is re-represented in the amygdala and that this re-representation accommodates flexible changes.

The amygdala might also mediate some aspect of incentive learning. In the conditioned reinforcement paradigm, excitotoxic lesions of the amygdala impair acquisition of lever-pressing supported by a conditioned reinforcer (Cador, Robbins and Everitt, 1989a) and retention of the response sustained by a conditioned reinforcer (Everitt et al., 1989a). The impairments are ameliorated by amphetamine infusions into the nucleus accumbens (Cador et al., 1989, Everitt et al., 1989a). Although these studies are suggestive, uncertainty remains. First, amygdala lesions produced only mild impairments in these studies. This might be due to the fact that the paradigm relies heavily on response learning, which is not impaired by amygdala lesions (Kemble and Beckman, 1970, Schwartzbaum, 1960). Alternatively, the location of the lesions might be inappropriate. In these studies, the excitotoxin was injected into the basolateral nucleus of the amygdala. Yet, damage was found in the basolateral nucleus, the central nucleus, and lateral nucleus (Cador et al., 1989, Everitt et al., 1989a, b). It is not clear which of these damaged subdivision may have been responsible for the deficits observed.

The present study

The literature shows that dopamine systems are involved in incentive learning. Since the site of action of amphetamine in establishing a CPP is reasonably well characterized, this paradigm seems to be a useful model to study the functional role of the mesolimbic dopamine system in incentive learning. Using the CPP paradigm, the present study investigates several unclarified aspects of this form of incentive learning. One is involvement of dopamine in the expression of the amphetamine CPP. There has been no study dealing with this issue, and some indirect evidence suggests conflicting hypotheses (Beninger and Hahn, 1983, Gold et al., 1989, Taylor and Robbins, 1986). The first set of experiments was concerned with this issue.

The second issue investigated is the roles of the two pharmacologically distinct dopamine pools. Although it seems clear that amphetamine interacts with the *a* -MPT-sensitive dopamine pool (Scheel-Kruger, 1971), it remains unclear what role, if any, is played by the other, reserpine sensitive, pool in the amphetamine CPP. The effects of *a* -MPT and reserpine on the expression of the amphetamine CPP were examined.

The third set of experiments examined the involvement of limbic systems in the amphetamine CPP. The hippocampus and the nucleus accumbens seem to be jointly involved in accumbens-mediated behavior (Mittleman et al., 1990) and hippocampal-type learning (Annett et al., 1989, Sutherland and Rodriguez, 1989). Yet little is known about roles of this system in incentive learning. Evidence also exists suggesting that the amygdala interacts with the nucleus accumbens with respect to the effect of conditioned incentive stimuli (Cador et al., 1989, Everitt et al., 1989a). Given

the heterogeneity of the amygdaloid nuclei (deOlmos, Alheid and Beltramino, 1985), it seems necessary to investigate the functional roles of distinct amygdaloid nuclei in incentive learning. Therefore, the effects of lesions to amygdaloid subdivisions were also examined.

CHAPTER 2

GENERAL METHODS

Subjects

The subjects were 691 experimentally naive, male Long Evans rats purchased from Charles River Canada, St-Constant, Quebec, weighing from 275 to 310 g at the start of the experiments. The animals were individually housed with food and water available *ad libitum*.

Apparatus

Conditioned place preference

The CPP apparatus was made of wood, with a Plexiglas front wall. It consisted of three different compartments, two of which were identical in size (45 x 45 x 30 cm). One compartment was painted white and had wood chips on a smooth floor. The other was painted black with white vertical stripes and had a floor of wire mesh. A few drops of vinegar (1ml 2% acetic acid) were placed on the floor of this compartment below the wire mesh. These two compartments were completely separated from each other by a wooden partition. The entrance to each compartment was at the rear of the apparatus, immediately adjacent to the partition. An unpainted tunnel (36 x 18 x 20cm), protruding from the rear of the large compartments, connected the two entrances. On conditioning days the entrances to the tunnel were blocked. The entrances were open on the pre-exposure and test days. In previous studies, it has been demonstrated that, on a group basis, rats do not exhibit a natural preference for either large compartment of this apparatus (Carr and White, 1983, Clarke, White and Franklin, 1990).

Locomotor activity

Locomotor activity was measured in three identical open-field boxes (41 x 41 x 28 cm) constructed of Plexiglas. The floors had nine 3 cm holes in them. Eight photocell beams, 4 in each direction located 3 cm above the floor, partitioned the box into 25 cells. The total number of photobeam interruptions during a test was taken as the locomotor activity score.

Cannulae

Guide cannulae were made from 20 ga (0.7 mm outer diameter) hypodermic needles. The plastic hubs were removed, and the needles were cut to a length of 12.2 mm. Inner cannulae, used for microinjections, were made from 30 ga needles. They were cut to a length of approximately 30.0 mm and bent so that the tip of the inner cannulae extended beyond the tip of the guide cannulae by 2.5 mm for intra-accumbens injections or 0.5 mm for intra-caudate/putamen injections. The inner cannulae were attached to a 5.0 *ul* Hamilton syringe with PE 10 tubing.

Electrodes

Nichrome electrodes (0.25 mm in diameter) with enamel insulation were used for electrolytic lesions. The tips (0.8 mm) of the electrodes were decoated with Strip X (GC Electronics).

Procedures

Conditioned place preference

The procedure required 6 sessions. On session 1 the rats were given a 10 min pre-exposure period each one was allowed to move freely in the

three compartments of the test apparatus. The next 4 sessions included 2 pairings with *d* -amphetamine (2.0 mg/kg, s.c.) and 2 pairings with saline (1.0 ml/kg, s.c.). Animals in each group were randomly assigned to the cells of a 2 x 2 factorial design. One factor was pairing compartment (black or white), and the other was injection order. Half of the rats received amphetamine injections before exposure to the white compartment, and the other half received amphetamine injections before exposure to the black compartment. Within each of these subgroups, half of the rats received *d* -amphetamine injections on even numbered sessions and saline injections on odd numbered sessions, the pattern was reversed for the remaining rats. In all CPP experiments, eight or more rats were used for each group. The animals were placed into the appropriate compartment immediately after receiving subcutaneous injections of amphetamine or saline, and left there for 30 min. The entrances to the compartments were blocked so that the animals were confined to the compartments.

The sixth session was the test day. No amphetamine was injected, and the entrances to the compartments were open. The animals were placed into the tunnel and allowed to move freely in the three compartments for 20 min. The amount of time spent in each of the two large compartments was recorded. This procedure normally produces a robust CPP with amphetamine: animals normally spend approximately 50 %, 20 %, and 30 % of the total test time in the amphetamine-paired compartment, saline paired compartment, and the tunnel, respectively.

Locomotor activity

Locomotor activity was measured for 20 min after animals were placed

into the locomotor activity boxes. Details of the procedures are described in each experiment.

Statistical Analysis

In the CPP paradigm, animals could spend time in either of the two large compartments or the tunnel on the test day. Thus, time spent in one of the two large compartments did not necessarily affect time spent in the other of the two large compartments. However, since each individual animal spent time in both compartments, the compartment factor was considered to be a repeated measure. Two-way ANOVAs with planned comparisons were applied to time spent in the amphetamine paired and unpaired compartments. Thus the independent variables were groups and compartments (repeated measure), and the dependent variable was time. Two types of planned comparisons were applied. One was a comparison between time spent in the paired and unpaired compartments for each group. This assessed the existence or absence of place conditioning for individual groups. The other was a comparison between groups using the time differences obtained by subtracting time spent in the unpaired compartment from that spent in the paired compartment. This analysis was used to examine if a given group differed from a control group.

Locomotor activity data were analyzed using ANOVAs. When a time course was analyzed, the ANOVA was followed by post-hoc tests (Scheffe method).

d-Amphetamine Sulphate

d -Amphetamine sulphate (Smith, Kline and French, Canada) was dissolved in physiological saline as 2.0 mg of salt/ml of the solution and

injected subcutaneously on the back. In all experiments, the dose used was 2.0 mg/kg.

CHAPTER 3

Acquisition of the amphetamine CPP is blocked by both selective D1 (Hoffman and Beninger, 1989; Leone and DiChiara, 1987) and D2 (Hoffman and Beninger, 1989, Spyraki et al., 1982a) dopamine antagonists, and pharmacological activation of either dopamine receptor subtype in the nucleus accumbens establishes CPPs (White et al. in press). These findings suggest that a CPP is established by activation of either receptor subtype provided the other subtype is at least tonically activated by endogenous dopamine. However, little is known about how the two types of dopamine receptors are involved in the expression of the amphetamine CPP. Also unknown is the involvement of the nigrostriatal dopamine system in CPP expression on the test day. The findings that dopamine depletion in the nucleus accumbens attenuated the amphetamine CPP (Spyraki et al., 1982a) and that microinjections of amphetamine into nucleus accumbens (Aulisi and Hoebel, 1983; Carr and White, 1983, 1986), but not into caudate/putamen (Carr and White, 1983, 1986), establish CPPs do not rule out the possibility that *expression* of the amphetamine CPP involves the nigrostriatal dopamine pathway.

The present set of experiments was designed to investigate the roles of dopamine receptor subtypes and of the nigrostriatal and mesolimbic dopamine pathways in both the acquisition and expression of the amphetamine CPP. First, the blocking effects of systemic D1 and D2 dopamine antagonists on acquisition and expression of the amphetamine CPP were examined. Second, selective D1 or D2 antagonists were injected into nucleus accumbens or caudate/putamen prior to testing to examine the relative roles of the D1 and D2 receptors of these two areas in the expression

of the amphetamine CPP. Third, sodium pentobarbital was used to examine the effect of reduced activity levels on expression of the CPP as a control for antagonist-induced motor impairment. Fourth, lesions were made in the dorsal caudate/putamen, the main target area of the nigrostriatal dopamine pathway.

Experiment 1

This experiment examined the effects of systemically injected selective D1 and D2 dopamine antagonists on the acquisition and the expression of the amphetamine CPP.

Method

Procedure. Two sets of groups (n=8 for each group) were used. One set of groups underwent the experimental procedure of the CPP, but also received dopamine antagonist injections before each of the four conditioning sessions, but not before the test session. The other set was given antagonist injections before the test session, but not before the conditioning sessions. The intervals between injections and the conditioning or test sessions were 30 min for SCH23390 (0.02-0.16 mg/kg), 150 min for *a* -flupenthixol (0.2-1.0 mg/kg), 45 min for metoclopramide (1.25-20 mg/kg), and 60 min for sulpiride (10-160 mg/kg). The antagonists were administered intraperitoneally.

Drugs. SCH23390 (Schering Corp.), *a* -flupenthixol and metoclopramide (Nordic Laboratories Inc.) were dissolved in saline. Sulpiride (Research Biochemicals Inc.) was dissolved in 0.1 N HCl and diluted with

distilled water. All the antagonists were adjusted to pH 6.5-7.0 with sodium hydroxide.

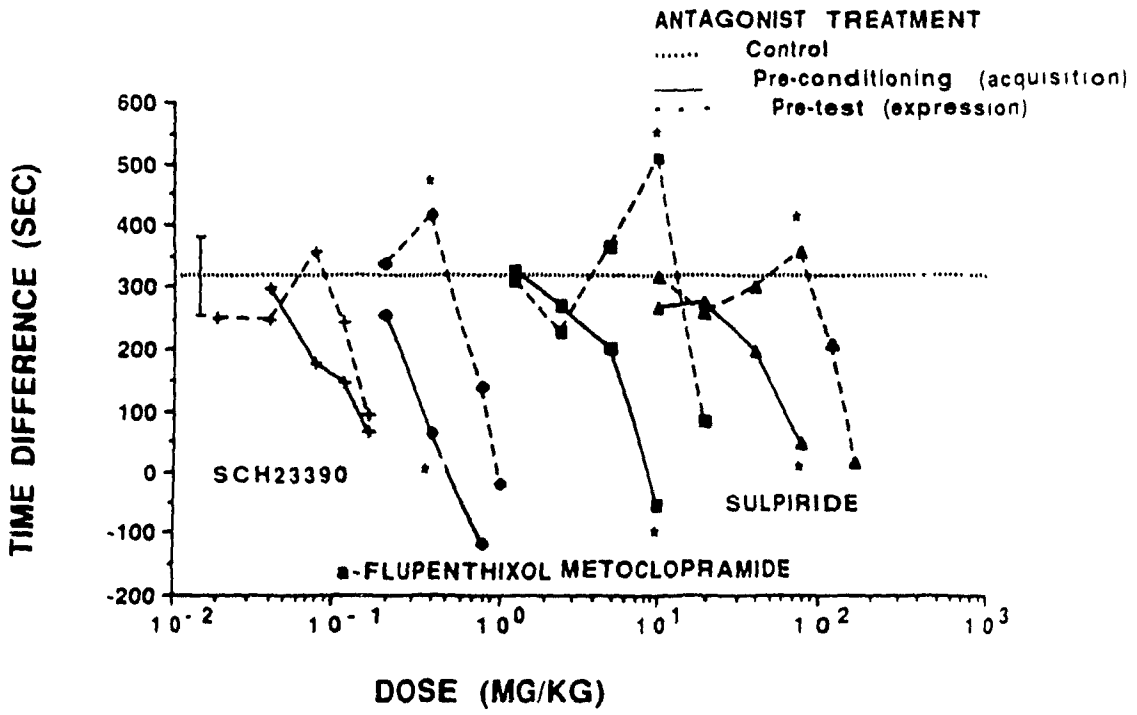
Results

Figure 1 shows the effects of dopamine antagonists on acquisition and expression of the amphetamine CPP. The control group showed a robust conditioned place preference ($p<0.05$). For clarity the figure shows the time difference obtained by subtracting the amount of time spent in the saline-paired compartment from that spent in the amphetamine-paired compartment. It is evident that as doses of the antagonists increased the time differences between the amphetamine-paired and saline-paired compartments decreased.

SCH23390. Both acquisition and expression of the CPP were blocked at approximately equivalent doses of this antagonist. As the figure reveals, the time differences approached zero as the doses increased. 0.16 mg/kg (the highest dose used) of the drug blocked both processes equipotently. For the groups given SCH23390 before the conditioning sessions, planned comparisons showed that there were significant differences between time spent in the paired and unpaired compartments at 0.04 mg/kg ($F(1,35)=14.17$, $p<0.01$) and 0.08 mg/kg ($F(1,35)=4.88$, $p<0.05$). These differences were not significant at 0.12 mg/kg ($F(1,35)=3.36$, $p>0.05$) and 0.16 mg/kg ($F(1,35)=0.71$, $p>0.05$). The time difference of the control group between the two large compartments was significantly different from that of the 0.12 mg/kg group ($F(1,70)=15.58$, $p<0.01$) and that of the 0.16 mg/kg group ($F(1,70)=32.86$, $p<0.01$). For the groups given this drug before testing, there were significant differences between time spent in the paired and

Figure 1

Dose response curves comparing the effects of dopamine receptor antagonists on the acquisition and the expression of the amphetamine CPP. The ordinate 'TIME DIFFERENCE' is the mean difference between the amount of time the rats in each group spent in the paired and unpaired compartments, the time difference was obtained by subtracting time spent in the unpaired compartment from that spent in the paired compartment for each group. SCH23390 (cross), *a* -flupenthixol (diamond), metoclopramide (square) and sulpiride (triangle). The solid lines represent the groups that received the antagonists before conditioning sessions and the broken lines represent the groups that received the antagonists before testing. The dotted horizontal line represents the mean time difference for the control group, the SEM of this group is indicated on the left side. For SCH23390, pre-conditioning doses were 0.04, 0.08, 0.12, and 0.16 mg/kg; pre-testing doses were 0.02, 0.04, 0.08, 0.12, and 0.16 mg/kg. For *a* -flupenthixol, pre-conditioning doses were 0.2, 0.4, and 0.8 mg/kg, pre-testing doses were 0.2, 0.4, 0.8, and 1.0 mg/kg. For metoclopramide, pre-conditioning doses were 1.25, 2.5, 5.0, and 10 mg/kg, pre-testing doses were 1.25, 2.5, 5.0, 10, and 20 mg/kg. For sulpiride, pre-conditioning doses were 10, 20, 40, and 80 mg/kg, pre testing doses were 10, 20, 40, 80, 120, and 160 mg/kg. Asterisks indicate the dose of each drug which blocked acquisition but not expression.


ANTAGONIST TREATMENT
Control
Pre-conditioning (acquisition)
Pre-test (expression)
TIME DIFFERENCE (SEC)
600
500
400
300
200
100
0
-100
-200
SCH23390
SULPIRIDE
a-FLUPENTHIXOL METOCLOPRAMIDE
10-2
10-1
100
101
102
103
DOSE (MG/KG)

unpaired compartments at 0.02 mg/kg ($F(1,42)=4.69$, $p<0.05$), 0.04 mg/kg ($F(1,42)=4.54$, $p<0.05$), 0.08 mg/kg ($F(1,42)=9.54$, $p<0.01$), and 0.12 mg/kg ($F(1,42)=4.38$, $p<0.05$), but not at 0.16 mg/kg ($F(1,42)=0.67$, $p>0.05$). The time difference of the 0.16 mg/kg group was significantly different from that of the control group ($F(1,84)=13.16$, $p<0.01$).

α-Flupenthixol. Both acquisition and expression were blocked by this antagonist; higher doses were required to block expression than to block acquisition. For the groups given this drug before the conditioning sessions, planned comparisons revealed that there were significant differences between time spent in the paired and unpaired compartments at 0.2 mg/kg ($F(1,28)=12.30$, $p<0.01$), but not at 0.4 mg/kg ($F(1,28)=0.74$, $p>0.05$) and 0.8 mg/kg ($F(1,28)=2.55$, $p>0.05$). The time difference of the control group was significantly different from that of the 0.4 mg/kg group ($F(1,56)=31.83$, $p<0.01$) and that of the 0.8 mg/kg group ($F(1,56)=91.65$, $p<0.01$). For the groups given this drug before the test session, there were significant differences between time spent in the paired and unpaired compartments at 0.2 mg/kg ($F(1,34)=5.11$, $p<0.05$) and 0.4 mg/kg ($F(1,34)=7.65$, $p<0.01$), but not at 0.8 mg/kg ($F(1,34)=0.82$, $p>0.05$) and 1.0 mg/kg ($F(1,34)$ 0.02, $p>0.05$). The time difference of the control group was significantly different from that of the 0.8 mg/kg group ($F(1,68)=5.11$, $p<0.05$) and that of the 1.0 mg/kg group ($F(1,68)=17.60$, $p<0.01$). A significant rightward shift of the antagonist curves from acquisition to expression is evidenced by the differential effects of 0.4 mg/kg (the second lowest dose) on acquisition and expression. This dose blocked acquisition but not expression.

Metoclopramide. For the groups that received this drug before the conditioning sessions, there were significant differences between time spent

in the paired and unpaired compartments at 1.25 mg/kg ($F(1,35)=17.18$, $p<0.01$), 2.5 mg/kg ($F(1,35)=12.00$, $p<0.01$), and 5.0 mg/kg ($F(1,35)=6.64$, $p<0.05$), but not at 10 mg/kg ($F(1,35)=0.52$, $p>0.05$). The time difference for the control group was significantly different from that of the 10 mg/kg group ($F(1,70)=73.93$, $p<0.01$). For the test day groups, there were significant differences between time spent in the paired and unpaired compartments at 1.25 mg/kg ($F(1,42)=7.14$, $p<0.05$), 5.0 mg/kg ($F(1,42)=9.95$, $p<0.01$) and 10.0 mg/kg ($F(1,42)=19.27$, $p<0.01$), but not at 2.5 mg/kg ($F(1,42)=3.77$, $p>0.05$) and 20 mg/kg ($F(1,42)=0.49$, $p>0.05$). The time difference for the control group was significantly different from that of the 20 mg/kg group ($F(1,84)=13.69$, $p<0.01$), but not from that of the 2.5 mg/kg group ($F(1,84)=2.09$, $p>0.05$). The highest pre-conditioning dose (10 mg/kg) blocked acquisition but not expression.

