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Role of the Dopamine D₁-like receptor in amphetamine-induced behavioral sensitization: A study using Dopamine D₁A-receptor deficient mice

Patrick Eugene Karper

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ROLE OF THE DOPAMINE D_{1}-LIKE RECEPTOR IN AMPHETAMINE-INDUCED
BEHAVIORAL SENSITIZATION: A STUDY USING DOPAMINE D_{1A}-
RECEPTOR DEFICIENT MICE

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts
in
Psychology

by
Patrick Eugene Karper
September 2000
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Approved by:

Sanders A. McDougall, Chair, Psychology
Cynthia A. Crawford, Psychology
Jeffrey D. Thompson, Biology

Date
5/29/00
ABSTRACT

The ability of the indirect dopamine agonist, amphetamine, to produce behavioral sensitization was assessed in adult D_{1A}-deficient and wild-type mice. It was originally predicted that: 1) dopamine (DA) D_{1}-like receptors are necessary for the occurrence of short- and long-term amphetamine-induced behavioral sensitization, 2) DA D_{1}-like receptors are necessary for environmental conditioning factors associated with amphetamine-induced behavioral sensitization, and 3) DA D_{5} receptors are required for amphetamine-induced behavioral sensitization. Locomotor activity and stereotyped sniffing were assessed in each of three experiments.

In Experiment 1, adult wild-type and D_{1A}-deficient mice were injected with amphetamine (1.0, 2.0, 4.0, or 8.0 mg/kg, i.p.) or saline for seven consecutive days and then challenged with amphetamine after three and seventeen abstinence days. In Experiment 2, wild-type and D_{1A}-deficient mice were injected with amphetamine (8.0 mg/kg, i.p.) or saline in either their home cage or testing chamber for seven consecutive days. Mice were then challenged with amphetamine (1.0 mg/kg, i.p.) after three abstinence days to determine whether the D_{1A} receptor is
necessary for the environmental conditioning factors associated with amphetamine-induced behavioral sensitization. After an additional day, all mice were challenged with saline to determine the influence of the D_{1A} receptor on conditioned activity. In Experiment 3, wild-type and D_{1A}-deficient mice were co-administered the DA D_{1}-like antagonist SCH-23390 (0.15, 0.50, or 1.5 mg/kg, i.p.) and either amphetamine (8.0 mg/kg, i.p.) or saline for seven consecutive days. Mice were then challenged with amphetamine (1.0 mg/kg, i.p.) in the testing chamber after three abstinence days to determine whether the D_{5} receptor is important for amphetamine-induced behavioral sensitization.

The results of the first experiment showed that the D_{1A}-receptor is not necessary for short- and long-term amphetamine-induced behavioral sensitization. In the second experiment, D_{1A}-deficient mice were not found to be heavily influenced by drug-paired cues (Pavlovian associations), but they did show pronounced conditioned activity when compared to wild-type controls. Results from the third experiment determined that the D_{1}-like receptor is necessary for amphetamine-induced behavioral sensitization in wild-type mice, but that neither D_{1A} nor D_{5} receptors are
required for amphetamine-induced behavioral sensitization in D_{1A}-deficient mice. When taken together, these results indicate that D_{1A}-deficient and wild-type mice are both able to express amphetamine-induced behavioral sensitization. Wild-type mice require the D_{1}-like receptor to express amphetamine-induced behavioral sensitization, whereas D_{1A}-deficient mice apparently developed a compensatory mechanism which enables them to express amphetamine-induced behavioral sensitization independent of both D_{1A} and D_{5} receptors. The nature of this compensatory mechanism is not yet understood.
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INTRODUCTION

It is now well established that dopamine (DA) has intimate ties with the basic underlying neurobiological mechanisms of drug addiction. In fact, the DA neurotransmitter system not only mediates reward, but also the locomotor activating effects of various drugs of abuse.

Several studies suggest that DA is the main neurotransmitter associated with reward and reinforced responding (Kalivas & Stewart, 1991; Nestler, 1992; Robinson & Becker, 1986; Robinson & Berridge, 1993; Wise & Bozarth, 1984). For example, various psychostimulants, such as cocaine and amphetamine, augment locomotor activity and reward by indirectly increasing DA concentrations in the synapse (Reith, Sershen, & Lajtha, 1980). This increase in DA has been shown to exert a euphoric-like effect, as well as induce other motivational factors associated with drug craving (Robinson & Berridge, 1993) and, consequently, psychostimulants are subject to abuse (Robinson & Berridge, 1993; Wise & Bozarth, 1987).

Dopamine Pathways and Input/Output Structures

Psychostimulant drugs, like amphetamine and cocaine, increase DA in the synapse by either blocking or reversing the DA reuptake pump. More specifically, cocaine
indirectly augments DA levels by blocking the DA reuptake pump, therefore enabling synaptic DA to persist in the synapse longer (Reith et al., 1980). Amphetamine reverses the reuptake pump, thus transporting newly synthesized DA into the synapse (Seiden, Sabol, & Ricuarte, 1993). As key systems regulating reward and behavioral activity, the mesolimbic and nigrostriatal DA pathways appear to be primary sites of action for psychostimulant drugs (Wise & Bozarth, 1984).

Mesolimbic Dopamine Pathway

The mesolimbic DA pathway is comprised of cell bodies in the ventral tegmental area which have axons that project from this midbrain structure to an area in the forebrain, known as the striatum (see Figure 1) (Pierce & Kalivas, 1997; Wise & Bozarth, 1987). The most anterior portion of the striatum is a structure commonly referred to as the nucleus accumbens (or ventral striatum). Fibers which originate in the ventral tegmental area and terminate in the nucleus accumbens modulate GABA-ergic efferents projecting to the prefrontal cortex and substantia nigra (Bedingfield, Calder, Thai, & Karler, 1997; Karler, Bedingfield, Thai, & Calder, 1997; Pierce & Kalivas, 1997; Smith & Bolam, 1990). Psychostimulant-induced modulation of
Figure 1. Mesolimbic Dopamine Pathway. DYN = Dynorphin; DA = Dopamine; k = k-Opioid receptors; GABA = γ-Amino Butyric Acid; EAA = Excitatory Amino Acid.
the mesolimbic DA tract has been theorized to mediate behavioral (i.e., locomotor) activity and the subjective effects of reward (Wise & Bozarth, 1987).

