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PRESYNAPTIC CATECHOLAMINE MECHANISMS IN AMPHETAMINE CONDITIONED BEHAVIORS

BY

SHERRY LYNN DILULLO

A THESIS

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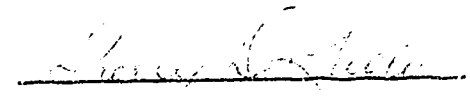

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled PRESYNAPTIC CATECHOLAMINE MECHANISMS IN AMPHETAMINE CONDITIONED BEHAVIORS by SHERRY LYNN DILULLO in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in PSYCHIATRY.

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Date October 30, 1991

DEDICATION

To the memory of my Father.

ABSTRACT

Rats that receive (+)-amphetamine (AMP) in a specific environment exhibit a conditioned psychomotor response when subsequently placed in that environment without drug treatment. Previous work has shown that while the direct effects of AMP can be blocked by dopamine (DA) D_1 or D_2 receptor antagonists, the establishment of a conditioned locomotor response is not influenced by DA receptor antagonists other than pimozide. This thesis investigates the possibility that a presynaptic neural event is conditioned during repeated AMP administration to produce increased locomotion. In the first of three investigations, a series of experiments investigated the role of presynaptic DA and noradrenaline (NA) neurotransmission in AMP-conditioned behaviors. α-Methyl-p-tyrosine (αMPT), a catecholamine synthesis inhibitor, blocked the direct effects of AMP, but did not block AMP-conditioned behaviors. N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4), a selective, long-lasting NA neurotoxin, did not affect either the direct or conditioned effects of AMP. In the second investigation, neither haloperidol, a selective DA D_2 antagonist, nor nimodipine, an L-type calcium channel antagonist, influenced AMP-conditioned locomotion. Concomitant treatment with haloperidol and nimodipine did attenuate the AMP-conditioned locomotor response. This observation suggests that DA plays a critical role in AMP-conditioning, though not necessarily an exclusive role. Pimozide's unique actions are likely due to its dual D_2 receptor antagonism and calcium channel blockade effects. In the final investigation, the role of presynaptic αMPT-sensitive and reserpine-sensitive processes in the establishment of AMP-conditioned locomotion was examined. Neither αMPT nor reserpine treatment during AMP-conditioning blocked AMP-conditioned locomotion. Combined treatment with αMPT and reserpine completely blocked the AMP-conditioned locomotor response. These observations suggest that either one of these presynaptic processes, both of which likely involve DA, is sufficient for the acquisition of the AMP-conditioned response, but neither is necessary; both of these processes must be blocked to block the establishment of the AMP-conditioned response.

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TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION 1
1.1. CLASSICAL CONDITIONING OF PSYCHOMOTOR STIMULANT EFFECTS 1
1.2. MECHANISMS OF DIRECT BEHAVIORAL EFFECTS OF PSYCHOMOTOR STIMULANTS 2
1.3. ANATOMICAL SPECIFICITY OF LOCOMOTOR EFFECTS 3
1.4. DA INVOLVEMENT IN THE REINFORCING EFFECTS OF PSYCHOMOTOR STIMULANTS 4
1.4.1. *Dose Effects of Self-Administration of Stimulants* 4
1.4.2. *Extinction of Self-Administration of Stimulants* 6
1.5. ANATOMICAL SPECIFICITY OF REINFORCING EFFECTS OF STIMULANTS 7
1.6. CONDITIONED REINFORCING EFFECTS OF STIMULANTS 8
1.7. SENSITIZATION TO PSYCHOMOTOR STIMULANTS 9
1.8. CONDITIONING OF DRUG EFFECTS AND DRUG ABUSE 13
1.9. MECHANISMS OF CONDITIONED LOCOMOTION 18
1.10. BIBLIOGRAPHY 25

CHAPTER 2. PRESYNAPTIC DOPAMINERGIC NEUROTRANSMISSION MEDIATES AMPHETAMINE-INDUCED UNCONDITIONED BUT NOT AMPHETAMINE-CONDITIONED LOCOMOTION AND DEFECATION IN THE RAT 38
2.1. INTRODUCTION 38
2.2. MATERIAL AND METHODS 42
2.2.1. Animals, apparatus and drugs 42
2.2.2. Procedures 43
2.3. RESULTS 46
2.3.1. Expt. 1: Effects of DSP4 and αMPT on AMP-induced unconditioned and conditioned locomotion. 46
2.3.2. Expt. 2: Effects of DSP4 on monoamines and acidic metabolites. .. 47
2.3.3. Expt. 3: Effects of αMPT on AMP-induced unconditioned and conditioned locomotion and defecation. 47
2.3.4. Expt. 4: Effects of αMPT administered 170 min prior to AMP treatment on AMP-induced unconditioned and conditioned locomotion and defecation. 48
2.4. DISCUSSION 61
2.5. BIBLIOGRAPHY 67

CHAPTER 3. CALCIUM CHANNEL BLOCKADE: A POTENTIAL ADJUNCTIVE TREATMENT WITH NEUROLEPTICS FOR STIMULANT ABUSE AND SCHIZOPHRENIA 72
3.1. INTRODUCTION 72
3.2. METHODS 75
3.3. RESULTS 76
3.4. DISCUSSION 79
3.5. BIBLIOGRAPHY 81

CHAPTER 4. EVIDENCE FOR PRESYNAPTIC DOPAMINE MECHANISMS UNDERLYING AMPHETAMINE-CONDITIONED LOCOMOTION 85
4.1. INTRODUCTION 85
4.2. MATERIALS AND METHODS 88
4.2.1. Animals and Drugs 88
4.2.2. *Procedures* 89
4.2.3. Statistics 91
4.3. RESULTS 91
4.4. DISCUSSION 96
4.5. BIBLIOGRAPHY 101

CHAPTER 5. GENERAL DISCUSSION 107
5.1. CONCLUSIONS 115
5.2. CLINICAL IMPLICATIONS 116
5.3. BIBLIOGRAPHY 118

LIST OF TABLES

Table 1. DSP4 effects on brain regional monoamine levels 52

LIST OF FIGURES

Figure 1. DSP4 and αMPT (25 mg/kg) effects on AMP-locomotion 50

Figure 2. DSP4 and αMPT effects on conditioned locomotion 51

Figure 3. αMPT (50 mg/kg) effects on AMP-locomotion 53

Figure 4. αMPT (50 mg/kg) effects on AMP-defecation 54

Figure 5. αMPT (50 mg/kg) effects on conditioned locomotion 55

Figure 6. αMPT (50 mg/kg) effects on conditioned defecation 56

Figure 7. Increased αMPT-AMP injection interval: locomotion 57

Figure 8. Increased αMPT-AMP injection interval: defecation 58

Figure 9. αMPT-AMP 170 min interval: conditioned locomotion 59

Figure 10. αMPT-AMP 170 min interval: conditioned defecation 60

Figure 11. HAL and NIM effects on AMP-locomotion 77

Figure 12. HAL and NIM effects on conditioned locomotion 78

Figure 13. RES and αMPT effects on spontaneous locomotion 93

Figure 14. RES and αMPT effects on AMP-locomotion 94

Figure 15. RES and αMPT effects on conditioned locomotion 95

LIST OF ABBREVIATIONS

1.	AMP	(+)-amphetamine
2.	αMPT	α-methyl-p-tyrosine methyl ester (hydrochloride)
3.	ANOVA	analysis of variance
4.	CR	conditioned response
5.	CS	conditioned stimulus
6.	DA	dopamine
7.	DOPAC	3,4-dihydroxyphenylacetic acid
8.	DSP4	N-2-chloroethyl-N-ethyl-2-bromobenzylamine
9.	HAL	haloperidol
10.	5-HIAA	5-hydroxyindoleacetic acid
11.	5-HT	serotonin
12.	NA	noradrenaline
13.	NIM	nimodipine
14.	6-OHDA	6-hydroxydopamine
15.	PHNO	(+)-4-propyl-9-hydroxynaphthoxazine
16.	RES	reserpine
17.	UCR	unconditioned response
18.	UCS	unconditioned stimulus
19.	VEH	vehicle

CHAPTER 1. GENERAL INTRODUCTION

1.1. CLASSICAL CONDITIONING OF PSYCHOMOTOR STIMULANT EFFECTS

Repeated administration of psychomotor stimulants, such as amphetamine (AMP), cocaine and methylphenidate, in a unique environment can impart stimulant-like activity upon that environment. The ability of the situational cues associated with the administration and/or the subsequent effects of psychomotor stimulants to induce a drug-related response is well established and has been demonstrated in human (O'Brien *et al.*, 1986; O'Brien *et al.*, 1988; Muntaner *et al.*, 1989) and non-human (Pickens and Crowder, 1967; Schiff, 1982; Spyraki *et al.*, 1982a,b; Barr *et al.*, 1983; Beninger and Hahn, 1983; Martin-Iverson *et al.*, 1985; Beninger and Herz, 1986; Mithani *et al.*, 1986; Miyamoto and Hada, 1987; Gold *et al.*, 1988; Hiroi and White, 1989, 1990; Carey, 1990; Drew and Glick, 1990; Martin-Iverson and McManus, 1990) animals. Pavlov (1927) first recognized that the circumstances under which a drug is administered may become the conditioned stimulus in a classical conditioning paradigm; whereby an event (conditioned stimulus, CS) which is temporally associated with an unconditioned stimulus (UCS) acquires the ability to induce a conditioned response (CR). The CR is usually similar to the unconditioned response (UCR) elicited by the UCS and therefore elicits the effects of the drug when presented in the drug's absence. Pavlov demonstrated that a tone (CS) could elicit the previously paired effects of morphine (UCS). Thus, the acquisition of drug-like properties by unique environmental stimuli is most likely a function of classical conditioning, and has most often been interpreted within this framework. The search for the mechanism underlying stimulant-conditioning has logically trailed the evidence which implicates dopamine (DA) in the activating and rewarding effects of psychomotor stimulants.

1.2. MECHANISMS OF DIRECT BEHAVIORAL EFFECTS OF PSYCHOMOTOR STIMULANTS

The central mechanism of action of psychomotor stimulants is of great interest, in part because stimulants can impart a psychosis virtually indistinguishable from paranoid schizophrenia (Connell, 1958) and because of the abuse potential of this class of drugs. Originally, it was thought that psychomotor stimulants exert their direct behavioral effects via their actions on noradrenaline (NA) (see an early review of neuronal mechanisms of AMP by Cole, 1978). AMP has three primary modes of action on dopaminergic neurons. AMP causes the release of newly synthesized DA from presynaptic nerve terminals (Chiueh and Moore, 1974) via what is most likely an exchange diffusion process (Westerink *et al.*, 1989). AMP blocks the uptake of DA into presynaptic terminals thereby prolonging the activation of postsynaptic DA receptors (Ferris *et al.*, 1972). Thirdly, AMP can inhibit the metabolism of DA by monoamine oxidase (Clarke, 1980). AMP also acts as a direct releaser, as well as a re-uptake inhibitor of NA [McMillen, 1983]. Though αMPT depletes the brain of DA and NA and abolishes AMP-induced behaviors (Weissman *et al.*, 1966; Stolk and Rech, 1970) dopamine-beta-hydroxylase inhibitors which deplete the brain of NA but not DA have, at most, only minor effects on these behaviors (Randrup and Scheel-Kruger, 1966; Thornburg and Moore, 1973; Corrodi *et al.*, 1970). Similarly, intraventricular (Creese and Iversen, 1975), intracisternal (Hollister *et al.*, 1974), or local stereotaxic-guided (Creese and Iversen, 1975) infusions of 6-hydroxydopamine (6-OHDA) which produce extensive destruction of dopaminergic systems in the brain can abolish AMP-induced locomotor activity and stereotyped behavior (Creese and Iversen, 1973; 1975; Hollister *et al.*, 1974). Destruction of noradrenergic neurons alone, either by stereotaxic-guided injection (Creese and Iversen, 1975; Roberts *et al.*, 1975) of 6-OHDA or by repeated intracisternal

administration of low doses (Hollister *et al.*, 1974) does not abolish AMP-induced locomotor activity or stereotypy (Creese and Iversen, 1975; Roberts *et al.*, 1975; Hollister *et al.*, 1974). Together, this indicates that the induced release of newly synthesized DA is AMP's primary mechanism of action. Cocaine, methylphenidate and nomifensine inhibit the uptake of DA from the synaptic cleft into the presynaptic nerve terminal, and may augment impulse-dependent DA release (Ferris *et al.*, 1972; Dyck, 1981; Reith *et al.*, 1989).

1.3. ANATOMICAL SPECIFICITY OF LOCOMOTOR EFFECTS

Anatomical analyses have implicated mesolimbic DA neurons in AMP- (Kelly and Iversen, 1976; Joyce *et al.*, 1983; Clark *et al.*, 1988) and cocaine- (Kelly and Iversen, 1976) induced activity. Locomotor stimulation produced by AMP (Kelly and Iversen, 1976; Joyce *et al.*, 1983) or cocaine (Kelly and Iversen, 1976) in rats is blocked by selective destruction of mesolimbic DA neurons (cell bodies in the ventral tegmental area projecting to the nucleus accumbens and olfactory tubercles) with 6-OHDA. Bilateral destruction of frontal cortical DA terminals has no effect on AMP-induced locomotion (Joyce *et al.*, 1983). More recently Clarke *et al.* (1988) replicated the blockade of AMP-induced activity by selectively depleting DA terminals in the nucleus accumbens using slow bilateral infusions of 6-OHDA. Lesions were consequently not compromised by DA depletions in either the olfactory tubercles or the medial prefrontal cortices as in the previous two studies (Kelly and Iversen, 1976; Joyce *et al.*, 1983). In these three investigations, subjects were pretreated systemically with desipramine to protect noradrenergic neurons from destruction by 6-OHDA and with pargyline to enhance dopaminergic depletions (Kelly and Iversen, 1976; Joyce *et al.*, 1983; Clark *et al.*, 1988). These studies have unequivocally demonstrated that actions on dopaminergic neurons are the primary mechanisms of the behavioral effects of psychomotor stimulants.

1.4. DA INVOLVEMENT IN THE REINFORCING EFFECTS OF PSYCHOMOTOR STIMULANTS

1.4.1. Dose Effects of Self-Administration of Stimulants

Psychomotor stimulants have been shown to produce reward-like effects. That is, giving rats injections of a stimulant contingent on the performance of a response can alter the probability of occurrence of that response. One of the variables which influences the initiation and maintenance of a given behavior is the reinforcer's magnitude. Animals with intravenous catheters connected to an infusion pump can be trained to depress a lever for infusions of certain types of drugs. This is strong evidence that particular drugs have reinforcing properties, providing that non-specific increases in bar-pressing is ruled out. Rats (Pickens and Harris, 1968; Yokel and Pickens, 1973, 1974) and nonhuman primates (Wilson *et al.,* 1971) tested within this paradigm will maintain a constant serum level of stimulant regardless of dose per injection or number of lever presses required. Thus, if the concentration of drug per infusion is reduced, animals will increase their rate of responding. On the other hand, if the drug concentration is increased animals will reduce responding (Pickens and Harris, 1968; Wilson *et al.,* 1971; Yokel and Pickens, 1973, 1974). If catecholamine synthesis is partially blocked by a low dose of α-methyl-p-tyrosine (αMPT) [Pickens and Harris, 1968] or if DA receptors are partially blocked by a low dose of a neuroleptic, such as chlorpromazine (Wilson and Schuster, 1972), pimozide (Yokel and Wise, 1975, 1976; deWit and Wise, 1977) or butaclamol (Yokel and Wise, 1976), rats will increase their rate of lever pressing for drug. This phenomenon indicates that there is a plasma or brain concentration of drug that is optimally rewarding. An animal acquires a satisfying level of drug and simply increases or decreases responding to achieve and maintain that reinforcing plasma or brain drug level.

This phenomenon is consistent with Timberlake and Allison's (1974) response deprivation theory of instrumental conditioning, a modification of Premack's original response theory of reinforcement, which states that if a behavior that normally occurs at a high frequency is made contingent on a less frequent behavior, the behavior with a higher baseline level of responding will become a reinforcer for the behavior with a lower baseline level of responding. The response deprivation theory has been extended to include stimulus deprivation (Heth and Warren, 1978). In this scheme, a behavior or stimulus does not have an absolute rewarding value. Rather, a contingency that reduces the duration of the contingent behavior below its optimal level, determined by pre-contingency baseline testing, produces an increase in the instrumental behavior (reinforcement). In other words, a contingent event (behavior or stimulus) is a reinforcer if the baseline level of the instrumental response deprives the animal of its baseline level of the contingent event. However, the contingent event is a negative reinforcer (a punisher) if the baseline level of the instrumental response produces the contingent event above the animal's operant level. For example, in the self-administration paradigm, reducing the dose of drug administered per lever press increases the stimulus deprivation established by the contingency, causing an increase in the frequency of the instrumental response, lever pressing. On the other hand, if the dose per injection is increased the contingency dependent stimulus (drug) deprivation is reduced and the frequency of lever pressing will decrease.

The effects of low doses of DA receptor antagonists, like those of reducing the concentration of stimulant per infusion, increase the deprivation of the reinforcer induced by the contingency. This is strong evidence that the reward-like effects of stimulants are mediated by DA.

1.4.2. Extinction of Self-Administration of Stimulants

After infusions of AMP as a consequence of pressing a lever are terminated, the rate of lever pressing initially increases dramatically before it is terminated for lack of reinforcement (Pickens and Harris, 1968). This phenomenon represents the first phase of operant extinction (Amsel, 1958). A similar response pattern occurs when the effectiveness of the dopaminergic system is blocked. When αMPT or neuroleptic doses are increased to completely block catecholamine synthesis (Pickens and Harris, 1968) or to completely block DA receptors (Wilson and Schuster, 1972; Yokel and Wise, 1975, 1976; de Wit and Wise, 1977), respectively, animals first increase and then stop responding. This effect appears similar to extinction of an operant response. Inhibition theories of extinction assert that during extinction animals learn that the relationship between their behavior and rewards are no longer valid and consequently learn to suppress the originally reinforced response (Mackintosh, 1974). This theory, however, does not explain the burst of responding usually apparent after the reinforcer has initially been withdrawn. Amsel's (1958) frustration theory seems to account for this reaction. Frustration theory is an interference theory of extinction which attributes the decline in a previously reinforced response during extinction to an increase in the probability of some other, competing response. Amsel (1958) suggests that the competing response elicited by non-reinforced responses during extinction is frustration. Frustration is apparent in the self-administration paradigm by an increase in the frequency of the operant response (lever pressing). The problem with the frustration theory of extinction is that the eventual disappearance of responding after the withdrawal of reinforcement is not accounted for. Mackintosh (1974) suggests that the disappearance of previously reinforced behavior is accounted for by the inhibitory theories of extinction. Frustration is not responsible for extinction; it is epiphenomenal to extinction. Both the appearance of

frustration responses and the disappearance of trained responses are a consequence of learning that the previously learned relationship between some behavior and some reward is no longer valid (Mackintosh, 1974).

1.5. ANATOMICAL SPECIFICITY OF REINFORCING EFFECTS OF STIMULANTS

The mesolimbic DA system has been implicated as the source of DA-containing neurons which mediates the reinforcing and euphoric effects of psychomotor stimulants (Roberts *et al.*, 1980; Spyraki *et al.*, 1982b). In rats trained to self-administer cocaine, extensive and selective bilateral dopaminergic lesions of the nucleus accumbens with 6-OHDA and systemic pargyline (a monoamine oxidase inhibitor which inhibits intraneuronal metabolism of 6-OHDA, consequently enhancing its effects) and desipramine (a NA uptake inhibitor which prevents the uptake of 6-OHDA and subsequent destruction of noradrenergic terminals) resulted in an increase followed by a cessation of responding (Roberts *et al.*, 1980). As previously described, this pattern of behavior resembles operant extinction. Although cocaine is self-administered directly into the medial prefrontal cortex but not into the nucleus accumbens (Goeders and Smith, 1983), bilateral 6-OHDA lesions of the medial prefrontal cortex do not affect intravenous cocaine self-administration (Martin-Iverson *et al.*, 1986). Thus, the nucleus accumbens and not the medial prefrontal cortex appears to mediate the rewarding effects of intravenously administered cocaine.

Noradrenergic blockade does not produce an extinction-like pattern of responding in rats self-administering AMP (Yokel and Wise, 1976) or cocaine (deWit and Wise, 1977) Together this evidence suggests that DA is the primary catecholamine involved in AMP- and cocaine-reward and euphoria.

1.6. CONDITIONED REINFORCING EFFECTS OF STIMULANTS

It has been demonstrated that euphoric effects of psychomotor stimulants can be conditioned to specific stimuli in humans (O'Brien *et al.*, 1986; Muntaner *et al.*, 1989)). In addition, apparent euphoric effects in animals have also been conditioned in the place preference procedure (Spyraki *et al.*, 1982a, b; Martin-Iverson *et al.*, 1985; Mithani *et al.*, 1986; Hiroi and White, 1990). In this paradigm, the initially neutral or less preferred side of a two-sided shuttle box is paired with a psychomotor stimulant. On subsequent placement in the test box without drug, rats will actively seek the side previously associated with the drug. This side preference has been explained as being due to the stimuli associated with the drug side becoming secondary reinforcers. Secondary reinforcement occurs when previously neutral or non-reinforcing stimuli acquire rewarding properties through a Pavlovian conditioning process (i.e. repeated pairings with a reinforcer). Thus, conditioned place preferences may be an example of conditioned reinforcement. Some investigators infer conditioned euphoria from conditioned place preferences; if rats experience euphoria as do humans, then this would parallel conditioned euphoria in human cocaine addicts.

It has been demonstrated that place preferences conditioned with AMP are mediated by DA (Spyraki *et al.*, 1982b; Mithani *et al.*, 1986). During conditioning with AMP, haloperidol pretreatment blocks the establishment of place preferences (Spyraki *et al.*, 1982b; Mithani *et al.*, 1986). In contrast, the establishment of conditioned place preferences with methylphenidate (Martin-Iverson *et al.*, 1985; Mithani *et al.*, 1986), nomifensine (Martin-Iverson *et al.*, 1985) and cocaine (Spyraki *et al.*, 1982a), stimulants whose mechanism of action is predominantly DA uptake inhibition, does not appear to be mediated by DA. Pretreatment with the neuroleptics, haloperidol (Spyraki *et al.*, 1982a; Martin-Iverson *et al.*, 1985; Mithani *et al.*, 1986) or pimozide (Spyraki *et al.*, 1982a) and

nonspecific or specific DA depletions using 6-OHDA intraventricularly (Martin-Iverson *et al.*, 1985) or in the nucleus accumbens (Spyraki *et al.*, 1982a), respectively, have no effect on conditioned place preferences produced by DA uptake inhibitors.