Sulpiride. For the groups that received this drug before the conditioning sessions, there were significant differences between time spent in the paired and unpaired compartments at 10 mg/kg ($F(1,35)=6.43$, $p<0.05$) and 20 mg/kg ($F(1,35)=6.79$, $p<0.05$), but not at 40 mg/kg ($F(1,35)=3.47$, $p>0.05$) and 80 mg/kg ($F(1,35)=0.19$, $p>0.05$). The time difference for the control group was significantly different from that of the 40 mg/kg group ($F(1,70)=4.82$, $p<0.05$) and that of the 80 mg/kg group ($F(1,70)=23.99$, $p<0.01$). For the groups that received this drug before the test session, there were significant differences between time spent in the paired and unpaired compartments at 10 mg/kg ($F(1,49)=10.39$, $p<0.01$), 20 mg/kg ($F(1,49)=6.81$, $p<0.05$), 40 mg/kg ($F(1,49)=9.31$, $p<0.01$), 80 mg/kg ($F(1,49)=12.99$, $p<0.01$), and 120 mg/kg ($F(1,49)=4.39$, $p<0.05$), but not at 160 mg/kg ($F(1,49)=0.02$, $p>0.05$). The time difference for the control group

was significantly different from that of the 160 mg/kg group ($F(1,98)$ 34.76, $p<0.01$). 80 mg/kg of sulpiride completely blocked acquisition but not expression.

When given before the test session, all the antagonists potentiated expression of the amphetamine CPP at certain doses. However, none of the potentiated preferences was significantly different from the preference observed for the control group, except for the 10 mg/kg metoclopramide group ($F(1,84)=8.94$, $p<0.01$).

Visual observation revealed that all the dopamine antagonists decreased locomotion. It is unlikely that the blockade of acquisition was due to this effect of the antagonists, however, since simple inhibition of locomotion does not prevent the establishment of the amphetamine CPP (Carr, Phillips and Fibiger, 1988). At the same time, the nature of the expression blockade remains unclear it is unknown how reduced locomotion affects the expression of the CPP. This issue is dealt with in Experiments 2 and 3.

In summary, the data show that SCH23390 blocked acquisition and expression within similar dose ranges, but that the expression-blocking dose ranges of the other antagonists were considerably higher than the acquisition-blocking dose ranges.

Experiment 2

Since studies have shown that the mesolimbic dopamine system projecting to the nucleus accumbens mediates acquisition of the amphetamine CPP (Carr and White, 1983, 1986, Spyraki et al., 1982a), it is

most likely that dopamine receptors in the nucleus accumbens are the site of action of the antagonists on acquisition. The site of action of the antagonists on expression remains unclear, however. Also, the possibility exists that the expression blockade was simply due to decreased locomotion produced by the antagonists. In this experiment, the effects of SCH23390 and sulpiride injected into the nucleus accumbens or the caudate/putamen on expression of he amphetamine CPP were compared in order to reveal the site of action of the antagonists on expression of the amphetamine CPP. A control experiment examined the degree of locomotor inhibition produced by intra-accumbens microinjections of the doses of the two antagonists which blocked the expression of the CPP.

Method

Surgery. Using standard stereotaxic techniques, rats were bilaterally implanted with guide cannulae under 65 mg/kg sodium pentobarbital anesthesia (A+1.7, L:+-1.5, V:-4.5mm) (Paxinos and Watson, 1982). The guide cannulae were filled with insect pins (00) cut to the length of the guide cannulae. Testing began after a one week recovery period.

Procedure. Two sets of groups (n=8 for each group) were used in the CPP paradigm. One set of groups received bilateral microinjections of vehicle, SCH23390 (0.0001, 0.001 or 0.01 *ug*/side), or sulpiride (0.01 or 0.1 *ug*/side) into the nucleus accumbens before testing. The other set of groups received vehicle, SCH23390 (0.01 *ug*/side), or sulpiride (0.1 *ug*/side) bilaterally into the caudate/putamen before testing.

Using different animals (n=6-8 for each group), vehicle, SCH23390

(0.01 ug/side), or sulpiride (0.1 ug/side) were bilaterally injected into the nucleus accumbens to examine their effects on spontaneous locomotor activity.

Nucleus accumbens injections were made by using inner cannulae that extended 2.5 mm below the tip of the guide cannulae, to coordinates (A +1.7, L+-1.5, V-7.0mm). Caudate/putamen injections were made using inner cannulae that extended 0.5 mm below the tip of the guide cannulae, to coordinates (A:+1.7, L:+-1.5, V:-5.0mm).

After the inner cannulae were inserted into the guide cannulae, the drugs were delivered over a 30 sec period. The inner cannulae were left in place for a further 60 sec. Testing began after a further 90 sec.

Histology. After the completion of testing, animals were perfused with saline and subsequently formol saline through the heart. Brains were removed and stored in formol saline. The brains were sectioned with 30 um slices. They were then stained with thionin.

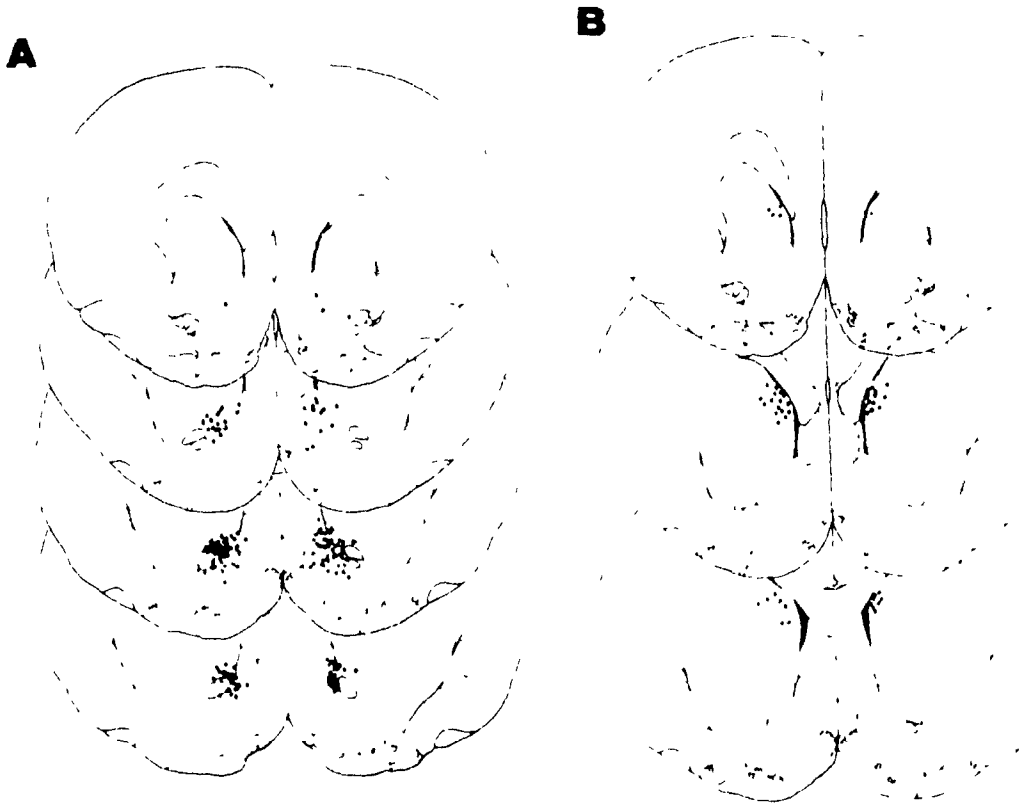
Drugs. SCH 23390 (Schering Corp.) was dissolved in saline. Sulpiride (Research Biochemicals Inc.) was dissolved in 0.1 N HCL and diluted with distilled water. The antagonists were adjusted to pH 6.5-7.0 with sodium hydroxide.

Results

Figure 2 shows the locations of the tips of the inner cannulae for the intra-accumbens (A) and intra-caudate/putamen (B) groups. The data from animals which received microinjections outside the nucleus accumbens or caudate/putamen were eliminated from the analysis. Animals were recruited

Figure 2

The locations of the tips of the inner cannulae for the intra-accumbens groups (A) and intra-caudate/putamen (B) groups.


A
B

until the counterbalanced design of the conditioned place preference was achieved.

The behavioral data are shown in Figure 3. The control group showed a robust conditioned place preference ($p<0.01$). 0.01 *ug* of SCH23390 and 0.1 *ug* of sulpiride injected into the nucleus accumbens on the test day completely blocked the expression of the amphetamine CPP.

For SCH23390, there were significant differences between time spent in the paired and unpaired compartments at 0.0001 *ug* ($F(1,27)=11.72$, $p<0.01$) and 0.001 *ug* ($F(1,27)=4.55$, $p<0.05$), but not at 0.01 *ug* ($F(1,27)=0.02$, $p>0.05$). The time difference of the control group was significantly different from that of the 0.01 *ug* SCH23390 group ($F(1,54)=20.04$, $p<0.01$). For the sulpiride-treated groups, there were no significant differences between time spent in the paired and unpaired compartments at 0.01 *ug* ($F(1,20)=1.52$, $p>0.05$) and 0.1 *ug* ($F(1,20)=0.02$, $p>0.05$). The time difference of the control was significantly different from that of the 0.1 *ug* sulpiride group ($F(1,40)=18.87$, $p<0.01$), but not from that of the 0.01 *ug* group ($F(1,40)=3.82$, $p>0.05$). As the figure reveals, 0.01 *ug* of SCH23390 and 0.1 *u-g* of sulpiride completely blocked expression of the amphetamine CPP.

The doses of SCH23390 and sulpiride which completely blocked the expression of the amphetamine CPP had no effect on this conditioned behavior when they were injected into the caudate/putamen (Figure 4). Planned comparisons revealed that there were significant differences between time spent in the paired and unpaired compartments for the vehicle ($F(1,21)=7.70$, $p<0.05$), SCH23390 ($F(1,21)=14.89$, $p<0.01$), and sulpiride ($F(1,21)=9.28$, $p<0.01$) groups. The time difference of the control was not different from that of the SCH23390 ($F(1,42)=3.64$, $p>0.05$) or sulpiride

Figure 3

Effects of SCH 23390 (0.0001, 0.001 and 0.01 *ug*/side) and sulpiride (0.01 and 0.1 *ug*/side) injected into the nucleus accumbens on the expression of amphetamine CPP. The ordinate represents the time spent in the two large compartments. PAIRED SIDE amphetamine-paired compartment, UNPAIRED SIDE saline-paired compartment. The numbers above the columns are the differences in time (in seconds) spent in the two large compartments.

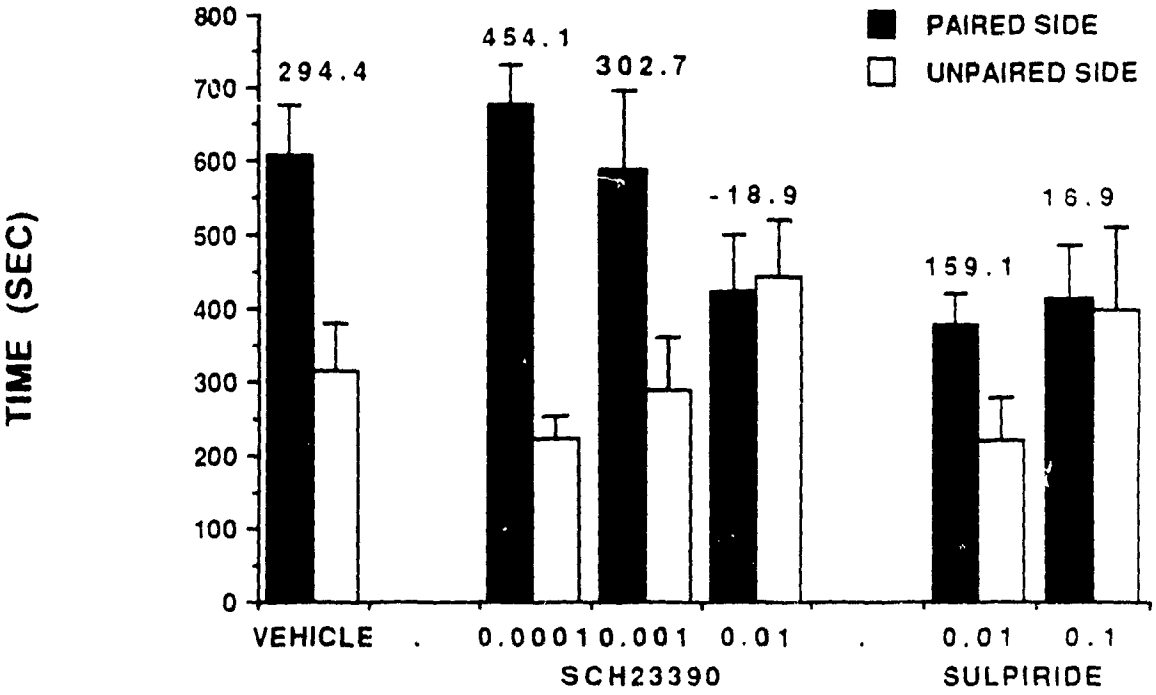

TIME (SEC)
800
700
600
500
400
300
200
100
0
294.4
454.1
302.7
-18.9
159.1
16.9
PAIRED SIDE
UNPAIRED SIDE
VEHICLE
0.0001
0.001
0.01
SCH23390
0.01
0.1
SULPIRIDE

Figure 4

Effects of vehicle, SCH23390 (0.01 ug/side), and sulpiride (0.1 u g/side) injected into the caudate/putamen on the expression of the amphetamine CPP.

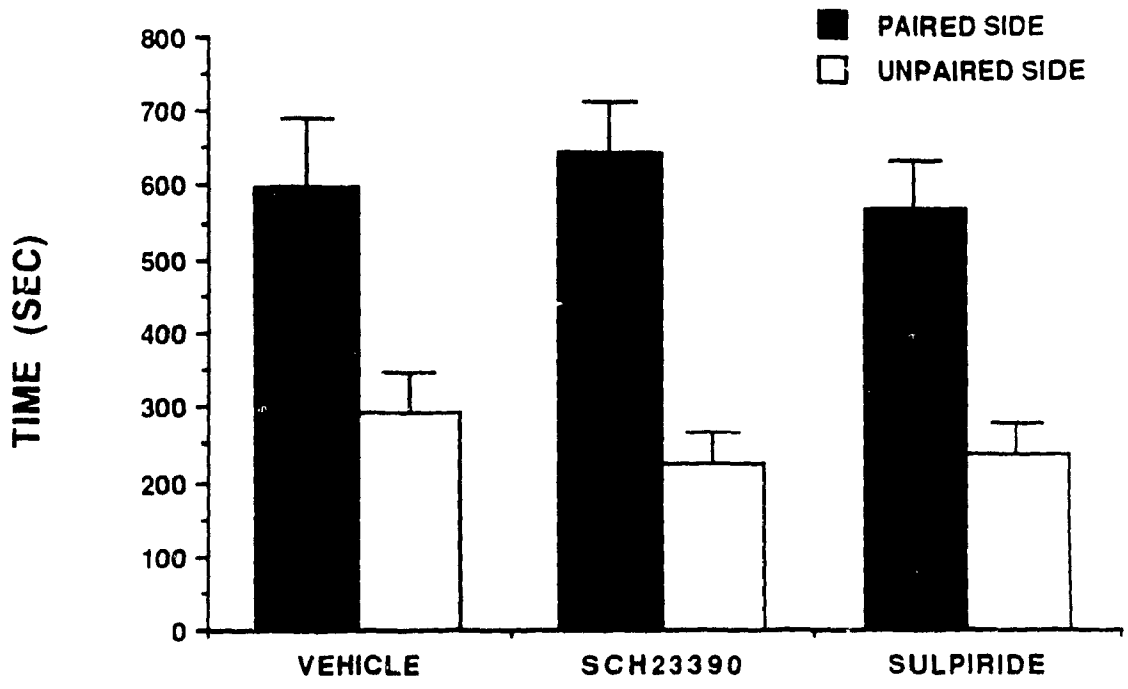
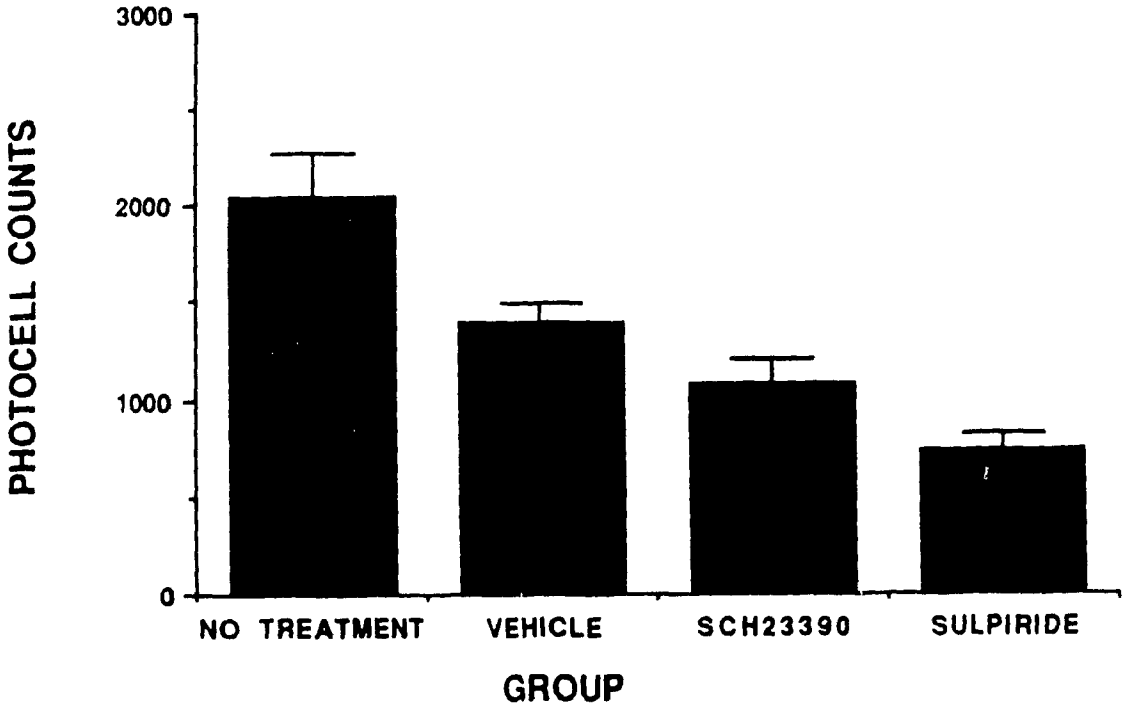

PAIRED SIDE
UNPAIRED SIDE
TIME (SEC)
800
700
600
500
400
300
200
100
0
VEHICLE
SCH23390
SULPIRIDE

Figure 5

Effects of microinjections of vehicle, SCH23390 (0.01 *ug*/side), and sulpiride (0.1 *ug*/side) into nucleus accumbens on spontaneous locomotor activity.