Evidence Indicating That Psychostimulant Drugs Affect the Mesolimbic Dopamine Pathway

DA receptors in the nucleus accumbens are stimulated by psychostimulant drugs through two mechanisms (Bjijou, Stinus, Le Moal, & Cador, 1996); Vezina, 1993; 1996). First, intra-cranial infusion of amphetamine into the ventral tegmental area produces an augmented concentration of DA in the nucleus accumbens (Vezina, 1993; 1996). Second, microinjecting cocaine or amphetamine into the nucleus accumbens produces robust behavioral activity by indirectly increasing DA levels (Delfs, Schreiber, & Kelley, 1990). Lesioning the nucleus accumbens substantially reduces behavioral activity induced by systemic amphetamine injections (Kelly & Iversen, 1976).

Since psychostimulant drugs have a high abuse potential in humans (Di Chiara, 1998; Robinson & Berridge, 1993) it is not surprising that intravenous self-administration of psychostimulant drugs has been intensely studied in animals. To this end, animals will readily self-administer amphetamine or cocaine directly into the nucleus
accumbens or the ventral tegmental area (Collins, Weeks, Cooper, Good, & Russell, 1984; Schuster & Thompson, 1969). When the mesolimbic DA system is lesioned by 6-hydroxydopamine (6-OHDA) or kainic acid, self-administration of cocaine (Pettit, Ettenberg, Bloom, & Koob, 1984; Roberts, Koob, Klonoff, & Fibiger, 1980) or amphetamine (Lyness, Friedle, & Moore, 1979) is abolished. Clearly then, DA receptor stimulation in the mesolimbic pathway mediates cocaine and amphetamine self-administration.

Nigrostriatal Dopamine Pathway

As opposed to the mesolimbic DA pathway, the nigrostriatal DA pathway primarily mediates behavioral stereotypy (Arnt, 1987). The nigrostriatal DA pathway includes two primary brain areas: the substantia nigra and the caudate/putamen (also called the dorsal striatum; see Figure 2). Specifically, DA neurons project from the substantia nigra to the caudate/putamen; Clark & White, 1987; Pierce & Kalivas, 1997). Accordingly then, DA receptors in the caudate/putamen are indirectly stimulated by psychostimulant drugs and, as a result, cause behavioral stereotypy.

Evidence Indicating That Psychostimulant Drugs Affect the
Figure 2. Nigrostriatal Dopamine Pathway. DYN = Dynorphin; DA = Dopamine; κ = κ-Opioid receptors; GABA = γ-Amino Butyric Acid.
Nigrostriatal Dopamine Pathway

DA receptors in the caudate/putamen are indirectly stimulated by psychostimulant drugs (Dickson, Lang, Hinton, & Kelly 1994; Staton & Solomon, 1984). Systemic administration of lower doses of amphetamine preferentially induces locomotor activity, presumably because of increased DA receptor stimulation in the nucleus accumbens. In contrast, systemic administration of higher doses of amphetamine produces stereotyped behaviors via stimulation of the caudate/putamen (Clark & White, 1987; Sharp, Zetterstrom, Ljungberg, & Ungerstedt, 1987). In fact, microinjecting amphetamine into the caudate/putamen induces intense oral stereotypies, consisting of bar biting, non-injurious self-biting and repetitive paw-to-mouth movements (Dickson et al., 1994), whereas microinjecting amphetamine into the nucleus accumbens only produces locomotor activity (Staton & Solomon, 1984).

Similarly, Stahl and colleagues have shown, using electroencephalograph (EEG) pattern recognition, that amphetamine affects different brain regions depending on dose administered (Stahl, Ferger, & Kuschinsky, 1997). In terms of stereotypy, low versus high doses of amphetamine cause a shift of activation from the mesolimbic DA system.
to the nigrostriatal DA system, respectively (Stahl et al., 1997). Additionally, other researchers suggest an additive relationship between the DA systems. Chronic amphetamine treatment produces augmented DA release in the nucleus accumbens and thereby induces locomotor activity and rearing (Robinson, Jurson, Bennett, & Bentgen, 1988), whereas the same chronic treatment produces augmented DA release in the caudate/putamen, resulting in intense oral stereotypies (Kelly, Seviour, & Iversen, 1975; Patrick, Thompson, Walker, & Patrick, 1991).

**Dopamine Receptor Classification**

As study of the DA neurotransmitter system progressed, the initial classification of DA receptors was revised to include a number of DA receptors subtypes. Generally, DA receptors belong to a class of seven-transmembrane domain, G-protein-coupled receptors (Seeman & Van Tol, 1994; Sokoloff & Schwartz, 1995). Based on sequence homology studies, six DA receptors have been cloned: D_{1A}, D_{2S}, D_{2L}, D_{3}, D_{4}, and D_{5} (D_{5} is sometimes referred to as D1B) (Seeman & Van Tol, 1994; Sokoloff & Schwartz, 1995). These receptors can be separated into two subfamilies, D_{1}-like and D_{2}-like, based on the original biochemical classification of Kebabian and Calne (1979). The D_{1}-like receptor family
includes the D₁ and D₅ receptors, whereas the D₂ family includes the D₂S, D₂L, D₃, and D₄ receptors (Clark & White, 1987).