There is also evidence that the mesolimbic DA system mediates AMP-induced conditioned place preferences. Rats received either bilateral 6-OHDA lesions to the nucleus accumbens (including olfactory tubercles) or the striatum, or systemic 6-OHDA injections before conditioning with AMP. In the rats with lesions in the nucleus accumbens the integrity of the DA depletion correlated positively with the lack of preference for either side of the shuttle box. Rats with striatal or sham lesions and rats with peripheral NA depletions showed preference for the side associated with AMP (Spyraki *et al.*, 1982b). In addition, the selective NA uptake inhibitor desipramine failed to induce conditioned place preferences at a variety of doses (Martin-Iverson *et al.*, 1985). These studies indicate that central mesolimbic DA terminals mediate the primary, and possibly the conditioned, rewarding effects produced by AMP (Roberts *et al.*, 1980; Spyraki *et al.*, 1982b; Martin-Iverson *et al.*, 1986). The rewarding effects produced by stimulants like cocaine which block DA uptake may be mediated by a different mechanism than AMP.

1.7. SENSITIZATION TO PSYCHOMOTOR STIMULANTS

Repeated intermittent administration of a constant dose of AMP produces a progressive and long-lasting augmentation of its effects. Several behaviors have been quantified including rearing, locomotion, sniffing, compulsive oral behaviors, rotation, drinking, acoustic startle and cage climbing (see Robinson and Becker, 1986 for a recent comprehensive review). Behaviors are augmented dose-dependently. Low intermittent doses of AMP will produce an augmentation of locomotion, sniffing and rearing; higher intermittent doses will produce an augmentation of stereotyped behaviors such as continuous

head and forelimb movements, sniffing, licking and gnawing. Locomotor activity initially augmented by repeated AMP administration may result in hypoactivity with the emergence of focused stereotypy. Stereotypy could therefore be mistaken for tolerance to the psychomotor stimulant effects of AMP. An acute injection of AMP will produce, dose-dependently, either hyperactivity, stereotypy or hyperactivity followed by stereotypy and then hyperactivity again as the central drug effects diminish. Stereotypy produced by AMP in a low-dose intermittent-AMP injection paradigm is an indication of behavioral sensitization (Robinson and Becker, 1986).

Behavioral sensitization to stimulants has been reported to occur when drug administration is associated with a unique environment (Tilson and Rech, 1973; Post *et al.*, 1981; Weiss *et al.*, 1989). Weiss and her colleagues (1989) have additionally demonstrated that sensitization to the effects of cocaine is context-dependent; it is only observed if the cocaine challenge is administered in an environment similar to that in which cocaine was previously given. Furthermore, they have demonstrated that haloperidol will block the establishment of sensitization to cocaine, if administered with cocaine on the conditioning days, but that haloperidol will not prevent the expression of sensitization if administered prior to the cocaine challenge. Similarly, cross sensitization, a phenomenon whereby preexposure to one drug (eg. AMP) causes an enhancement of the activity associated with the second drug (eg. morphine), has been reported to occur only when both drugs are administered in the same environment. Cross sensitization to morphine is not apparent in animals preexposed to AMP in an environment different from that in which they later received morphine (Krank and Bennett, 1987; Stewart and Vezina, 1987).

There is strong evidence against environment-dependent (conditioned) AMP-induced effects being the only factor in sensitization. Daily administration of AMP in rats

continuously housed in the same environment, or housed in different chambers throughout the AMP treatment sessions, thus diminishing the environmental-specificity of the stimulant effect, still results in AMP-produced behavioral sensitization (Segal and Mandel, 1974; Segal, 1975; Brown and Segal, 1977; Rebec and Segal, 1979). Rats continuously infused with DA agonists, such as AMP (Martin-Iverson, 1991), and (+)-4-propyl -9-hydroxynaphthoxazine (PHNO), a DA D_2 receptor agonist (Martin-Iverson *et al.*, 1987, 1988a, b; Martin-Iverson, 1991), in their home cages, using subcutaneous osmotic minipumps, show sensitization of the psychomotor stimulant effects during the night and tolerance during the day. Combined PHNO and SKF 38393, a DA D_2 agonist produces day and nighttime sensitization to locomotor and rearing stimulant effects. The continuous infusion model of sensitization demonstrates that specific periods of locomotor stimulation conditioned with a unique environment are not required to produce behavioral sensitization to psychomotor stimulants. The sensitization phenomenon is such that drug-induced behaviors evolve into more intense and often qualitatively different behaviors. It has been argued by Segal (1975) that such an effect would not be apparent if conditioning mediated it. 'Conditioning' implies that the original unconditioned response and the conditioned response are the same.

Other factors have been proposed to mediate stimulant-induced behavioral sensitization including receptor supersensitivity (Klawans and Margolin, 1975; Klawans *et al.*, 1979), augmented release of DA produced by repeated *in vivo* AMP treatment (Antelman *et al.*, 1980; Robinson and Becker, 1982; Robinson *et al.*, 1982; Kolta *et al.*, 1985; Castenada *et al.*, 1988), increased activation of supersensitive DA D_1 receptors by DA (Martin-Iverson *et al.*, 1988a, b) and steroid endocrine systems (Lewis and Smith, 1983; D'Orban, 1989; Cole *et al.*, 1990). However, as evident in two comprehensive reviews (Robinson and

Becker, 1986; Johanson and Fischman, 1989), changes in pre- and postsynaptic receptors following repeated intermittent stimulant treatments are inconsistent. In fact, much of the evidence suggests the opposite, i.e. that postsynaptic DA receptors are down-regulated (Robertson, 1982, 1983) and hyposensitive (Kamata and Rebec, 1985). Although animals which receive repeated intermittent *in vivo* treatments with AMP consistently show a subsequent increase of *in vitro* AMP-, stressor-, KCl-, and electrical field stimulated-DA release (Antelman *et al.*, 1980; Robinson and Becker, 1982; Robinson *et al.*, 1982; Kolta *et al.*, 1985; Castenada *et al.*, 1988), there is no evidence that enhancement of stimulated DA release does not occur after stimulant injection regimens that do not produce daytime behavioral sensitization (i.e. continuous infusions with osmotic minipumps). Sensitization to AMP may develop through an interaction between DA D_1 and D_2 receptors. Treatment with SCH 23390, a DA D_1 receptor antagonist, during an intermittent treatment regimen with AMP has been reported to attenuate the development of sensitization to AMP (Stewart and Vezina, 1989; Drew and Glick, 1990). Martin-Iverson *et al.* (1988) have similarly provided evidence that supersensitive DA D_1 receptors may be required to develop sensitization to AMP.

The development of AMP-induced sensitization has been blocked with the antiserum to corticotropin-releasing factor (Cole *et al.*, 1990). Further, the ability of steroid hormones to induce psychoses in humans (Lewis and Smith, 1983; D'Orban, 1989) and the evidence which supports the idea that behavioral sensitization provides a model of AMP psychosis (Robinson and Becker, 1986), suggests steroid hormones may mediate stimulant sensitization.

1.8. CONDITIONING OF DRUG EFFECTS AND DRUG ABUSE

Conditioned responses to drug-related cues appear to be related to the maintenance of stimulant addiction. Behavioral sensitization induced by repeated intermittent doses of psychomotor stimulants can be enhanced if the drug-induced state is repeatedly paired with a particular environment (Post *et al.*, 1981). As such, it is likely that stimuli repeatedly associated with drug effects may become conditioned and thus contribute to the motivational properties of stimuli associated with drug craving. In humans, both the subjective euphoria and the physiological arousal produced by cocaine have been demonstrated to be conditioned to situational stimuli (O'Brien *et al.*, 1986; Muntaner *et al.*, 1989). Drugs tend to be used repeatedly under similar conditions so that the environment, including signs, smells, sounds, mood, people and places, become a part of the drug-taking ritual. The situational and environmental stimuli may become so deeply associated with taking drugs that the mere occurrence of a drug-related stimulus can elicit conditioned responses such as heart rate changes and subjective "highs"; these responses appear to induce drug cravings (Gawin, 1988; O'Brien *et al.*, 1988; Lee and Ellinwood, 1989).

After standard drug-detoxification and drug-rehabilitation treatment when post-cocaine anhedonia and anergia have been surpassed, former cocaine users continue to exhibit strong cravings for cocaine and physiological reactions when presented with cocaine-related stimuli (O'Brien *et al.*, 1988). Subjective and physiological drug-conditioned responses have been reported by cocaine addicts to occur prior to relapse both during the initial withdrawal period (Gawin, 1988) and after drug-rehabilitation (O'Brien *et al.*, 1988). Drug treatments have been tried to alleviate the craving which often precedes relapse. Many of the subjects in clinical trials to treat cocaineabuse and addiction have been opioid addicts who mix morphine, heroin or methadone with cocaine. These

drug combinations have been called "speedballs" and have potent euphoric effects. Buprenorphine, a mixed opioid agonist/antagonist has been reported to decrease the speedball effect. There is significantly less (8-10 fold) cocaine abuse among buprenorphine-maintained opioid addicts compared to methadone-maintained opioid addicts (Kosten *et al.*, 1989). In contrast, a recent animal study using the conditioned place preference paradigm suggests that buprenorphine may act synergistically with cocaine to enhance rather than attenuate the rewarding properties of cocaine (Brown *et al.*, 1991). Subthreshold doses of cocaine and buprenorphine, themselves incapable of producing conditioned place preferences, produce conditioned place preferences when given together. Similarly, moderate doses of cocaine and buprenorphine, individually capable of producing conditioned place preferences, produced a significantly larger effect when given in combination (Brown *et al.*, 1991). Buprenorphine dosage has an inverse correlation with continued cocaine abuse, apparent by cocaine metabolites in the urine samples of buprenorphine-maintained opiate-abusing outpatients (Kosten *et al.*, 1989). These studies suggest that in the treatment of cocaine and cocaine *and* opioid addiction the dose of buprenorphine must be carefully monitored because of its ability to either enhance or antagonize euphoria produced by cocaine.

Using the same philosophy underlying the treatment of opioid addiction, drug substitution therapy, i.e. substitution with antidepressants and direct and indirect DA agonists, which increase the availability of catecholamines in the synapse, has been tried to treat cocaine addiction. Preliminary evidence suggests that tricyclic antidepressants may decrease cocaine craving and abuse, although not dramatically (O'Brien *et al.*, 1988). Desipramine, a tricyclic antidepressant, has produced mixed results. Depression is commonly experienced during withdrawal and as a consequence the substance abuser is

often diagnosed with both major depression and a substance-abuse disorder. The rationale behind antidepressant drug therapy may be to treat the depression during withdrawal which may help the addict to avoid relapse or to treat the depression which instigated the stimulant abuse (Lee and Ellinwood, 1989). Desipramine has been reported to reduce post-cocaine anhedonia during drug detoxification but has also been reported to worsen cocaine craving in a population of abstinent cocaine addicts (Lee and Ellinwood, 1989). Desipramine and other tricyclics may be effective only for newly withdrawn, anhedonic patients. Buproprion, a "second generation" antidepressant, with few cardiovascular and anticholinergic side effects has been reported to reduce cocaine abuse in methadone-maintained opiate *and* cocaine addicts. Patients treated with buproprion and methadone reported fewer cravings although physiolcgical reactivity to drug cues was apparent. Upon cessation of buproprion, craving for cocaine was reinstated (Margolin *et al.*, 1991). Lithium is not effective in the treatment of cocaine abuse (Gawin and Kleber, 1984). Open trials with bromocriptine, a partial DA D2 receptor agonist, have resulted in reduced cocaine craving and use (Gawin, 1988). However, several patients in this study dropped out due to side effects such as nausea and headache. Substitution therapy with methylphenidate, a piperidine derivative structurally and mechanistically similar to AMP is also ineffective for treating cocaine abuse as it shares the same abuse potential as cocaine and AMP (Gawin, 1988; Lee and Ellinwood, 1989). L-Dopa, a precursor for DA which crosses the blood-brain barrier, in a small open study with no control, suppressed cocaine craving for two weeks, after which craving reappeared (Wolfsohn and Angrist, 1990). Amantadine, a releaser of central DA, has been similarly reported in an uncontrolled study to immediately reduce cocaine craving and use (Gawin, 1988). Amantadine has since been shown to be ineffective in maintaining cocaine abstinence (Giannini et al., 1989).

Few clinical trials using DA receptor blockers have been tried for the treatment of stimulant addiction. Patient compliance is a general problem (Gawin, 1986), the possibility of extrapyramidal side-effects, unpleasant subjective experiences associated with the administration of these drugs, and the fact that drug abuse therapists are usually not psychiatrists, are all factors that may explain the lack of treatment with neuroleptics. Haloperidol, administered to volunteer cocaine addicts prior to a four hour continuous infusion of cocaine, was ineffective against the initial "rush" but antagonized subjective "high" produced throughout the infusion (Sherer, 1988). Similarly, chlorpromazine and haloperidol eliminated cocaine-induced paranoia but not euphoria during episodal binges by four cocaine abusers. Patients reported no paranoia during binges and longer binging episodes with continued euphoria (Gawin, 1986).

Euphoria may not have been blocked by neuroleptics in each of these cases because "experienced" cocaine users may have both unconditioned and conditioned euphoria, possibly mediated by different neuronal mechanisms. Direct reward-like effects produced by cocaine have been demonstrated to be antagonized by DA receptor blockers in drug naive nonhuman primates (Wilson and Schuster, 1972; Bergman *et al.*, 1990). Antagonism with neuroleptics of cocaine-induced euphoria in stimulant-naive human subjects would support an anti-dopaminergic hypothesis for conditioned-euphoria and stimulant-addiction.

Psychosocial therapy has been of little significant clinical benefit to the stimulant abuser (Kang *et al.*, 1991). Abstinence rates of less than twenty percent after group- or family- therapy or psychotherapy probably represents spontaneous remission among patients motivated to seek treatment (Kang *et al.*, 1991).

As conditioned cues have been recently recognized to play a role in the initiation and the maintenance of stimulant addiction, behavioral treatment approaches have been

redesigned to try to extinguish the link between drug-associated cues and euphoria produced by cocaine and AMP-like compounds. During extinction proceedings the patient is required to remain abstinent when conditioned drug cravings occur (Gawin, 1988; O'Brien *et al.*, 1988; Lee and Ellinwood, 1989). Stimuli that often provoke impulses to use drugs are presented to the patient in an environment where the patient is protected from using drugs. The patient is given insight into his responses and is given suggestions on ways to resist resuming drug use. When cues are experienced and are not reinforced by euphoria, their potency in producing craving should theoretically dissipate. However, several practitioners have attributed cocaine as one of the most powerful reinforcing agents known (Gawin and Kleber, 1984; Gawin and Ellinwood, 1988; Lee and Ellinwood, 1989) and that the extinction phase in the treatment of stimulant-addiction is life-long (Gawin, 1988; O'Brien *et al.*, 1988). One on-going study demonstrated how apparently successful extinguished cues can suddenly re-trigger craving and conditioned-physiological "high" (O'Brien *et al.*, 1988). Patients released into their own environments after apparently successful extinction therapy are often overwhelmed by drug cravings and consequently relapse (O'Brien *et al.*, 1988). In addition, addicts in long-term remission have reportedly relapsed after a single "taste" of cocaine. The resultant euphoria "greased the slide" and re-triggered previously extinguished conditioned cues that originally took several stimulus-euphoria pairings to evolve (O'Brien *et al.*, 1988). Relapse has similarly been modelled in animals. In a self-administration paradigm, rats trained to self-administer cocaine and then behaviorally extinguished will restore responding after a single cocaine priming injection (deWit and Stewart, 1981). Behavioral extinction alone appears to be ineffective. Blockade of the neuronal mechanism of conditioned craving alone or in combination with behavioral extinction procedures may provide a more effective treatment for stimulant addiction.

1.9. MECHANISMS OF CONDITIONED LOCOMOTION

Although the activating (Kelly and Iverson, 1976; Joyce *et al.*, 1983; Clark *et al.*, 1988) and reinforcing (Pickens and Harris, 1968; Wilson and Schuster, 1972; Yokel and Wise, 1975, 1976; deWit and Wise, 1977; Roberts *et al.*, 1980; Spyraki *et al.*, 1982b) effects of psychomotor stimulants are dependent on dopaminergic mechanisms, the role of DA neurotransmission in stimulant conditioned responses is not well understood. Schiff (1982) initially investigated the possibility that DA agonists produce environment-specific conditioned stimulant responses through a DA mediated mechanism. Several AMP-induced behaviors were quantified during drug administration to rats in a unique environment. Only AMP-induced head-bobbing and sniffing were significantly conditioned. After reconditioning, haloperidol, a DA D2 receptor antagonist administered on a drug-free test day blocked the head-bobbing and sniffing components of the conditioned response (Schiff, 1982). Hyperactivity measured by photobeam interruptions is an easier measure of locomotor stimulant effects than observer-rated scores of individual behaviors. Similar methods are consequently utilized by several investigators to quantify stimulant-conditioned responses. Beninger and Hahn (1983) and Beninger and Herz (1986) investigated the role of DA in both the establishment and the expression of AMP- and cocaine-induced locomotion. Pimozide, a selective DA D_2 receptor antagonist blocked the 'establishment' of conditioning when it was administered concomitantly with either AMP (Beninger and Hahn, 1983) or cocaine (Beninger and Herz, 1986) during a 10-day training period but did not block the 'expression' of pre-established environment-specific conditioned locomotion when it was administered on the stimulant-free test days. Pimozide, when administered during the conditioning phase of the experiments, was noted to have blocked the unconditioned locomotor effects of AMP (Beninger and Hahn, 1983) and cocaine (Beninger

and Herz, 1986). This observation and the ability of pimozide to block the establishment but not the expression of the stimulant-conditioned responses are suggested by Beninger and his colleagues (1983, 1986) to imply that during conditioning the activation of dopaminergic neurons produces a change in the brain that subsequently influences behavior which can, once established, be expressed even when DA receptors are blocked. DA receptors must, however, be functional in order for this 'change' to occur.

The role of DA in the establishment of stimulant-conditioned behaviors has not been as actively investigated as its role in the expression of stimulant-conditioned behaviors. The ability of pimozide to block the establishment of stimulant-conditioned behaviors was replicated by Hiroi and White (1989) using both AMP and apomorphine. The anticholinergic drug scopolamine shows a conditioned effect that is not blocked by pimozide (Mazurski and Beninger, 1988). This suggests that environment-specific conditioned-activity produced by drugs which affect different neurotransmitter systems are not mediated by a common mechanism.

While haloperidol blocks the establishment of AMP-conditioned place preference (Spyraki *et al.*, 1982b; Mithani *et al.*, 1986) neither haloperidol nor SCH 23390, a DA D1 receptor antagonist, block the establishment of AMP- (Mithani *et al.*, 1986; Martin-Iverson and McManus, 1990) or PHNO- (Martin-Iverson and McManus, 1990) conditioned locomotion. This suggests that different behaviors may be conditioned through different neuronal mechanisms.

The mechanism underlying stimulant-conditioning is not by any means simple. Psychomotor stimulants such as AMP, cocaine, methylphenidate and nomifensine, which all affect DA neurotransmission, are differentially affected by the same treatments during environment-specific conditioning. For example, the establishment of AMP-conditioned

place preference is blocked by both haloperidol and 6-OHDA lesions to the nucleus accumbens (Spyraki *et al.*, 1982b). In contrast, the establishment of conditioned place preference produced by cocaine (Spyraki *et al.*, 1982a), methylphenidate (Martin-Iverson *et al.*, 1985; Mithani *et al.*, 1986) and nomifensine (Martin-Iverson *et al.*, 1985) is not blocked by DA receptor antagonists (Spyraki *et al.*, 1982a; Martin-Iverson *et al.*, 1985; Mithani *et al.*, 1986) or by central DA depletions with 6-OHDA (Spyraki *et al.*, 1982a; Martin-Iverson *et al.*, 1985). In direct contrast to Beninger and colleagues (1983, 1986) speculation that the establishment of stimulant-conditioning requires a functional dopaminergic system to mediate some neuronal change to produce a conditioned response, it has been shown repeatedly that stimulant-induced behaviors can be completely blocked with DA antagonists during conditioning without effecting the conditioned response (Martin-Iverson *et al.*, 1985; Mithani *et al.*, 1986; Martin-Iverson and McManus, 1990). This observation provided direct evidence against a postsynaptic dopaminergic mechanism in the establishment of stimulant conditioning.

The mechanism which mediates the expression of stimulant-conditioned behaviors is as poorly understood as that which mediates the establishment of stimulant-conditioned behaviors. As with the establishment of stimulant-conditioned activity, there appears to be a differential effect of pimozide on the expression of conditioned behaviors induced by different stimulants. Pimozide, having no effect on the expression of AMP (Beninger and Hahn, 1983) or cocaine (Beninger and Herz, 1986) conditioned locomotion, has been reported to completely block the expression of apomorphine-conditioned stereotypy (Hiroi and White, 1989) and to attenuate the expression of morphine- (Neisewander and Bardo, 1987) and methamphetamine- (Miyamoto and Hada, 1987) conditioned stereotypy (Miyamoto and Hada, 1987; Hiroi and White, 1989) and locomotion (Miyamoto and Hada,

1987; Neisewander and Bardo, 1987; Hiroi and White, 1989).

Poncelet *et al.* (1987) replicated Beninger and Hahn's (1983) original finding that pimozide does not block the expression of stimulant-induced locomotion but found that other DA D2 receptor antagonists, haloperidol and sulpiride, do block this effect. The ability of other DA D_2 receptor antagonists to block stimulant-conditioned activities has been replicated using haloperidol (Miyamoto and Hada, 1987), chlorpromazine (Miyamoto and Hada, 1987) and metoclopramide (Drew and Glick, 1990). In agreement with the results of Beninger and Hahn (1983) and Beninger and Herz (1986), the expression of apomorphine-conditioned contralateral rotation in rats with unilateral 6-OHDA lesions is not attenuated by either separate or combined DA D1 and DA D2 receptor blockade (Carey, 1990).

There is evidence for a DA D_1 receptor-mediated postsynaptic process in the expression of AMP-conditioned turning (Drew and Glick, 1990). There is also evidence against involvement of postsynaptic DA D_1 receptors, alone or in combination with DA D_2 receptors in the establishment of stimulant-conditioned locomotion (Martin-Iverson and McManus, 1990) or contralateral rotation (Carey, 1990).