PHOTOCELL COUNTS
3000
2000
1000
0
NO TREATMENT
VEHICLE
SCH23390
SULPIRIDE
GROUP

($F(1,42)=0.23$, $p>0.05$) group. These data indicate that injections of dopamine receptor antagonists into the caudate/putamen have no effect on expression of the CPP and that the blockade of expression of the amphetamine CPP by intra-accumbens microinjections of SCH23390 or sulpiride is not due to spread of the drugs into the caudate/putamen.

In the experiment on spontaneous locomotor activity, microinjections of the highest doses of SCH23390 and sulpiride into the nucleus accumbens suppressed activity to 53 and 36 % of the level of the no-treatment group, respectively (Figure 5). A one-way ANOVA revealed that the drug effect was significant ($F(3,22)=14.41$, $p<0.01$).

Experiment 3

In both Experiments 1 and 2, injections of SCH23390 and sulpiride reduced spontaneous locomotor activity and blocked the expression of the CPP. The possibility exists that the blockade of expression of the amphetamine CPP is due to this reduction of motor activity. To establish the effect of this type of behavioral change on the CPP, the effects of sodium pentobarbital on spontaneous locomotor activity and the expression of the amphetamine CPP were examined.

Methods

Procedure. Five groups of animals (n=8-12) were given sodium pentobarbital (0.0, 10, 15, 17.5, or 20 mg/kg, i.p.) and placed into the activity boxes 10 min later. Spontaneous locomotor activity was measured for 20 min. Another two groups (n=8 for each) received either vehicle or the dose of sodium pentobarbital (17.5 mg/kg), which produced the same degree of

reduction of spontaneous locomotor activity as intra-accumbens injections of sulpiride, 10 min before the test session of the CPP.

Drugs. Sodium pentobarbital was dissolved in a solution of 10 % ethanol in 40 % propylene glycol.

Results

Sodium pentobarbital decreased spontaneous locomotor activity in a dose-dependent manner (Figure 6). A one-way ANOVA showed a significant group effect ($F(4,18)=6.62$, $p<0.01$). 17.5 mg/kg of sodium pentobarbital decreased spontaneous locomotor activity to 37 % of control.

When this dose of sodium pentobarbital was given before testing for the CPP, animals showed normal CPPs (Figure 7). Planned comparisons using groups as one factor and compartments as the other (repeated factor) showed that there were significant differences between time spent in the paired and unpaired compartments for the vehicle- ($F(1,14)=11.61$, $p<0.01$) and pentobarbital- ($F(1,14)=16.77$, $p<0.01$) treated groups. The time difference of the pentobarbital-treated group was not statistically different from that of the control group ($F(1,28)=0.19$, $p>0.05$). These findings suggest that drug-produced decreases in spontaneous locomotion do not, in and of themselves, affect the expression of amphetamine CPP.

Experiment 4

The results of Experiment 2 showed that microinjections of the dopamine antagonists into the caudate/putamen did not impair expression of

Figure 6

Effects of sodium pentobarbital (0.0, 10, 15, 17.5, and 20 mg/kg, i.p.) on spontaneous locomotor activity.

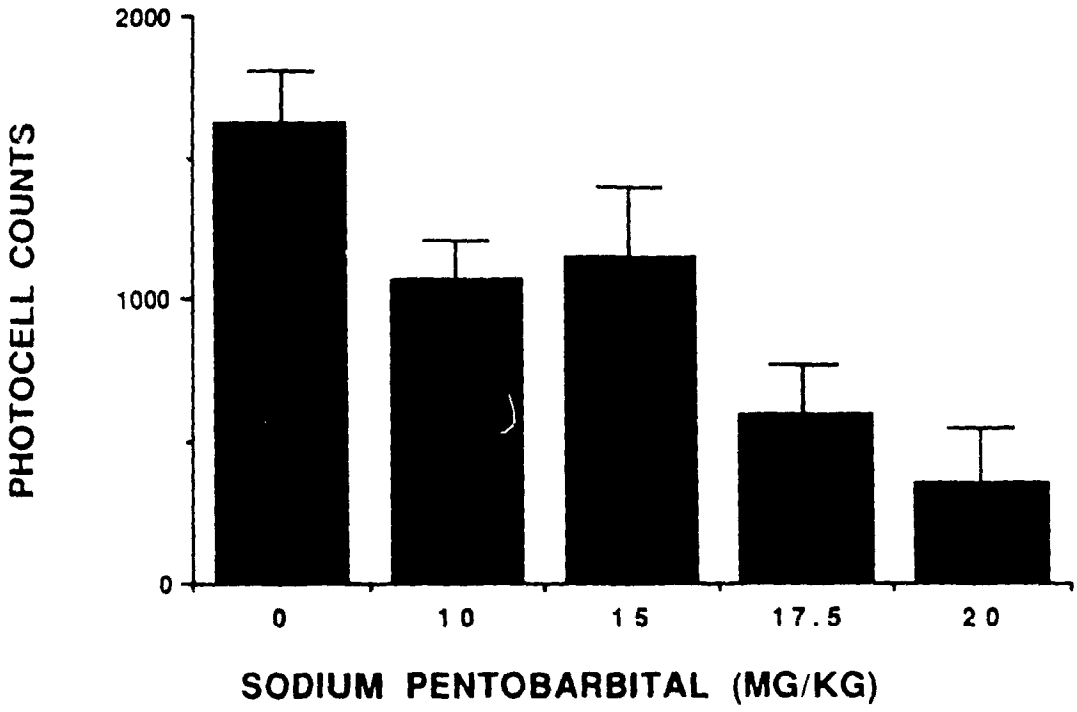
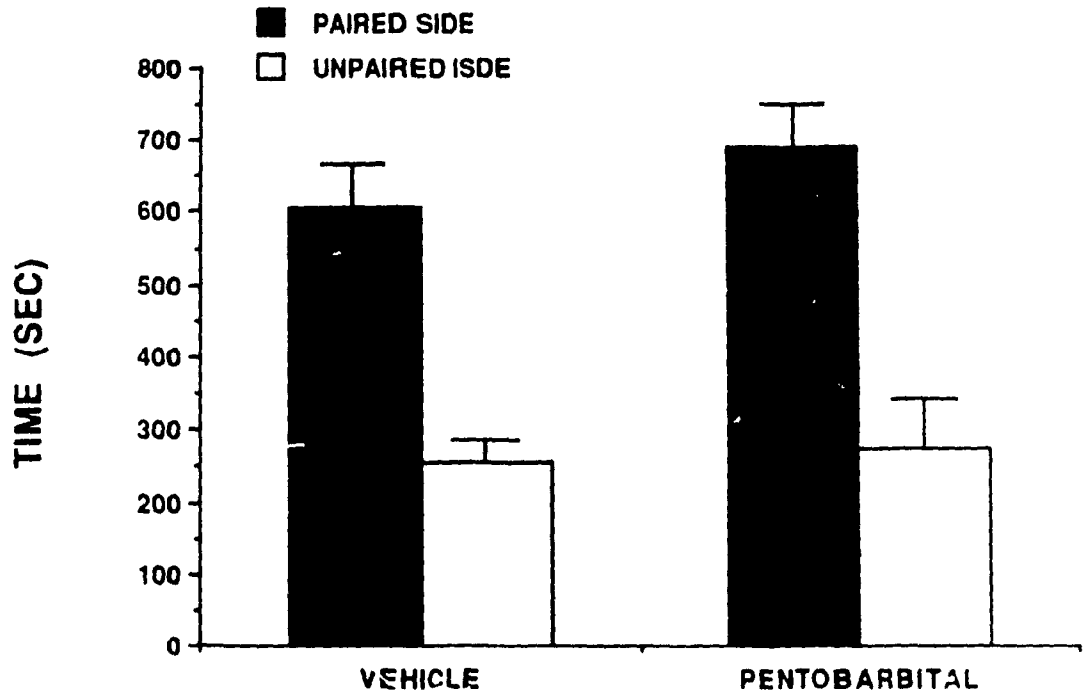

2000
1000
0
PHOTOCELL COUNTS
0
10
15
17.5
20
SODIUM PENTOBARBITAL (MG/KG)

Figure 7

Effect of sodium pentobarbital (17.5 mg/kg, i.p.) on the expression of the amphetamine CPP. Sodium pentobarbital was given 10 min before the test session.


PAIRED SIDE
UNPAIRED ISDE
TIME (SEC)
800
700
600
500
400
300
200
100
0
VEHICLE
PENTOBARBITAL

the amphetamine CPP, suggesting that the nigrostriatal dopamine system is not involved in this behavior. This experiment examined the effect of substantial damage to the dorsal caudate/putamen on the amphetamine CPP in order to confirm this conclusion.

Methods

Procedure. One group of rats (n=12) received bilateral electrolytic lesions (2.5 mA for 20 sec) of the caudate/putamen (A +1.0, L +2.8, V -5.5mm); the other (n=8) received sham lesions. After surgery, the animals' weights were monitored, and they were fed wet mash mixed with sucrose. At the end of a 1 week recovery session, there was no significant difference between the mean weights of the group with lesions (Average 316.0 g, SEM=4.2) and of the group with sham lesions (Average=322.1 g, SEM 4.1). After the recovery period, the CPP experiment began.

Results

The extent of lesions is shown in Figure 8. They were mainly confined to the dorsal section of the caudate/putamen.

Electrolytic lesions of the dorsal caudate/putamen potentiated the amphetamine CPP (Figure 9). A two-way ANOVA with planned comparisons showed that there were significant differences in time spent in the two compartments for the control group ($F(1,18)=11.30$, $p<0.01$) and for the lesioned group ($F(1,18)=31.96$, $p<0.01$). The time difference for the lesioned group was significantly different from that for the control group ($F(1,36)=8.79$, $p<0.01$).

Figure 8

The extent of electrolytic lesions of the dorsal caudate/putamen. The shade indicates the maximum extent of all lesions in all rats.

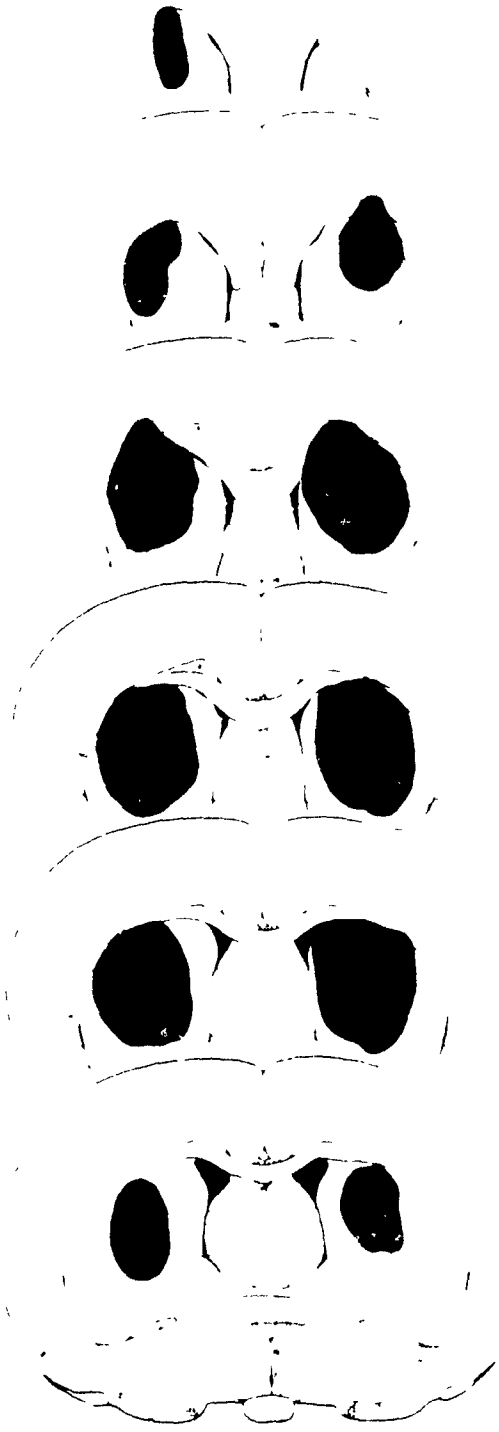
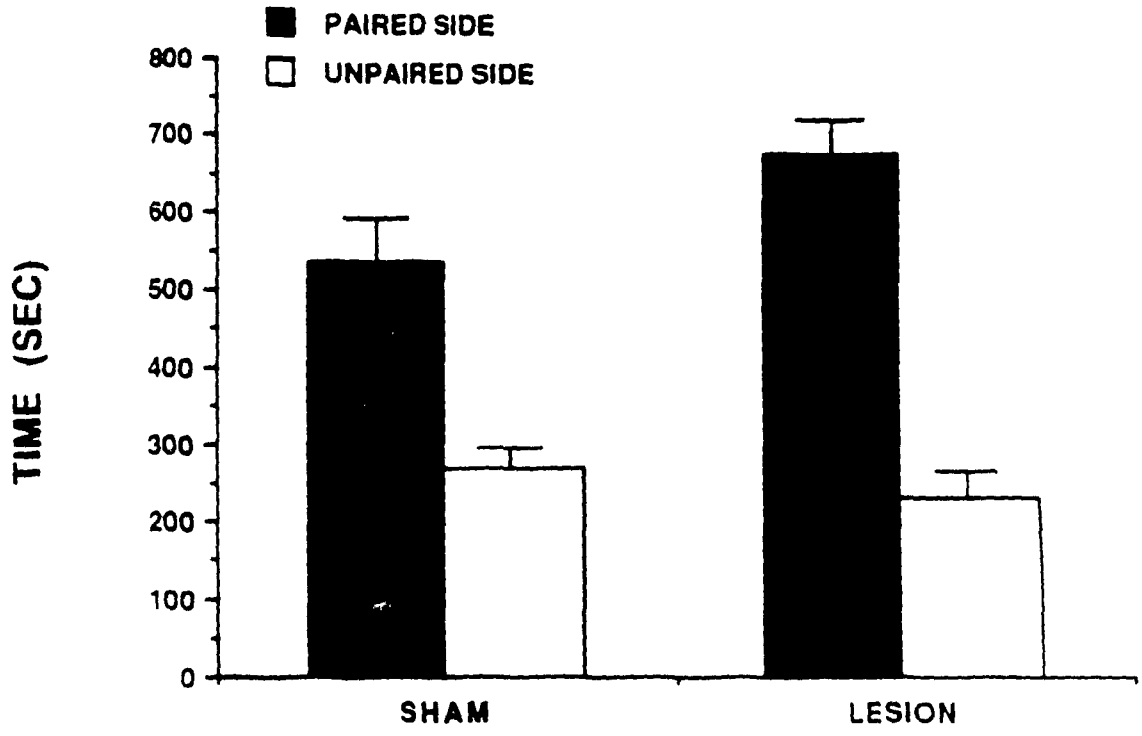


Figure 9

Effects of electrolytic lesions of the dorsal caudate/putamen on the amphetamine CPP. Sham sham lesioned group, Lesion dorsal caudate/putamen lesioned group.


PAIRED SIDE
UNPAIRED SIDE
TIME (SEC)
800
700
600
500
400
300
200
100
0
SHAM
LESION

Discussion

The present set of experiments show that systemically injected selective D1 and D2 dopamine antagonists have different effects on the acquisition and the expression of the amphetamine CPP. The selective D1 antagonist SCH23390 blocked acquisition and expression within similar dose ranges. The antagonists with high affinity for D2 dopamine receptors had no effect on expression at doses which completely blocked acquisition. Yet higher doses of these D2 antagonists blocked expression. Microinjections of SCH23390 or sulpiride into the nucleus accumbens also blocked expression of the amphetamine CPP, microinjections of the antagonists into the caudate/putamen were without effect. Although intra-accumbens microinjections of SCH23390 and sulpiride reduced spontaneous locomotor activity, sodium pentobarbital (17.5 mg/kg) produced the same degree of reduction of spontaneous locomotor activity without having an effect on the expression of the amphetamine CPP. Finally, electrolytic lesions of the dorsal caudate/putamen potentiated the amphetamine CPP.

Acquisition of the amphetamine CPP

Although amphetamine induces dopamine release unconditionally in the nucleus accumbens and caudate/putamen (Butcher, Fairbrother, Kelly and Arbuthnott, 1988, Teviel and Guibert, 1987, Wood, Kim and Marien, 1987), dopamine release in the caudate/putamen does not seem to be relevant to acquisition of the amphetamine CPP since lesions of the dorsal caudate/putamen failed to impair the amphetamine CPP in the present study. Together with previous demonstrations that dopamine depletion in the nucleus accumbens attenuated the amphetamine CPP (Spyraki et al., 1982a)

and that microinjections of amphetamine into nucleus accumbens, but not into caudate/putamen, establish CPPs (Aulisi and Hoebel 1983, Carr and White, 1983, 1986), the mesolimbic dopamine system in the nucleus accumbens seems to be a critical neural system for the establishment of this type of learning.

The selectivity of the antagonists to dopamine receptors is maintained within the dose ranges used in the acquisition and expression parts of this study (Andersen, 1988, Andersen, Nielsen, Gronvald and Braestrup, 1986). Although SCH23390 also binds to 5HT-2 receptors (Bischoff, Heinrich, Sonntag and Krauss, 1986, Hicks, Schoemaker and Langer, 1984), the blocking effect of SCH23390 on the amphetamine CPP does not seem to be due to this action of this drug. First, depletion of serotonin in the nucleus accumbens has no effect on the amphetamine CPP (Spyraki, Nomikos, Galanopoulou and Daifotis, 1988). Second, the doses of SCH23390 used in the present study have no effect on 5HT-2 binding sites (Bischoff et al., 1986) or on 5HT mediated behaviors (Pugh et al., 1985). Thus, the present finding that both D1 and D2 dopamine antagonists blocked acquisition suggests that activation of both D1 and D2 dopamine receptors is required for the establishment of the amphetamine CPP. This is consistent with previous studies showing that the establishment of the amphetamine CPP was blocked by SCH23390 (Hoffman and Beninger, 1989, Leone and DiChiara, 1987), *a* flupenthixol (MacKay and van der Kooy, 1985), and metoclopramide (Hoffman and Beninger, 1989).

One possible explanation of the present findings is based upon the facts that metoclopramide establishes CPPs (Hoffman and Beninger, 1989) and SCH23390 establishes conditioned place aversions (CPAs) (Shippenberg

and Herz, 1987, 1988). However, in the present studies, the antagonists were given before both amphetamine-pairing and saline-pairing sessions. Even if metoclopramide established a CPP, it would have been added to both conditions, and the amphetamine-paired compartment would still have been uniquely paired with the amphetamine effect. In this hypothetical situation, animals might still be expected to choose the amphetamine-metoclopramide paired compartment over the metoclopramide paired compartment if the CPP-establishing effect of amphetamine was not blocked by the antagonist. Similarly, if SCH23390 simply produced a CPA without blocking the effect of amphetamine, animals might be expected to choose the amphetamine-SCH23390 paired compartment over the saline-SCH23390 paired compartment. The argument that CPPs or CPAs produced by the antagonists cannot explain the blockade of the amphetamine CPP is further strengthened by evidence that the dose of *a* -flupenthixol which completely blocked the establishment of the amphetamine CPP in this study does not produce a CPP or CPA (Mackay and Van der Kooy, 1985). Thus, it seems that both D1 and D2 dopamine antagonists directly antagonize the action of amphetamine.