As the importance of these subtypes became known, pharmacological compounds for these DA receptor subtypes were developed. Using selective DA agonists and antagonists, a number of receptor-specific behavioral profiles have been determined. For example, D₁-like receptor stimulation dramatically augments grooming, rearing, and non-stereotyped locomotor activity, yet has little effect on yawning and sniffing (Arnt, 1987; Braun & Chase, 1986; Molloy & Waddington, 1987). On the other hand, D₂-like receptor activation produces stereotyped locomotor activity, yawning and sniffing (Arnt, 1987; Johansson, Levin, Gunne, & Ellison, 1987; Longoni, Spina, & Di Chiara, 1987; White, Bednarz, Wachtel, Hjorth, & Brooderson, 1988). In addition, D₁-like receptor stimulation by SKF-38393 (a D₁-like receptor agonist) induces locomotor and grooming activity, while quinpirole-induced stimulation of D₂-like receptors produces only stereotyped locomotor activity and sniffing (Page & Terry, 1997; Hooks et al., 1994). Systemic injections of N-propylyorapomorphine (NPA; a full dopamine
receptor agonist) induce robust stereotypy with minimal locomotor activity (Bordi, Carr, & Meller, 1989).

Logically then, Clark and White (1987) hypothesized that D₁-like receptors mediate low-intensity behaviors, but hypothesized that high-intensity behaviors require the co-activation of both D₁-like and D₂-like receptors.

Other studies indicate that tonic activation of D₁-like receptors is essential for the full behavioral expression of D₂-like receptor mediated behaviors. Accordingly, Molloy and Waddington (1987) have shown that antagonizing D₁-like receptors with SCH-23390 attenuates D₂-like agonist-induced locomotion. D₁-like receptor-mediated behaviors, on the other hand, do not need co-activation of D₂-like receptors (White et al., 1988). To affirm this, D₂-like receptor blockade failed to influence D₁-like receptor-mediated behaviors, perhaps indicating a one-way synergistic role between the D₁ and D₂ receptor families (White et al., 1988).

**Brain Structures Associated With D₁-Like and D₂-Like Receptor-Mediated Behaviors**

Although stimulation of DA receptors induces a wide range of behaviors, several DA receptor-specific behaviors have been linked to particular brain areas. As such, D₁-
like receptor-mediated behaviors (grooming, rearing and locomotor activity) are thought to be induced via stimulation of the nucleus accumbens, while D₂-like receptor-mediated behaviors (stereotyped sniffing and yawning) are thought to be primarily induced through stimulation of the caudate/putamen (Bordi et al., 1989; Delfs et al., 1990; Dickson et al., 1994; Staton & Solomon, 1984).

Psychostimulant drugs induce many of the same behaviors that are produced by DA D₁-like- and D₂-like-receptor agonist drugs. In fact, low doses of amphetamine elicit locomotor activity and sniffing, whereas higher doses tend to reduce locomotor activity while promoting intense oral stereotypies (Kelly, Seviour, & Iversen, 1975). As a result, amphetamine displays a broad behavioral profile, as it can produce different intensities of behavior in a dose-dependent manner (Dickson et al., 1994).

Through EEG pattern recognition, Ferger, Kropf, and Kuschinsky (1994) revealed that cocaine and amphetamine preferentially affect D₁-like receptors. Thus acute treatment with low doses of amphetamine produce EEG patterns precisely resembling D₁-like receptor agonist EEG patterns, suggesting mesolimbic DA system activity. After
repeated amphetamine injections, or after an acute injection with a high dose of amphetamine, both D<sub>1</sub>-like and D<sub>2</sub>-like receptor EEG patterns were evident, suggesting a synergistic relationship between mesolimbic and nigrostriatal systems. These results are consistent with other studies showing that low doses of amphetamine and cocaine produce low-intensity behaviors, such as locomotor activity and rearing (a D<sub>1</sub>-like effect), whereas high doses produce high-intensity behaviors, such as oral stereotypies (a combined D<sub>1</sub>-like and D<sub>2</sub>-like effect; Clark & White, 1987; White et al., 1988). What is more, D<sub>1</sub>-like receptor EEG patterns were fully attenuated by the DA D<sub>1</sub>-like receptor antagonist SCH-23390, while D<sub>2</sub>-like EEG patterns were only reduced (Stahl et al., 1997).

Dopamine D<sub>1</sub>-Like and D<sub>2</sub>-Like Receptor Interaction

Research suggesting a synergistic interaction between DA D<sub>1</sub>-like and D<sub>2</sub>-like receptors implies that stimulation of D<sub>1</sub>-like receptors somehow 'facilitates' or 'enables' D<sub>2</sub>-like receptor-mediated behaviors. Because amphetamine can produce intense stereotypy at high doses (Callaway, Kuczenski, & Segal, 1989; Clark & White, 1987), amphetamine must also stimulate DA D<sub>2</sub>-like receptors, albeit indirectly. This conclusion is not surprising. In point of fact, both
spiperone and sulpiride (D$_2$-like receptor antagonists) block amphetamine-induced stereotypy, but leave locomotor activity relatively unaffected. This indicates that the D$_2$-like receptor is necessary for amphetamine-induced stereotypy (Bedingfield et al., 1997).

The potent and specific D$_2$-like receptor agonist, quinpirole, induces a wide range of behaviors, like locomotor activity, sniffing and yawning (Dall’Olio, Gandolfi, Vaccheri, Roncada, & Montanaro, 1988; Longoni et al., 1987; Molloy & Waddington, 1987). Quinpirole-induced behaviors are intensified by the D$_1$-like receptor agonist, SKF-38393, converting normal D$_2$-like receptor-mediated behaviors to more intense and focused forms of stereotypy (licking and gnawing) (Dall’Olio et al., 1988; Starr, 1988; White et al., 1988). In fact, after systemic DA depletion by α-methyl-p-tyrosine (AMPT; a tyrosine hydroxylase inhibitor), quinpirole-induced behaviors are abolished, yet the same behaviors are reinstated when SKF-38393 is administered (White et al., 1988). The reciprocal relationship, however, does not exist, as quinpirole fails to alter SKF-38393-induced grooming, further indicating that D$_1$-like receptor stimulation enables D$_2$-like receptor
mediated behavior (White et al., 1988).