Recently the role of mesolimbic DA in stimulant-conditioning has been investigated. 6-OHDA-induced lesions of the nucleus accumbens block the conditioned effects of AMP in rats, when the lesions are made either pre- (Spyraki *et al.*, 1982b; Gold *et al.*, 1988) or post-conditioning (Gold *et al.*, 1988). Rats with 6-OHDA-induced lesions of the nucleus accumbens made prior to conditioning with apomorphine do not show a conditioned locomotor response, although during conditioning they exhibit locomotor hyperactivity (Gold *et al.*, 1988). Bilateral microinjections of α-flupenthixol, a DA receptor blocker, into the nucleus accumbens before the test session have been reported to block the expression

of AMP-conditioned place preferences (Hiroi and White, 1990). Bilateral microinjections of αMPT into the nucleus accumbens on the test day have no effect on the conditioned response (Hiroi and White, 1990). Reserpine, which depletes catecholamines from granular storage pools in the presynaptic terminal, administered systemically before the test session has been reported to block the expression of conditioned place preferences (Hiroi and White, 1990). These results suggest that the expression of conditioned behaviors are mediated by DA released in the nucleus accumbens. Moreover, DA released from the reserpine-sensitive pool appears to be important. This is in contrast to the direct effects of AMP, which are not blocked by reserpine (See Section 1.2 of the Introduction).

Though consistent with other studies, there is a logical fault with the conclusion drawn after 6-OHDA lesions preceding stimulant-conditioning (Gold *et al.*, 1988). Although 6-OHDA lesions blocked AMP-conditioned responses, it is impossible to separate the establishment from the expression in this case; DA would have to be replaced on the test day in order to do so. Thus, it is not clear if DA release in the nucleus accumbens is necessary for the *establishment* of stimulant-conditioning.

NA may be important in the expression of stimulant-conditioned behaviors since clonidine, a NA α_2 receptor agonist, has been reported to block the expression of AMP-conditioned locomotion (Poncelet *et al.*, 1987). However, clonidine itself produces sedation which was not ruled out in this particular study and could explain the reported finding. In addition, any effect of NA depletions in studies which have utilized 6-OHDA has been ruled out. Desipramine pretreatment, which spares NA fibers if utilized prior to 6-OHDA treatment, does not affect the blockade of AMP-conditioning (Spyraki *et al.*, 1982b). Any role of peripheral NA has similarly been ruled out. Systemic injections of 6-OHDA do not effect either the establishment of AMP- (Spyraki *et al.*, 1982b) or cocaine-

(Spyraki *et al.*, 1982a) conditioned place preferences.

Although the ability of pimozide to block the establishment (Beninger and Hahn, 1983; Beninger and Herz, 1986; Hiroi and White, 1989) and not the expression (Beninger and Hahn, 1983; Beninger and Herz, 1986; Poncelet *et al.*, 1987; Hiroi and White, 1989) of stimulant-conditioned behaviors has been replicated, the mechanism to which this has been attributed, DA D_2 receptor antagonism, is likely incorrect. The strongest evidence against this interpretation is the inability to replicate these effects using other DA D_2 receptor antagonists. For example, haloperidol fails to block the establishment of AMP-, PHNO- (Martin-Iverson and McManus, 1990) and apomorphine- (Carey, 1990) conditioned locomotor activity. Other investigators have contradicted the evidence that DA D_2 receptor activation is not required for the expression of pre-established stimulant-conditioned activity. Haloperidol (Schiff, 1982; Miyamoto and Hada, 1987; Poncelet *et al.*, 1987), chlorpromazine (Miyamoto and Hada, 1987), sulpiride (Poncelet *et al.*, 1987) and metoclopramide (Drew and Glick, 1990) are reported to block the expression of stimulant-conditioned behaviors.

Pimozide is a diphenylbutylpiperidine antipsychotic that has been demonstrated to antagonize DA D_2 receptors, L-type calcium channels (Cohen *et al.*, 1986; Tecott *et al.*, 1986; Enyeart *et al.*, 1987b) and 5-HT_2 receptors (Cohen *et al.*, 1986; Tecott *et al.*, 1986) equipotently. Drug competition studies at DA D_2 (Tecott *et al.*, 1986), calcium channel (Tecott *et al.*, 1986) and 5-HT_2 (Cohen *et al.*, 1986) binding sites revealed that pimozide displays a high affinity for DA D_2 (IC_{50} = 8.9 +/- 2 nM), calcium channel (IC_{50} = 38 +/- 10 nM) and 5-HT_2 (IC_{50} = 2.7 +/- 1.5 nM) binding sites *in vitro*. Haloperidol, in contrast shows very low affinity for calcium channel binding sites (IC_{50} = 6400 +/- 1000 nM) and high

affinity for DA D_2 binding sites (IC_{50} = 3.9 +/- 1 nM) [Tecott *et al.*, 1986]. This indicates that the rather large number of studies utilizing pimozide to draw conclusions concerning the role of DA D_2 receptors in mediating behaviors should be re-evaluated.

Pimozide has been demonstrated to inhibit depolarization-dependent calcium uptake through both slowly (Enyeart *et al.*, 1987b) and rapidly (Enyeart *et al.*, 1987a) inactivating calcium channels. It is possible that pimozide's ability to block the establishment of AMP- (Beninger and Hahn, 1983) and cocaine- (Beninger and Herz, 1986) conditioned locomotion is through a depolarization-dependent calcium-mediated mechanism, possibly the exocytotic release of a RES-sensitive compartment of DA. This hypothesis fits logically with a recent finding by Hiroi and White (1990). Systemic injections of reserpine made after AMP-conditioned place preferences were established blocked the expression of conditioned place preferences.

This thesis addresses 3 questions concerning the establishment of AMP-conditioned behaviors: 1) Is presynaptic DA or NA release the neural event being conditioned in AMP-conditioning? 2) Is the unique ability of pimozide to block the acquisition of conditioned locomotor effects due to concomitant DA D_2 receptor and calcium channel antagonism? This would indicate that activation of D_2 receptors by DA released via a calcium-dependent mechanism mediates conditioning. 3) Is the release of DA from the reserpine-sensitive pool alone or in conjunction with DA release from the αMPT-sensitive pool the neural event conditioned during AMP administration in a unique environment?

1.10. BIBLIOGRAPHY

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CHAPTER 2. PRESYNAPTIC DOPAMINERGIC NEUROTRANSMISSION MEDIATES AMPHETAMINE-INDUCED UNCONDITIONED BUT NOT AMPHETAMINE-CONDITIONED LOCOMOTION AND DEFECATION IN THE RAT[1]

2.1. INTRODUCTION

Rats repeatedly administered AMP in a unique environment exhibit increased locomotion when placed in that environment without drug treatment (Pickens and Crowder, 1967; Schiff, 1982; Martin-Iverson and McManus, 1990). This environmentally-specific increase in locomotion has been attributed to classical (Pavlovian) conditioning of amphetamine's motor stimulant effects. Conditioning effects likely contribute to the 'sensitization' phenomenon, a gradual augmentation of the behavioral effects of psychomotor stimulants which has been suggested by a number of investigators to be related to stimulant-induced psychosis (for review see Robinson and Becker, 1986). Classical conditioning of stimulant effects may also contribute to the maintenance of addictive behaviors, as suggested by both animal (de Wit and Stewart, 1981) and clinical (Gawin, 1988) studies.

Several researchers have investigated the roles of DA and NA in AMP-produced environment-specific conditioning. These experiments differentiate between the establishment and the expression of stimulant-conditioned behaviors by giving animals treatments that might interfere with the conditioning of drug effects either during the process of pairing the effects of stimulants with a unique environment (establishment), or after pairing but before a stimulant-free test (expression). Sometimes the stimulant is given on

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1 A version of this chapter has been accepted for publication. Brain Research.

the test day as well, and conditioned behaviors are demonstrated by an increased behavioral response relative to animals with a similar stimulant treatment history, but in an environment different from the environment previously paired with the drugs.

Beninger and Hahn (1983) and Beninger and Herz (1986) investigated the role of dopaminergic neurotransmission in AMP- and cocaine-induced conditioned locomotion using pimozide, a neuroleptic. Pimozide, a selective DA D_2 antagonist which is also an antagonist of calcium channels (Cohen *et al.*, 1986; Tecott *et al.*, 1986; Enyeart *et al.*, 1987) and serotonin$_2$ (5-HT_2) receptors (Cohen *et al.*, 1986; Tecott *et al.*, 1986), blocked conditioning when pimozide was administered during conditioning with stimulants but did not block conditioned locomotion when pimozide was administered only on the AMP-free test day. It was concluded that DA mediates the establishment but not the expression of stimulant-conditioned locomotion (Beninger and Hahn, 1983; Beninger and Herz, 1986). Poncelet et al. (1987) replicated Beninger and Hahn's (1983) finding that pimozide does not block the *expression* of AMP-conditioned locomotion, but found that other DA D_2 receptor antagonists (sulpiride and haloperidol) did block this conditioned effect. Schiff (1982) has also reported that haloperidol blocks the expression of AMP-conditioned sniffing and head-bobbing. Furthermore, pimozide has been reported to block the expression of apomorphine-conditioned stereotypy and to attenuate the expression of AMP-conditioned stereotypy (Hiroi and White, 1989).

Drew and Glick (1990) found that both SCH 23390, a D1 antagonist and metoclopramide, a D2 antagonist, blocked the expression of AMP-conditioned turning in rats. However, conclusions as to the role of DA in the expression of conditioned turning cannot be safely drawn from this study since non-specific sedating effects were not ruled out. Carey (1990) showed that neither SCH 23390 nor haloperidol blocked the expression

of conditioned apomorphine-induced contralateral turning in unilaterally DA depleted rats.

Gold et al. (1988) found that depletions of both DA and NA in the nucleus accumbens using the neurotoxin, 6-OHDA, blocked the AMP-conditioned locomotor response if made prior to testing for conditioned effects. NA may be important in the expression of conditioned locomotion since clonidine, an NA α_2 receptor agonist blocked the expression of AMP-conditioned locomotion (Poncelet, 1987). However, as noted previously (Hiroi and White, 1989; Martin-Iverson and McManus, 1990), clonidine itself produces sedation, and this nonspecific action could explain the obtained results. Research on the involvement of DA and NA in the expression of stimulant-conditioned behaviors has therefore been contradictory and conclusions cannot be confidently drawn at this time.

Relatively less work has been done on the involvement of DA in the establishment of stimulant-conditioned behaviors since the work of Beninger and his colleagues showing that pimozide blocks the establishment of AMP- and cocaine-conditioned behaviors (Beninger and Hahn, 1983; Beninger and Herz, 1986). DA depletions in the nucleus accumbens made prior to conditioning blocked AMP-conditioned locomotion (Gold *et al.*, 1988), but since these animals were also lesioned at the time of the testing and this was sufficient to block conditioned locomotion, no conclusion regarding the importance of DA during conditioning can be confidently drawn from this study. Martin-Iverson and McManus (1990) examined the effects of concomitant injections of DA receptor antagonists (haloperidol, a D_2 antagonist, and SCH 23390, a D_1 antagonist) with AMP during conditioning. Surprisingly, the establishment of stimulant-induced conditioned locomotion was unaffected by antagonists of DA D_1 or DA D_2 receptors given separately or together, although unconditioned stimulant-induced locomotion was blocked by either antagonist (Martin-Iverson and McManus, 1990). This finding is similar to an earlier report that

haloperidol did not block AMP- or methylphenidate-induced conditioned locomotion (Mithani *et al.*, 1986). These studies lead to the conclusion that either DA neurotransmission, responsible for the reinforcing (Roberts *et al.*, 1980; Spyraki *et al.*, 1982b) and activating (Kelly and Iversen, 1976) effects of stimulants is not responsible for the establishment of stimulant-conditioned behaviors or that conditioning of stimulant effects is a function of neurochemical events occurring presynaptically at DA terminals or cell bodies, at a level prior to receptor action.

The possibility that AMP's effects on presynaptic NA- or DA-releasing terminals are the actions that are conditioned was investigated in the present experiments. A catecholamine synthesis inhibitor, αMPT, was used to inhibit AMP-induced presynaptic release of DA. αMPT has been previously shown to block the behavioral effects of AMP (Weissman *et al.*, 1966; Scheel-Kruger, 1971) which depend upon the presence of a compartment of newly-synthesized DA. αMPT inhibits the synthesis of both NA and DA by blocking the action of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines. To separate the role of DA from NA in the establishment of AMP-conditioned behaviors, the effects of selective destruction of forebrain NA-containing terminals were examined using N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4), a neurotoxin specific for NA terminals (Ross and Renyi, 1976; Archer *et al.*, 1982). In addition, we report that AMP-increased defecation can also be conditioned, and tested the possibility that the effects of αMPT pretreatment on conditioned locomotion might be unique to this behavior by assessing the effects of αMPT on defecation.

Four experiments were conducted in total. Expt. 1 was designed to determine if DSP4 or αMPT could block the unconditioned and conditioned locomotor stimulant effects of AMP. Expt. 2 verified that DSP4 in our hands produced NA depletions in various forebrain

regions. The effect of a higher dose of αMPT on AMP-induced unconditioned and conditioned locomotion and defecation was investigated in Expt. 3. The effects of αMPT on AMP unconditioned and conditioned behaviors were further replicated in Expt. 4 and extended to test the influence of the injection interval between αMPT and AMP administration, to ensure that an adequate injection interval was chosen in the previous experiments (Scheel-Kruger, 1971; Braestrup, 1977).

2.2. MATERIAL AND METHODS

2.2.1. Animals, apparatus and drugs

Male Sprague-Dawley rats (n=11 or 12 for each group in Expts. 1, 3 and 4 and n=8 for each group in Expt. 2) weighing 250-350 g with *ad libitum* access to food and water and maintained on a 12:12 h light cycle (lights on 0700-1900) were used in all experiments. Locomotor activity counts were made in conditioning boxes equipped with two infrared photocell assemblies in each, with a sensitivity set so that rapid interruptions (less than 1 sec apart) did not register. This setting was chosen so that only gross locomotor activity would be counted; rapid movements of the head, paws or tail were not counted. The conditioning boxes (48 in total) were made of stainless steel, with one wall being Plexiglas and the floor made of steel mesh. The dimensions of these boxes were 25 (w) x 25 (h) x 30 (l) cm, with the two photocells placed 3 cm from the floor on the side walls, spaced 14 cm apart, and equidistant from the end walls. All drugs were dissolved with double-distilled water. AMP sulfate was provided courtesy of SmithKline Beecham Pharma and was dissolved into a 1.5 mg/ml solution. DSP4 was purchased from Research Biochemicals Inc. and was dissolved into a 50 mg/ml solution. αMPT was purchased from Sigma and was dissolved into a 25 mg/ml solution for Expt. 1 and a 50 mg/ml solution for Expts. 3 and 4. Doses of the drugs used were chosen on the basis of the literature. The dose of AMP was

chosen to produce optimal photobeam interruption counts, over the course of the 10 days of treatment, and has previously been shown to elicit robust conditioned locomotion (Mithani *et al.*, 1986, Martin-Iverson and McManus, 1990). The dose of DSP4 was chosen in order to adequately deplete NA terminals according to the literature (Ross and Renyi, 1976; Archer *et al.*, 1982). The doses of αMPT were chosen as ones which would adequately block catecholamine synthesis, based on acute effects (Scheel-Kruger, 1971). The 30 min time-interval between αMPT and placement of rats into the testing apparatus was chosen on the basis of in vitro studies that indicate that maximal inhibition of tyrosine hydroxylase occurs at this time (Braestrup, 1977). All drug weights are expressed as salts.

2.2.2. Procedures

Four experiments were conducted as follows: Expt. 1 consisted of three phases: a pretreatment phase, a drug conditioning phase and a test phase. Expts. 3 and 4 each consisted of two phases: a drug conditioning phase and a test phase. The pretreatment phase of Expt. 1 consisted of injecting rats with either DSP4 or vehicle (VEH) seven days prior to the conditioning phase. During the conditioning phase (lasting 10 consecutive days) of Expts. 1, 3 and 4, all rats received two injections (VEH or αMPT and VEH or AMP) followed by placements into the conditioning boxes for 60 min. The test day occurred on the fourth day after the last conditioning day injection (i.e. 3 intervening treatment-free days), to allow for drug clearance. On this day, all animals received an injection of VEH prior to placement for 60 min in the conditioning boxes.

Expt. 1 consisted of 6 treatment groups with each animal receiving three treatments: DSP4 (50 mg/kg, i.p.) or double-distilled water (VEH, 1 ml/kg, i.p.) 7 days before conditioning, αMPT (25 mg/kg, s.c.) or VEH (1 ml/kg, s.c.) 30 min before being placed into the conditioning boxes, and AMP (1.5 mg/kg, s.c.) or VEH (1 ml/kg, s.c.) 10 min before

being placed into the conditioning boxes. The latter two treatments occurred each day for 10 consecutive days. The groups were as follows: VEH-VEH-VEH, VEH-VEH-AMP, DSP4-VEH-VEH, DSP4-VEH-AMP, VEH-αMPT-VEH, and VEH-αMPT-AMP. As there were 48 conditioning boxes (4 rows of 12, arranged vertically) and 72 rats, 6 rats from each group of 12 were randomly chosen to be conditioned either in the morning or in the afternoon. Each rat was placed into the same test box at the same time each day, with the box chosen following a pseudorandom counterbalanced procedure. One rat from each treatment group was assigned to one box from each set of 6 boxes so that equal numbers of each treatment group would be in each row to control for the different distances of the boxes from the ceiling illumination. Distance from the source of illumination has been observed in this laboratory to influence locomotion in rats (personal observation).

Expt. 2 was an acute drug treatment study to determine the degree of forebrain depletions of NA, DA and 5-HT produced by DSP4 (50 mg/kg, i.p.). Two treatment groups of 8 animals (n=16) received one injection each of DSP4 (50 mg/kg, i.p.) or double distilled water (1 ml/kg, i.p.) and were killed by guillotine decapitation on the 17th day after drug administration, the day representative of the 10th and final conditioning day in Expt. 1. The brains were rapidly removed and placed on ice. The cortex, olfactory tubercles, hippocampus and hypothalamus were dissected out and frozen immediately with solid carbon dioxide and then kept at -80°C until the time of the assay. Each pair of bilateral regions was analyzed separately for content of monoamines and metabolites. Levels of DA and its acid metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) and NA were assayed using high performance liquid chromatography with electrochemical detection, following the procedure of Baker *et al.* (1987).

The possibility that the effects of αMPT treatment on unconditioned and conditioned locomotion might be unique to this behavior rather than a general conditioning phenomenon was investigated in Expt. 3 by assessing the effect of a higher dose of αMPT (50 mg/kg, s.c.) on AMP-induced defecation in addition to locomotion. A previous pilot study in this laboratory revealed that defecation was increased by AMP. A higher dose of αMPT was used to test the possibility that the effects observed in Expt. 1 were due to an inadequate dose of αMPT.

Expt. 3 consisted of 4 treatment groups with each group (n=12) receiving two injections, consisting of αMPT (50 mg/kg, s.c.) or VEH (1 ml/kg, s.c.) 30 min before placements in the conditioning boxes and AMP (1.5 mg/kg, s.c.) or VEH (1 ml/kg, s.c.) 10 min prior to placements in the conditioning boxes daily, for 10 consecutive days. The groups were as follows: VEH-VEH, VEH-AMP, αMPT-VEH and αMPT-AMP. Test boxes were cleaned of any fecal matter before putting rats into the apparatus, and the total number of feces at the end of each 60 min session was counted.

A fourth experiment was devised to test the possibility that increasing the injection interval between αMPT and AMP administration might provide a better block of the conditioned effects of AMP. It has been indicated that it might take longer than 30 min to deplete DA from an AMP-sensitive pool (Scheel-Kruger, 1971), since maximum block of AMP-induced stereotypy by αMPT occurs approximately 2-1/2 hours after maximal *in vitro* catecholamine synthesis inhibition occurs (Braestrup, 1977). Expt. 4 was a replication of Expt. 3 with the exception that the appropriate animals were injected with αMPT (50 mg/kg) 170 min prior to administration of AMP, as opposed to 20 min as in Expt. 3. Rat feces were counted as in Expt. 3.

For Expts. 1, 3 and 4, the photobeam interruptions for each rat were recorded in 5 min blocks which were collapsed for each rat to produce daily totals. The mean of these daily totals for each rat were subjected to analysis of variance (ANOVA) with post-hoc comparisons between groups by Tukey's test. The total number of feces produced in the test boxes over the 10 days of conditioning was similarly analyzed.

2.3. RESULTS

2.3.1. Expt. 1: Effects of DSP4 and αMPT on AMP-induced unconditioned and conditioned locomotion.

The results of the conditioning phase of this experiment were subjected to ANOVA with 2 independent factors: (1) pretreatment (PRE) consisting of 3 levels (VEH, DSP4 or αMPT) and (2) AMP treatment consisting of 2 levels (VEH or 1.5 mg/kg). One of the animals from the DSP4-VEH-VEH group died before conditioning began, reducing this group to 11 subjects. AMP significantly increased locomotion depending on the pretreatment, as can be seen in Fig. 1 (PRE x AMP interaction $F_{2,65} = 5.53$, $p < 0.01$). To determine whether the PRE effect was due to DSP4 or αMPT, the results were subjected to a second and third ANOVA, each excluding one level (DSP4 or αMPT) of the PRE factor. DSP4 had no effect on AMP-induced locomotion. αMPT significantly attenuated AMP-induced locomotion ($F_{1,44} = 6.12$, $p < 0.02$). Thus, AMP-induces locomotion, and this effect is attenuated by αMPT but not by DSP4.

Figure 2 displays the conditioned activity (i.e. locomotion induced by exposure to the test boxes only, with a single VEH injection being the only treatment) of the 6 groups. All rats previously treated with AMP displayed significant increases in locomotion, relative to the control group (VEH-VEH-VEH), regardless of any other drug treatments that they received during conditioning. Only the main effect of AMP was significant ($F_{1,64} = 56.03$,

$p < 0.001$). Therefore, NA terminal depletions by DSP4 or catecholamine synthesis inhibition by αMPT prior to conditioning trials had no effect on AMP-conditioned locomotion. Conditioned locomotion was observed in the VEH-αMPT-AMP treatment group in spite of the fact that αMPT blocked the unconditioned motor stimulation produced by AMP during the conditioning trials.