Expression of the amphetamine CPP

Systemically injected dopamine antagonists blocked expression of previously established amphetamine CPPs when given on the test day. Microinjections of SCH23390 and sulpiride into the nucleus accumbens, but not the caudate/putamen, also blocked this behavior. It is unlikely that the antagonists blocked this conditioned behavior simply by impairing performance. Although microinjections of SCH23390 or sulpiride reduced locomotion, the same degree of motor retardation produced by sodium

pentobarbital had no effect on the expression of the amphetamine CPP. Since sodium pentobarbital produces strong aversive effects measured in the place conditioning paradigm (Mucha and Iversen, 1984), the finding that this drug injected on the test day had no effect on expression of the amphetamine CPP also indicates that the blockade of the expression of the CPP by the antagonists was not due to any aversive effects they might have (Shippenberg and Herz, 1987, 1988). Together with the finding that lesions of the dorsal caudate/putamen did not impair the amphetamine CPP, it can be concluded that dopamine released from the mesolimbic, rather than the nigrostriatal, dopamine pathway has a crucial role in expressing the CPP.

The data also reveal different effects of systemically injected SCH 23390 and the other antagonists. While the D1 antagonist was equally effective in blocking the acquisition and expression of the amphetamine CPP within the dose range that maintains selectivity for the D1 dopamine receptor (Andersen, 1988), higher doses of the other antagonists were required to block expression than acquisition. The latter drugs share the property of higher affinity to the D2 than the D1 dopamine receptor. In vivo, *a* -flupenthixol and sulpiride have 2 and 7 times higher affinity for D2 receptors than D1 receptors, respectively (Andersen, 1988, Waddington and O'Boyle, 1989). These ratios coincide approximately with the degree of the rightward shift of the antagonism curves from acquisition to expression (Figure 1). For example, 0.4 mg/kg and 0.8 mg/kg of *a* flupenthixol produced complete blockade of the acquisition and expression of the amphetamine CPP, respectively. Since the high doses of the D2 antagonists used in this study may bind to D1 receptors in vivo (Andersen, 1988), the observed blocking effects of the high doses of the D2 antagonists on

expression might be due to blockade of D1 dopamine receptors. If the blockade of the expression of the CPP by systemic sulpiride was due to blockade of D1 dopamine receptors, then the blockade of the expression of the amphetamine CPP by intra-accumbens injections of sulpiride might also have been due to this drug's binding to the D1 dopamine receptor in the nucleus accumbens.

In summary, the data in this chapter suggest that the acquisition of the amphetamine CPP relies on co-activation of D1 and D2 dopamine receptors. Expression of this conditioned behavior is more effectively blocked by the D1 dopamine receptor antagonist than the antagonists with higher affinity for D2 than D1 receptors. This might suggest that the expression of the amphetamine CPP is mediated by activation of D1, rather than D2, dopamine receptors. However, the possibility that activation of nucleus accumbens D2 dopamine receptors is also required for expression cannot be ruled out on the basis of the available data.

CHAPTER 4

The existence of two dopamine pools has been suggested on the grounds that *a* -MPT and reserpine exert differential effects on stimulant induced behaviors. The tyrosine hydroxylase inhibitor *a* -MPT effectively blocks behavioral activation induced by amphetamine, it has a weak or no effect on behavioral activation produced by pipradrol (Scheel-Kruger, 1971) The vesicle depletor reserpine blocks the behavioral effect of pipradrol, but not of amphetamine (Scheel-Kruger, 1971). Thus it may be that amphetamine acts to release dopamine from the pool which is dependent on a constant supply of newly synthesized dopamine, while pipradrol acts to release dopamine from the vesicle pool (Glowinski, 1970, 1973).

Accordingly, it can be hypothesized that amphetamine induces dopamine release from the *a* -MPT-sensitive dopamine pool when establishing a CPP. The first set of experiments in Chapter 3 suggest that expression of the amphetamine CPP in the absence of amphetamine also involves dopamine release. This raises the question of which pool is involved in this dopamine release. In this chapter, this question is investigated by examining the effects of *a* -MPT and reserpine on expression of the amphetamine CPP.

Experiment 5

This experiment tested the hypothesis that the dopamine receptor activation mediating expression of the amphetamine CPP is produced by dopamine released from the *a* MPT sensitive dopamine pool First, the effects of intra-accumbens *a* -MPT injection on amphetamine and pipradrol induced locomotor activity were examined, and a dose of *a* MPT which

completely blocked amphetamine-, but not pipradrol-induced locomotor activity was identified. Second, the effect of this dose of *a* -MPT on expression of the amphetamine CPP was determined by injecting it into nucleus accumbens on the test day of the CPP paradigm.

Method

Surgery. Stereotaxic surgery using standard techniques was performed under 65 mg/kg sodium pentobarbital anesthesia on rats to implant the guide cannulae. Using the atlas of Paxinos and Watson (1982), the guide cannulae were aimed at coordinates (A+1.7, L+-1.5, V -4.5mm). The guide cannulae were filled with insect pins (00) cut to the length of the guide cannulae. The experiment started after a one-week recovery period.

Procedure. Six groups of rats were used for the locomotor activity experiment. Three of the six groups were used for testing the effect of intra-accumbens *a* MPT microinjections (110 *ug*/side) on amphetamine (2.0 mg/kg, s.c.)-induced locomotor activity. These groups received either vehicle-amphetamine (n=5), *a* -MPT-amphetamine (n=5), or vehicle-vehicle (n=5) treatment. The other three groups were used for testing the effect of intra-accumbens *a* -MPT microinjections (110 *ug*/side) on pipradrol (25 mg/kg, s.c.) induced locomotor activity. These received either vehicle-pipradrol (n 5), *a* -MPT-pipradrol (n=-5), or vehicle-vehicle (n=5) treatment. All rats received bilateral microinjections of *a* -MPT or its vehicle (phosphate buffer) followed 3 min later by systemic injections of the stimulants or their vehicles.

The effect of 110 *ug* of *a* -MPT on expression of the amphetamine CPP was also tested with different animals. Two groups (N = 13 and 8) received intra accumbens injections of *a* -MPT or its vehicle 3 min before the CPP test.

Nucleus accumbens injections were made by using inner cannulae that extended 2.5 mm below the tip of the guide cannulae, to coordinates (A +1.7, L +-1.5, V -7.0mm). After the inner cannulae were inserted into the guide cannulae the drug or vehicle was delivered over a 30 sec period. The inner cannulae were left in place for a further 60 sec. Testing began after a further 90 sec. Thus the interval between the onset of microinjections and testing was 3 min.

Histology. After the completion of behavioral testing, the animals were perfused with saline and formol saline. The brains were removed, sliced, and stained with thionin.

Drugs. Pipradrol was dissolved in diluted propylene glycol. alpha Methyl-DL-para-tyrosine (*a* -MPT) (Sigma Chemical Company) was dissolved in phosphate buffer as 220 mg/ml of the solution. *a* MPT or phosphate buffer was bilaterally injected into nucleus accumbens in a volume of 0.5 *u* l/side.

Results

Figure 10 shows the location of the tips of the inner cannulae. The locations were confined to the nucleus accumbens in all cases used in the data analysis.

Figure 11 shows the effects of intra-accumbens injections of *a* MPT on amphetamine(A)- and pipradrol(B)- induced locomotor activity. While *a* -MPT-treated animals did not show amphetamine induced locomotor activity, they showed a considerable amount of pipradrol induced locomotor activity.

For the amphetamine groups, a two way ANOVA with groups as one

Figure 10

Brain sections showing the locations of the tips of the inner cannulae.

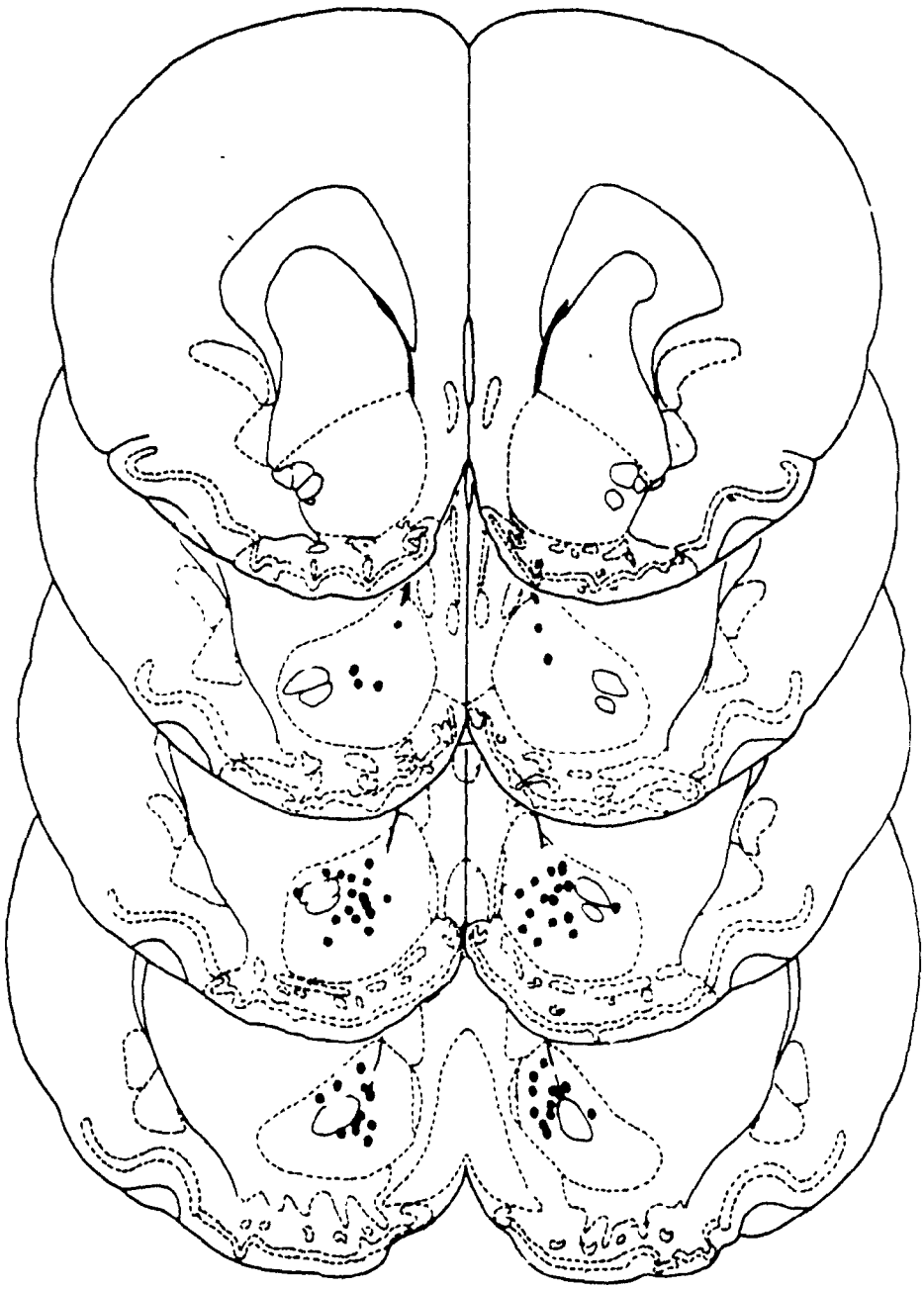
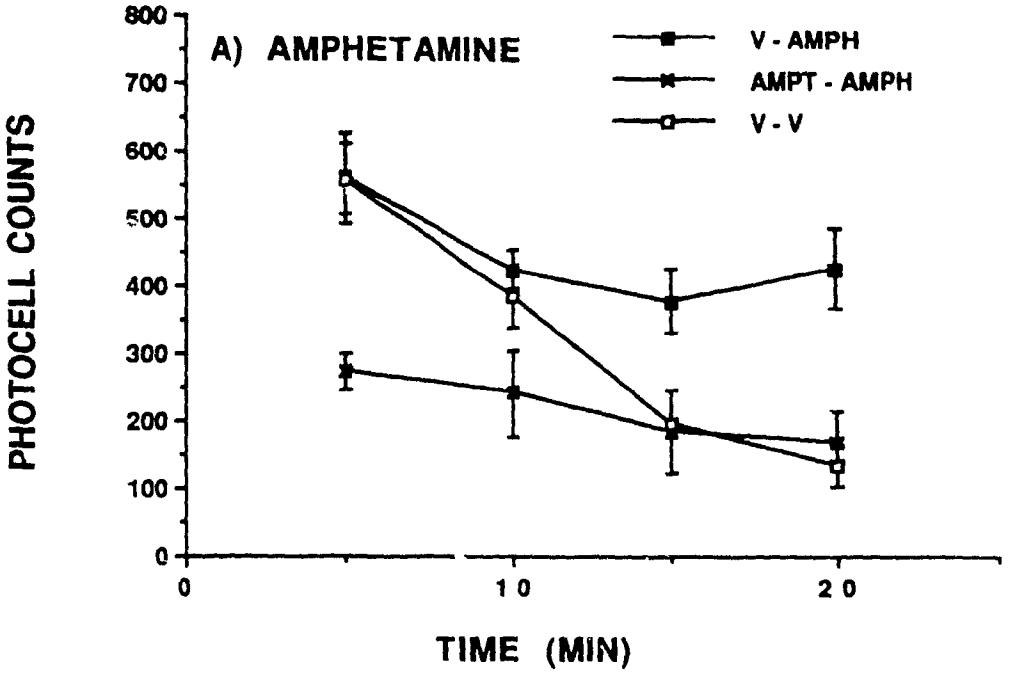
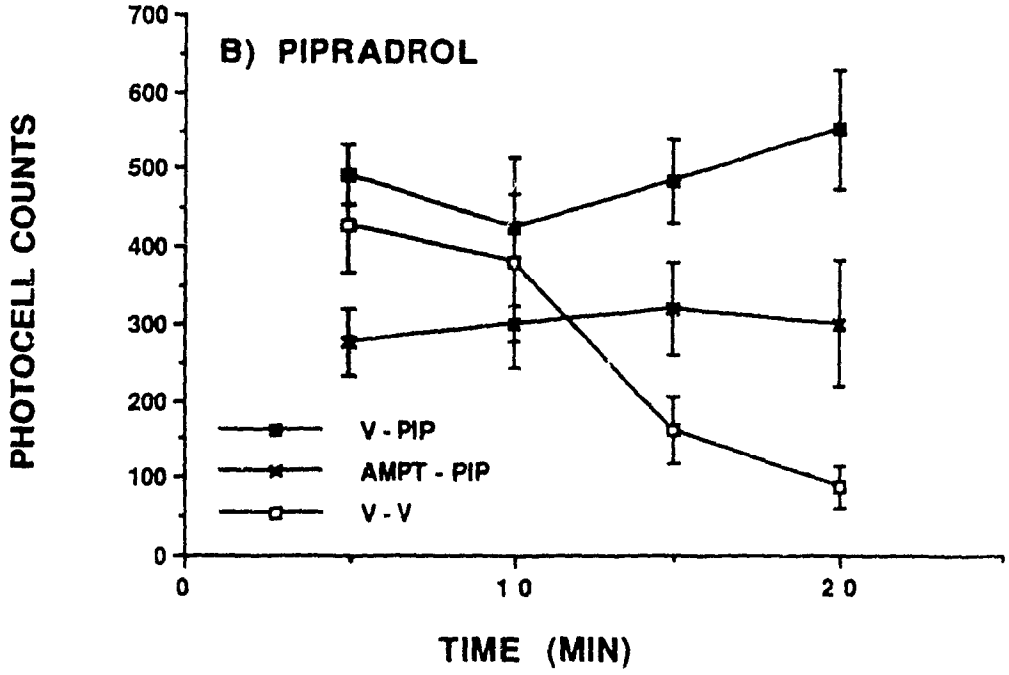


Figure 11

Effects of intra-accumbens injections of *a* -MPT (110 *u*g/side) on amphetamine- (A) and pipradrol- (B) induced locomotor activity. V vehicle, AMPH amphetamine, AMPT *a* -MPT, PIP pipradrol.


A) AMPHETAMINE
V - AMPH
AMPT - AMPH
V - V
PHOTOCELL COUNTS
800
700
600
500
400
300
200
100
0
0
10
20
TIME (MIN)


B) PIPRADROL
V - PIP
AMPT - PIP
V - V
PHOTOCELL COUNTS
700
600
500
400
300
200
100
0
0
10
20
TIME (MIN)

factor and intervals as the other (repeated measure) revealed a significant interaction effect ($F(6,33)=4.84$, $p<0.01$). One-way ANOVAs applied to each interval showed that there were significant group effects at the first ($F(2,11)=10.96$, $p<0.01$), second ($F(2,11)=4.01$, $p<0.05$), third ($F(2,11)=5.35$, $p<0.05$), and fourth ($F(2,11)=11.25$, $p<0.01$) intervals. Scheffe tests revealed that the *a* MPT-amphetamine and vehicle-vehicle groups did not differ at the second ($F(2,11)=3.95$, $p>0.05$), third ($F(2,11)=0.03$, $p>0.05$), and fourth ($F(2,11)=0.27$, $p>0.05$) intervals. At the first interval, the photocell count of the *a* MPT-amphetamine group was significantly lower than that of the vehicle-vehicle group ($F(2,11)=15.09$, $p<0.01$). The *a* -MPT-amphetamine group showed significantly lower locomotor activity counts than the vehicle-amphetamine group at the first ($F(2,11)=17.11$, $p<0.01$), third ($F(2,11)=8.80$, $p<0.05$), and fourth ($F(2,11)=15.32$, $p<0.01$) intervals. At the second interval, there was no significant difference between the two groups ($F(2,11)=7.37$, $p>0.05$).

A two-way ANOVA applied to the pipradrol groups showed that there was a significant interaction effect ($F(6,36)=5.03$, $p<0.01$). One-way ANOVAs revealed that the group effect was not significant at the first ($F(2,12)=3.37$, $p>0.05$) and second ($F(2,12)=0.71$, $p>0.05$) intervals. Significant group effects were found at the third ($F(2,12)=8.48$, $p<0.01$) and fourth ($F(2,12)=9.53$, $p<0.01$) intervals. Scheffe tests showed that the *a* -MPT-pipradrol group did not differ from the vehicle-pipradrol group at the third ($F(2,12)=1.19$, $p>0.05$) and fourth ($F(2,12)=1.86$, $p>0.05$) intervals. The *a* MPT pipradrol group and the vehicle-vehicle group differed at the third ($F(2,12)=8.36$, $p<0.05$) and fourth ($F(2,12)=8.47$, $p<0.05$) intervals.

These findings are consistent with the hypothesis that *a* -MPT and

amphetamine act on the same dopamine pool.

On the CPP test day, both the *a* -MPT and vehicle groups exhibited robust CPPs (Figure 12). A two-way ANOVA with groups as one factor and compartments as the other (repeated measure) revealed that the compartment factor was the only significant effect ($F(1,14)$ 6.70, $p<0.05$). Further analysis by planned comparisons revealed that the time difference of the control group was not statistically different from that of the *a* MPT group ($F(1,28)=0.34$, $p>0.05$). Therefore, the dose of *a* -MPT that completely blocked amphetamine-induced locomotor activity had no effect on expression of the amphetamine CPP.