Other neurotransmitter systems (e.g., excitatory amino acids and GABA) modulate amphetamine-induced behaviors. That is, CPP (an NMDA receptor antagonist) and THIP (a GABA_A receptor agonist) attenuate amphetamine-induced stereotypy (Karler et al., 1997). Not surprisingly, NMDA and GABA_A receptors are co-expressed on nigrostriatal and striatonigral DA neurons, suggesting that the mechanisms mediating amphetamine-induced stereotypy are much more complex than originally thought (Karler, Calder, Chaudhry, & Turkanis, 1989; Karler, Calder, & Turkanis, 1991; Pierce & Kalivas, 1997; Wolf & Jeziorski, 1993).

In sum, chronic treatment with psychostimulant drugs has been shown to alter dopaminergic, GABA-ergic, and glutaminergic systems (Cador, Bbijou, Cailhol, & Stinus, 1999; Vezina, 1993; Vezina & Stewart, 1989; Wolf, 1998). Because these neurotransmitter systems are involved in drug craving, addiction, and reward, the impact of chronic amphetamine treatment on these systems has been intensely investigated. As a result, over the past decade, several theories describing psychostimulant abuse have been developed, most of them focusing on the effects of chronic amphetamine treatment.
Amphetamine and Sensitization

Chronic amphetamine use in humans can result in two major disorders: drug addiction and amphetamine-induced psychosis (Kalivas & Stewart, 1991; Lett, 1989; Robinson & Becker, 1986; Robinson & Berridge, 1993; Sato, 1986). The most studied is drug addiction, as this phenomenon can be described as a persistent and intense involvement with stress upon a single behavior pattern, with a minimization of exclusion of other behavior patterns. Chronic amphetamine use can also result in a condition called amphetamine psychosis, a state similar to paranoid schizophrenia (Sato, 1986). Although the symptoms of amphetamine psychosis often disappear after cessation of drug taking, craving for the drug and hypersensitivity to the psychomimetic effects remain for years (Robinson & Berridge, 1993; Sato, 1986).

In animal research, a similar phenomenon is termed behavioral sensitization, and it occurs after chronic treatment with amphetamine, cocaine, methylphenidate (Ritalin), methamphetamine and many other psychostimulant drugs (Akimoto, Hamamura, Kazahaya, Akiyama, & Otsuki, 1990; Crawford, McDougall, Meier, Collins, & Watson, 1998; Kalivas & Duffy, 1990; McDougall, Collins, Karper, Watson,
Behavioral sensitization is characterized by a progressive and enduring enhancement of drug-induced behavioral effects of psychostimulant compounds (Kalivas & Stewart, 1991; Robinson & Becker, 1986; Robinson & Berridge, 1993). More specifically, sensitization is viewed as the initial series of behavioral responses that may lead to drug addiction (Robinson & Berridge, 1993). Accordingly, sensitization has often been depicted as a major component to the animal model of drug addiction, since it has been shown to persist for up to a year after a single drug administration (Paulson, Camp, & Robinson, 1991). Because sensitization has similar characteristics to that of paranoid schizophrenia and the initial stages of drug addiction, a better understanding of the processes involved would prove beneficial (Kalivas & Stewart, 1991; Robinson & Becker, 1986; Robinson & Berridge, 1993).

Sensitization in Terms of Associative Learning (Environmental Conditioning)

Robinson and Berridge (1993) suggests an Incentive-Sensitization Theory of drug addiction to explain: (1) the
intense craving and compulsive drug-seeking behavior or 'wanting' of the drug, as opposed to 'liking' the drug, (2) why drug craving often persists, and can be reinstated, long after the discontinuation of drug use, and (3) why drug addicts continue to use and 'want' psychostimulant drugs, despite the decreasing pleasurable effects experienced after repeated use (Robinson & Berridge, 1993).

The term 'incentive' suggests that while taking psychostimulant drugs, certain stimuli associated with the drug-taking environment become salient and induce a psychological process called 'incentive-salience'. Thus stimuli (e.g., rooms, paraphernalia, friends, smells, etc.) that are continuously, contiguously, and frequently associated with drug-taking become attractive to the user and, therefore, psychologically induce 'craving' and 'wanting' for the drug (Kozlowski & Wilkinson, 1987; Robinson & Berridge, 1993). These powerful incentive stimuli, in turn, create an uncontrollable craving and wanting for the drug, spawning repeated use that may develop into drug addiction (Robinson & Berridge, 1993; Wise, 1988). In essence, the drug-paired environment produces a psychological incentive to take more drug.

The Incentive-Sensitization theory indicates that
repeated bouts of drug-taking produce incremental neuroadaptations in the mesolimbic DA system, rendering it, perhaps, permanently hypersensitive (sensitized) to these drug-associated stimuli (Robinson & Becker, 1986; Robinson & Berridge, 1993). Therefore, drug-associated stimuli are (perhaps) permanently destined, via these neuroadaptations in the mesolimbic DA system, to induce drug-taking behaviors (Robinson & Berridge, 1993).

The foundation under which DA sensitization is built is through the process of associative learning (Robinson & Berridge, 1993). Specifically, the drug-taking environment elicits drug-taking behaviors. Consequently, the drug-associated stimuli imbue the drug-taker with incentive-salience and therefore make the act of drug-taking attractive and irresistible to the user.

This theory posits that repeated drug use does not come from the sensitization of the rewarding effects of the drug, but from the environmental cues that have become associated with drug-taking (Robinson & Berridge, 1993). Granted, psychostimulant drugs produce a euphoric-like effect (Wise & Bozarth, 1987, Wise & Rompre, 1989), however this theory makes a clear distinction between 'wanting' (i.e., craving) and 'liking' (i.e., rewarding) the
psychostimulant drug (Robinson & Berridge, 1993). Testimonials of ex-drug abusers reflect this, as they express a generalized dislike for the drug after extended use, but they continue to use the drug and risk everything to obtain the drug, despite the diminishing pleasurable effects of the drug (Robinson & Berridge, 1993; Sato, 1986). Therefore, drug addiction may not be sustained via the rewarding characteristics of the drug, but through the craving and wanting which develop as a result of incentive-salience.