2.3.2. Expt. 2: Effects of DSP4 on monoamines and acidic metabolites.

The data were analyzed by ANOVA for each brain region with the drug as one factor with two levels (VEH, DSP4) and are presented in Table 1. DSP4 significantly reduced the mean level of NA in all brain regions assayed: cortex ($F_{1,14} = 198$, $p < 0.001$); hippocampus ($F_{1,13} = 312$, $p < 0.001$); olfactory tubercles ($F_{1,14} = 250$, $p < 0.001$); and hypothalamus ($F_{1,14} = 79.3$, $p < 0.001$). The levels of 5-HIAA in the same four areas were also significantly reduced: cortex ($F_{1,14} = 16.4$, $p < 0.001$); hippocampus ($F_{1,13} = 14.1$, $p < 0.002$); olfactory tubercles ($F_{1,14} = 11.6$, $p < 0.005$) and hypothalamus ($F_{1,14} = 10.1$, $p < 0.01$). The mean levels of 5-HT were also reduced by DSP4: cortex ($F_{1,14} = 40.1$, $p < 0.001$); hippocampus ($F_{1,13} = 19.7$, $p < 0.001$); olfactory tubercles ($F_{1,14} = 12.0$, $p < 0.005$); and hypothalamus ($F_{1,14} = 19.2$, $p < 0.001$). Levels of DOPAC and DA were not significantly affected by DSP4 in any of the brain regions.

2.3.3. Expt. 3: Effects of αMPT on AMP-induced unconditioned and conditioned locomotion and defecation.

The results of the conditioning phase of Expt. 3 were subjected to ANOVA with two independent factors. The independent factors were: (1) αMPT consisting of 2 levels (VEH or 50 mg/kg) and (2) AMP treatment consisting of 2 levels (VEH or 1.5 mg/kg). As in Expt. 1, increases in locomotion induced with AMP were dependent on αMPT, and a similar

dependency was observed for AMP-induced defecation. αMPT significantly attenuated AMP-induced locomotion ($F_{1,44}$ = 15.8, $p < 0.001$; Fig. 3) and defecation ($F_{1,44}$ = 6.8, $p < 0.02$; Fig. 4).

Rats conditioned with AMP exhibited increased locomotion (Fig. 5) and defecation (Fig. 6) when placed in the testing boxes without drug treatments (a conditioned effect). This effect occurred for both behaviors independently of pretreatment with αMPT during conditioning. ANOVA verified this observation, since there was a significant main effect of prior AMP-test box pairing on locomotion ($F_{1,44}$ = 92.2, $p < 0.001$) and defecation ($F_{1,44}$ = 6.5, $p < 0.02$, on data after square root tranformation). There was no statistically significant effect of prior treatment with αMPT during conditioning on either conditioned locomotion (Fig. 5) or conditioned defecation (Fig. 6). Note that the large variance in defecation counts are shown in Fig. 6 without square root transformations.

2.3.4. Expt. 4: Effects of αMPT administered 170 min prior to AMP treatment on AMP-induced unconditioned and conditioned locomotion and defecation.

The results of the conditioning phase of Expt. 4 were subjected to ANOVA with two independent factors. The independent factors were: (1) αMPT consisting of 2 levels (VEH or 50 mg/kg) and (2) AMP treatment consisting of 2 levels (VEH or 1.5 mg/kg). The unconditioned defecation results were subjected to ANOVA after square root transformation to produce homogeniety of variance. As in Expts. 1 and 3, AMP increased locomotion ($F_{1,44}$ = 46.0, $p < 0.001$) and defecation ($F_{1,44}$ = 36.0, $p < 0.001$) and αMPT significantly attenuated AMP-induced locomotion ($F_{1,44}$ = 14.7, $p < 0.001$; Fig. 7) and defecation ($F_{1,44}$ = 4.1, $p < 0.05$; Fig. 8).

The conditioned locomotion and defecation test day results were subjected to ANOVA as in Expts. 1 and 3 and are displayed in Figures 9 and 10, respectively. All groups receiving AMP exhibited significant conditioned locomotion ($F_{1,44} = 55.9$, $p < 0.001$) and conditioned defecation ($F_{1,44} = 33.6$, $p < 0.001$), regardless of prior αMPT treatment.

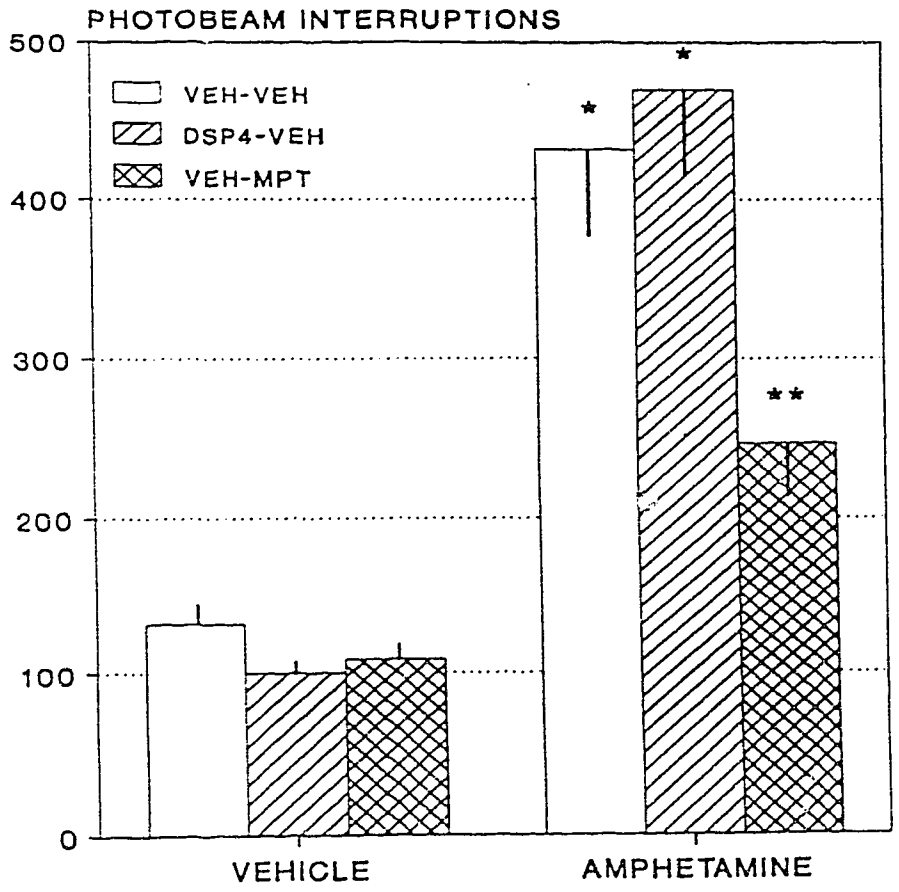

Fig. 1: The effects of injections of VEH or DSP4 (50 mg/kg, i.p.) and VEH or αMPT (MPT, 25 mg/kg, s.c.) and vehicle or amphetamine (1.5 mg/kg, s.c.) collapsed across 10 consecutive days on locomotion (photobeam interruptions) during 60 min periods. Error bars represent the standard error of the mean.

* Significantly different from the control group (VEH-VEH-VEH), $p < 0.01$.

** Significantly different from the VEH-VEH-amphetamine group ($p < 0.02$).

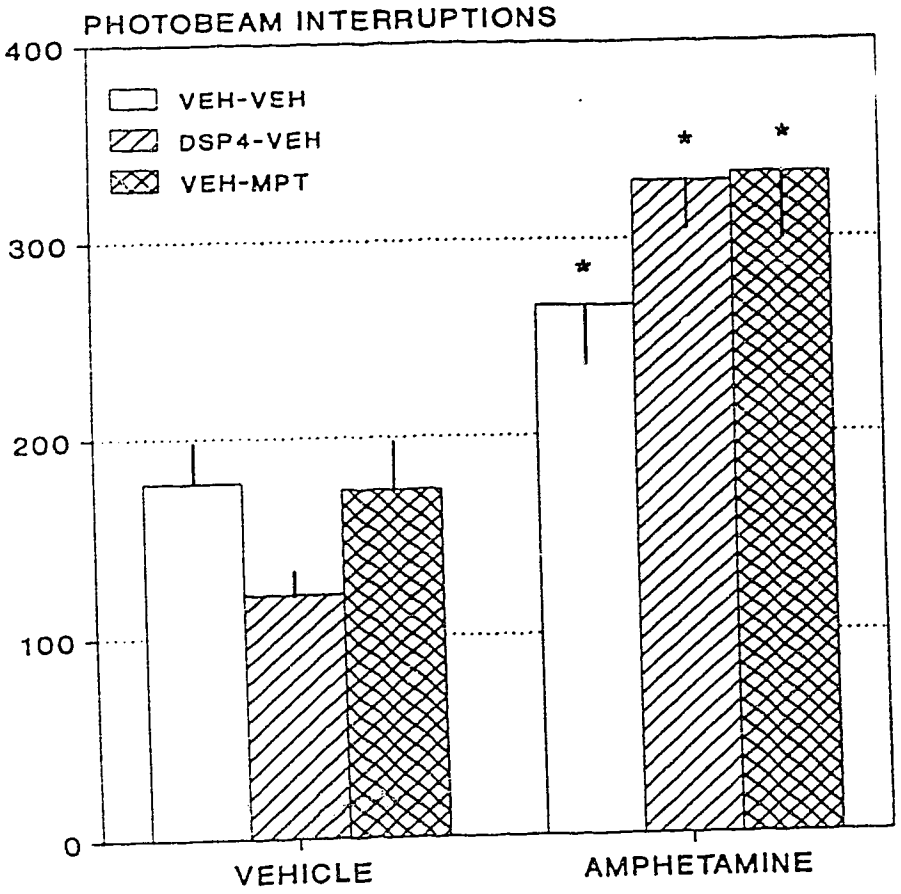

Fig. 2: The effects of previous treatments with VEH or DSP4 (50 i mg/kg, i.p.) or 10 days of treatment with VEH or αMPT (MPT, 25 mg/kg, s.c.) and vehicle or amphetamine (1.5 mg/kg, s.c.) paired with the conditioning boxes on locomotion (photobeam interruptions) on the test day with no drug treatments. Previous treatment with AMP produces increases in locomotion on the test day. Conditioned locomotion occurred with all 3 treatments. Error bars represent the standard error of the mean.

* Significantly different from the control group (VEH-VEH-VEH), $p < 0.001$.

TABLE 1. Mean brain regional monoamine and acidic metabolite levels (ng/g tissue ± SEM in parentheses) in rats treated with VEH or DSP4 (50 mg/kg,i.p.) 17 days prior to dissection. NA = noradrenaline, 5-HT = serotonin, 5-HIAA = 5-hydroxyindoleacetic acid, DA = dopamine, DOPAC = 3,4-dihydroxyphenylacetic acid. Results from DSP4-treated rats are also presented as % of vehicle.

Brain Region	Drug	NA	5-HT	5-HIAA	DA	DOPAC
Frontal Cortex:	Vehicle	226 (9)	820 (33)	497 (24)	120 (31)	57 (9)
	DSP4	32 (10)*	525 (32)*	383 (14)*	86 (16)	40 (5)
	% Vehicle	14	64	77	72	70
Hippocampus:	Vehicle	229 (12)	907 (49)	850 (40)	5 (.6)	6 (.4)
	DSP4	3 (3)*	606 (46)*	656 (30)*	4 (.5)	9 (1.8)
	% Vehicle	1.3	67	77	80	150
Hypothalamus:	Vehicle	1422 (62)	1175 (95)	596 (71)	160 (23)	67 (17)
	DSP4	766 (40)*	687 (57)*	357 (25)*	148 (17)	44 (15)
	% Vehicle	54	58	60	93	66
Olfactory Tubercle:	Vehicle	204 (7)	990 (90)	2075 (227)	777 (121)	352 (100)
	DSP4	56 (6)*	642 (45)*	1269 (66)*	598 (80)	214 (32)
	% Vehicle	27	65	61	77	61

* Significantly different from vehicle, $p < 0.01$.

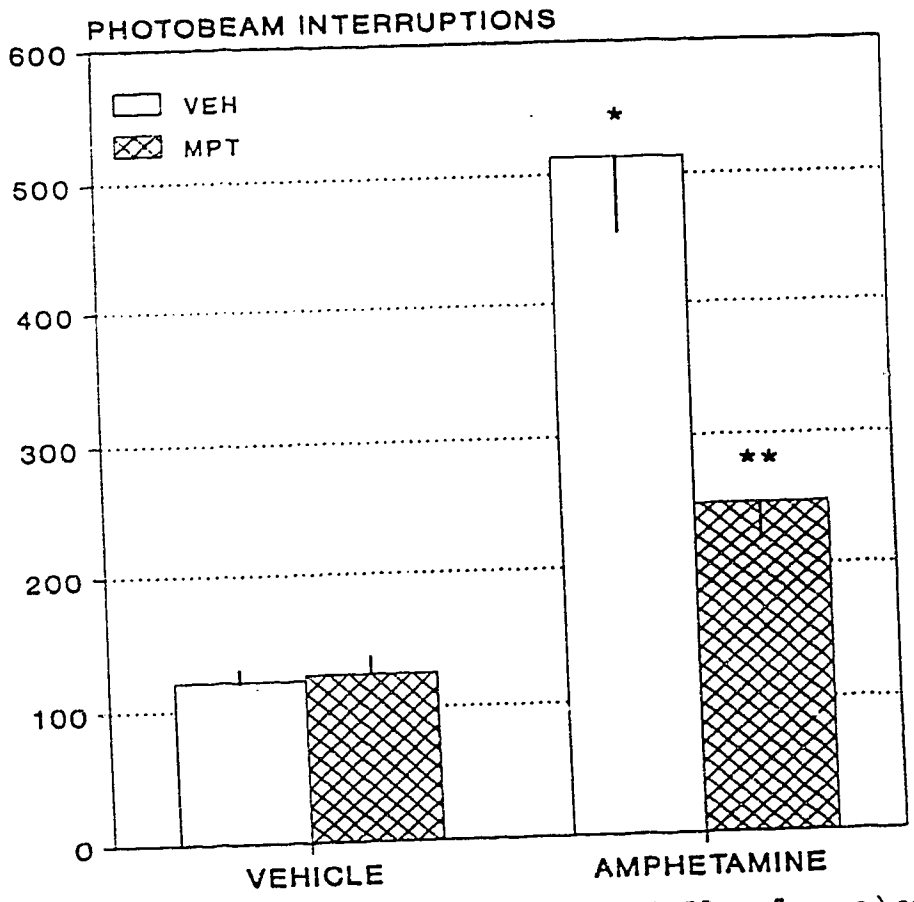

Fig. 3: The effects of injections of VEH or αMPT (MPT, 50 mg/kg, s.c.) and vehicle or amphetamine (1.5 mg/kg, s.c.) collapsed across 10 consecutive days on locomotion (photobeam interruptions) during 60 min periods. Error bars represent the standard error of the mean.

* Significantly different from the control group (VEH-VEH), $p < 0.05$.

** Significantly different from the VEH-AMP group ($p < 0.001$).

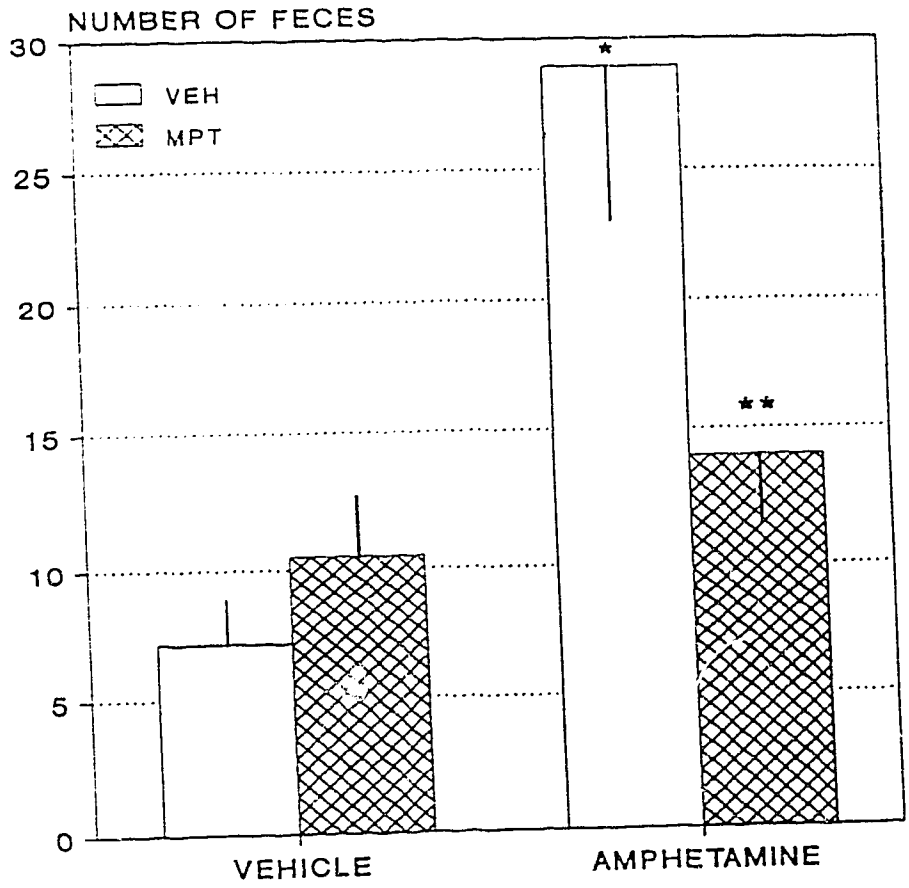

Fig. 4: The effects of injections of VEH or αMPT (MPT, 50 mg/kg, s.c.) and vehicle or amphetamine (1.5 mg/kg, s.c.) collapsed across 10 consecutive days on defecation (fecal counts) during 60 min periods. Error bars represent the standard error of the mean.

* Significantly different from the control group (VEH-VEH), $p < 0.05$.

** Significantly different from the VEH-AMP group ($p < 0.001$).

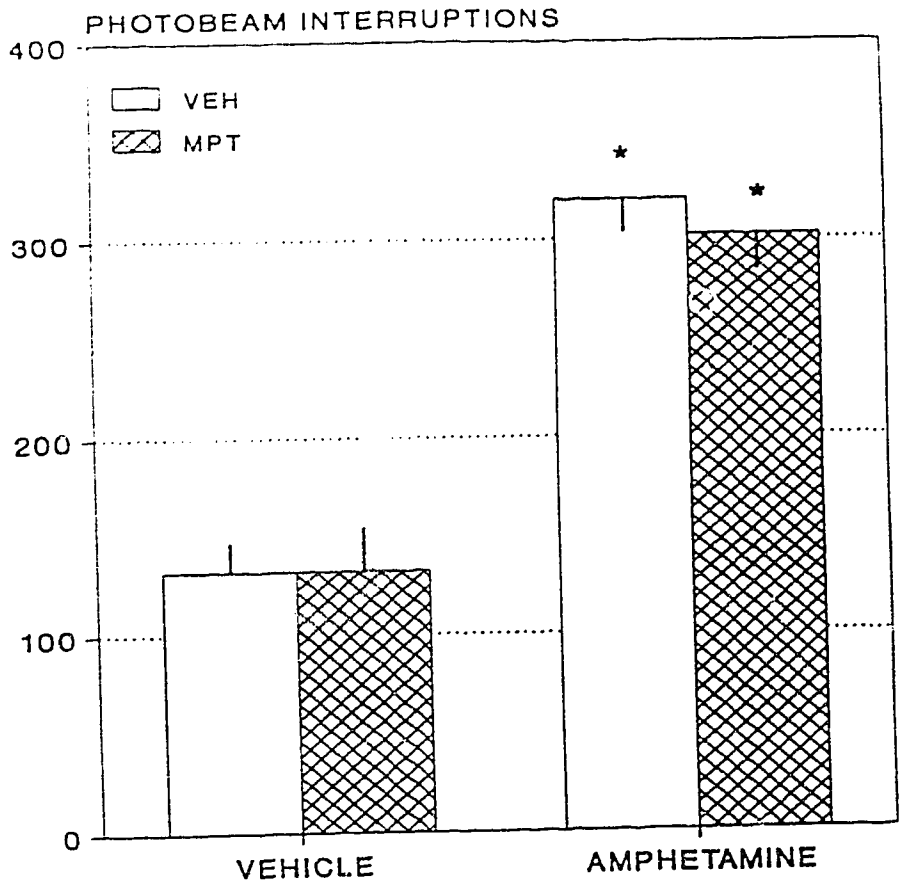

Fig. 5: The effects of 10 days of previous treatment with VEH or αMPT (MPT, 50 mg/kg, s.c.) and vehicle or amphetamine (1.5 mg/kg, s.c.) paired with the conditioning boxes on locomotion (photobeam interruptions) on the test day with no drug treatment. Previous treatment with AMP produces increases in locomotion on the test day. Conditioned locomotion occurred in both AMP-treated groups. Error bars represent the standard error of the mean.

* Significantly different from the control group (VEH-VEH), $p < 0.001$.

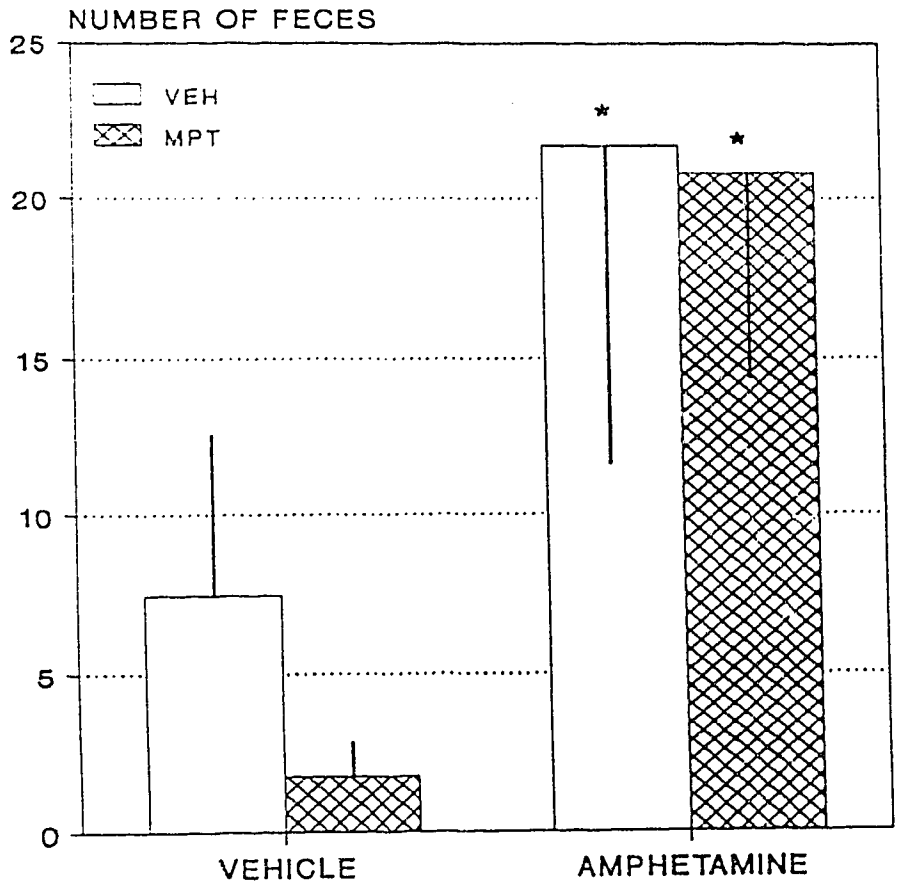

Fig. 6: The effects of 10 days of previous treatment with VEH or αMPT (MPT, 50 mg/kg, s.c.) and vehicle or amphetamine (1.5 mg/kg, s.c.) paired with the conditioning boxes on defecation (fecal counts) on the test day with no drug treatments. Previous treatment with AMP produces increases in defecation on the test day. Conditioned defecation occurred in both AMP-treated groups. Error bars represent the standard error of the mean. Note that the statistics were performed after square root transformations of the data, but the raw scores are depicted in this figure.