Experiment 6

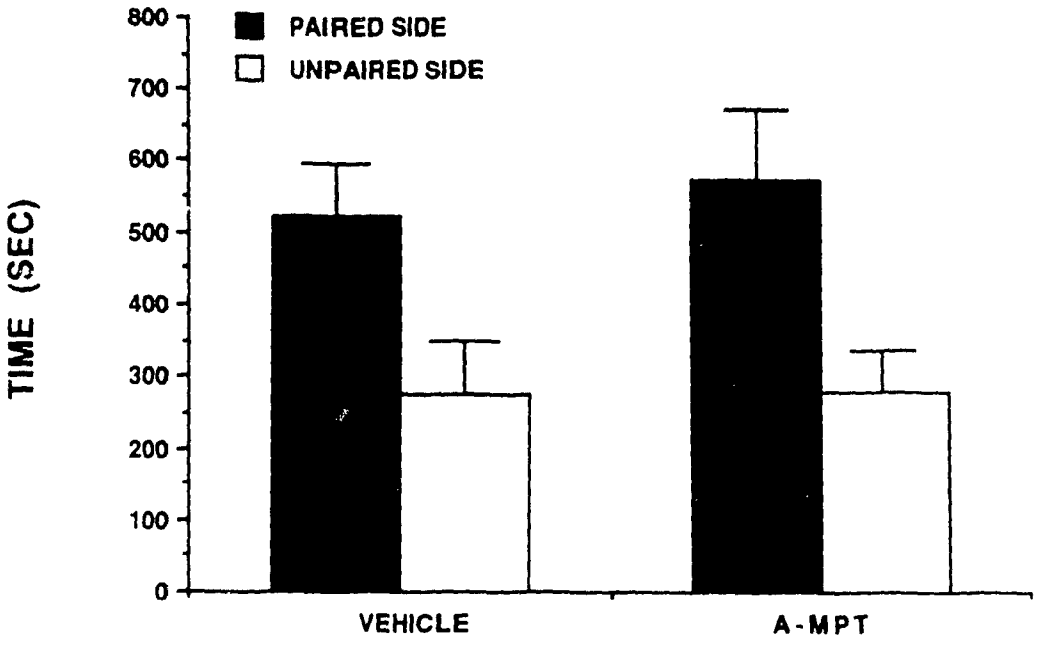
This experiment tested the hypothesis that expression of the amphetamine CPP is mediated by dopamine release from the reserpine sensitive pool. First, the effect of reserpine on the expression of the CPP was examined Second, the effect of reserpine on amphetamine and pipradrol induced locomotor activity was investigated. This test behaviorally assessed the availability of the *a* -MPT-sensitive and reserpine sensitive pools after reserpine treatments. Third, the effect of reserpine on catecholamine concentrations in the nucleus accumbens was assessed by high performance liquid chromatography (HPLC) analysis to determine the availability of catecholamines in the nucleus accumbens after reserpine treatments

Method

Procedure. In the CPP paradigm (N 8 for each group), reserpine (0.0, 2.0, 4.0, and 6.0 mg/kg, s.c.) was injected 4 hours after the last conditioning

Figure 12

Effects of α-MPT (110 μg/side) microinjections into nucleus accumbens on the expression of the amphetamine CPP.


800
700
600
500
400
300
200
100
0
TIME (SEC)
PAIRED SIDE
UNPAIRED SIDE
VEHICLE
A-MPT

session. The animals were tested 48 hours later.

A single dose of reserpine (6.0 mg/kg) was used to determine the effect of this drug on amphetamine (2.0 mg/kg, s.c.)- and pipradrol (25 mg/kg, s.c.)-induced locomotor activity. Forty-eight hours after systemic reserpine injections, the effects of the stimulants and their vehicles on locomotor activity were tested. Three groups received either vehicle-amphetamine (n=13), reserpine-amphetamine (n=6), or vehicle-vehicle (n=7) treatment. Another three groups received either vehicle-pipradrol (n=4), reserpine-pipradrol (n=4), or vehicle-vehicle (n=4) treatment.

Catecholamine depletion in the nucleus accumbens induced by reserpine (0.0, 1.0, 2.0, 4.0, and 6.0 mg/kg, s.c.) was determined. The drug was injected 48 hours before the animals (n= 29) were decapitated with a guillotine. Their brains were removed, and a section between approximately +2.7 mm and +0.7 mm in the anterior-posterior plane (Paxinos and Watson, 1982) was cut using a cooled cutter on a cold plate. A piece of tissue 1.5 mm in diameter consisting solely of nucleus accumbens was punched out of each side of the section using a modified syringe barrel. After homogenizing, the samples were analyzed by HPLC.

The tissue samples were homogenized in a volume of solvent consisting of 135 *ul* of 0.1M perchloric acid containing 50 mg/l EDTA and 15 *ul* of dihydroxybenzylamine hydrochloride (DHBA). The homogenate was centrifuged for 15 min at 15,000 RPM at 0°C. 20 or 25 *ul* of the eluate was injected by an automatic sample injector (Shimadsu, Sil-6A Autoinjector) into the chromatograph with a 2 ml/min flow rate. Electrochemical detection was by Amperometric detector LC-4B (Bioanalytical Systems) with an applied voltage of 0.680. The mobile phase was 0.1M sodium acetate and 0.02 M

citric acid with pH adjusted to 4 with glacial acetic acid. 0.02M sodium octyl sulfate, 50 mg/l EDTA, and 2% of methanol were added. Dopamine, norepinephrine, and DHBA were identified by comparing retention times of their peaks to those of the standards. Standard curves were calculated from six internal standards which consisted of DHBA, six concentrations of dopamine, and six concentrations of norepinephrine. Concentrations of sample dopamine and norepinephrine were estimated from the curves and expressed as nmol/g wet tissue weight.

Drugs. Reserpine (Sigma) was dissolved in acetic acid and diluted with distilled water, as 1.0, 2.0, and 3.0 mg/ml of the solution. The solutions were adjusted with sodium hydroxide to pH 4. For the 4.0 mg/kg and 6.0 mg/kg doses, the solutions were subcutaneously injected in a volume of 2.0 ml/kg. The other doses, 1.0 and 2.0 mg/kg, were injected in a volume of 1.0 ml/kg.

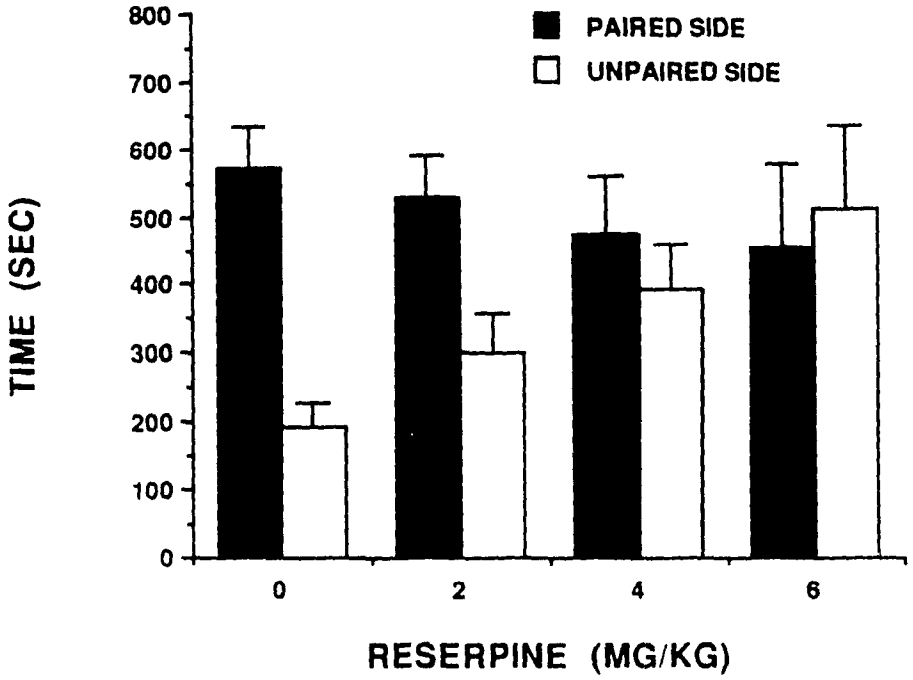
Results

Figure 13 shows that reserpine produced a dose dependent reduction of expression of the amphetamine CPP. A two way ANOVA with planned comparisons revealed that there was a significant difference between time spent in the two compartments for the vehicle treated group ($F(1,28)=5.71$, $p<0.05$), the differences were not significant at 2.0 mg/kg ($F(1,28)=2.07$, $p>0.05$), 4.0 mg/kg ($F(1,28)=0.25$, $p>0.05$), and 6.0 mg/kg ($F(1,28)=0.14$, $p>0.05$). The time difference of the control group was significantly different from that of the 4.0 mg/kg group ($F(1,56)=13.10$, $p<0.01$) and that of the 6.0 mg/kg group ($F(1,56)=27.88$, $p<0.01$), but not from that of the 2.0 mg/kg group ($F(1,56)=3.29$, $p>0.05$).

The dose of reserpine (6.0 mg/kg), which completely blocked the

Figure 13

Effects of systemic reserpine (0.0, 2.0, 4.0, and 6.0 mg/kg, s.c.) injections on the expression of the amphetamine CPP.


PAIRED SIDE
UNPAIRED SIDE
TIME (SEC)
800
700
600
500
400
300
200
100
0
0
2
4
6
RESERPINE (MG/KG)

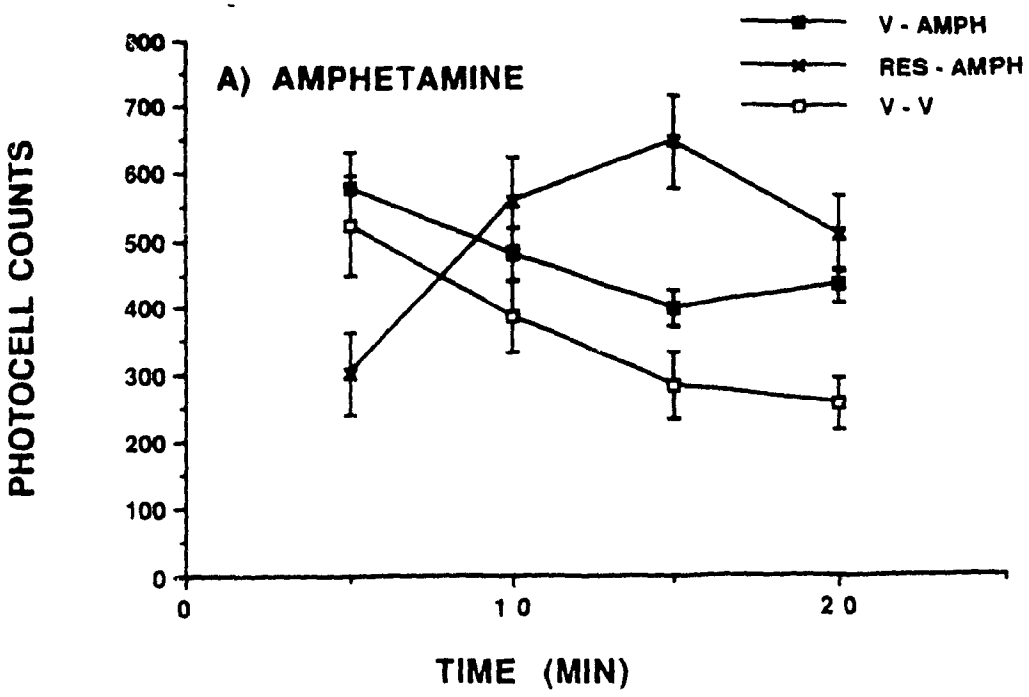
expression of the amphetamine CPP, exerted differential effects on amphetamine- (Figure 14-A) and pipradrol- (Figure 14-B) induced locomotor activity. Reserpine either had no effect on or potentiated amphetamine-induced locomotor activity, but completely blocked pipradrol-induced locomotor activity.

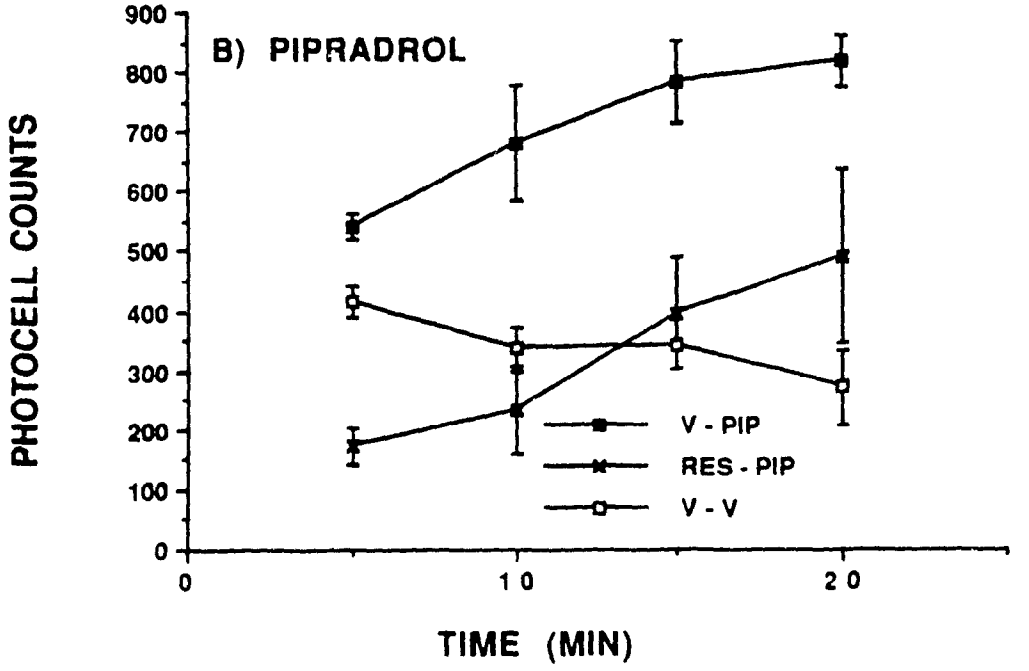
For the amphetamine groups, a two-way ANOVA showed a significant interaction effect ($F(6,69)=12.18$, $p<0.01$). One-way ANOVAs showed that the group effect was significant at the first ($F(2,23)=4.48$, $p<0.05$), third ($F(2,23)=13.86$, $p<0.01$), and fourth ($F(2,23)=10.41$, $p<0.01$) intervals, but not at the second interval ($F(2,23)=2.19$, $p>0.05$). Scheffe tests revealed that at the first interval the reserpine-amphetamine group significantly differed from the vehicle-amphetamine group ($F(2,23)=8.80$, $p<0.05$), but not from the vehicle-vehicle group ($F(2,23)=4.46$, $p>0.05$). At the third interval, the reserpine-amphetamine group had a significantly higher photocell count than the vehicle-amphetamine group ($F(2,23)=15.52$, $p<0.01$) and the vehicle-vehicle group ($F(2,23)=26.69$, $p<0.01$). At the fourth interval, the reserpine-amphetamine was different from the vehicle-vehicle group ($F(2,23)=18.72$, $p<0.01$), but not from the vehicle-amphetamine group ($F(2,23)=2.18$, $p>0.05$).

For the pipradrol groups, a two-way ANOVA showed a significant interaction effect ($F(6,27)=3.72$, $p<0.01$). One-way ANOVAs revealed significant group effects at the first ($F(2,9)=47.89$, $p<0.01$), second ($F(2,9)=10.01$, $p<0.01$), third ($F(2,9)=12.81$, $p<0.01$), and fourth ($F(2,9)=8.43$, $p<0.01$) intervals. Scheffe tests showed that there were no significant differences between the reserpine pipradrol and vehicle-vehicle groups at the second ($F(2,9)=0.95$, $p>0.05$), third ($F(2,9)=0.31$, $p>0.05$), and fourth

Figure 14

Effects of systemic reserpine (6.0 mg/kg, s.c.) injections on amphetamine- (A) and pipradrol- (B) induced locomotor activity. V vehicle, AMPH amphetamine, RES reserpine, PIP pipradrol.


A) AMPHETAMINE
V - AMPH
RES - AMPH
V - V
PHOTOCELL COUNTS
800
700
600
500
400
300
200
100
0
0
10
20
TIME (MIN)


B) PIPRADROL
V - PIP
RES - PIP
V - V
PHOTOCELL COUNTS
900
800
700
600
500
400
300
200
100
0
0
10
20
TIME (MIN)

(F(2,9)=2.68, p>0.05) intervals. At the first interval, the reserpine-pipradrol group showed a significantly lower photocell count than the vehicle-vehicle group (F(2,9)=40.21, p<0.01). There were significant differences between the reserpine-pipradrol and vehicle-pipradrol groups at the first (F(2,9) 92.66, p<0.01), second (F(2,9)=18.23, p<0.01), and third (F(2,9) 16.63, p<0.01) intervals. The difference was not statistically significant at the fourth interval (F(2,9)=5.96, p>0.05).

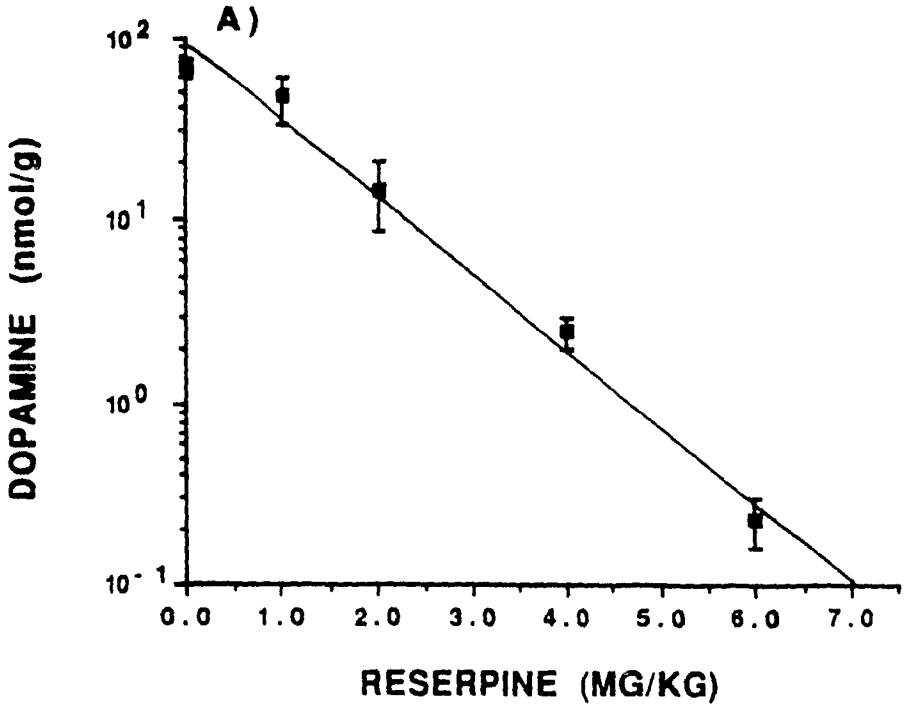
Figure 15 shows the effects of reserpine on catecholamine concentrations in the nucleus accumbens. Reserpine depleted dopamine and norepinephrine in a dose-dependent fashion. The decreases were exponential, it was significant for both dopamine (F(4,24) 31.05, p<0.01) and norepinephrine (F(3,18)=7.11, p<0.01).