Two other theories of addiction have tried to explain the uncontrollable urge to take drugs: the positive-reinforcement model and the negative-reinforcement model. Specifically, the negative-reinforcement model posits that drugs are taken to avoid the symptoms of withdrawal, whereas the positive-reinforcement model posits that drugs are taken for their reinforcing effects (Robinson & Becker, 1986; Robinson & Berridge, 1993; Wise & Bozarth, 1987, Wise & Rompre, 1989). Importantly, both theories cannot explain why abusers relapse even after the symptoms of withdrawal have subsided and after many years of abstinence. Moreover, neither of these theories can explain why previous drug abusers continue to crave their specific
drug, even though the pleasurable effects of the drug became attenuated (Stewart, de Wit, & Eikelboom, 1984; Wise, 1988).

In sum, the incentive-sensitization model clarifies how these long-term drug effects occur: by the process of associative learning. More specifically, with incentive-salience, the drug-associated stimuli become attractive to the user and this causes repeated use. Through continuous drug use specific neuroadaptations occur in the mesolimbic DA system causing these associations to become permanently hypersensitive (or sensitized). Therefore, the user craves drug-associated stimuli, and not the pleasurable effects of the drugs (as in the positive reinforcement model) or the avoidance of withdrawal symptoms (as in the negative reinforcement model). The importance of associative learning, as suggested by the incentive-sensitization model, is supported by other research, since environmental conditions influence the magnitude of sensitization to psychostimulant compounds (Badiani, Camp, & Robinson, 1997; Campbell & Raskin, 1978; Mattingly & Gotsick, 1989).

Conditioning Factors (i.e., Pavlovian Associations) in Amphetamine Sensitization

The neuropharmacological actions of amphetamine and
cocaine have been well characterized. These drugs substantially increase synaptic DA by their action on the DA transporter (or reuptake pump) (Seiden et al., 1993). Sensitization to these drugs is a result of increased DA in the synapse and, from repeated use, produce neuroadaptations in the neural system where these drugs have their action. However, several reports have argued that behavioral sensitization may develop, not only through the persistent use of psychostimulant drugs, but also by associating the drug-taking environment with drug administration (i.e., Pavlovian Associations) (Anagnostaras & Robinson, 1996; Badiani et al., 1997; Badiani, Anagnostaras, & Robinson, 1995; Badiani, Browman, & Robinson, 1995; Campbell & Raskin, 1978; Einat et al., 1996; Hoffman & Wise, 1992; Lienau & Kuschinsky, 1997; Mattingly & Gotsick, 1989). For example, recent work by Robinson and colleagues indicates that the environmental conditions of the testing chamber alter the acute effects of amphetamine and the magnitude of amphetamine and cocaine sensitization (Badiani, Browman, & Robinson, 1995). Specifically, if the drug treatment is paired with the animal's testing environment (but the home cage environment is different), a greater rate of sensitization would occur
than if the drug treatment is paired with the home environment (Anagnostaras & Robinson, 1996; Badiani, Anagnostaras, & Robinson, 1995; Badiani, Browman, & Robinson, 1995; Lienau & Kuschinsky, 1997; Mattingly & Gotsick, 1989). This would suggest that environmental cues surrounding the drug treatment have a much stronger influence on the rate of sensitization than what was previously thought. This may be potentially important for sensitization research and drug addiction, since the subjective and behavioral effects of addictive drugs largely depend on the environmental context during drug administration (Badiani, Browman, & Robinson, 1995; Carlin, Bakker, Halpern, & Post, 1972; Chait, 1993).

Although environmental conditioning factors may affect the overall strength of sensitization, these cues only act in an additive manner. That is, sensitization still occurs when environmental cues are eliminated, albeit the sensitization is not as robust (Badiani, Browman, & Robinson, 1995). Thus, the environmental cues work in tandem with the effects of the psychostimulant drug to promote craving for the drug (in the human model) or induce a sensitized increase in behavioral responding (as in the animal model).
The Involvement of Excitatory Amino Acids in Behavioral Sensitization: A Neural Basis of Behavioral Sensitization

Historically, sensitization research has focused on the idea that changes in the DA system are responsible for the occurrence of behavioral sensitization (Robinson & Becker, 1986; Robinson & Berridge, 1993; Wise & Bozarth, 1987). However, recent work has shown that excitatory amino acids (EAAs) may play an important role in the development of behavioral sensitization (Wolf, 1998). In fact, EAAs and DA complement each other in a number of ways. That is, EAAs possess a regulatory function over DA, as antagonism of various EAAs attenuates DA release (Karler et al., 1989; 1991). What is more, several reports suggest an important role for EAAs in amphetamine- and cocaine-induced behavioral sensitization, as antagonism of specific EAA subtypes eliminates sensitization to these drugs (Karler et al., 1989; Wolf & Jeziorski, 1993).

In her theory, Marina Wolf (1998) posited that DA plays a secondary role to EAAs in behavioral sensitization. Because EAAs exert a regulatory role over DA, EAAs control the underlying circuitry responsible for the development of behavioral sensitization, and therefore make the involvement of DA less significant than previously thought.
This is not to say that DA is not important for the occurrence of behavioral sensitization, since behavioral sensitization cannot be produced without the presence of endogenous DA (Wolf, 1998).

Wolf (1998) provides evidence to support the involvement of EAA neurotransmitter systems in sensitization. Blockade of NMDA receptors by MK-801 (a non-competitive antagonist of the NMDA glutamate receptor) prevents amphetamine- and cocaine-induced behavioral sensitization in mice (Karler et al., 1989). In fact, blockade of NMDA receptors has also been shown to prevent D₁-like agonist-induced sensitization (Wolf, White, & Hu, 1994). This evidence clearly supports the involvement of NMDA receptors in amphetamine-induced behavioral sensitization.