* Significantly different from the control group (VEH-VEH), $p < 0.02$.

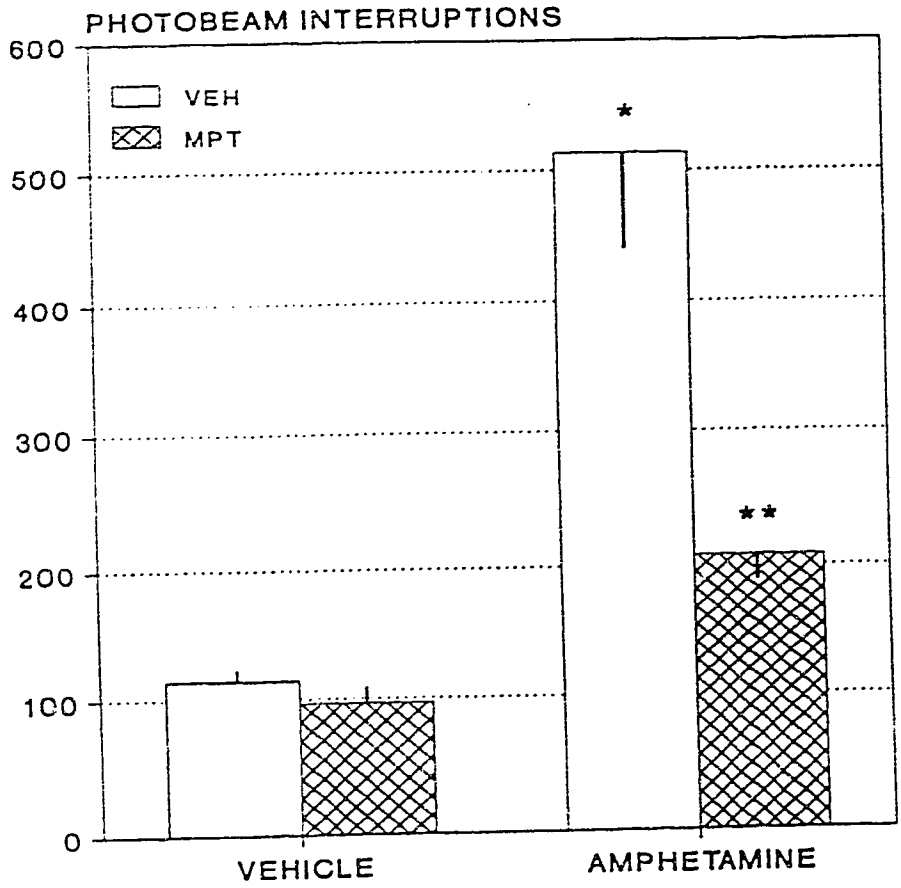

Fig. 7: The effects of injections of vehicle (VEH) or αMPT (MPT, 50 mg/kg, s.c.) 170 min prior to vehicle or amphetamine (1.5 mg/kg, s.c.) on locomotion (photobeam interruptions) during 60 min periods collapsed across 10 consecutive days. Error bars represent the standard error of the mean.

* Significantly different from the control group (VEH-VEH), $p < 0.05$.

** Significantly different from the VEH-AMP group ($p < 0.001$).

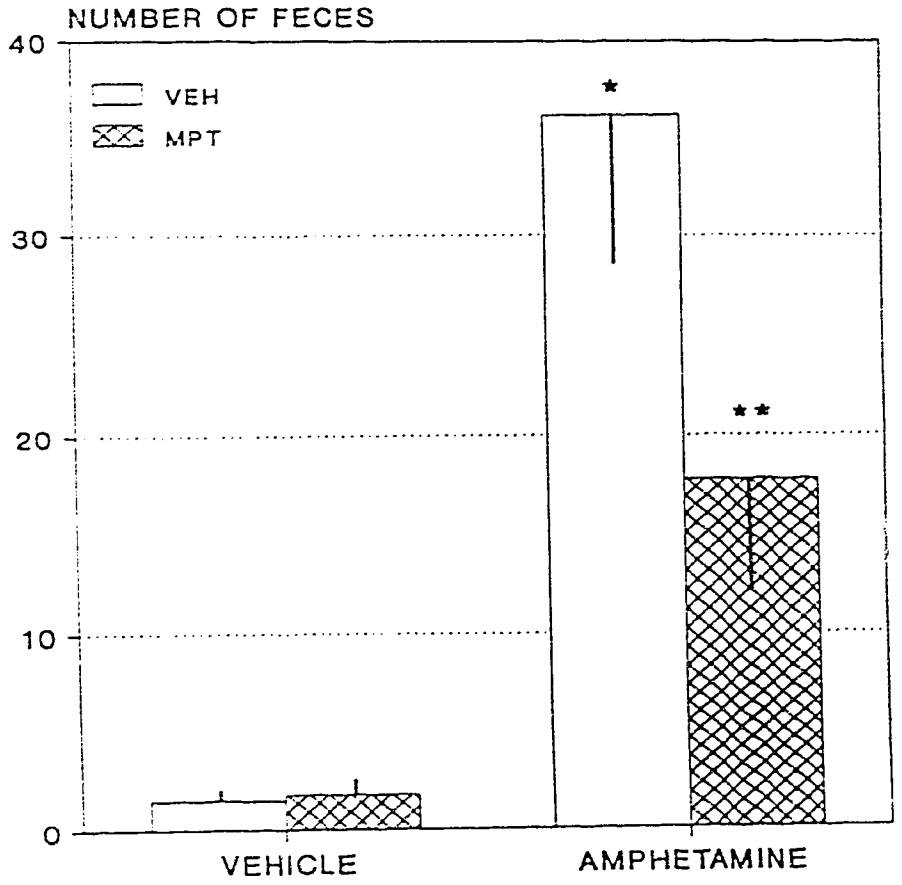

Fig. 8: The effects of injections of VEH or αMPT (MPT, 50 mg/kg, s.c.) 170 min prior to vehicle or amphetamine (1.5 mg/kg, s.c.) on defecation (fecal counts) during 60 min periods collapsed across 10 consecutive days. Error bars represent the standard error of the mean.

* Significantly different from the control group (VEH-VEH), $p < 0.05$.

** Significantly different than the VEH-AMP group ($p < 0.001$).

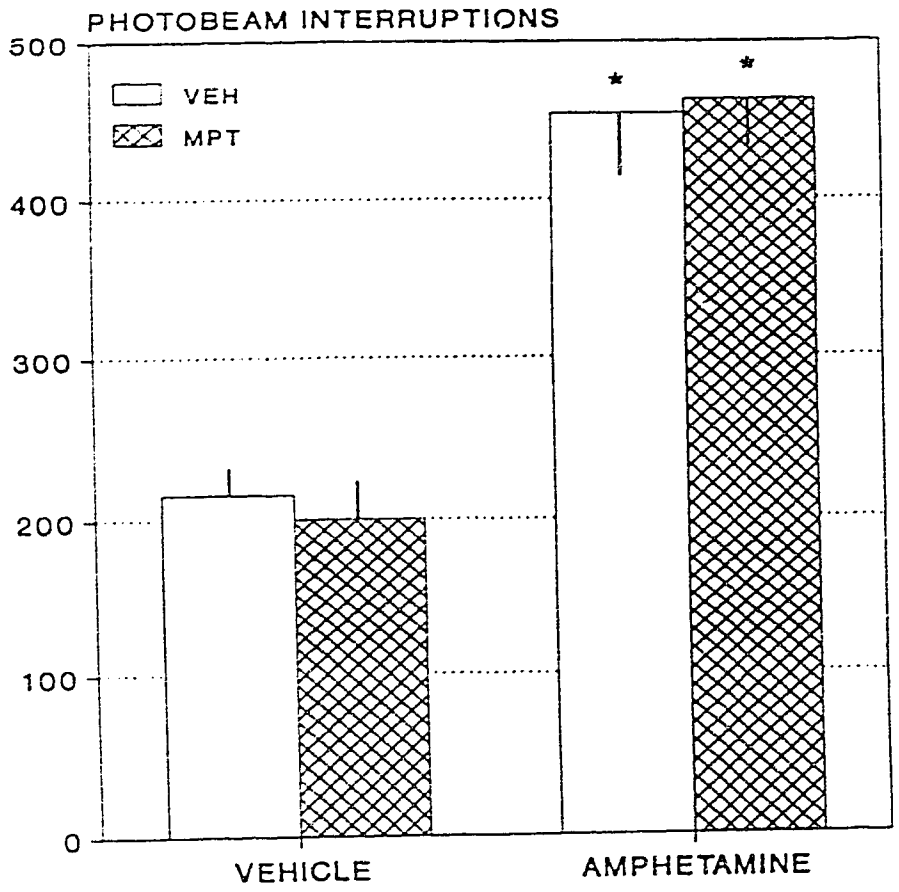

Fig. 9: The effects of 10 days of previous treatment with VEH or αMPT (MPT, 50 mg/kg, s.c.) 170 min prior to vehicle or amphetamine (1.5 mg/kg, s.c.) paired with the conditioning boxes on locomotion (photobeam interruptions) on the test day with no drug treatments. Previous treatment with AMP produces increases in locomotion on the test day. Conditioned locomotion occurred in both AMP-treated groups. Error bars represent the standard error of the mean.

* Significantly different from the control group (VEH-VEH), $p < 0.001$.

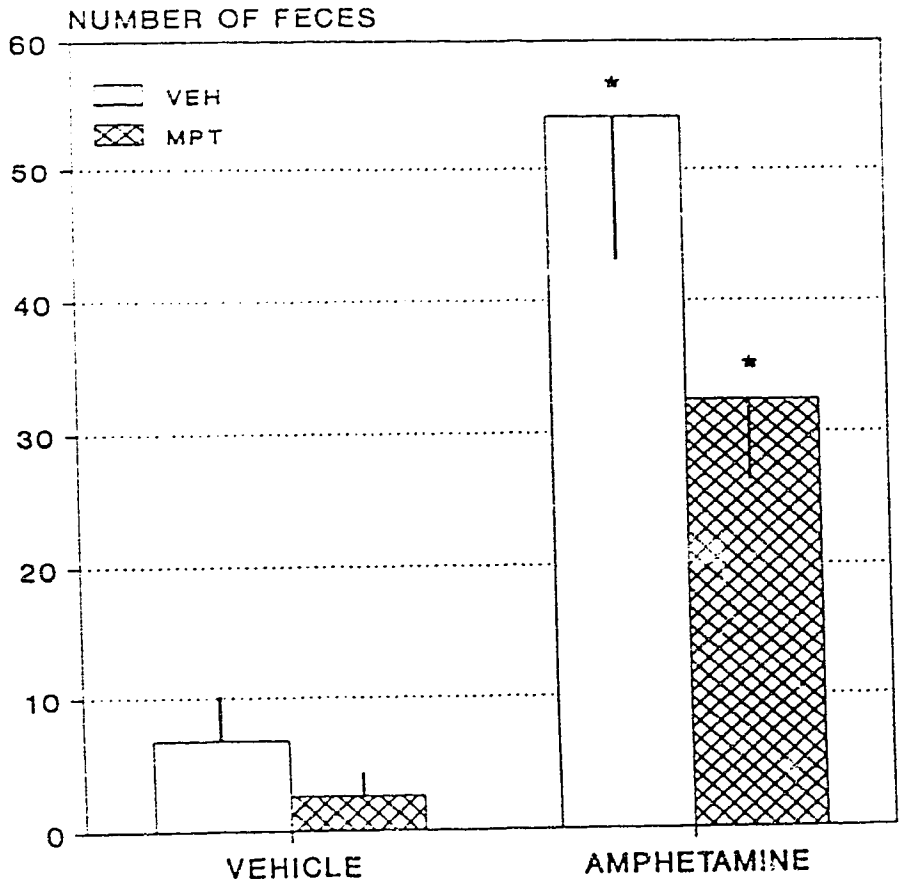

Fig. 10: The effects of 10 days of previous treatment with VEH or αMPT (MPT, 50 mg/kg, s.c.) 170 min prior to vehicle or amphetamine (1.5 mg/kg, s.c.) paired with the conditioning boxes on defecation (fecal counts) on the test day with no drug treatments. Previous treatment with AMP produces increases in defecation on the test day. Conditioned defecation occurred in both AMP-treated groups. Error bars represent the standard error of the mean.

* Significantly different from the control group (VEH-VEH), $p < 0.001$.

2.4. DISCUSSION

Several investigators have demonstrated that the behavioral effects of stimulants can be classically conditioned to the unique environment in which the drugs are administered (Pickens and Crowder, 1967; Schiff, 1982; Beninger and Hahn, 1983; Beninger and Herz, 1986; Gold *et al.*, 1988; Martin-Iverson and McManus, 1990), such that exposure to the environment alone can elicit the drug effects. This series of experiments verified this observation: AMP injections to rats paired with exposure to a unique environment resulted in an AMP-like response when the rats were placed in the environment without AMP treatment. In addition, the present experiments demonstrated that AMP increases defecation, and that defecation can be conditioned to environmental stimuli. The AMP-conditioned behaviors were tested relative to rats that received "conditioning" with vehicle injections. Previous work in this laboratory, following the same procedures as used in the present experiments determined that the conditioned AMP-like response is unique to the testing environment; rats receiving AMP while in their home cages 3 h after conditioning trials do not exhibit environment-specific increases of locomotion to the conditioning boxes (Martin-Iverson and McManus, 1990). Thus, the present results are likely not a function of an unconditioned increase in spontaneous locomotion induced by prior treatment with AMP.

The inability of DA receptor antagonists to block the establishment of stimulant-conditioned locomotion (Martin-Iverson and McManus, 1990) led us to hypothesize that perhaps the critical event conditioned with AMP was a non-receptor mediated presynaptic process, such as AMP-induced DA or NA release. A second possibility was that the increase in locomotion observed on the test day in rats previously given AMP paired with the testing environment was due to some process unique to locomotion. These

two possibilities were tested in the present experiments.

It has been well-established that AMP-induced DA release occurs via actions on a compartment of newly-synthesized DA which can be blocked by synthesis inhibition with αMPT (Weissman *et al.*, 1966; Scheel-Kruger, 1971; Braestrup, 1977; McMillan, 1983; Finn *et al.*, 1990,). The effects of two doses of α-MPT given only during the conditioning procedure on the unconditioned and subsequent conditioned effects of AMP were investigated in the first and third experiments. αMPT was given to rats 30 min prior to placement in the test boxes since maximal DA synthesis inhibition by αMPT occurs 30 min after treatment (Braestrup, 1977). However, it has been reported that maximal blockade of the *behavioral* effects of AMP requires a longer time interval, possibility because sufficient DA remains in the newly synthesized, AMP-sensitive compartment after a shorter time interval (Scheel-Kruger, 1971; Braestrup, 1977). The effect of the higher dose of αMPT after a longer time interval was investigated in the fourth experiment. Since αMPT blocks the synthesis of both DA and NA, and since there is some evidence that NA may be involved in AMP-unconditioned (Ogren *et al.*, 1983) and conditioned locomotion (Poncelet, 1987), some rats were treated with DSP4, a neurotoxin that produces selective NA depletions, prior to conditioning. This was done to determine if any effects of αMPT on conditioned locomotion were due to actions on dopaminergic or noradrenergic systems. These experiments provided a direct test of the hypothesis that either AMP-induced DA release or an increase in NA neurotransmission is the critical event that becomes conditioned after pairing of AMP treatments with a unique environment. AMP-induced defecation was also assessed in some of the present experiments to determine if AMP-induced defecation can be conditioned and if the effects of the drug treatments are specific to locomotion or can be generalized to other behaviors.

Depletion of forebrain NA with DSP4 had no effect on either unconditioned or conditioned locomotion, although levels of NA in forebrain regions were depleted to 1-14% of controls and were down to 27-54% in diencephalic regions. The lack of effect of DSP4 on AMP-induced locomotion contrasts with the results of a previous report (Ogren *et al.*, 1983). Similar doses, but different routes of administration of AMP, (i.p. vs. s.c. in the present study) were used. Since DSP4 does not affect locomotion after higher doses of AMP (Ogren *et al.*, 1983) and s.c. injections likely avoid first-pass metabolism, the difference in route of administration of AMP may explain the discrepancy.

Small 5-HT depletions (10-30%) are often seen following DSP4 treatment (Archer, 1982). In the present experiment (see Table 1), consistent 5-HT and 5-HIAA decreases in this range were observed (Table 1). These decreases are likely secondary effects to NA depletion, rather than primary effects of DSP4 on 5-HT neurons. Archer (1982) has demonstrated that the 5-HT depletion is not reduced by co-administration with a 5-HT uptake inhibitor, but is blocked with a NA uptake inhibitor. These findings indicate that NA tonically increases the synthesis and metabolism of 5-HT.

In all three experiments, αMPT attenuated AMP-induced <u>unconditioned</u> locomotion and defecation. However, in no case was <u>conditioned</u> locomotion or defecation reduced. It can therefore be concluded that both locomotion and defecation induced by AMP are dependent upon the release of DA from newly-synthesized DA. On the other hand, the establishment of conditioned behaviors is <u>not</u> blocked by catecholamine synthesis inhibition. Taken together with evidence that DA D_1 and/or D_2 receptor antagonists also block unconditioned but not conditioned behaviors (Martin-Iverson and McManus, 1990), neither presynaptic nor postsynaptic dopaminergic mechanisms can account for the conditioning of stimulant-induced behaviors. This interpretation is strengthened by the finding that the

establishment of conditioned locomotion by a direct postsynaptic DA D_2 agonist is not blocked by a DA receptor antagonist, although the direct locomotor stimulant effects are (Martin-Iverson and McManus, 1990).

Conflicting results have been obtained with regard to the expression and the establishment of drug-conditioned behaviors. The expression of stimulant-conditioned locomotion has been demonstrated to be blocked by pimozide (Beninger and Hahn, 1983; Beninger and Herz, 1986). Others have been unable to block (Poncelet *et al.*, 1987) or to completely block (Hiroi and White, 1989) the expression of stimulant-conditioned stereotypy with pimozide. Pimozide, a selective DA D_2 receptor antagonist which also blocks rapidly-inactivating calcium channels (Cohen *et al.*, 1986, Tecott *et al.*, 1986; Enyeart *et al.*, 1987) and 5-HT_2 receptors (Cohen *et al.*, 1986, Tecott *et al.*, 1986), has been demonstrated to attenuate the expression of morphine-conditioned hyperactivity (Neisewander and Bardo, 1987). Other DA antagonists have similarly been shown to either block (Schiff, 1982; Drew and Glick, 1990) or have no effect (Carey, 1990) on the expression of stimulant-conditioned behaviors other than locomotion. Although there have been reports that pimozide can block the establishment of AMP- and cocaine-conditioned locomotion (Beninger and Hahn, 1983; Beninger and Herz, 1986), experiments using other DA D_2 and D_1 antagonists have been unable to demonstrate blockade of the establishment of conditioned locomotion (Martin-Iverson and McManus, 1990) produced with AMP or PHNO, a direct DA D_2 agonist. The conflicting effects of these studies on the expression and the establishment of stimulant-conditioned behaviors may be a result of the use of different DA antagonists with varying pharmacological profiles, different experimental procedures or differences in the behaviors measured.

The findings obtained in the present study and in other studies (Mithani *et al.*, 1986; Martin-Iverson and McManus, 1990) indicate dopaminergic neurotransmission is not responsible for the conditioning of stimulant effects. The lack of blockade of conditioning occurs even though the direct unconditioned psychomotor stimulant effects are blocked during the conditioning training. It is probable therefore that the effects of pimozide on stimulant-conditioned behaviors (Beninger and Hahn, 1983; Beninger and Herz, 1986; Neisewander and Bardo, 1987; Hiroi and White, 1989) are dependent on actions on something other than DA receptors. Calcium channel blockade is a likely candidate as an explanation for pimozide's unique profile. Reserpine disrupts presynaptic granular storage pools of DA which are apparently released calcium dependently. Reserpine, injected directly into the nucleus accumbens after conditioning, has been recently demonstrated to block the expression of conditioned place preference.

Stimulant conditioning has been implicated as a contributing factor in the development of behavioral sensitization to repeated stimulant treatment (Post *et al.*, 1981; Weiss *et al.*, 1989) and in stimulant addiction (de Wit and Stewart, 1981; Gawin, 1988; O'Brien *et al.*, 1988). The observations that blockade of dopaminergic systems fails to block stimulant-conditioning indicates that DA receptor blockade with neuroleptics may not be sufficient to block conditioned euphoric effects of stimulants. Gawin (1986) reported that neither chlorpromazine nor haloperidol attenuated cocaine-induced euphoria reported by cocaine addicts. Since the reinforcing effects of cocaine in animals requires an intact mesolimbic DA system (Roberts *et al.*, 1980; Spyraki *et al.*, 1982b), it may be that conditioned euphoric effects were sufficient to account for the lack of effect of neuroleptics on cocaine-induced reward in these experienced users. It is of interest to note that cocaine-, methylphenidate- and nomifensine-conditioned place preferences are not blocked by

haloperidol or by 6-OHDA-induced lesions of central DA systems when given during or before conditioning (Spyraki *et al.*, 1982a; Martin-Iverson *et al.*, 1985; Mithani *et al.*, 1986). The present observations suggest that some aspects of AMP-sensitization may be independent of AMP-induced release of DA, and also provide further evidence for a non-dopaminergic mechanism of stimulant-conditioned behaviors. What this alternative mechanism may be is presently unknown.