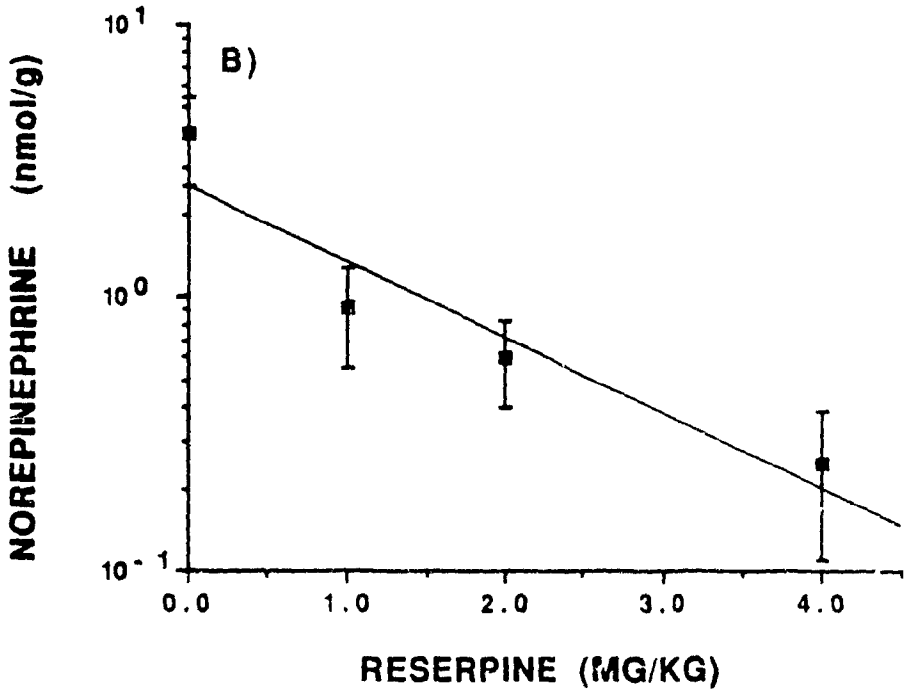
Discussion

Reserpine did not attenuate amphetamine-induced locomotor activity, but completely blocked pipradrol-induced locomotor activity. This is consistent with neurochemical and behavioral data showing that the efflux of dopamine by amphetamine is not affected (Callaway, Kuczenski and Segal, 1989) or is enhanced by reserpine (Chiueh and Moore, 1975) leading to enhanced amphetamine locomotor stimulation (Stolk and Rech, 1967, 1968). In contrast, microinjections of *a* -MPT into the nucleus accumbens blocked amphetamine-induced locomotor activity while sparing a considerable amount of pipradrol-induced locomotor activity. These findings support the hypothesis that amphetamine releases dopamine from the *a* -MPT sensitive pool and that pipradrol induces dopamine release from the reserpine sensitive pool (Glowinski 1970, 1973, Scheel-Kruger, 1971).

Figure 15

Effects of reserpine (0.0, 1.0, 2.0, 4.0, and 6.0 mg/kg, s.c.) on the catecholamine concentrations in the nucleus accumbens. Dopamine (A) and norepinephrine(B) depletion are shown.


A)
10^2
10^1
10^0
10^{-1}
DOPAMINE (nmol/g)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
RESERPINE (MG/KG)


B)
10^1
10^0
10^{-1}
NOREPINEPHRINE (nmol/g)
0.0
1.0
2.0
3.0
4.0
RESERPINE (MG/KG)

Reserpine blocked the expression of the amphetamine CPP in a dose dependent manner, and the dose of reserpine which completely blocked expression did not block amphetamine-induced locomotor activity (Experiment 6). Reserpine depletes monoamines including catecholamines (Experiment 6) and serotonin (Weiner, 1980). It is unlikely, however, that the expression of the amphetamine CPP is mediated by systems other than dopamine. First, as shown in Chapter 3, selective dopamine antagonists blocked expression. This tends to rule out the possible involvement of norepinephrine and serotonin pools that are sensitive to reserpine. Second, lesions of the serotonergic system in the nucleus accumbens with 5,7-dihydroxytryptamine do not affect the amphetamine CPP (Spyraki et al., 1988), indicating that the serotonin system in the nucleus accumbens is involved in neither acquisition nor expression of the amphetamine CPP.

a -MPT did not affect expression of the amphetamine CPP at the dose which blocked amphetamine-induced locomotor activity. Although *a* -MPT decreases the concentration of *a* -MPT-sensitive dopamine and norepinephrine, neither catecholamine in the *a* -MPT-sensitive pool seems to be necessary for the expression of the CPP. This conclusion is supported by the finding that even though amphetamine-induced locomotor activity was intact after reserpine treatment, the expression of the CPP was completely blocked by reserpine.

Taken together, these findings clearly suggest that the expression of the amphetamine CPP is mediated by dopamine release from the reserpine sensitive pool in the nucleus accumbens. Given that amphetamine interacts with the *a* -MPT-sensitive dopamine pool it was an unexpected finding that the expression of the CPP was blocked by reserpine rather than by *a* MPT.

This finding suggests that although the establishment of the CPP depends upon dopamine released from the *a*-MPT-sensitive pool, the expression of the CPP on the test day depends upon dopamine released from the reserpine-sensitive pool. Therefore, in the case of the amphetamine CPP, different mechanisms may produce dopamine release on the conditioning and test days.

CHAPTER 5

The previous two chapters revealed the critical role of the mesolimbic dopamine projection to the nucleus accumbens in the expression of the amphetamine CPP. The nucleus accumbens is a target of massive afferents from the prefrontal (Beckstead, 1979, Phillipson and Griffiths, 1985) and entorhinal (Krayniak et al., 1981; Phillipson and Griffiths, 1985, Sorensen and Witter, 1983) cortices, the subiculum (Kelley and Domesick, 1982; Phillipson and Griffiths, 1985; Swanson and Cowan, 1977), the hippocampus (Kelley and Domesick, 1982; Phillipson and Griffiths, 1985, Raisman et al., 1966, Siegel et al., 1974; Swanson and Cowan, 1977), and the amygdala (Kelley et al., 1982, Phillipson and Griffiths, 1985). Behavioral studies have also shown functional connections between the basal ganglia and limbic systems (Annett et al., 1989, Cador et al., 1989, Everitt et al., 1989a, Magnus and Lammers, 1956, Mittleman et al., 1990; Shealy and Peele, 1957, Turner, 1970, Ursin and Kaada, 1960). Given the anatomical and behavioral evidence, some link between limbic systems and dopamine systems in the basal ganglia might exist for the CPP.

The present study was designed to investigate involvement of the hippocampal system and the amygdala in the amphetamine CPP. Since the hippocampus and subiculum project to the nucleus accumbens through the fornix/fimbria (Kelley and Domesick, 1982, Raisman et al., 1966, Swanson and Cowan, 1977, Totterdell and Smith, 1989) and lesions of the fornix/fimbria and the hippocampus produce similar deficits in rats in many experiments (Jarrard, 1978a, b), lesions were made to this fiber bundle. The other structure studied, the amygdala, is made up of heterogeneous nuclei Most workers studying the behavioral roles of the amygdala have used lesions

to the basolateral complex and its adjacent areas in rats (Cador et al., 1989, Everitt et al., 1989a, Sutherland and McDonald, 1990) and gross amygdalectomy in monkeys (Jones and Mishkin, 1972; Murray and Mishkin, 1985, Parkinson, Murray and Mishkin, 1988; Zola-Morgan, Squire and Amaral, 1985). Because amygdaloid nuclei have different projections (de Olmos, Alheid and Beltramino, 1985), small electrolytic lesions were made to either the central nucleus, basolateral nucleus, or lateral nucleus of the amygdala. Since the lateral nucleus lesions appeared to attenuate the CPP and this nucleus is surrounded by fibers of passage which are functionally unrelated to it, lesions were made to the lateral nucleus using the excitotoxin N-methyl-d-aspartic acid (NMDA), which destroys intrinsic neurons, but not fibers of passage (Mayer and Westbrook, 1987). Two additional areas, the endopyriform nucleus and ventral hippocampus, which were affected by NMDA lesions of the lateral nucleus, were also electrolytically damaged.

Experiment 7

This experiment was designed to determine involvement of the hippocampus and the amygdala in the amphetamine CPP.

Method.

Surgery. Lesions were made 1 week before the CPP procedure began. Rats were anesthetized with 65 mg/kg of sodium pentobarbital and subjected to lesions using standard stereotaxic techniques, with coordinates based on the atlas of Paxinos and Watson (1982).

The three amygdaloid nuclei and their two adjacent structures were each damaged electrolytically. Seven groups, each consisting of eight rats,

received bilateral electrolytic lesions of the lateral (A-3.5,L+-5.5,V-8.5mm), central (A:-2.3,L:+-4.5,V.-8.5mm), or basolateral (A-2.8,L+-5.0,V-9.0mm) nucleus of amygdala, endopyriform nucleus (A.-4.3,L+-6.0,V-8.5mm), or ventral hippocampus (A-4.8,L:+-5.5,V:-8.0mm). The lesion parameters were 1.5 mA for 20 sec.

Pilot studies revealed that radiofrequency lesions produced more complete damage to fornix/fimbria than electrolytic lesions. Therefore, the fornix/fimbria (A:-1.5,L:+-1.0 and +-2.2,V.-4.5mm) was damaged bilaterally by radiofrequency lesions. The parameters were 6 mA for 40 sec.

Two groups (n=12 or 8) received bilateral injections of NMDA (0.25 M in phosphate buffer, pH 7.0) or vehicle into the lateral nucleus of the amygdala. Cannulae loaded with either NMDA or phosphate buffer were lowered to the lateral nucleus (A:-3.5,L+-5.5,V:-8.0mm). The solutions (0.3 *ul*) were infused over a 330 sec period by a Harvard minipump, and the cannulae were left in position for a further 120 sec. After surgery, the lesioned animals were monitored. When early signs of epileptic seizure were noted, additional injections of sodium pentobarbital (3.25 mg) were given.

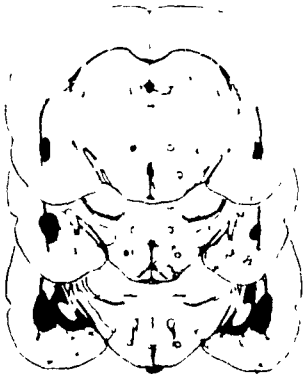
Histology. After completion of behavioral testing, the animals were killed with an overdose of chloral hydrate, their brains were removed, fixed in formol saline, sliced, and stained with Luxol fast blue and neutral red

Results

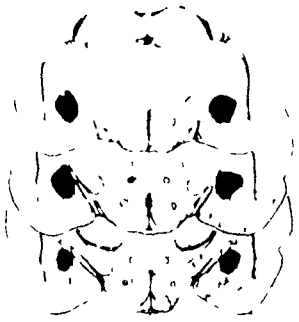
Histology. The extent of each of the six lesions is shown in Figure 16 Electrolytic lesions aimed at the lateral nucleus of the amygdala produced damage to the middle and posterior parts of this nucleus in all cases In a few

Figure 16

Electrolytic and radio-frequency lesions of limbic structures. The shaded areas represent the maximum extent of all lesions in all rats.

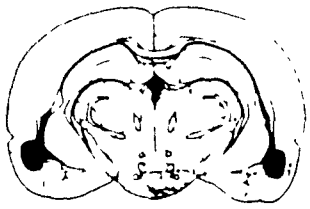


LATERAL NUCLEUS

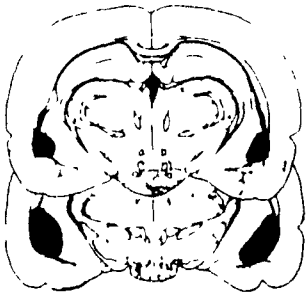


CENTRAL NUCLEUS

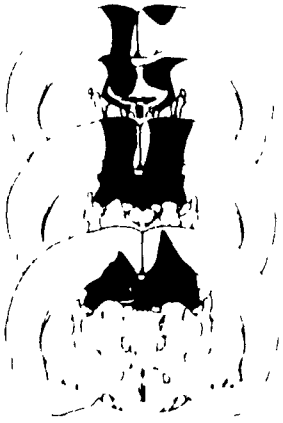
BASOLATERAL NUCLEUS



ENDOPYRIFORM NUCLEUS



VENTRAL HIPPOCAMPUS



FORNIX/FIMBRIA

cases, the lesions extended into the endopyriform nucleus. Lesions aimed at the central amygdaloid nucleus were well-confined to this structure in all cases. Lesions aimed at the basolateral amygdaloid nucleus mostly damaged the middle and posterior parts of this nucleus and the basomedial nucleus. These lesions did not extend into the lateral nucleus of the amygdala. Lesions of the endopyriform nucleus damaged posterior parts of the nucleus, which are located posterolaterally to the lateral nucleus of the amygdala. The lesions did not extend to the lateral amygdaloid nucleus or the basolateral nucleus of amygdala. Lesions aimed at the ventral hippocampus, which is located posteromedially to the lateral amygdaloid nucleus, produced damage in ventrolateral parts of the structure. Radiofrequency lesions of the fornix/fimbria produced substantial damage to the target. The cortex and cingulum were also damaged in some of the cases.

Figure 17 shows the extent of NMDA lesions aimed at the lateral nucleus of the amygdala. There was substantial neuronal cell damage in the lateral nucleus in all cases. Most rats also had substantial damage to the basolateral nucleus. In some of these cases, the lesions extended to the basomedial nucleus bilaterally or unilaterally. In all cases, the endopyriform nucleus was damaged. A few rats had partial damage to the central nucleus. The dorsolateral part of the primary olfactory cortex was damaged in a few rats. In all cases, a part of the hippocampus located posteromedially to the lateral nucleus across the lateral ventricle was also damaged. This damaged area corresponded to the part of the ventral hippocampus damaged by electrolytic lesions.

Representative photomicrographs are shown in Figure 18. The control brain (Figure 18A) shows well-delineated lateral, basolateral, and

Figure 17

The extent of NMDA lesions of the lateral nucleus of the amygdala.

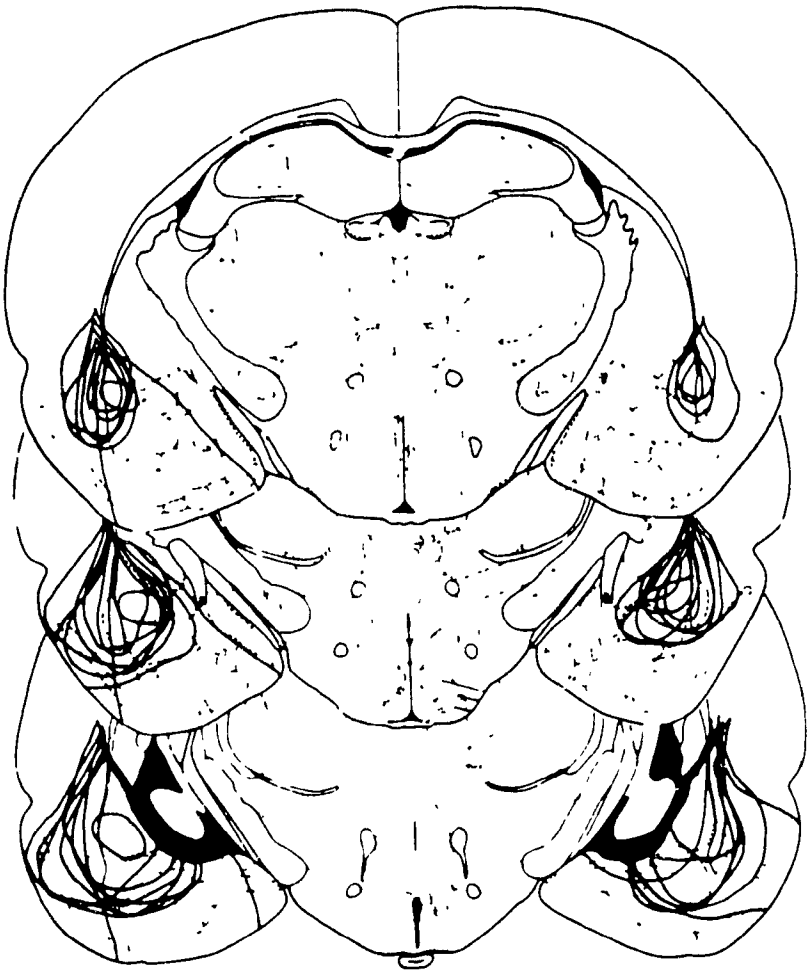
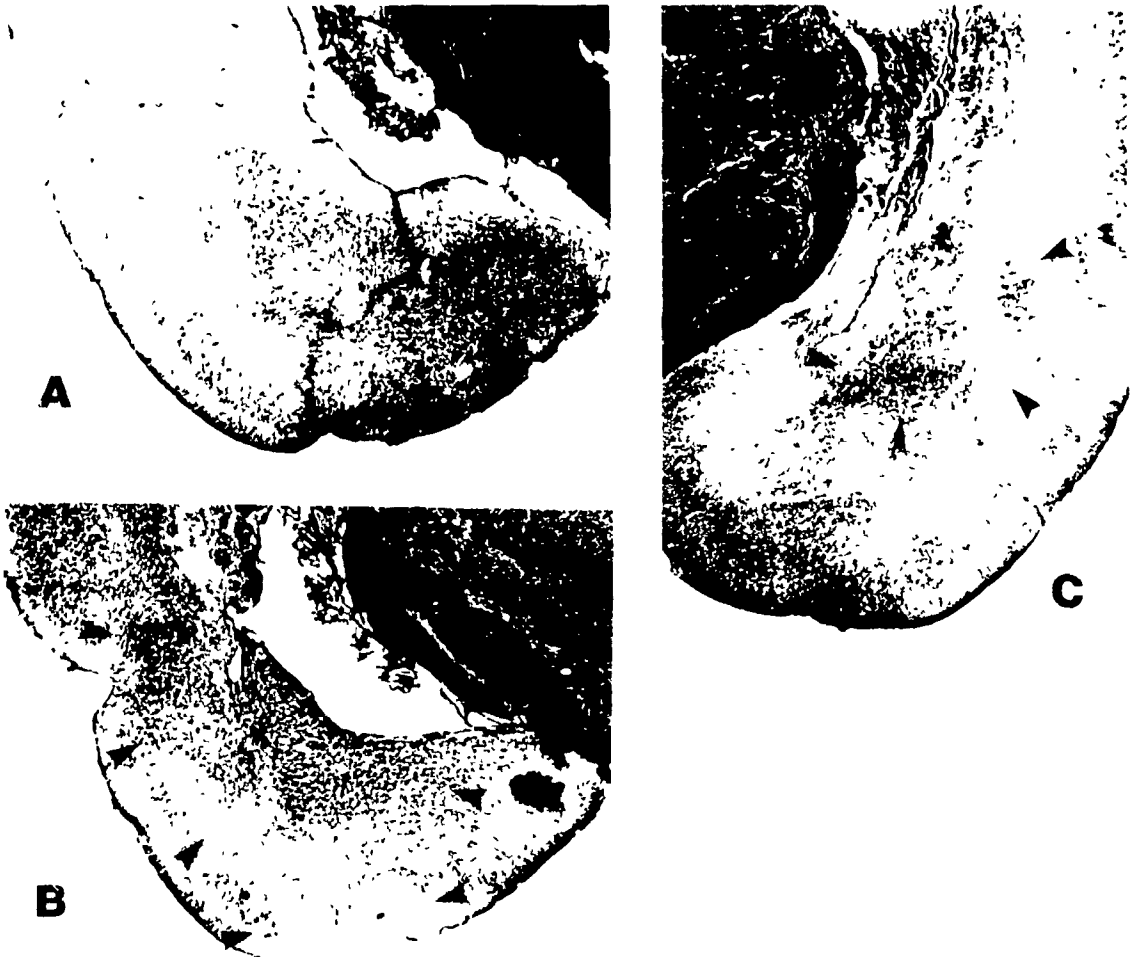


Figure 18

Representative brain sections showing the extent of NMDA lesions of the lateral nucleus of amygdala. The arrows delineate the affected areas which showed cell loss and extensive gliosis. A) Control, B) large lesion, and C) small lesion.