In addition to NMDA receptors, other glutamate receptor subtypes are important for amphetamine sensitization. For instance, the selective AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxalin (DNQX) blocks the development of sensitization to amphetamine-induced stereotypy and locomotor activity (Li, Vartanian, White, Xue, & Wolf,
1997). In addition, cocaine sensitization was blocked by the AMPA receptor antagonist 6,7 dinitroquinoxaline-2,3-dione (NBQX; Li et al., 1997). Other AMPA antagonists, such as 6-cyano-7-nitroqinoxaline-2,3-dione (CNQX), block expression of cocaine-induced sensitization when microinjected into the core of the nucleus accumbens (Pierce, Bell, Duffy, & Kalivas, 1996).

So when this evidence is taken together, EAA and DA neurotransmitters appear to interact when mediating behavioral sensitization, since NMDA and AMPA antagonists block DA agonist-induced behavioral sensitization. The Involvement of Dopamine D₁-Like Receptors in Behavioral Sensitization

In addition to Robinson and Berridge’s (1993) and Wolf’s (1998) theories on behavioral sensitization, Vezina (1996) has developed a theory of behavioral sensitization that primarily focuses on the importance of the DA D₁ family of receptors. Paul Vezina (1996) showed that behavioral sensitization to amphetamine can be eliminated by antagonizing the D₁ family of receptors with the potent and specific D₁-like receptor antagonist SCH-23390. According to Vezina’s model, amphetamine causes somatodendritic DA release in the ventral tegmental area. This DA stimulates
Di-like receptors located on the presynaptic terminals of EAA and GABA projections synapsing on DA neurons. Therefore, amphetamine indirectly affects the activation of mesolimbic DA neurons by modulating GABA and glutamate neurotransmission in the ventral tegmental area (Vezina, 1996).

The location and function of DA D_1-like receptors establishes their importance for behavioral sensitization, because repeatedly microinjecting amphetamine into the ventral tegmental area: 1) produces a sensitized behavioral response to a systemic challenge of amphetamine (Vezina, 1996); 2) produces a significant increase in nucleus accumbens DA when challenged with a systemic injection of amphetamine (Vezina, 1996); 3) produces a sensitized behavioral response to a local infusion of amphetamine into the nucleus accumbens (Kalivas & Duffy, 1993a; 1993b; Vezina, 1996); and 4) does not induce behavioral sensitization when co-administered with SCH-23390 (Bjijou et al., 1996; Pierre & Vezina, 1998; Vezina, 1996). When taken together, it is clear that amphetamine-induced locomotor sensitization requires the activation of DA D_1-like receptors, thus supporting Vezina's (1996) theory that DA D_1-like receptor stimulation in the ventral tegmental
area is necessary for the occurrence of amphetamine-induced behavioral sensitization.

Currently, the DA D₁ family of receptors has only two members: D₁A and D₅ (or D₁B). As previously indicated, Vezina (1996) hypothesized that the D₁ family of receptors was responsible for the induction of amphetamine-induced behavioral sensitization. Recent work on the DA D₁ family of receptors has indicated that both D₁A and D₅ receptors stimulate adenylyl cyclase activity and have a similar affinity for SCH-23390 (Baldessarini & Tarazi, 1996; Clark & White, 1987). But while the D₁A receptor may exist in greater numbers throughout limbic and basal ganglia circuits, the D₅ receptor, when compared to the D₁A receptor, has a much higher affinity for endogenous DA (Baldessarini & Tarazi, 1996; Kostrzewa, 1995).

The likelihood of a DA D₅ influence on amphetamine-induced behavioral sensitization is plausible, since repeated administration of psychostimulants has behavioral impact in D₁A-deficient mice. For example, Crawford, Drago, Watson, and Levine (1997) showed that amphetamine initiates less locomotor activity in D₁A-deficient mice than wild-type controls during pretreatment, but D₁A-deficient mice still exhibited a sensitized response when challenged with a low
dose of amphetamine three days after pretreatment. DA D_{1A}-deficient mice also show a lessened responsiveness after acute cocaine treatment, as cocaine-induced hyperactivity was blunted in mice lacking the DA D_{1A} receptor (Miner, Drago, Chamberlain, Donovan, & Uhl, 1995; Xu, Hu, Cooper, Moratalla, Graybiel, White & Tonegawa, 1994). This is important since both amphetamine and cocaine function as indirect DA agonists (Reith et al., 1980). Therefore, these data suggest that the DA D_{1A} receptor is necessary for the induction of amphetamine-induced locomotor sensitization.

Dopamine D_{5} Receptor Subtype in Amphetamine Sensitization

The fact that D_{1A}-deficient mice are capable of expressing locomotor sensitization (Crawford et al., 1997) suggests that the DA D_{5} receptor may be importantly involved in amphetamine-induced sensitization. Previously, it has been shown that complete blockade of the DA D_{1}-like receptor eliminates the occurrence of amphetamine-induced sensitization (Vezina, 1996) and that serotonergic and DA D_{2}-like receptor systems are not involved in this elimination (Bjijou et al., 1996). Therefore, the DA D_{5} receptor may be critically important for amphetamine-induced behavioral sensitization.
Genetic Tools For Studying Receptor Function

Several lines of research establish the importance of the DA D₁-like family of receptors for the occurrence of amphetamine sensitization (Bjijou et al., 1996; Crawford et al., 1997; Pierre & Vezina, 1998; Vezina, 1996). Yet because the DA D₁ family of receptors includes two different receptors, it has been impossible to determine the precise role that DA D₁A and D₅ receptors play in amphetamine sensitization. Specifically, available ligands are not selective enough to distinguish between the D₁A and D₅ receptors. Fortunately, a DA D₁A receptor deficient mouse was engineered to make the answer to this question more accessible. Therefore, the current project will use the D₁A-deficient mouse to assess the importance of DA D₁A and D₅ receptors for amphetamine-induced behavioral sensitization.