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CHAPTER 3. CALCIUM CHANNEL BLOCKADE: A POTENTIAL ADJUNCTIVE TREATMENT WITH NEUROLEPTICS FOR STIMULANT ABUSE AND SCHIZOPHRENIA[2]

3.1. INTRODUCTION

Former addicts of psychomotor stimulants continue to exhibit strong cravings and drug-like physiological responses when presented with drug-related cues (O'Brien *et al.*, 1988; Muntaner *et al.*, 1989). Such responses are not affected by usual drug-detoxification or drug-rehabilitation programs (O'Brien *et al.*, 1988). In humans, both the subjective euphoric and cardiovascular effects of cocaine can be conditioned to situational stimuli (O'Brien *et al.*, 1986; Muntaner *et al.*, 1989). These observations have led some researchers to propose that drug-conditioned responses may contribute to the maintenance of addictive behaviors (O'Brien *et al.*, 1988). O'Brien and his colleagues have attempted to extinguish the conditioned responses to drug-related stimuli in cocaine addicts. Repeated exposure to individualized drug-associated cues diminishes conditioned-craving responses. Addicts, however, remain vulnerable to relapse of drug cravings in response to stimulus exposure (O'Brien *et al.*, 1988). The conditioned stimulant-cravings and physiological responses appear to be difficult to extinguish.

Because the reinforcing (Roberts *et al.*, 1980; Spyraki *et al.*, 1982) and activating (Kelly and Iversen, 1976; DiLullo and Martin-Iverson, in press) effects of stimulants are mediated by DA neurons in the brain, the role of DA neurotransmission in stimulant-conditioning has been actively investigated. Beninger and Hahn (1983) and Beninger and Herz (1986) investigated the role of DA in AMP- and cocaine-induced

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2 A version of this chapter has been submitted for publication in Biological Psychiatry.

conditioned locomotion in rats using pimozide, a neuroleptic. Pimozide, a selective DA D_2 receptor antagonist and equipotent antagonist of L-type calcium channels (Cohen *et al.*, 1986), blocked conditioning when pimozide was administered during conditioning with stimulants. It was concluded that DA mediates the establishment of stimulant-conditioned locomotion (Beninger and Hahn, 1983; Beninger and Herz, 1986). Some studies have since replicated the original findings of Beninger and his research group with pimozide (Poncelet *et al.*, 1987; Hiroi and White, 1989), but not with other DA antagonists (Poncelet *et al.*, 1987; Carey, 1990; Drew and Glick, 1990; Martin-Iverson and McManus, 1990). The relative inability of other DA receptor antagonists (Martin-Iverson and McManus, 1990) and of the catecholamine synthesis inhibitor αMPT (DiLullo and Martin-Iverson, in press) to block the establishment of stimulant-conditioned locomotion has led us to a different hypothesis. The ability of pimozide to block the establishment of stimulant-conditioned locomotion may be dependent on its action as an antagonist at calcium channels, either exclusively, or in conjunction with its DA D_2 receptor blockade.

That calcium channel antagonists can attenuate the psychomotor (Grebb, 1986; Trouve and Nahas, 1986; Pani *et al.*, 1990), physiological (Trouve and Nahas, 1986; Rowbotham *et al.*, 1987), central (Pani *et al.*, 1990) and discriminative stimulus properties (Nencini and Woolverton, 1988) of psychomotor stimulants has recently been recognized. Calcium channel antagonism, though able to diminish the effects of cocaine in naive animals, does not alter cocaine-induced responses by experienced human users (Rowbotham *et al.*, 1987). Animal (Trouve and Nahas, 1986; Nencini and Woolverton, 1988) and clinical (Bloom *et al.*, 1987; Jacques and Cox, 1991; Stedman et al., 1991) research suggest the efficacy of calcium channel blockers in the treatment of stimulant addiction (Nencini and Woolverton,

1988; Pani *et al.*, 1990), cocaine-induced cardiac toxicity (Trouve and Nahas, 1986; Hale *et al.*, 1991) and psychotic (Bloom *et al.*, 1987; Bartko *et al.*, 1991; Jacques and Cox, 1991; Stedman *et al.*, 1991) disorders.

Animal studies using DA receptor blockade with neuroleptics (Spyraki *et al.*, 1982; Mithani *et al.*, 1986) and dopaminergic depletions of the nucleus accumbens with 6-OHDA (Roberts *et al.*, 1980; Spyraki *et al.*, 1982) in the conditioned place preference (Spyraki *et al.*, 1982; Mithani *et al.*, 1986) and self-administration (Robert *et al.*, 1980) paradigms indicate that mesolimbic DA neurotransmission mediates AMP- (Spyraki *et al.*, 1986; Mithani *et al.*, 1986) and cocaine-induced (Roberts *et al.*, 1980) induced reinforcement. The inability of neuroleptics to block cocaine-induced euphoria in drug addicts (Gawin, 1986; Sherer, 1988) suggests that the euphoria produced by cocaine in experienced users is mediated by a non-dopaminergic mechanism which could be DA-independent Pavlovian conditioning.

Combined pharmacotherapy with DA D_2 and calcium channel antagonists appears to be an attractive treatment alternative for stimulant addiction. Diphenylbutylpiperidines, neuroleptics which antagonize DA D_2 receptors and L-type calcium channels, appear to be more effective than selective DA D_2 receptor antagonism in the treatment of unresponsive chronic schizophrenia and the alleviation of negative schizophrenia syndrome (Lapierre, 1978; Gould *et al.*, 1982; Feinberg *et al.*, 1988).

The purpose of this experiment was to investigate the potential of nimodipine (NIM), a calcium channel blocker, to attenuate the establishment of environment-specific amphetamine-conditioned activity of amphetamine, when given alone or in conjunction with haloperidol (HAL), a neuroleptic with DA D_2 receptor antagonistic activity.

3.2. METHODS

Male Sprague-Dawley rats (n=12 or 24 for each group), weighing 250-350 g with *ad libitum* access to food and water and maintained on a 12:12 h light cycle (lights on 0700-1900) were used. Locomotor activity was measured by counting interruptions of infra-red photobeams in test boxes described previously (Martin-Iverson and McManus, 1990; DiLullo and Martin-Iverson, in press). AMP sulfate was provided courtesy of SmithKline Beecham Pharma and was dissolved into a 1.5 mg/ml solution. NIM was provided courtesy of Dr. Miles Scriabine of Miles Inc. and was dissolved into a 10 mg/kg solution. HAL was purchased from McNeil and was diluted into 0.05 and 0.2 mg/kg solutions. The vehicle for AMP and HAL was double-distilled water and for NIM was polyethylene glycol 400.

The experiment consisted of 2 phases, a drug conditioning phase and a test phase. During the conditioning phase (lasting 10 consecutive days) all rats received three injections (VEH or NIM, VEH or HAL, and VEH or AMP) followed by placements into the conditioning boxes for 60 min. The test day occurred on the fourth day after the last conditioning day injection (i.e. 3 intervening treatment-free days), to allow for drug clearance. On this day, all animals received an injection of VEH prior to placement for 60 min in the conditioning boxes.

There were 12 treatment groups with each animal receiving three treatments daily, consisting of NIM (10 mg/kg, sc) or VEH (1 ml/kg, sc) 120 min before placements in the conditioning boxes, HAL (0.05 or 0.2 mg/kg, ip) or VEH (1 ml/kg, ip) 70 min before placements in the conditioning boxes, and AMP (1.5 mg/kg, sc) or VEH (1 ml/kg, sc) 30 min prior to placements in the conditioning boxes for 10 consecutive days. The groups were as follows: VEH-VEH-VEH, VEH-VEH-AMP, NIM-VEH-VEH, NIM-VEH-AMP, VEH-HAL(0.05)-VEH, VEH-HAL(0.05)-AMP, VEH-HAL(0.2)-VEH,

VEH-HAL(0.2)-AMP, NIM-HAL(0.05)-VEH, NIM-HAL(0.05)-AMP, NIM-HAL(0.2)-VEH and NIM-HAL(0.2)-AMP. There were 12 animals in each group which received HAL, and 24 in all other groups.

Locomotor activity for each rat during both phases of the experiment was recorded in 5 min blocks and summed to produce individual daily totals. The means of the individual rat's daily total number of locomotor counts were subjected to ANOVA with planned comparisons between individual groups.

3.3. RESULTS

The results of the conditioning phase of the experiment were subjected to ANOVA with 3 independent factors: (1) NIM pretreatment, consisting of 2 levels (VEH or NIM); (2) HAL pretreatment, consisting of 3 levels (VEH, 0.05 mg/kg or 0.2 mg/kg); and (3) AMP treatment, consisting of 2 levels (VEH or AMP). Fig. 11 represents the unconditioned locomotion collapsed across days. AMP significantly increased locomotion depending on the pretreatment. Both low (0.05) and high (0.2 mg/kg) doses of HAL attenuated AMP-induced locomotion (HAL x AMP interaction: $F_{2,180}=18.8$, $p<0.001$). ANOVA revealed a significant interaction between NIM and AMP ($F_{1,180}=6.0$, $p<0.02$).

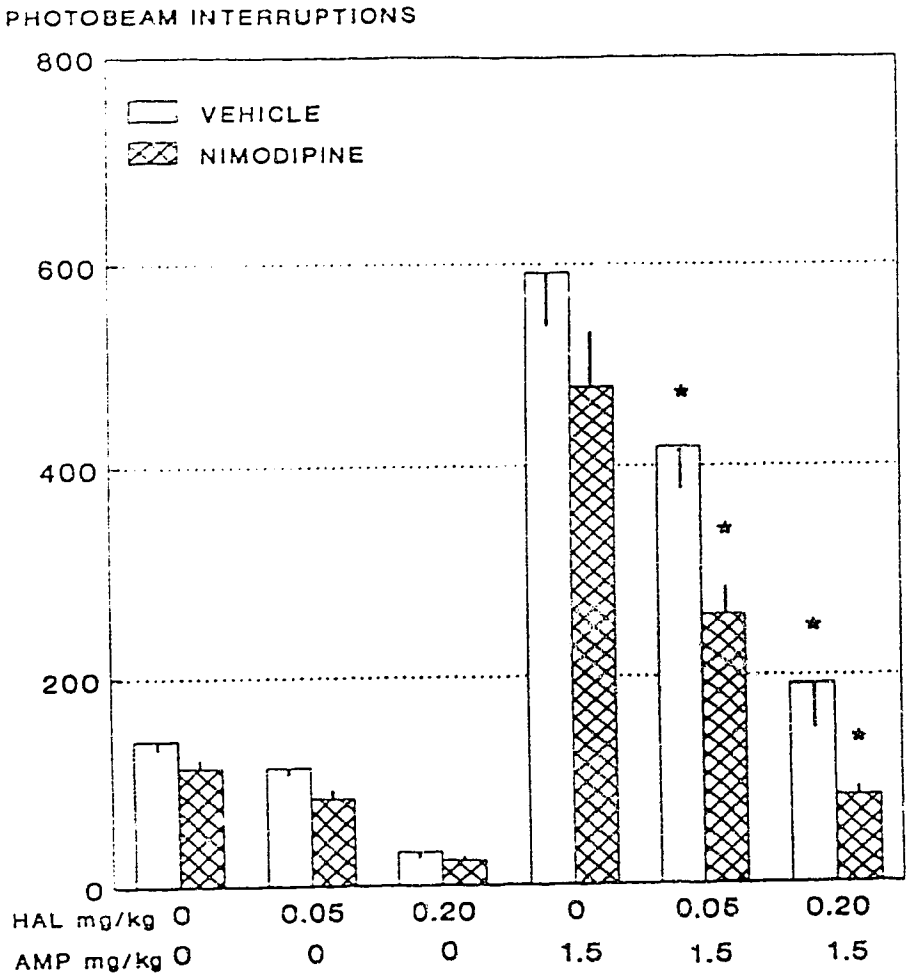

Fig. 11: The effects of 3 daily injections of VEH or nimodipine (10 mg/kg, s.c.), VEH or one of two doses of HAL (i.p.), and VEH or AMP (s.c.) on mean photobeam interruptions (unconditioned locomotion) averaged over 10 consecutive days of 60 min tests.

* Significantly different from VEH-VEH-AMP group, $p < 0.05$, Tukey's Test.

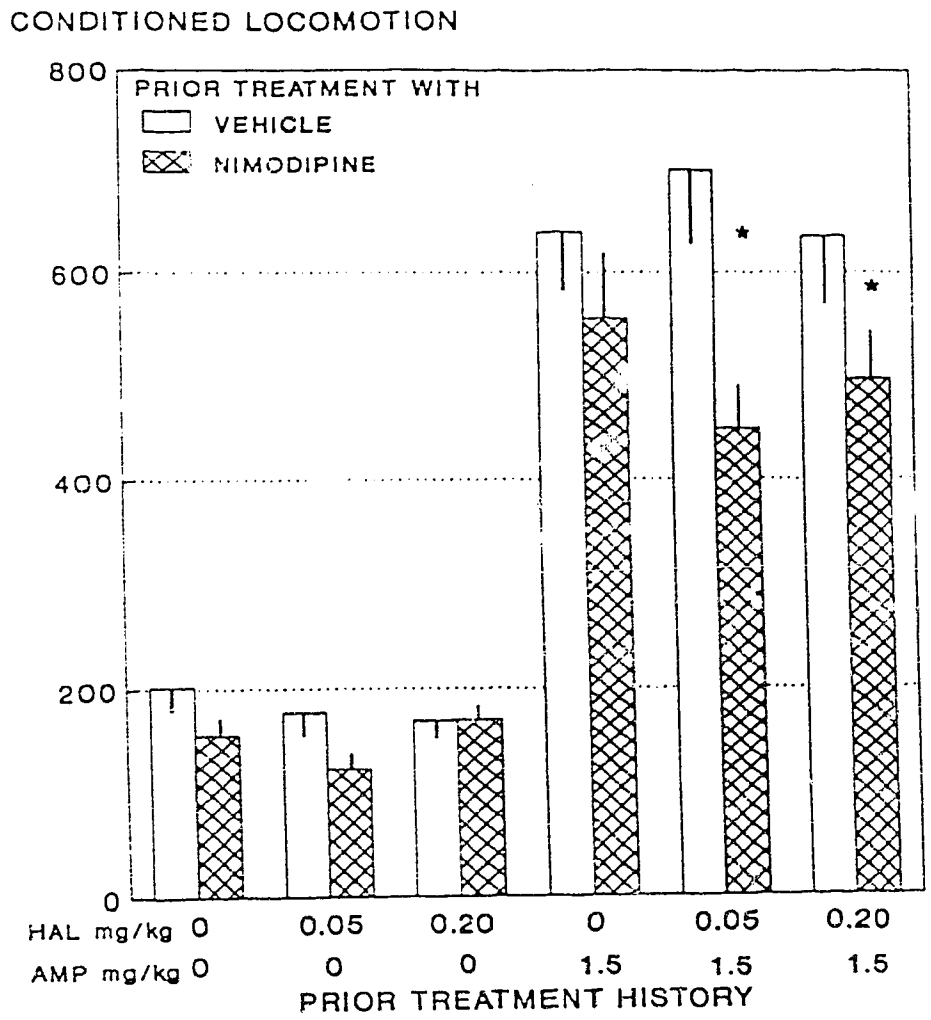

Fig. 12: The same groups of rats as in Fig. 11, 4 days after the last drug treatments, were all given a placebo (s.c.) injection and were tested 10 min later in the environment previously paired with drug treatments for 60 min. Conditioned locomotion is evident in the AMP-treated groups as an increase in photobeam interruptions relative to controls ($p<0.001$). Previous combined treatments with nimodipine and HAL significantly attenuated AMP-conditioned locomotion.

* Significantly different from vehicle (+) nimodipine) control, $p<0.05$, Tukey's Test.

Planned comparisons show that NIM significantly ($p<0.05$) augmented the decrease in locomotion produced by HAL but had no effect when NIM was given without HAL.

The test phase of the experiment was subjected to ANOVA with the same independent factors as in the conditioning phase. A significant conditioning effect with AMP was observed (main effect: $F_{1,180}=226.6$, $p<0.001$, see Fig. 12). ANOVA revealed a significant interaction between NIM and AMP ($F_{1,80}=4.8$, $p<0.03$). Planned comparisons show that neither HAL nor NIM, when given independently influenced locomotion on the test day. AMP-conditioned locomotion was significantly attenuated with combined HAL and NIM pretreatment ($p<0.05$). This effect as seen in Fig. 2 was not dependent on the dose of HAL pretreatment.

3.4. DISCUSSION

Recent studies have made DA-calcium synergism an attractive target for the treatment of psychoses (Bloom *et al.*, 1987; Bartko *et al.*, 1991; Jacques and Cox, 1991; Stedman *et al.*, 1991) and stimulant addiction (Rowbotham *et al.*, 1987; Nencini and Woolverton, 1988; Pani *et al.*, 1990). Clinical trials, although uncontrolled, have demonstrated the efficacy of combined L-type calcium channel blockade and DA D_2 receptor blockade in the treatment of chronic schizophrenia and negative schizophrenic symptoms (Lapierre, 1978; Gould *et al.*, 1983; Feinberg *et al.*, 1988). In addition, schizophrenics with periodic psychosis show episodal increases of serum calcium levels (Carman and Wyatt, 1979).

The observation that NIM augmented the HAL-produced decrease in direct AMP-induced locomotion indicates the potential for calcium channel blockade as an adjunctive therapy with neuroleptics for the treatment of psychosis. Effective doses of most neuroleptics can cause extrapyramidal side effects, as well as inducing tardive dyskinesia after long-term treatment. The augmentation of HAL's effects by NIM, independent of the

high dose of HAL used, indicates that adjunctive treatment of schizophrenia with calcium channel blockers may allow clinicans to lower the therapeutic dose-range of neuroleptics, thereby decreasing the incidence of extrapyramidal side effects. Indeed, after the administration of the dihydropyridine nifedipine, four of 10 patients in an open study showed increases in plasma neuroleptic activity with a reduction in Abnormal Movement Scale scores associated with tardive dyskinesia (Stedman *et al.*, 1991).

Based on the observations of the present study, it appears that diphenylbutylpiperidine antipsychotic drugs, which combine DA D_2 receptor and calcium channel blockade in a single drug, or combined therapy with a calcium antagonist and a neuroleptic may be clinically efficacious for the treatment of stimulant addiction. Gawin (1986) reported that neither chlorpromazine nor HAL attenuates cocaine-induced euphoria reported by cocaine addicts. It has similarly been reported that HAL pretreatment does not block the rush produced by infusion of cocaine in cocaine addicts (Sherer, 1988). Since the euphoric effects of cocaine in animals require an intact mesolimbic DA system (Roberts *et al.*, 1980; Spyraki *et al.*, 1982), it is likely that conditioned euphoric effects are sufficient to account for the lack of effect of neuroleptics on cocaine-induced euphoria in experienced cocaine users. Similarly, the conditioned physiological effects but not the subjective "high" of cocaine have been shown to be blocked by calcium channel antagonism (Rowbotham *et al.*, 1987). The observations from this study suggest that concomitant DA and calcium channel antagonism can attenuate or block both the unconditioned and the conditioned "high" produced by stimulants.

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CHAPTER 4. EVIDENCE FOR PRESYNAPTIC DOPAMINE MECHANISMS UNDERLYING AMPHETAMINE-CONDITIONED LOCOMOTION[3]

4.1. INTRODUCTION

Rats exhibit classically conditioned psychomotor stimulant responses when placed into an environment in which they have a history of repeated AMP treatment (Pickens and Crowder, 1967, Schiff, 1982; Martin-Iverson and McManus, 1990; DiLullo and Martin-Iverson, in press). In humans, both the euphoric and physiological effects of stimulants can be conditioned to situational stimuli (O'Brien *et al.*, 1986; O'Brien *et al.*, 1988; Muntaner *et al.*, 1989). Former cocaine addicts continue to exhibit strong 'cravings' and drug-like physiological responses when presented with drug-related cues (O'Brien *et al.*, 1988; Muntaner *et al.*, 1989). These apparent conditioned responses are not affected by drug-detoxification or drug-rehabilitation programs (O'Brien *et al.*, 1988). Therefore, conditioned stimulant effects likely contribute to the resistance of addicts to these treatments or to psychosocial therapy (Kang *et al.*, 1991), and may account for the high rate of relapse among addicts of cocaine and AMP-like compounds. Similarly, stimulant-induced euphoria in chronic cocaine abusers has been shown to be unaffected by DA receptor antagonists (Gawin 1986). This observation seems to complicate the role of DA in the mediation of the reinforcing (Roberts *et al.*, 1980; Spyraki *et al.*, 1982b) and activating (Kelly and Iversen, 1976) effects of stimulants established in the non-human literature. It may be that the conditioned euphoric effects of cocaine were sufficient to account for the apparent lack of effect of neuroleptics on cocaine-induced euphoria by the experienced stimulant users in

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3 A version of this chapter has been accepted for publication. Brain Research.

this study. This hypothesis is supported by the observations that neuroleptics block the unconditioned but not the conditioned effects of stimulants in rats (Carey, 1990; Martin-Iverson and McManus, 1990), and that cocaine-, methylphenidate- and nomifensine-conditioned place preferences in rats are not blocked by neuroleptics or DA depletions (Spyraki *et al.*, 1982a; Martin-Iverson et al., 1985; Mithani *et al.*, 1986).

Although many of the direct behavioral effects of psychomotor stimulants are dependent on dopaminergic mechanisms, the role of DA neurotransmission in conditioning of stimulant effects is not well understood. Beninger and Hahn (1983) and Beninger and Herz (1986) initially investigated the role of DA in AMP- and cocaine-induced conditioned locomotion in rats in an elegant series of experiments. Pimozide, a selective DA D_2 receptor antagonist blocked the 'establishment' of conditioning when it was administered concomitantly with AMP (Beninger and Hahn, 1983) or cocaine (Beninger and Herz, 1986) during a 10-day training period but did not block the 'expression' of conditioning when it was administered only on the stimulant-free test days.

While the ability of pimozide to block the establishment (Hiroi and White, 1989) and not the expression (Poncelet *et al.*, 1987:Hiroi and White, 1989) of stimulant-conditioned behaviors has been replicated, the mechanism which this has been attributed to, DA D_2 antagonism, may be incorrect. Pimozide is an equipotent antagonist of L-type calcium channels (Cohen *et al.*, 1986; Tecott *et al.*, 1986; Enyeart *et al.*, 1987) and has other antagonistic effects on serotonin$_2$ receptors (Cohen *et al.*, 1986; Tecott *et al.*, 1986), α_1 receptors (Cohen *et al.*, 1986), prolactin secretion (Enyeart *et al.*, 1987) and calmodulin (Cohen *et al.*, 1986). There is strong evidence against the interpretation that the effects of pimozide on stimulant-conditioning are due to DA D_2 receptor blockade; namely, other DA D_2 antagonists do not have similar actions. DA D_2 antagonists such as metoclopramide

(Drew and Glick, 1990), HAL (Schiff, 1982; Miyamoto and Hada, 1987; Poncelet *et al.*, 1987), chlorpromazine (Miyamoto and Hada, 1987) and sulpiride (Poncelet *et al.*, 1987) block the expression of stimulant-conditioned behaviors. However, conclusions as to the role of DA D_2 receptors in the expression of conditioning cannot be safely drawn. Non-specific sedating effects of DA receptor antagonism were not ruled out in at least one investigation (Drew and Glick, 1990). In addition, Carey (1990) recently demonstrated that haloperidol does not block the expression of apomorphine-conditioned contralateral turning in unilaterally DA depleted rats.