A
B
C

endopyriform nuclei in the posterior amygdala, which are surrounded by a layer of cell bodies of the primary olfactory cortex. The lesioned areas (Figures 18-B and C) are characterized by neuronal cell loss appearing as pale areas and extensive gliosis appearing as intensely dark areas. Due to these changes, distinctions among nuclei are blurred. In the case of a large lesion (Figure 18-B), the damaged area covered the lateral and basolateral amygdaloid nuclei, endopyriform nucleus, part of the primary olfactory cortex, and the posterolateral cortical amygdaloid nucleus. Small lesions (Figure 18-C) damaged the lateral nucleus of amygdala substantially and its adjacent nuclei slightly.

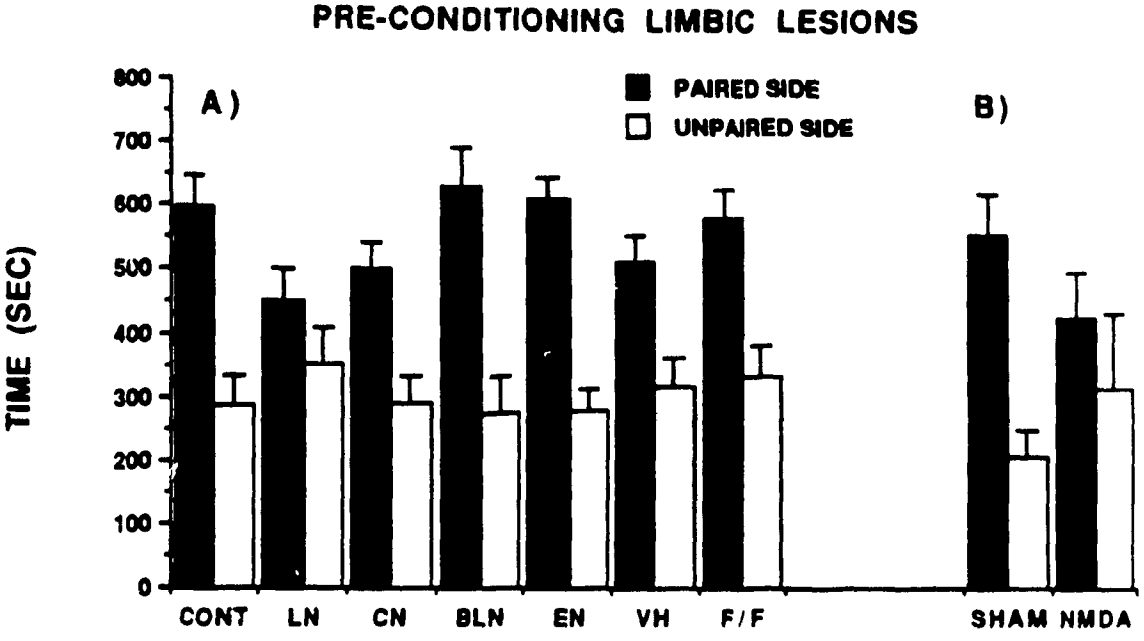
In summary, the electrolytic lesions were well confined to their intended target areas of the amygdala. NMDA lesions produced neuronal cell damage affecting the lateral, central, basolateral, and basomedial amygdaloid nuclei, endopyriform nucleus, and ventral hippocampus. Thus these two lesion techniques achieved complementary effects. Electrolytic lesions achieved good regional localization. NMDA lesions damaged neuronal cell bodies, but not fibers of passage.

Behavior. Figure 19 shows the effects of these lesions on the amphetamine CPP. For the electrolytic and radiofrequency lesions (Figure 19-A), a two-way ANOVA with planned comparisons revealed significant differences between the amount of time spent in the paired and unpaired compartments for the control ($F(1,49)=11.81$, $p<0.01$), central nucleus ($F(1,49)=5.59$, $p<0.05$), basolateral nucleus ($F(1,49)=15.73$, $p<0.01$), endopyriform nucleus ($F(1,49)=14.38$, $p<0.01$), ventral hippocampus ($F(1,49)=4.73$, $p<0.05$), and fornix/fimbria ($F(1,49)=7.52$, $p<0.01$) groups. There was no significant difference in time spent in the two compartments

Figure 19

A) Effects of pre-conditioning lesions of the limbic structures on the amphetamine CPP. CONT Control, LN: Lateral nucleus, CN· Central nucleus, BLN. Basolateral nucleus, EN. Endopyriform nucleus, VH: Ventral hippocampus, F/F Fornix/fimbria.

B) Effect of pre-conditioning NMDA lesions of the lateral nucleus of amygdala on the amphetamine CPP. SHAM: Vehicle-infused group, NMDA NMDA-infused group.


PRE-CONDITIONING LIMBIC LESIONS
PAIRED SIDE
UNPAIRED SIDE
A)
B)
TIME (SEC)
800
700
600
500
400
300
200
100
0
CONT
LN
CN
BLN
EN
VH
F/F
SHAM
NMDA

for the lateral nucleus group ($F(1,49)=1.30$, $p>0.05$). The time difference of the lateral nucleus group was significantly different from that of the control group ($F(1,98)=18.99$, $p<0.01$). The blockade was obtained as a result of decreased time spent in the amphetamine-paired compartment and increased time spent in the amphetamine-unpaired compartment. The control and the lateral nucleus groups spent 26.1 and 33 % of the total test time in the tunnel, respectively.

The effect of NMDA lesions of the lateral nucleus of the amygdala on the amphetamine CPP is shown in Figure 19-B. Planned comparisons revealed that time spent in the amphetamine-paired compartment was significantly different from that spent in the amphetamine-unpaired compartment for the sham group ($F(1,14)=5.70$, $p<0.05$), but not for the group with the NMDA lesions ($F(1,14)=0.54$, $p>0.05$). The time difference of the control was significantly different from that of the NMDA-lesioned group ($F(1,28)=9.33$, $p<0.01$). The control and the NMDA-lesioned groups spent 36.6 and 38.4 % of the total test time in the tunnel, respectively.

These findings clearly implicate the lateral nucleus of the amygdala in the mediation of the amphetamine CPP. However, as the lesions were made before conditioning, the present findings do not permit a conclusion about possible differences in the involvement of the lateral nucleus of the amygdala in acquisition or expression.

Experiment 8

This experiment was designed to examine the effects of electrolytic and NMDA lesions of the lateral nucleus of the amygdala on the expression of the amphetamine CPP.

Method

Animals were operated 24 hours after the last conditioning session and tested after a 1 week recovery period. Two groups of animals received either sham or electrolytic lesions of the lateral nucleus of the amygdala. Another two groups received bilateral injections of NMDA (0.25 M in phosphate buffer, pH 7.0) or vehicle into the lateral nucleus of the amygdala. The surgical and histological procedures were identical to those described in Experiment 7.

Results

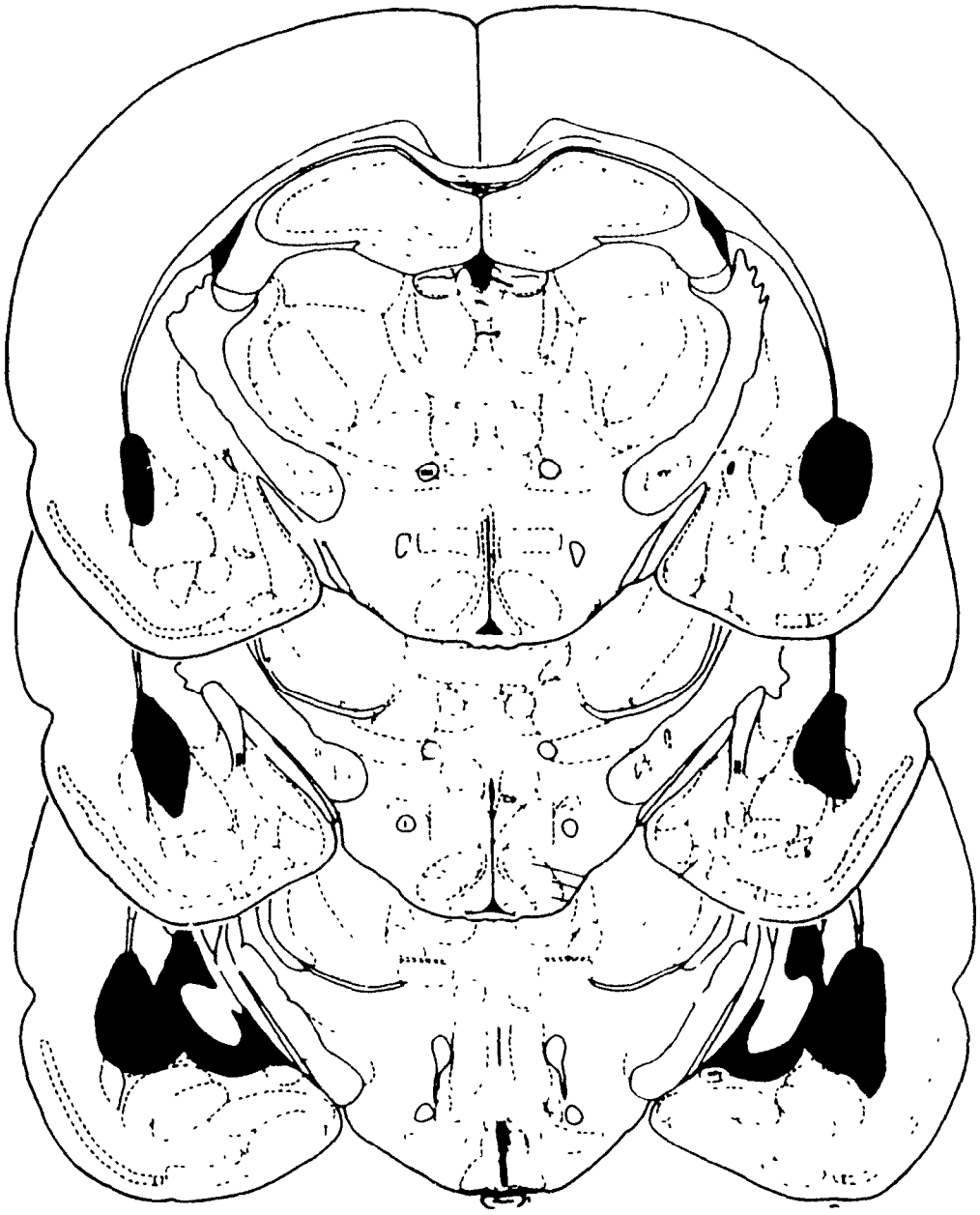
The electrolytic (Figure 20) and NMDA (Figure 21) lesions were comparable to those of Experiment 7.

Electrolytic lesions of the lateral nucleus attenuated the expression of the CPP (Figure 22-A). Planned comparisons showed that there was a significant difference between time spent in the paired and unpaired compartments for the sham group ($F(1,14)=7.45$, $p<0.05$), but not for the lesioned group ($F(1,14)=1.70$, $p>0.05$). The time difference of the sham group was significantly different from that of the lesioned group ($F(1,28)=7.49$, $p<0.05$). The control and the lesioned groups spent 25.7 and 25.9 % of the total test time in the tunnel, respectively.

The NMDA lesions also attenuated the expression of the CPP (Figure 22-B). Planned comparisons revealed a significant difference between time spent in the amphetamine-paired and unpaired compartments for the control ($F(1,19)=10.60$, $p<0.01$), but not for the NMDA-lesioned group ($F(1,19)=1.75$, $p>0.05$). The time difference of the control was significantly different from that of the NMDA-lesioned group ($F(1,38)=8.43$, $p<0.01$). The control and the NMDA-lesioned groups spent 25.8 and 36.1 % of the total test time in the

Figure 20

The maximum extent of post-conditioning electrolytic lesions of the lateral nucleus of the amygdala in all the rats.



LATERAL NUCLEUS

Figure 21

The extent of post-conditioning NMDA lesions of the lateral nucleus of the amygdala.

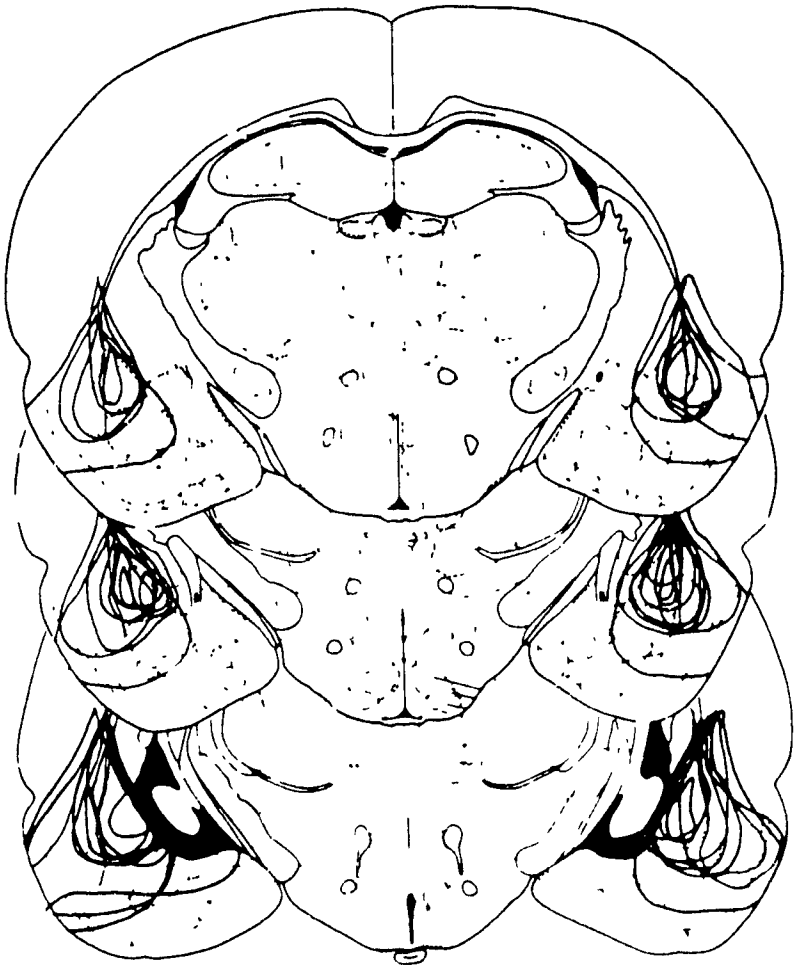
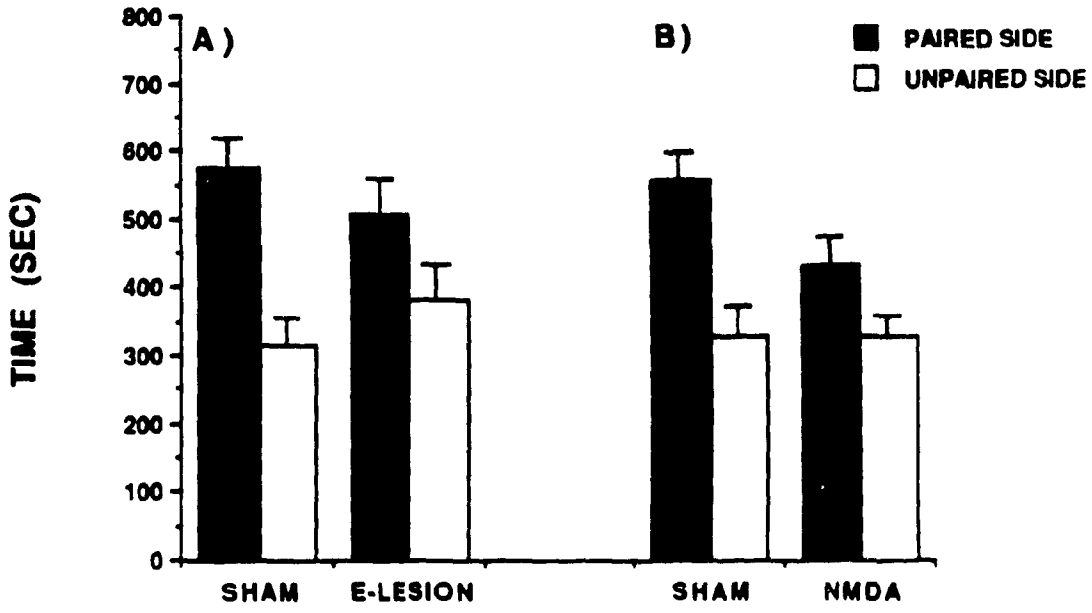


Figure 22

Effects of post-conditioning A) electrolytic and B) NMDA lesions of the lateral nucleus of amygdala on the expression of the amphetamine CPP. A) SHAM: Sham-operated group, E-LESION. Electrolytic lesion group. B) SHAM Vehicle-infused group, NMDA NMDA-infused group.

POST-CONDITIONING LESIONS OF THE LATERAL NUCLEUS



tunnel, respectively.

Discussion

The present set of experiments shows that the amphetamine CPP was attenuated by pre-conditioning electrolytic or NMDA lesions of the lateral nucleus of the amygdala, but not by electrolytic lesions of its adjacent areas or by radiofrequency lesions of the fornix/fimbria. Furthermore, when the lateral nucleus of the amygdala was damaged by electrolytic or NMDA lesions after conditioning, animals failed to express amphetamine CPPs. Although NMDA lesions aimed at the lateral nucleus of the amygdala also affected the central, basolateral, and basomedial amygdaloid nuclei, endopyriform nucleus, and ventral hippocampus, electrolytic lesions confined to each of these structures produced no impairments.

The finding that pre-conditioning lesions of the lateral nucleus attenuated the amphetamine CPP does not necessarily provide clear evidence that this nucleus is involved in acquisition because pre-conditioning lesions would affect both acquisition and expression, and post-conditioning lesions, which affect expression only, attenuated the CPP. Nevertheless these results clearly show that intrinsic neurons of the lateral nucleus of the amygdala mediate the expression of the amphetamine CPP and suggest that the central, basolateral, and basomedial amygdaloid nuclei, the endopyriform nucleus, and the hippocampal-accumbens projection are probably not involved in mediating acquisition or expression of this behavior.

Since amygdala lesions are known to affect behaviors normally exhibited in the presence of novel stimuli (Nachman and Ashe, 1974, Peinado-Manzano, 1988, Rolls and Rolls, 1973), the possibility that the CPP measures

responses to novelty which were eliminated by lesions of the lateral nucleus must be considered. If, by some unknown mechanism, amphetamine acted to preserve the novelty of the stimuli in the paired compartment, the drug might produce a preference for this compartment over the unpaired compartment (Scoles and Siegel, 1986), simply because rats tend to explore novel stimuli (Bardo, Neisewander and Pierce, 1989). However, since amygdala lesions potentiate this tendency to explore novel stimuli (Nachman and Ashe, 1974, Peinado-Manzano, 1988, Rolls and Rolls, 1973), and the effect of the lesions in the present study was a reduction in preference, this cannot be the explanation of the observed effects. The notion that increased tendency to explore novel stimuli is not the cause of the blockade of the CPP is further supported by the finding that the blockade was not due to increased time spent in the tunnel, to which animals were exposed only once, the amount of time spent in the tunnel on the test day ranged between 25.7 and 36.6 % for the control groups and between 25.9 and 38.4 % for the lateral nucleus groups.