Summary

In general, the results of these studies can be summarized as follows: 1) Drug addiction involves the DA system; 2) Psychostimulants such as cocaine and amphetamine increase DA levels and are often abused; 3) The functioning of the mesolimbic and nigrostriatal DA systems is influenced by psychostimulants; 4) Psychostimulants indirectly stimulate DA receptors in the ventral tegmental
area, which causes augmented DA release in the nucleus accumbens; 5) Destruction of the mesolimbic DA system, either by lesioning the ventral tegmental area or the nucleus accumbens, prevents cocaine- and amphetamine-induced locomotor effects; 6) DA D₁-like receptor activation mediates locomotor activity and rearing, whereas DA D₂-like receptor activation mediates stereotyped behaviors; 7) The induction of amphetamine-induced locomotor sensitization is primarily mediated by actions in the ventral tegmental area, whereas expression of amphetamine-induced behavioral sensitization is primarily mediated by the nucleus accumbens; 8) The induction of amphetamine-induced locomotor sensitization can be blocked by D₁-like receptor antagonism; and 9) Persistence of amphetamine-induced locomotor sensitization has been shown in mice lacking the D₁A receptor. When taken together, these findings suggest the involvement of the DA D₁ family of receptors in amphetamine-induced behavioral sensitization.

Hypothesis

Therefore, I proposed that: 1) the DA D₁-like receptor is critically important for the induction and long-term expression of amphetamine-induced behavioral sensitization, 2) environmental conditioning factors associated with
amphetamine sensitization are negatively impacted by the lack of the D\textsubscript{1A} receptor; and 3) pretreatment with SCH-23390 would block amphetamine-induced behavioral sensitization in D\textsubscript{1A}-deficient mice, indicating that the D\textsubscript{3} receptor is important for the occurrence of amphetamine-induced locomotor sensitization.

**Experimental Plan**

To test these ideas, I conducted three experiments. In the first experiment, I injected D\textsubscript{1A}-deficient and wild-type mice with various doses of amphetamine or saline for seven consecutive days. I challenged these mice with amphetamine after three and seventeen abstinence days. This experiment determined whether the DA D\textsubscript{1A} receptor was responsible for short- and long-term amphetamine-induced behavioral sensitization. In the second experiment, I manipulated the conditioning environment. Specifically, half the D\textsubscript{1A}-deficient and wild-type mice received amphetamine in the home cage for seven days and the other half received amphetamine in the testing chamber for seven days. After three abstinence days, I challenged all animals with amphetamine in the activity chamber. On the following day, I challenged all mice with saline to assess conditioned activity. This experiment determined whether
environmental conditioning factors influenced amphetamine-induced sensitization of D_{1A}-deficient and wild-type mice. In the third experiment, I injected D_{1A}-deficient and wild-type mice for seven days with varied doses of the D_{1}-like antagonist SCH-23390 or saline 30-min prior to amphetamine treatment. After three abstinence days, I challenged all animals with amphetamine in the activity chamber. This experiment determined whether the DA D_{5} receptor mediates amphetamine-induced behavioral sensitization.
GENERAL METHOD

Subjects

Subjects were 271 adult, wild type (+/+) and D1A-deficient (-/-) C57BL-6 mice. The subjects were bred at California State University, San Bernardino in a room maintained at 22-24°C on a 12-hour light/dark cycle. Litters were culled to a maximum of 12 pups at seven days of age. Mice were housed with their dam and sire until 21 days of age after which they were separated by gender until testing. Care was taken to ensure that a nearly equal number of male and female mice were assigned to each treatment group and that no more than one animal from each litter was placed into any particular group. Subjects were conditioned and tested during the light cycle between the ages of 90 and 120 days. The Animal Care and Use Committee at California State University, San Bernardino approved protocol for the experimental procedures.

Apparatus

Behavior was assessed in Coulbourn Instruments, Tru-Scan Photobeam Activity Chambers (25.5 × 25.5 × 41 cm). The chambers were made of Plexiglas and have two sets of 16
pulse-modulated infrared photo beams spaced 1.6 cm apart with dark gray removable floor trays and an open top.

**Drugs**

*S(+)*-amphetamine sulphate and SCH-23990 (Research Biochemicals, Natick, MA) were dissolved in saline and injected intraperitoneally (i.p.) at a volume of 5 ml/kg.

**Statistical Analysis**

Repeated measures analyses of variance (ANOVARs) were used for analyzing the locomotor activity and stereotyped sniffing data. Significant three- and four-way interactions were further analyzed using lower-order ANOVAs. Additional analysis of the data was made using Tukey tests \((p < .05)\).

**General Procedure**

In each of the three experiments there were seven conditioning days followed by either one or two challenge days. On drug pretreatment days, all animals were given either amphetamine or saline and then conditioned for 60 min. On challenge days, all animals were challenged with either amphetamine or saline to assess the presence or absence of behavioral sensitization or conditioned activity. Length of the testing sessions and number of drug abstinence days (time between the last drug pretreatment day and the drug challenge day) varied according to the experiment.
On all pretreatment and test days, behavioral assessment took place immediately after amphetamine or saline treatment. Locomotor activity was recorded continuously across the testing session, whereas stereotyped sniffing was assessed using a fixed interval momentary time sampling method (Cameron, Crosby, & Crocker, 1988). Essentially the presence or absence of stereotyped sniffing was determined in 30-s intervals. After behavioral assessment, all animals were immediately placed back in their home cage and returned to the animal colony room.