Pimozide has been shown to block the establishment of AMP- and apomorphine-conditioned stereotypy and to block the expression of apomorphine- but not AMP-conditioned stereotypy (Hiroi and White, 1989). In addition, HAL fails to block the establishment of AMP-conditioned locomotion (Martin-Iverson and McManus, 1990), but does attenuate it when given with a calcium channel blocker (Di Lullo and Martin-Iverson, submitted). This indicates that pimozide's unique profile is likely due to its combined DA D_2 receptor antagonism and calcium channel blockade. The unique profile of pimozide in this behavioral procedure indicates that the rather large number of behavioral studies utilizing pimozide to draw conclusions concerning the role of DA D_2 in mediating behaviors should be re-evaluated.

In addition to the evidence against a DA D_2 receptor-mediated postsynaptic process being conditioned with AMP, there is evidence against involvement of postsynaptic D_1 receptors, alone or in combination with D_2 receptors (Martin-Iverson and McManus, 1990). Furthermore, there is evidence against the view that some presynaptic DA-related process is conditioned after repeated pairings of AMP with a unique environment. αMPT, a catecholamine synthesis inhibitor, co-administered with AMP during environment-specific

conditioning to inhibit the AMP-induced release of DA, significantly attenuated AMP-induced locomotion and defecation, but did not block either AMP-conditioned locomotion or defecation (DiLullo and Martin-Iverson, in press).

Recently, Hiroi and White (1990) blocked the expression of AMP-conditioned place-preferences using microinjections of reserpine (RES) into the nucleus accumbens. The possible role of DA from granular stores, which may be released via a calcium-dependent exocytotic mechanism, in AMP-conditioned behaviors fits logically with a recent finding by DiLullo and Martin-Iverson (submitted). Neither HAL nor NIM (an impulse-dependent L-type calcium channel blocker) influenced AMP-conditioned locomotion when given independently. However, in conjunction with HAL, NIM attenuated AMP-conditioned locomotion, an effect similar to that seen with pimozide, a drug which combines DA D_2 receptor antagonism with calcium channel blockade. This suggests that behaviors conditioned with AMP are dependent at least in part on an impulse-dependent calcium-mediated mechanism, which could be exocytotic DA release from a RES-sensitive pool.

The objective of this study was to examine the roles of the αMPT-sensitive and the RES-sensitive DA pools in AMP-conditioned and unconditioned behaviors in the rat. This was accomplished by measuring the effects of these agents *given during the conditioning process* on AMP-induced conditioned locomotion.

4.2. MATERIALS AND METHODS

4.2.1. Animals and Drugs

Experimentally naive male Sprague-Dawley rats (n=10-12 for each group) weighing 250-300 g at the start of the experiment were maintained on a 12:12 h light cycle (lights on 0700-1900) with *ad libitum* access to food and water. Locomotor activity (infrared

photobeam interruptions) was measured in conditioning boxes following a procedure described previously (Martin-Iverson and McManus, 1990; DiLullo and Martin-Iverson, in press). AMP sulfate, provided courtesy of SmithKline Beecham Pharma, and αMPT, purchased from Sigma, were dissolved with double-distilled water into 1.5 mg/ml and 50 mg/ml solutions, respectively. RES, purchased from Aldrich, was dissolved using a few drops of glacial acetic acid, and then diluted with a 50 mM glucose solution into a 2.5 or 1.25 mg/ml solution and the pH was adjusted to 4.0 with 10 N sodium hydroxide. All drug weights are expressed as salts. Doses of the drugs used were chosen on the basis of the literature. The dose of AMP was chosen to produce optimal photobeam interruptions over the course of several days and to elicit conditioned locomotion (Martin-Iverson and McManus, 1990; DiLullo and Martin-Iverson; in press). The dose of αMPT was chosen to block catecholamine synthesis, based on acute effects (Scheel-Kruger, 1971). The 170 min time-interval between αMPT and AMP administration was chosen on the basis of previous experience in this laboratory with αMPT during AMP-conditioning (DiLullo and Martin-Iverson, in press) and the literature (Scheel-Kruger, 1971; Braestrup, 1977) which indicates that although maximal inhibition of tyrosine hydroxylase occurs 30 min after αMPT administration (Braestrup, 1977), the psychomotor stimulant effects of AMP are maximally reduced after a longer interval. The dose of RES was chosen to deplete the granular stores of DA (Martin-Iverson et al., submitted).

4.2.2. Procedures

The experiment consisted of two phases: a drug conditioning phase and a drug-free test phase. During the drug conditioning phase (lasting 8 consecutive days) the rats received three daily injections (VEH_R or RES, VEH_α or αMPT and VEH_A or AMP) after which they were placed into the conditioning boxes for 60 min. The test day occurred on the sixth day

after the last conditioning-day injection to allow for drug clearance and recovery from DA depletions. On this day, all animals received two consecutive injections of VEH (VEH_R, i.p. and VEH_A, s.c.) prior to placement for 60 min into the conditioning boxes.

There were 8 treatment groups with each animal receiving three treatments daily. The dosing schedule for RES was quite complex because the repeated injections produced marked aphagia, adipsia and akinesia, especially in those rats co-administered αMPT. RES or VEH_R (i.p.) was administered 24 h before being placed into the conditioning boxes. For all rats given RES, this drug was given at a dose of 2.5 mg/kg for the first 4 days (Day 0-3), and 1.25 mg/kg for Days 4, 5, 7 and 8. VEH_R was injected in all groups on Day 6, due to the marked effects of the prior RES treatments. The criterion whereby the dose of RES was reduced was a decrease in body weight to 85% of its pre-experimental weight; when this occurred on Day 4 for all but 2 RES-treated rats, the dose of RES was reduced by half for all rats. On Day 6, a number of animals' weights dropped to 80% of pre-experimental weights, so the dose of RES for all rats was skipped for this one day. αMPT (50 mg/kg, s.c.) or VEH_α (s.c.) was injected 180 min before being placed into the conditioning boxes and AMP (1.5 mg/kg, s.c.) or VEH_A (s.c.) was injected 10 min before being placed into the conditioning boxes. The groups were as follows: VEH_R-VEH_α-VEH_A, VEH_R-VEH_α-AMP, RES-VEH_α-VEH_A, RES-VEH_α-AMP, VEH_R-αMPT-VEH_A, VEH_R-αMPT-AMP, RES-αMPT-VEH_A and RES-αMPT-AMP. Three rats (1 from the RES-αMPT-VEH_A and 2 from the RES-αMPT-AMP treatment groups) were killed during the experiment (prior to the test phase) because their weights dropped below our criteria for euthanasia. Rats exhibiting weight loss were fed wet mash to encourage feeding.

4.2.3. Statistics

Locomotor activity for each rat during both phases of the experiment was recorded in 5 min blocks and summed to produce individual daily totals. The means of the individual rat's daily totals were subjected to ANOVA with planned comparisons between individual groups. Since ANOVA is not reliable with more than two repeated measures unless there is homogeniety of variances and covariances (a condition that is seldom met due to order effects), in all cases in which repeated measures were used the data were further subjected to a variety of multivariate tests (Pillais Trace, Hotellings T test and Wilks Lambda; these tests are automatically run in cases of repeated measures by the statistical software package used). Only the results of the ANOVA are reported if they were in agreement with the multivariate tests. If consensus between ANOVA and the multivariate tests was not achieved, both results are reported.

4.3. RESULTS

The results of the conditioning phase of the experiment were subjected to ANOVA with 3 independent factors: 1) RES pretreatment, consisting of 2 levels (VEH_R or RES); 2) αMPT pretreatment, consisting of 2 levels (VEH_α or αMPT); and 3) AMP treatment, consisting of 2 levels (VEH_A or AMP). Fig. 13 represents the unconditioned locomotion by groups receiving VEH_A. On Days 3 and 4, locomotor activity in the RES pretreated groups was significantly lower than the VEH_R-VEH_α-VEH_A group, but did not differ significantly on other days. AMP significantly increased locomotion depending on the pretreatment, as can be seen in Fig. 14 (RES x AMP x DAYS: $F_{7,616} = 11.2$, $p < 0.001$: αMPT x AMP x DAYS: $F_{7,616} = 3.41$, $p < 0.01$). RES significantly decreased AMP-induced locomotion on Days 1-3, but augmented AMP-induced locomotion on Days 6-8. Although αMPT treatment alone completely blocked AMP-induced locomotion throughout the 8

treatment days, αMPT in conjunction with RES blocked AMP-induced locomotion only for the first 4 days, after which locomotion recovered to levels commiserate with those of the VEH_R-VEH_α-AMP group. These results indicate that AMP-induced locomotion is augmented by repeated RES and blocked by repeated αMPT, and that the αMPT blockade can be reversed with repeated RES treatments.

The test phase of the experiment was subjected to ANOVA with the same independent factors as in the conditioning phase. Fig. 15 displays the conditioned locomotor activity induced by exposure to the boxes after 2 placebo injections. All rats previously treated with AMP, except the group treated with both RES and αMPT, displayed AMP-conditioned locomotion, relative to the VEH_R-VEHα-VEH_A control group. Overall, RES pretreatment decreased locomotion on this day (main effect: $F_{1,85}=9.5$, $p<0.005$) but did not influence AMP-conditioned locomotion. There was a significant interaction between αMPT and AMP ($F_{1,85}=9.1$, $p<0.003$). αMPT significantly decreased (but did not block) AMP-conditioned locomotion. AMP-conditioned locomotion was blocked completely by combined RES and αMPT treatment during conditioning.

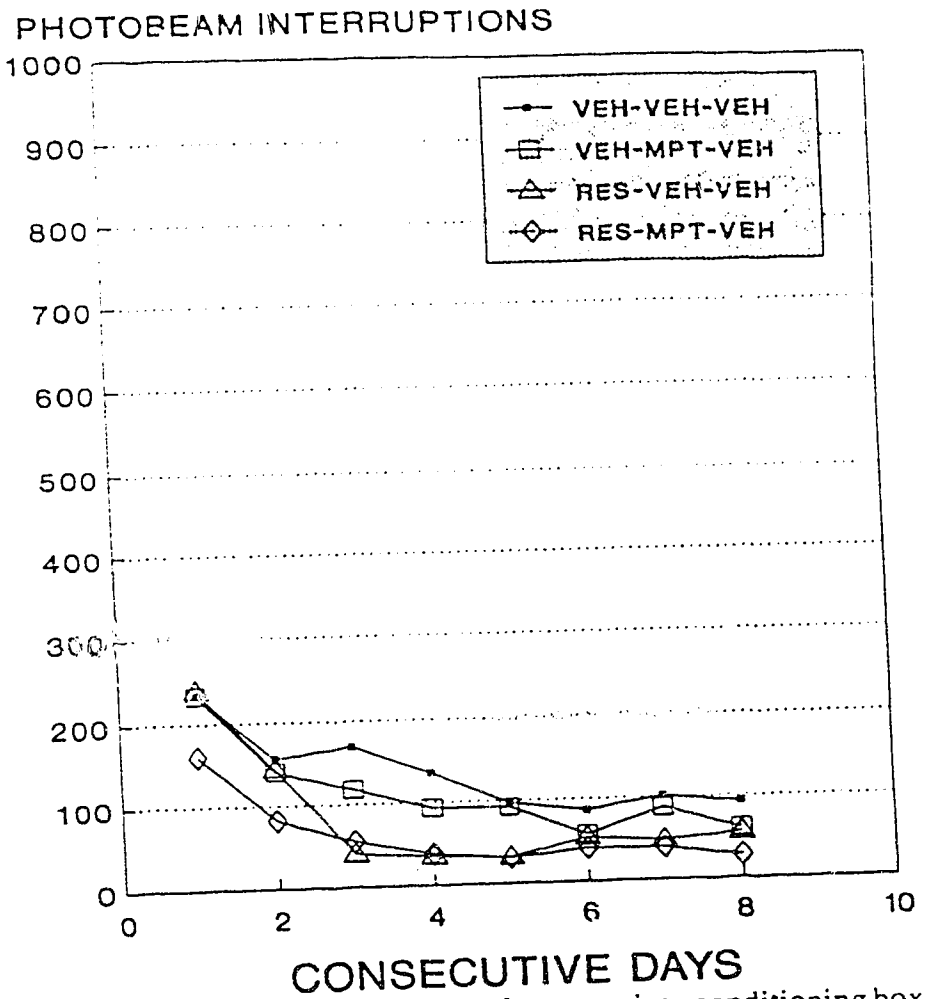

Fig. 13: The effects of daily injections before placement into conditioning boxes of VEH_R or RES (2.5-1.25 mg/kg, i.p.), VEH_α or αMPT (MPT, 50 mg/kg, s.c.) and VEH_A (1.0 ml/kg, s.c.) on locomotor activity (photobeam interruptions) over 8 consecutive days of 60 min periods. The critical difference between groups (planned comparisons, $p < 0.05$) is 100 photobeam interruptions.

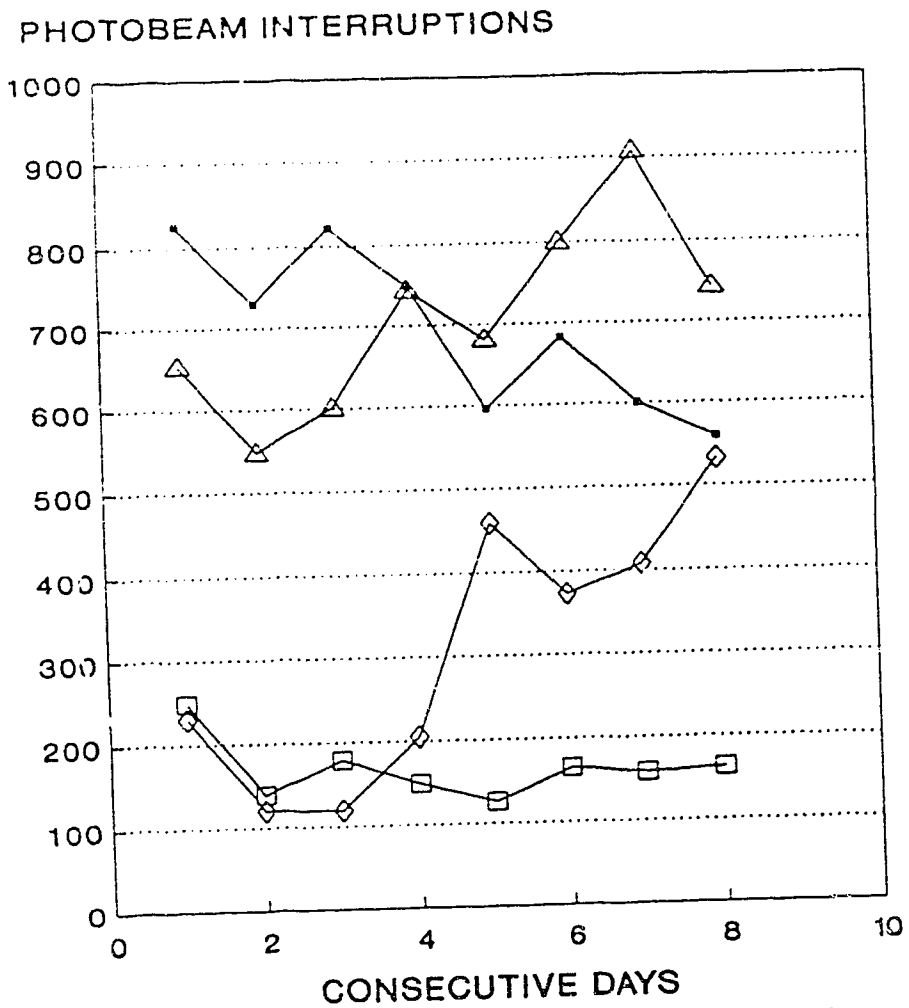

Fig. 14: The effects of daily injections before placement into conditioning boxes of VEH_R or RES (2.5-1.25 mg/kg, i.p.), VEH_α or αMPT (MPT, 50 mg/kg, s.c.) and AMP (1.5 mg/kg, s.c.) on locomotor activity (photobeam interruptions) over 8 consecutive days of 60 min periods. αMPT blocked AMP-induced locomotion. RES significantly augmented AMP-induced locomotion. The critical difference between groups (planned comparisons, $P < 0.05$) is 100 photobeam interruptions. Small boxes represent the VEH-VEH-AMP group, large boxes represent the VEH-MPT-AMP group, triangles represent the RES-VEH-AMP group and diamonds represent the RES-MPT-AMP group.

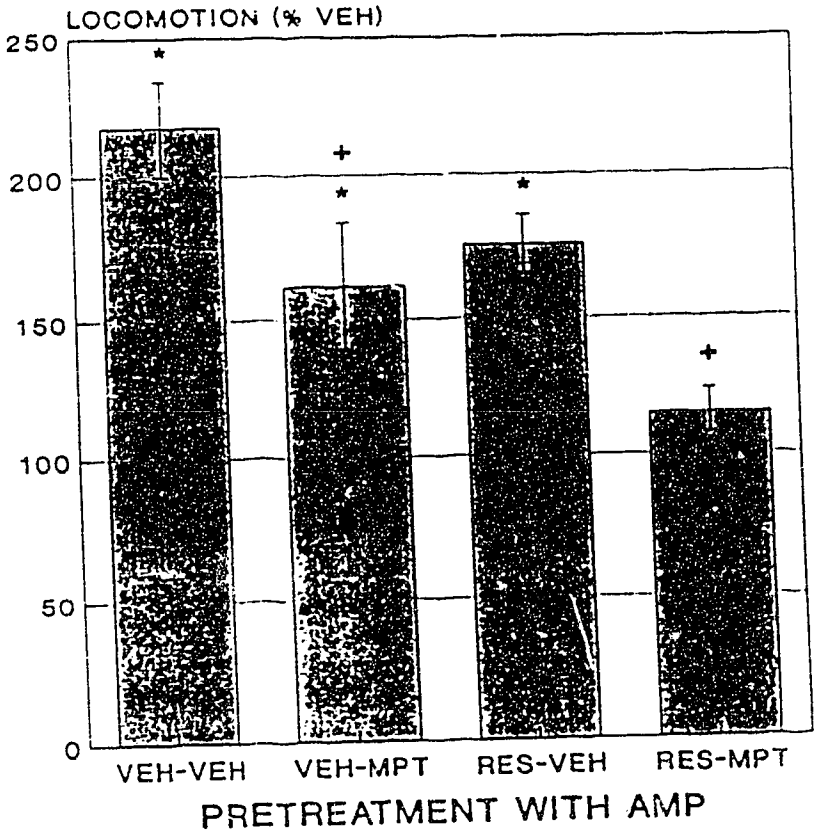

Fig. 15: The effects of 2 placebo injections to the same groups of rats as in Figs. 13 and 14, after a 5 day drug-free rest, on conditioned locomotion (photobeam interruptions) during a 60 min period. αMPT (MPT) attenuated AMP-conditioned locomotion. Combined αMPT and RES treatment blocked AMP-conditioned locomotion. Error bars represent the standard error of the mean.

* Significantly different from the VEH_R-VEH_α-VEH_A control group, $p<0.001$.

+ Significantly different from the VEH_R-VEH_α-AMP group, $p<0.003$.

4.4. DISCUSSION

Several investigations have demonstrated that the psychomotor stimulant effects of AMP-like compounds are mediated by the αMPT-sensitive pool of newly synthesized DA (Weissman *et al.*, 1966; Braestrup, 1977; McMillan. 1983; Finn *et al.*, 1990; DiLullo and Martin-Iverson, in press), potentiated by depletion of the RES-sensitive granular storage pools of DA (Weissman *et al.*, 1966; Stolk and Rech, 1968; Scheel-Kruger, 1971; McMillan, 1983) and can be conditioned to a unique environment in which the drugs are administered (Pickens and Crowder, 1967; Beninger and Hahn, 1983; Beninger and Herz, 1986; Poncelet *et al.*, 1987; Martin-Iverson and McManus, 1990, DiLullo and Martin-Iverson, in press). This experiment verified these observations: catecholamine synthesis inhibition with αMPT over 8 days completely blocked AMP-induced locomotion, AMP-induced locomotion was ultimately potentiated by repeated RES injections, and repeated AMP injections prior to placement in a unique environment produced the ability of the environment itself to induce AMP-like effects. The results of a previous report that blockade of AMP-induced locomotion with αMPT failed to block AMP-conditioned locomotion (DiLullo and Martin-Iverson, in press), was also replicated. In addition, two novel observations were made. RES failed to block AMP-conditioned locomotion, but did block AMP-conditioned locomotion when combined with αMPT. This latter finding is especially interesting since the combination of RES and αMPT did not block the direct locomotor effects of AMP on the last 4 days of treatment.

Depletion of granular storage pools of DA with repeated RES injections initially decreased but ultimately potentiated AMP-induced locomotion. This biphasic action may be due to the dose of AMP chosen; maximal locomotion is found with this dose in this laboratory such that initial potentiation of AMP's effects may produce stereotyped licking

or sniffing in one location at the expense of locomotion (Fray *et al.*, 1980). The emergence of a potentiation of AMP-induced locomotion may reflect a tolerance to some of reserpine's effects. A similar mechanism may explain the gradual decrease in the effect of AMP-induced locomotion. Daily injections produce a gradual augmentation of the effects of AMP (Robinson and Becker, 1986); since a maximal locomotor dose was used, the observed decrease in locomotion over days may represent the emergence of stereotyped behaviors.

AMP injections paired with exposure to a unique environment resulted in an AMP-like locomotor response when rats were placed into the environment without AMP treatment. Although a pseudo-conditioning control was not included in this report, previous work in this laboratory following the same AMP-conditioning procedures but with pseudo-conditioning controls, determined that the AMP-like response is unique to the testing environment. Rats which receive AMP while in their home cages 3 h after conditioning trials do not exhibit increases of locomotion when exposed to the conditioning boxes (Martin-Iverson and McManus, 1990). The present results are therefore not likely a function of an unconditioned spontaneous increase in activity due to previous AMP treatment.