An alternative possibility is that amphetamine, again by some unknown mechanism, might increase the familiarity of the stimuli in the paired compartment (Swerdlow and Koob, 1984) leading the animals to avoid the unpaired compartment because of neophobia for the relatively less familiar stimuli in that compartment. However, the fact that the animals had experienced 3 sessions of exposure to the unpaired compartment before the test trial makes it highly unlikely that the level of novelty represented by these stimuli would be high enough to induce neophobia. In fact, as conditioning progressed, animals often lay on the belly in the unpaired compartment and showed no neophobic responses, suggesting that they did

not perceive the unpaired compartment as novel. Therefore, it seems very unlikely that the effects of amygdala lesions on responses to novelty can explain the findings of the present experiments.

There are several other possible explanations for the nature of the deficit observed in the present experiment. The deficit could be due to an impairment of perception of the sensory stimuli, impairment of the effect of amphetamine, and/or impairment of acquisition and expression of conditioning.

Although amygdala lesions produce "psychic blindness", which implies indiscriminate behaviors toward objects in the environment (Kluver and Bucy, 1937, 1939, Schreiner and Kling, 1953, 1956), it should be noted that "psychic blindness" is clearly distinguishable from purely perceptual blindness. Animals with total amygdalectomy clearly locate environmental stimuli and objects (Kluver and Bucy, 1937, 1939; Schreiner and Kling, 1956) and show normal visual acuity (Kluver and Bucy, 1937, 1939). Moreover, lesions of the lateral nucleus of the amygdala do not produce somatosensory neglect (Turner, 1973).

The effect of amphetamine in releasing dopamine and establishing the CPP does not directly involve the amygdala. Although dopamine terminals are present in the amygdala, they are distributed in the central nucleus, and the other nuclei have extremely low concentrations of dopamine (Ben-Ari, Zigmond and Moore, 1975). Although systemically injected amphetamine would act on dopamine terminals in the nucleus accumbens as well as the amygdala, the fact that 6-OHDA lesions of nucleus accumbens attenuate the CPP induced by systemic injections of amphetamine (Spyraki et al., 1982a) suggests that dopamine release in the amygdala is not sufficient to establish

CPPs. Furthermore, microinjections of amphetamine aimed at the central nucleus of the amygdala do not establish CPPs (Carr and White, 1986), and microinjections of amphetamine into nucleus accumbens are sufficient to establish CPPs (Carr and White, 1983, 1986). These findings suggest that dopamine release in the amygdala is not a necessary event for establishing the CPP. Thus, the deficit observed in the present study is probably not due to an impaired amphetamine effect *per se*.

The remaining explanation for the effect of lateral amygdaloid nucleus lesions observed in the present study is that they disrupted acquisition and/or expression of memory involved in this conditioning. I shall further discuss this issue in the General Discussion.

It should be noted that in no case was a complete blockade of the CPP observed, even though groups of animals with lateral nucleus lesions did not show statistically significant CPPs. This might be due to the fact that the olfactory system redundantly gives rise to direct and indirect projections to almost all amygdaloid nuclei (deOlmos et al., 1985, Switzer, deOlmos and Heimer, 1985). Due to this redundancy, the lateral nucleus-lesioned animals may have been able to identify the drug-paired odor, while they were unable to identify the drug-paired visual and somatosensory stimuli. The survival of some conditioning to the olfactory stimuli in the apparatus is a possible explanation for the small, though insignificant, CPPs that persist after the lateral nucleus lesions.

CHAPTER 6

GENERAL DISCUSSION

The neural basis of the amphetamine CPP

There is little doubt that some effect of amphetamine establishes a CPP. Previous studies have provided ample evidence that this effect is mediated by dopamine release in the nucleus accumbens (Aulisi and Hoebel, 1983, Carr and White, 1983, 1986, Spyraki et al., 1982a). At the same time, there has been little attention paid to the neural basis for the *expression* of the CPP, which occurs in the absence of amphetamine during the test session. The present investigation provides new information about the roles of the nigrostriatal and mesolimbic dopamine systems, dopamine receptor subtypes, dopamine pools, and limbic system in this conditioned behavior.

Nigrostriatal and mesolimbic dopamine systems. The nigrostriatal dopamine system does not seem to have any role in either acquisition or expression of the amphetamine CPP. Pre-conditioning lesions of the dorsal caudate/putamen did not impair the amphetamine CPP (Experiment 4). This is consistent with previous findings that microinjections of amphetamine into caudate/putamen fail to establish a CPP (Carr and White, 1983, 1986). The finding that microinjections of dopamine antagonists into the caudate/putamen had no effect on expression (Experiment 2) complements this by suggesting that the expression of the amphetamine CPP does not involve the nigrostriatal dopamine system.

On the other hand, the present study shows the critical role of the mesolimbic dopamine system for the expression of the amphetamine CPP. Microinjections of dopamine antagonists into nucleus accumbens abolished

expression (Experiment 2), which was not due to reduced activity levels (Experiment 3) or spread of dopamine antagonists into the caudate/putamen (Experiments 2 and 4). Thus, when animals express an amphetamine CPP in the absence of amphetamine, dopamine release and dopamine receptor activation in the nucleus accumbens seem to be critical.

Dopamine receptor subtypes. Previous studies have shown that the acquisition of the amphetamine CPP is blocked by systemically injected D1 (Hoffman and Beninger, 1989, Leone and DiChiara, 1987) or D2 (Hoffman and Beninger, 1989; Spyraki et al., 1982a) dopamine antagonists. The results of Experiment 1 are consistent with this line of evidence both selective D1 and D2 dopamine antagonists blocked acquisition of the amphetamine CPP in a dose-dependent fashion. Although blockade of either receptor subtype is sufficient to antagonize the CPP-establishing effect of amphetamine, CPPs are established by systemically injected D2 but not D1 dopamine agonists (Hoffman and Beninger, 1988). This might simply be due to the relative inability of D1 agonists to cross the blood-brain barrier, since microinjections of either D1 or D2 dopamine agonists into nucleus accumbens establish CPPs (White, Packard and Hiroi, in press). Taken together, it might be concluded that a CPP is established when two dopamine receptor subtypes are activated with at least one of the subtypes in a state of supernormal activation.

The results of Experiment 1 further showed that the D1 dopamine receptor antagonist effectively blocked both acquisition and expression of the amphetamine CPP, but that the antagonists with higher affinity for the D2 than the D1 dopamine receptor did not block expression at doses that blocked acquisition, suggesting that D1 receptor activation may be more important for expression of the amphetamine CPP.

Dopamine pools. The results in Chapter 4 demonstrated that *a* -MPT and reserpine blocked amphetamine- and pipradrol-induced locomotor activity, respectively and that reserpine, but not *a* -MPT, blocked the expression of the amphetamine CPP. This suggests that dopamine release from the reserpine-sensitive pool is a critical event for the expression of the amphetamine CPP. Given that amphetamine seems to establish a CPP by releasing dopamine from the *a* -MPT-sensitive pool, it might be that the two dopamine pools are differentially involved in acquisition and expression of the CPP.

Involvement of limbic systems. The findings in Chapter 5 provide information about the involvement of limbic structures in the amphetamine CPP. Complete lesions of the fornix/fimbria had no effect on the amphetamine CPP (Experiment 7), suggesting that the hippocampal-accumbens system is not involved in the CPP. In contrast, electrolytic lesions of the lateral amygdaloid nucleus, but not the central or basolateral amygdaloid nucleus, impaired the amphetamine CPP (Experiment 7). NMDA injected into the lateral amygdaloid nucleus produced substantial neuronal cell damage in the region and impaired the amphetamine CPP (Experiment 7). Furthermore, post-conditioning electrolytic and NMDA lesions of the lateral amygdaloid nucleus impaired the expression of the amphetamine CPP. Thus, it can be concluded that the expression of the amphetamine CPP --at least the version used in the present study-- involves intrinsic neurons of the lateral amygdaloid nucleus.

Neural mechanisms of acquisition of incentive learning

In this section, I shall discuss how a completely internal action of amphetamine (dopamine release in the nucleus accumbens) establishes conditioned approach toward external stimuli and hypothesize about possible underlying neural events for the acquisition of incentive learning.

An explanation of the mechanism for the establishment of a CPP towards an external stimulus by dopamine release in the nucleus accumbens can begin by considering the fact that the presence of a natural incentive stimulus such as food activates the mesolimbic dopaminergic projection to the nucleus accumbens (Blackburn et al., 1986, Heffner et al., 1980, Holmes et al., 1989; Radhakishun et al., 1988). Incentive behaviors are facilitated by amphetamine injected into the nucleus accumbens (Evans and Vaccarino, 1986, 1990), and depletion of accumbens dopamine abolishes appetitive incentive behaviors (Kelley and Stinus, 1985; Koob et al., 1978). Moreover, direct pharmacological activation of the mesolimbic dopamine system produces behaviors reminiscent of appetitive incentive behaviors (Costall and Naylor, 1975, 1976, Jackson et al., 1975; Pijnenberg and Van Rossum, 1973), and these drug-induced behaviors are abolished by depletion of dopamine in the nucleus accumbens (Costall et al., 1977; Fink and Smith, 1980, Kelly and Iversen, 1976; Kelly et al., 1975). Thus, stimulants seem to produce approach toward external stimuli by mimicking the effect of natural incentive stimuli at a neural level.

The presence of natural incentive stimuli causes the establishment of CPPs (Papp, 1988, Spyraki et al., 1982c; Tombaugh et al., 1982), and this is prevented by a systemically injected dopamine antagonist (Spyraki et al, 1982c). It is not known which dopamine system is critical for CPPs

established with natural incentive stimuli. Nonetheless, given that pharmacological activation of the mesolimbic dopamine system reliably establishes CPPs (Aulisi and Hoebel, 1983; Carr and White, 1983, 1986), it is likely that this dopamine system is also involved in the establishment of CPPs with natural incentive stimuli.

This suggests that the way in which animals respond to originally neutral sensory stimuli is altered in a similar manner if the mesolimbic dopamine system is activated either by natural incentive stimuli or by stimulants. Through this process, sensory stimuli might acquire the properties of incentive stimuli to induce approach, evoke hyperactivity, and establish and maintain responses.

It is worth mentioning that the existence of such a learning process has previously been suggested (Bindra, 1969, 1972, 1974, 1978; Bolles, 1972). It has also been postulated that such learning relies on the amygdala (Jones and Mishkin, 1972).

A variety of behavioral tasks have been used to assess the mnemonic role of the amygdala in most paradigms, animals are required to discriminate among stimuli, to respond differentially to stimuli, and, in some cases, to reverse what they have learned (Jones and Mishkin, 1972, Peinado-Manzano, 1987, 1988, 1989, 1990, Spiegler and Mishkin, 1981). These tasks may involve discrimination, reversal, and all the possible associative linkages proposed by Estes (1969): stimulus-reward, response-reward, and stimulus-response. Although amygdala lesions produce impairments on these tasks (Jones and Mishkin, 1972, Peinado-Manzano, 1987, 1988, 1989, 1990; Spiegler and Mishkin, 1981), the large number of variables involved in the paradigms used leaves us unable to interpret these impairments in terms of a deficit to a

single process, such as the one by which neutral stimuli become associated with incentive stimuli.

In contrast, incentive learning paradigms seem to provide an ideal experimental setting to test hypotheses concerning the role of the amygdala in such an association process. The CPP paradigm, for example, involves pairings of neutral sensory stimuli with incentive stimuli independently of an animal's responses. The finding that pre-conditioning lesions of the lateral amygdaloid nucleus impaired the CPP (Chapter 5) is therefore consistent with the notion the amygdala is involved in the association of neutral sensory stimuli with incentive stimuli, although this finding does not unequivocally show that the deficit was due to impaired acquisition of such a learning process. Nonetheless, the lateral amygdaloid nucleus is an excellent candidate structure for the acquisition process since all sensory modalities have neuroanatomical access to it (deOlmos et al., 1985, Turner, 1981, Turner and Zimmer, 1984, Switzer et al., 1985). The precise way in which the mesolimbic dopamine system and the lateral amygdaloid nucleus interact to establish incentive learning remains to be established.

Possible mechanisms of expression of incentive learning

While the neural basis of the acquisition of incentive learning remains relatively unclear except for the role of the mesolimbic dopamine system, the present study is particularly informative in elucidating the neural mechanisms for the expression of this type of learning. The expression of incentive learning, which is initiated by the effect of conditioned incentive stimuli, seems to be mediated by the reserpine-sensitive dopamine pool, the D1 dopamine receptor in the nucleus accumbens, and the lateral amygdaloid

nucleus.

This suggests the possible involvement of a neuroanatomical system revealed by recent anatomical and neurochemical studies. The amygdaloid projection to the nucleus accumbens is mainly directed to the striosomes (Ragsdale and Graybiel, 1988), an area of the caudate/putamen and nucleus accumbens characterized by weaker acetylcholinestrase activity than the extra-striosomal matrix (Graybiel and Ragsdale, 1978). Dopamine terminals which are not sensitive to *a* -MPT (Olson, Seiger and Fuxe, 1972) are also distributed in the striosomes (Ferrante and Kowall, 1987, Graybiel, 1984; Graybiel, Nastuk and Agid, 1987, Jimenez-Costellonos and Graybiel, 1987). Compared to the extra-striosomal matrix, the striosomes have higher D1 (Besson, Graybiel and Nastuk, 1988) and lower D2 dopamine receptor densities (Joyce, Sapp and Marshall, 1986; Loopuijt, Sebens and Korf, 1987). Although the striosomal organization in the nucleus accumbens is not as clear as it is in the caudate/putamen, the amygdaloid projection to the striosomes in the nucleus accumbens might be a route through which conditioned incentive stimuli influence behavior.

It remains unclear exactly how this route mediates the expression of conditioned incentive behaviors. One possible mechanism could be that memory of the altered incentive values of originally neutral sensory stimuli stored in or around the lateral amygdaloid nucleus is activated when animals encounter conditioned incentive stimuli, and this causes activation of the terminals of mesolimbic dopamine neurons through the projection from the lateral amygdaloid nucleus to the nucleus accumbens. Alternatively, conditioned incentive stimuli may act directly to increase dopamine release in the nucleus accumbens, and this dopamine release may add high incentive

value to signals concerning neutral sensory stimuli sent from the lateral amygdaloid nucleus to the nucleus accumbens.

In the case of the amphetamine CPP, the *a* MPT-sensitive and reserpine-sensitive dopamine pools seem to be involved in acquisition and expression, respectively. It might be that this relationship also holds when natural unconditioned and conditioned incentive stimuli exert their effects. Thus, it may be that the hypothetical effect of the input from the amygdala promotes dopamine release from the reserpine-sensitive pool when signalling the presence of conditioned incentive stimuli. Further research will be required to test this hypothesis.

It has been suggested that the amygdala is involved in the process by which sensory stimuli are monitored based on past experience of their motivational significance (Gloor et al., 1981). The present investigation considerably extends this view by suggesting that the effect of conditioned incentive stimuli might be mediated by the lateral amygdaloid nucleus-striosomal accumbens unit.

Implications of the neural mechanism for other incentive learning

The neural mechanism suggested above explains a number of findings on the effect of conditioned incentive stimuli in other forms of incentive learning.

D1 and D2 dopamine receptors. It has been suggested that once established, amphetamine conditioned behaviors are expressed independently of dopamine functions (Beninger, 1983) on the ground that a dose of pimozide which completely blocks amphetamine unconditioned locomotor activity has no effect on the conditioned locomotor activity (CLA) (Beninger

and Hahn, 1983).

The studies consistent with this suggestion used D2 dopamine antagonists such as pimozide and haloperidol (Beninger and Hahn 1983; Hiroi and White, 1989, Poncelet et al., 1987, Schiff, 1982). These drugs have a higher affinity for D2 than D1 receptors in vivo (Andersen, 1988, Waddington and O'Boyle, 1989). The present study suggests that their failure to block the expression of amphetamine conditioned behaviors may be due to the weak effect of these antagonists on D1 receptors. Thus, the present findings call into question the notion that conditioned behaviors can be expressed even when dopaminergic function is disrupted and provide an explanation as to why accumbens dopamine depletion abolishes the expression of amphetamine CLA (Gold et al., 1988) while some neuroleptics at certain doses do not.

Two dopamine pools. It has been shown that pipradrol and methylphenidate potentiate the response acquisition supported by a conditioned reinforcer more effectively than amphetamine (Robbins, 1978; Robbins et al., 1983). Given that a conditioned reinforcer is, in fact, a conditioned incentive stimulus, the different effects of these two classes of stimulants might be explained in light of the hypothesis that the reserpine-sensitive pool, with which pipradrol and methylphenidate interact, is involved in the effects of conditioned incentive stimuli.

The role of the lateral amygdaloid nucleus. It has been reported that excitotoxic lesions of the basolateral nucleus of the amygdala impaired the ability of conditioned reinforcers to establish a new response (Cador et al., 1989) and to maintain an established response (Everitt et al., 1989a). These findings are inconsistent with the present finding that the effect of a

conditioned incentive stimulus depends on the lateral amygdaloid nucleus. It is unlikely that this discrepancy is due to differences between the conditioned reinforcement and CPP paradigms because the same group of investigators also showed that ibotenic acid injected into the basolateral nucleus produced impairment in the expression of a food CPP (Everitt et al., 1989b). This raises the possibility that the basolateral and lateral nuclei of the amygdala are involved in incentive learning with natural incentive stimuli and amphetamine, respectively.

Another possible explanation for the discrepancy is based on the fact that excitotoxic lesions cannot be confined to a single nucleus in the amygdala. The histological data of Cador et al. (1989) and Everitt et al. (1989a, b) show that their lesions impinged on the central, basolateral, basomedial, and lateral nuclei of the amygdala. The impairment found in those studies might, therefore, be due to lesions of the lateral nucleus of the amygdala. In fact, recent findings show that the food CPP is impaired by small electrolytic lesions confined to the lateral nucleus of the amygdala and by NMDA injected into (but not confined to) the lateral amygdaloid nucleus (Hiroi, McDonald and White, 1990).

Implication for stimulant self-administration in humans

According to the present hypothesis, approach to a drug-paired environment is mediated by a similar neural event to that produced by the acute amphetamine effect. Thus, the present study suggests the view that drug-seeking behaviors are produced because drug-associated environmental stimuli mimic acute drug actions (Stewart, deWit and Eikelboom, 1984).

If, as suggested by the present investigation, conditioning is a factor in

human stimulant self-administration, an effective treatment would be an extinction procedure. Addicts might quickly stop drug-seeking behaviors if they were exposed to the conditioned incentive stimuli associated with their drug-taking behavior while the reserpine-sensitive dopamine pool was depleted by reserpine, a treatment which would effectively eliminate the action of the conditioned incentive stimuli.

The present investigation also provides a new perspective on drug-seeking behavior. In the present thesis, I have argued that amphetamine activates the mesolimbic dopamine system, which normally mediates natural incentive behaviors. Since activation of the mesolimbic dopamine system by amphetamine would be far greater in amplitude than that produced by natural incentive stimuli, the drug might act at a neural level as though it had an extremely high value for survival. It is ironic that amphetamine dramatically reduces the chances of an individual's survival at a behavioral level.

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