There are at least two pharmacologically distinct DA pools. One pool is quickly depleted by αMPT, due to its rapid turnover rate. This 'αMPT-sensitive' pool of DA is mobilized and released independently of calcium (Tecott *et al.*, 1986) by the AMP class of stimulants (Braestrup, 1977; McMillan, 1983). The psychomotor stimulant effects of AMP have been demonstrated by several investigators to be blocked by αMPT (Stolk and Rech, 1970; Scheel-Kruger, 1971; DiLullo and Martin-Iverson, in press). The second pool of DA is depleted by reserpine (RES) and its impulse-dependent release is potentiated by the methylphenidate class (e.g. methylphenidate, cocaine, nomifensine, amfonelic acid) of stimulants (Braestrup, 1977; McMillan, 1983), probably via calcium-dependent exocytosis

(Shore, 1976). The psychomotor stimulant effects of non-AMP stimulants are blocked by RES. Rats administered RES prior to AMP show an enhanced behavioral response to AMP (Stolk and Rech, 1968). The potentiation of AMP by RES may be attributed to supersensitivity of postsynaptic DA receptors, the re-routing of DA that normally fills RES-sensitive vesicles into the AMP-releasable pool (Martin-Iverson *et al.*, submitted), and/or alteration in agonist-sensitivity of post-synaptic receptors as a function of history of receptor occupancy (Clark *et al.*, 1985a, b).

Previous work has shown that DA D_1 and D_2 receptor antagonists (Martin-Iverson and McManus, 1990) and αMPT (DiLullo and Martin-Iverson, in press) block the direct behaviorial effects of AMP, but fail to block AMP-conditioned locomotion. This indicates that neither post-synaptic events nor the direct pre-synaptic actions of AMP are responsible for the classical conditioning of AMP-induced locomotion. However, recent findings have provided evidence for a synergistic role of blockade of impulse-dependent calcium channels with DA D_2 receptor antagonism in AMP-conditioned locomotion (Di Lullo and Martin-Iverson, submitted). In addition, it has been reported that while αMPT fails to block AMP-conditioned place preferences, RES injected directly into the nucleus accumbens blocks this conditioned preference (Hiroi and White, 1990). These observations led us to hypothesize that the critical event conditioned with AMP is the impulse-dependent release of DA from presynaptic granular storage pools or a synergistic action between the αMPT- and RES-sensitive pools of DA in stimulant-conditioning. These two possibilities were tested in the present experiment.

αMPT attenuated (but did not completely block) the establishment of AMP-conditioned locomotion. This observation is somewhat different from that in an earlier report from this laboratory using the same dose of αMPT during AMP-conditioning in which

no attenuation was observed (DiLullo and Martin-Iverson, in press). In the present case, this dose of αMPT completely blocked unconditioned locomotion, while in the previous report the blockade of direct AMP-induced locomotion was not complete, due largely to some recovery of AMP effects on the last 2 days of the 10 treatment days. In the present experiment, conditioning occurred over only 8 days, and no recovery was apparent. Thus, when AMP-induced locomotion was completely blocked some attenuation of conditioned-locomotion occurred.

RES treatment alone did not selectively affect AMP-conditioned behaviors. However, in combination with αMPT, RES completely blocked the establishment of AMP-conditioned locomotion. This blockade occurred in spite of the recovery of AMP-induced locomotion over the last 4 days in this treatment group. Previous publications have shown intact conditioning with blockade of the direct effects of AMP (Martin-Iverson and McManus, 1990; DiLullo and Martin-Iverson, in press). In the present case, direct stimulant effects of AMP were observed in the absence of conditioned effects. These results suggest that the psychomotor stimulant effects induced by AMP are expressed when DA from the αMPT-sensitive pool is released and that the establishment of AMP-conditioned behaviors is mediated by the RES- and αMPT-sensitive pools of DA. Blockade of one of these pools of DA is not sufficient to block the establishment of stimulant-conditioning.

RES disrupts vesicles containing noradrenaline (NA) and 5-hydroxytryptamine (5-HT), as well as those containing DA. It is possible therefore that the blockade of conditioning is dependent upon actions on one of of these neurotransmitter systems. Since destruction of forebrain NA-containing terminals with the neurotoxin, DSP-4, does not affect the establishment of AMP-conditioned locomotion (DiLullo and Martin-Iverson, in press), it is unlikely that the effects of combined treatment with αMPT and reserpine are mediated

by actions on NA systems. However, effects on 5-HT cannot presently be ruled out. Decreases in 5-HT generally augment the behavioral effects of AMP (Martin-Iverson *et al.*, 1983); the possibility that RES-induced depletion of 5-HT mediates the blockade of AMP-conditioned locomotion is not consistent with this observation. On the other hand, destruction of DA-containing terminals with 6-OHDA has been reported to block AMP-conditioned locomotion (Gold *et al.*, 1988). It is therefore most likely that the blockade of AMP-conditioned locomotion with combined treatment with αMPT and RES is a function of effects on DA-containing neuronal terminals.

One question that remains is whether or not the conditioned effects mediated by the RES-sensitive pool are impulse-dependent. The observation that an impulse-dependent calcium channel blocker, nimodipine, in combination with a DA D_2 receptor antagonist, haloperidol, attenuates AMP-conditioned locomotion (Di Lullo and Martin-Iverson, submitted) indicates that this may be true, since this compound should block impulse-dependent DA release.

To summarize, neither αMPT nor RES given independently during the conditioning of AMP's locomotor effects to environmental stimuli completely blocked the establishment of AMP-conditioned locomotion. However, the two drugs given together did block the establishment of AMP-conditioned locomotion. Together with other evidence, this observation indicates that AMP effects on either newly-synthesized DA or on vesicular stores of DA are sufficient for conditioning of AMP's effects. Both processes must be blocked to block conditioning.

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CHAPTER 5. GENERAL DISCUSSION

The primary behavioral effects (Pickens and Crowder, 1967; Schiff, 1982; Beninger and Hahn, 1983; Beninger and Herz, 1986; Gold *et al.*, 1988; Hiroi and White, 1989; Carey, 1990; Drew and Glick, 1990; Martin-Iverson and McManus, 1990) and the reinforcing effects (Spyraki *et al.*, 1982a, 1982b; Martin-Iverson *et al.*, 1985; Mithani *et al.*, 1986; Hiroi and White, 1990)) of psychomotor stimulants can be conditioned to a unique environment with which the drug effects have been repeatedly associated. This thesis investigates the possibility that a presynaptic mechanism mediates the establishment of environment-specific stimulant-induced conditioning. As conditioning has been suggested to have a role in the initiation and maintenance of stimulant addiction, the results of the investigations in this thesis may provide the practitioner with an alternative treatment to relatively ineffective drug-detoxification and drug-rehabilitation programs that are currently available for the treatment of stimulant addiction.

It has been demonstrated that actions on DA receptors are not conditioned with AMP to produce conditioned behaviors (Mithani *et al.*, 1986; Carey, 1990; Martin-Iverson and McManus, 1990). The possibility that presynaptic DA or NA release is the neural event conditioned with repeated intermittent AMP administration to a unique environment was examined in the first of three investigations in this thesis. AMP-induced locomotor stimulation is a consequence of AMP-induced DA release (Scheel-Kruger, 1971; Braestrup, 1977; Finn *et al.*, 1990; Hiroi and White, 1990) from a compartment of newly-synthesized DA (Weissman *et al.*, 1966; Scheel-Kruger, 1971; Braestrup, 1977; McMillan, 1983; Finn *et al.*, 1990). Both locomotion and DA release produced by AMP can be blocked by synthesis inhibition with αMPT. Therefore, αMPT was administered during the conditioning process with AMP to determine if catecholamine release from newly synthesized pools is also

required to produce the conditioned response. αMPT has been reported to produce maximal synthesis inhibition of catecholamines within 30 min of administration (Braestrup, 1977). Two different doses of αMPT (25 and 50 mg/kg), administered 30 min prior to AMP for 10 consecutive days, blocked the direct effects of AMP (locomotion and defecation) independent of dose. However, neither dose of αMPT blocked the establishment of AMP-conditioned locomotion or defecation.

Reports that maximal blockade of the behavioral effects of AMP by αMPT requires a longer time interval between αMPT and AMP administration (Scheel-Kruger, 1971; Braestrup, 1977) indicated the possibility that a longer time interval between αMPT and AMP administration during the conditioning procedures might be required to interfere with the conditioning process. αMPT (50 mg/kg) administered 170 min prior to AMP produced a more complete block of the direct effects of AMP during conditioning but did not attenuate AMP-conditioned locomotion or defecation.

Since αMPT inhibits the synthesis of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of both DA and NA, and since there is some evidence that NA may be involved in AMP-unconditioned (Ogren *et al.*, 1983) and conditioned locomotion (Poncelet *et al.*, 1987), the role of presynaptic NA release in the unconditioned and conditioned locomotor effects of AMP was investigated. Rats were treated with DSP4, a neurotoxin that selectively depletes forebrain NA, 7 days prior to conditioning with AMP. DSP4 depleted NA from forebrain regions to between 1and 14 percent of controls but had no effect on either the direct locomotor effects or on conditioned locomotion produced by AMP.

The conclusion drawn from this set of experiments was that neither presynaptic DA nor presynaptic NA release are conditioned with AMP to produce conditioned behavioral responses. In addition it was concluded that [illegible] and not NA, released from αMPT-sensitive pools, mediates the direct psychomotor stimulant effects of AMP.

A second investigation examined the possibility that pimozide's ability to block the establishment of stimulant-conditioned responses (Beninger and Hahn, 1983; Beninger and Herz, 1986; Hiroi and White, 1989) is due to its unique profile as an antagonist of DA D_2 receptors and impulse-dependent L-type calcium channels. NIM, a dihydropyridine which blocks impulse-dependent L-type calcium channels, was given in combination with or separate from HAL, a selective DA D_2 receptor antagonist, for 10 consecutive days during conditioning with AMP. NIM had no effect when given alone on AMP-induced locomotion, but augmented the decrease of AMP-induced locomotion produced by HAL. Neither HAL nor NIM, when given independently, influenced AMP-conditioned locomotor activity. AMP-conditioned locomotion was significantly attenuated with combined HAL and NIM pretreatment. This effect did not appear to be dose-dependent on HAL; the dose of HAL pretreatment when increased from 0.05 to 0.2 mg/kg did not further augment the attenuation of the AMP-conditioned response.

The ability of combined DA D_2 receptor antagonism and calcium channel antagonism to attenuate the establishment of the AMP-conditioned response indicates that pimozide's ability to block the establishment of AMP-conditioned locomotion (Beninger and Hahn, 1983) is likely due to its equipotent ability to block calcium channels and DA D_2 receptors. In addition, this observation suggests that calcium channel blockade may provide an adjunctive treatment with neuroleptics for the treatment of cocaine addiction and schizophrenia. NIM's ability to reduce AMP-conditioned locomotor activity in conjunction

with a low dose of HAL indicates th[illegible] dose-range of neuroleptics for schizophrenia, and may alleviate conditioned drug craving in addicts without a high risk of developing extrapyramidal side effects and tardive dyskinesia

A third investigation examined the roles of the αMPT-sensitive and the RES-sensitive DA pools in the direct behavioral effects of AMP and in the establishment of AMP-conditioned locomotor activation. This was accomplished by measuring the separate and combined effects of αMPT and RES treatment on AMP-induced locomotor activity during 8 consecutive treatment days and on AMP-conditioned locomotor activity apparent on a drug-free test day. αMPT treatment alone completely blocked AMP-induced locomotion and attenuated AMP-conditioned locomotion. The observation that αMPT attenuated the establishment of AMP-conditioned locomotion is different from that in the first investigation using the same dose and treatment interval (50 mg/kg, administered 170 min prior to AMP during the conditioning phase of the experiment). In the previous report, blockade of AMP-induced locomotion was not complete. Some recovery of AMP-induced locomotion was apparent in the last 2 days of AMP treatment. In the present experiment αMPT completely blocked AMP-induced locomotion without apparent recovery, probably due to the decreased days of AMP treatment. This may explain differences in the degree of AMP-conditioned locomotion exhibited by the αMPT-treated groups.

RES treatment alone potentiated AMP-induced locomotor activity during the last 3 days of treatment and had no effect on AMP-conditioned locomotion. RES treatment in conjunction with αMPT treatment blocked AMP-induced locomotion for the first 4 days of conditioning only, with full recovery of locomotion by the last treatment day. Combined RES and αMPT treatment completely blocked AMP-conditioned locomotion.

induced by AMP was blocked with αMPT, the acquisition of the AMP-conditioned response was unaffected. In the third investigation, the direct stimulant effects of AMP were not blocked by concomitant treatment with αMPT and RES, but the AMP-conditioned response was completely blocked. αMPT alone completely blocked the direct effects of AMP, but only slightly attenuated conditioned locomotion. This double-dissociation suggests that the direct effects and the conditioned effects of psychomotor stimulants are mediated by different neural processes. On the other hand, the ultimate recovery of the unconditioned AMP-induced locomotor response during treatment with αMPT and RES may be a consequence of RES-induced augmentation of the AMP response, which was only partially blocked by αMPT.

The results from these three investigations support earlier studies which suggest psychomotor stimulant effects induced by AMP are expressed when DA from the αMPT-sensitive pool is released (Weissman *et al.*, 1966; Stolk and Rech, 1970; Scheel-Kruger, 1971; Chiueh and Moore 1974; McMillen, 1974; Braestrup, 1977), probably via an AMP-activated carrier mechanism which exchanges AMP for DA across the presynaptic membrane (Westerink *et al.*, 1989). Most importantly these studies suggest that the establishment of AMP-conditioned behaviors is mediated by conditioned DA release from both the αMPT-sensitive pool and the RES-sensitive pool. It appears that DA release from *both* compartments must be blocked during AMP-conditioning to block the establishment of conditioned DA release, and the subsequent conditioned increase in locomotor activity.

The results in this thesis suggest that there are two presynaptic processes which mediate the establishment of AMP-conditioned behaviors. One of the processes is suggested to

involve the presynaptic αMPT-sensitive pool of catecholamines, the other process is suggested to involve the presynaptic RES-sensitive pool of amines. In addition, these experiments have demonstrated that if any one of these processes is blocked during the administration of AMP, the establishment of the conditioned response will be at most only slightly attenuated. Complete blockade of the conditioned response requires inhibition of both processes.

Based on the observations in these and other investigations, DA is likely the neurotransmitter which mediates both the αMPT and the RES-sensitive presynaptic processes in the establishment of AMP-conditioned behaviors. αMPT, a catecholamine (DA, NA and adrenaline) synthesis inhibitor was shown to block stimulant-conditioned behaviors, providing it was co-administered with RES. This effect is likely not a function of NA depletions since selective noradrenergic forebrain depletions, with DSP4, have no affect on the establishment of conditioned locomotion. Other investigators similarly argue against a role of NA in AMP-conditioned responses. Rats with selective 6-OHDA depletions of dopaminergic terminals (Spyraki *et al.*, 1982b) show an attenuation of the AMP-conditioned response. In addition, the selective NA uptake inhibitor desipramine fails to induce conditioned place preferences (Martin-Iverson et al., 1985), which also suggests that NA does not mediate the conditioned response. However, the possibility that NA depletions in conjunction with some other treatment (e.g. 5-HT depletions) would block conditioning cannot presently be ruled out.

Although there are significant depletions of NA and 5-HT, as well as of DA, by RES (Radouco-Thomas *et al.*, 1971; Finn *et al.*, 1990), it is unlikely that actions on noradrenergic and serotonergic transmitter systems mediate conditioning. Destruction of forebrain NA-containing terminals with DSP4 does not affect the establishment of AMP-conditioned

locomotion and decreases in 5-HT generally augment the behavioral effects of AMP (Martin-Iverson *et al.*, 1983). Effects on 5-HT cannot be ruled out however. Recall that pimozide has equipotent antagonistic effects on DA D_2 receptors, calcium channels and 5-HT_2 receptors. Its ability to block the establishment of stimulant-conditioned behaviors may be due to any combination of these effects. To be absolutely certain that dopaminergic processes from both the αMPT and the RES-sensitive pools mediate the establishment of stimulant-conditioning other permutations using other neurotransmitter antagonists should be tested. For example the effect of concomitant blockade of the αMPT-sensitive pool of catecholamines and granular 5-HT_2 receptor antagonists on AMP-conditioned locomotion would be interesting.

The observation that selective DA D_2 receptor antagonism with concomitant blockade of calcium channels supports the view that DA plays a critical, albeit not necessarily an exclusive, role in AMP-conditioning.

One question which remains unclear is whether or not conditioned DA release from the RES-sensitive DA pool is both calcium and impulse dependent. In the second investigation, the observation that NIM in combination with HAL attenuates AMP-conditioned locomotion, indicates that this may be true. There is, however, a third type of DA release which is calcium-dependent and impulse-independent (Westerink *et al.*, 1989).

It appears that the ability by DA D_2 antagonists to block the establishment of stimulant-conditioned locomotion (Beninger and Hahn, 1983; Beninger and Herz, 1986; Hiroi and White, 1989; Mithani *et al.*, 1986; Carey, 1990; Martin-Iverson and McManus, 1990;) and the inability of DA D_2 antagonists to block the expression of stimulant-conditioned behaviors (Schiff, 1982; Beninger and Hahn, 1983; Beninger and

Herz, 1986; Miyamoto and Hada, 1987; Poncelet *et al.*, 1987; Drew and Glick, 1990) are specific to pimozide. The discrepency between the effects of pimozide and other DA D_2 antagonists are likely due to the unique pharmacological profile of pimozide. Pimozide is a diphenylbutylpiperidine neuroleptic which selectively and equipotently blocks DA D_2 receptors (Cohen *et al.*, 1986; Tecott *et al.*, 1986; Enyeart *et al.*, 1987b), impulse-dependent L-type calcium channels (Cohen *et al.*, 1986; Tecott *et al.*, 1986; Enyeart *et al.*, 1987b) and 5-HT_2 receptors (Cohen *et al.*, 1986; Tecott *et al.*, 1986).

The observation in the second investigation that pretreatment with NIM and HAL attenuates the establishment of AMP-conditioned locomotion supports the hypothesis that the unique ability of pimozide to block the establishment of stimulant-conditioned behaviors is due to its synergistic effect on DA D_2 receptors and calcium channels.

As alluded to earlier, a higher dose of HAL does not further augment the attenuation of AMP-conditioned locomotion produced by NIM and HAL pretreatment. What remains to be seen is if this response is dose-dependent for NIM. It has been reported that the dose-response curve for NIM resembles an inverted-u (Kurtz, 1990). As such the effect of both higher and lower doses of NIM in combination with a low dose of HAL on the establishment of the AMP-conditioned response should be investigated.

Pseudo-conditioning refers to the possibility that the unconditioned response to the unconditioned stimulus may come to be elicited by other stimuli in spite of the absence of any association between them (Mackintosh, 1974). With this possibility in mind, experimenters studying environment-specific classical conditioning have routinely used a pseudo-conditioning home cage control to ensure that any change in the behavior of an experimental group, exposed to paired presentations of conditioned environmental stimuli and unconditioned stimuli, can be unambiguously attributed to the formation of an

association between the two. In addition, there is a possibility that repeated treaments with AMP may increase subsequent spontaneous locomotion. None of the investigations presented in this thesis utilized pseudo-conditioning home cage controls. There are two reasons for this: 1) other investigators in this laboratory using the identical treatment procedures and conditioning and testing apparatus have demonstrated that the environment-conditioned response to AMP does not occur in pseudo-conditioned home cage control groups (Martin-Iverson and McManus, 1990); and 2) psuedo-conditioning home control groups would necessitate either reducing the number of rats in each of the treatment groups by half or doubling the number of rats in each of these investigations. The disadvantages of this are two-fold. Reducing the number of animals per treatment group reduces the overall reliability of the experiment. Doubling the total number of animals in the experiment for unnecessary control purposes is unethical and costly.

5.1. CONCLUSIONS

The results of the investigations in this thesis indicate that there are two presynaptic processes involved in the establishment of AMP-conditioned behaviors. One of the processes can be blocked with αMPT and the other process can be blocked with RES. Either one of these processes is sufficient for the acquisition of the AMP-conditioned response but not necessary; *both* of these processes must be blocked to block the establishment of the conditioned response. Furthermore, AMP-conditioned locomotion can be attenuated with concomitant treatment with a DA D_2 receptor antagonist and a calcium channel antagonist. It is likely that the calcium channel antagonist affects the same process as RES, and that the DA D_2 receptor antagonist affects the same process as αMPT, but on the other side of the synapse. Based on the observations in this thesis and in other investigations, it is likely that the neurotransmitter which mediates both of these processes is DA.

5.2. CLINICAL IMPLICATIONS

Environment-specific stimulant conditioning appears to contribute to the maintenance of stimulant addiction. Former cocaine addicts continue to exhibit strong 'cravings' and drug-like physiological responses when presented with drug-related cues (O'Brien *et al.*, 1988; Muntaner *et al.*, 1989). These apparent conditioned responses are not affected by usual drug-detoxification or drug-rehabilitation programs, nor do they appear to be affected by new behavioral treatment approaches designed to extinguish the link between drug-associated cues and euphoria produced by cocaine and AMP-like compounds (O'Brien *et al.*, 1988). Though conditioned stimulant-cravings and physiological responses are apparently extinguishable in the protective environment of the treatment center, success rates for stimulant abstinence, upon reentry into their own environments, remain low. Low success rates are likely related to the inability of the extinction procedures used in the protective hospital settings to extinguish social cues (eg. friends) inherent in the individual patient's environment. Blockade of the neuronal mechanism of conditioned-craving alone or in combination with behavioral extinction procedures will likely provide a more effective treatment for stimulant addiction. Without medicinal efforts the extinction phase in the treatment of stimulant-addiction appears to be life-long (Gawin, 1988; O'Brien *et al.*, 1988) and relapse is probable. The precise mechanism which mediates conditioned drug cravings and physiological arousal produced by drug-related cues remains unclear although evidence from this thesis suggests that αMPT and RES sensitive DA release or some other αMPT and RES sensitive presynaptic processes may be involved.

The effects of pharmacological treatments during the stimulant-environment pairing procedures, on the 'establishment' of conditioned responses elicited by drug-related stimuli, may have a direct relationship to the maintenance of stimulant addiction and potential drug

therapy for out-patients. It is apparent that drugs which block the reinforcing effects of stimulants in naive nonhuman primates (Wilson and Schust.., 1972; Bergman *et al.*, 1990) do not block the euphoric effects in "experienced" drug users (Gawin, 1986; Sherer, 1988). This implies that a treatment is required which will block both the unconditioned and the conditioned euphoria produced by stimulants. This way the addict will experience neither type of euphoria. The effects of pharmacological treatments on the 'expression' of pre-established conditioned responses to drug-related cues, may have a direct relationship to drug-cravings experienced during withdrawal and potential drug therapy during detoxification. If the expression of conditioned drug-cravings can be blocked then detoxification should be more efficacious.

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