Genotyping

D1-deficient mice were generated as described in Drago et al. (1994) from embryonic stem cells, where one of the D1 receptor alleles was targeted in vitro by homologous recombination. Briefly, a targeting construct containing a neomycin phosphotransferase gene was inserted into a region of the D1 receptor gene encoding the fifth transmembrane domain. A gene sequence (0.75 kb) downstream from the insertion site was excised. This excised gene sequence codes for the third intracytoplasmic loop. The insertion of the targeting construct and the removal of the gene sequence generates an inactive gene product. Positive clones were used to create chimeric mice. Chimeric males were then
mated to female C57BL-6 mice to create heterozygotes. Heterozygous mice have one disrupted D₁ receptor allele, while D₁-deficient mice have both alleles disrupted (wild-type mice have two normal D₁ receptor alleles). Receptor binding studies indicate that heterozygous mice have fewer than half the typical number of striatal D₁-like receptors (Drago et al., 1994). Despite the reduced number of D₁-like receptors, heterozygous mice tend to respond like wild-type controls on behavioral tasks (Drago, Gerfen, Westphal, & Steiner, 1996; Miner et al., 1995).

All mice were genotyped using polymerase chain reaction (PCR) as described previously (Bender, Drago, & Rivkees, 1997; Miner et al., 1995). The genomic DNA for the assays was obtained from tail biopsies (done before any behavioral assessment) and extracted using the PureGene DNA isolation kit (Gentra Systems, Minneapolis, MN). Two independent PCR reactions were performed for genotyping. The first reaction determined the presence of the neomycin containing transgene. This reaction used a forward primer (D1.5; 5'-ctgattagcgtagcatggactttgtc-3') and a reverse primer (PGK1; 5'-tggatgtggaatgtgtgcgag-3'). PCR conditions were 35 cycles of 94°C (20 min), 58°C (20 min), 72°C (1
min), followed by 72°C (6 min). PCR products were separated on a 1.5% agarose gel, with a 330 bp band indicating the presence of at least one transgenic allele. The second reaction determined the presence of the normal D1 gene. It used a forward primer (JD.27; 5'aaagttccttaagatgtcct-3') and a reverse primer (JD.26; 5'-tggtggctggaaaacatcaga-3'). PCR conditions were the same as in the first reaction with the exception that the annealing temperature was 55°C instead of 58°C. The presence of a 350 bp band indicated at least one wild-type allele.
EXPERIMENT 1

Prior research has indicated that the DA D₁ family of receptors are intimately linked to the induction of amphetamine sensitization (Bjijou et al., 1996; Crawford et al., 1997; Vezina, 1996). More specifically, Crawford et al. (1997) demonstrated that mice lacking the DA D₁A receptor exhibited short-term behavioral sensitization to amphetamine, however long-term amphetamine sensitization was not assessed. Therefore, the purpose of the first experiment was to determine whether DA D₁A-deficient mice exhibit amphetamine-induced behavioral sensitization after either a short (3-day) or long (17-day) drug abstinence period.

Method

Subjects. Subjects were 92 C57BL-6 DA D₁A-deficient and wild-type mice.

Procedure. The apparatus and procedure described in the General Methods were used with the following exceptions. Subjects were injected with amphetamine (1.0, 2.0, 4.0 or 8.0 mg/kg, i.p.) or saline for seven consecutive days. Each conditioning session lasted for 60 min. After three abstinence days, a challenge injection (i.p.) of 1.0 mg/kg amphetamine or saline was given to all mice to assess the
occurrence of short-term amphetamine-induced behavioral sensitization. To assess the occurrence of long-term amphetamine-induced behavioral sensitization, mice were challenged with amphetamine (1.0 mg/kg i.p.) after a 17-day drug abstinence period. Locomotor activity and stereotyped sniffing were assessed for a total of 150 min. In summary, D1A-deficient and wild-type mice received one of the following six sequences (PRETREATMENT/TEST DAY 1/TEST DAY 2) of amphetamine or saline during the pretreatment phase and on the first and second test day (doses are in parentheses): SAL/SAL/AMPH, SAL/AMPH/AMPH, AMPH(1.0)/AMPH/AMPH, AMPH(2.0)/AMPH/AMPH, AMPH(4.0)/AMPH/AMPH, or AMPH(8.0)/AMPH/AMPH.

Results

Drug Pretreatment Phase: Locomotor Activity. Overall, mice pretreated with 4 or 8 mg/kg amphetamine had larger distance traveled scores than saline controls (see Figure 3) [pretreatment main effect, $\textit{F}(4, 82) = 32.57$, $p < .001$; and Tukey tests, $p < .05$]. On all seven pretreatment days, wild-type and D1A-deficient mice given 4 mg/kg amphetamine (filled triangles) exhibited more locomotor activity than saline-pretreated mice (open circles) [wild-type:
Figure 3. Mean distance traveled (±SEM) of adult wild-type and Δ₁₅A-
deficient mice (n = 6 - 10 per group) administered saline (SAL) or amphetamine (AMPH; 1.0, 2.0, 4.0, or 8.0 mg/kg, i.p.) for seven consecutive pretreatment days. Behavioral assessment lasted for 60 min and occurred immediately after injection. ○ = SAL; ● = 1.0 mg/kg AMPH; ♦ = 2.0 mg/kg AMPH; ▲ = 4.0 mg/kg AMPH; ■ = 8.0 mg/kg AMPH. * Significantly different from the SAL group (p < .05).
pretreatment × day interaction, $F(24,294) = 4.38$, $p < .001$; D$_{1A}$-deficient: pretreatment × day interaction, $F(24,198) = 3.79$, $p < .001$; and Tukey tests, $p < .05$]. Wild-type and D$_{1A}$-deficient mice pretreated with 8 mg/kg amphetamine (filled squares) had more distance traveled on time blocks 1 and 2 than saline controls [Tukey tests, $p < .05$].

Drug Pretreatment Phase: Stereotyped Sniffing. During the pretreatment phase, mice given 8 mg/kg amphetamine sniffed more than mice given saline (see Figure 4) [pretreatment main effect, $F(4,82) = 12.06$, $p < .001$; and Tukey tests, $p < .05$]. This drug effect varied according to both genotype and pretreatment day. On day 1, wild-type mice pretreated with 4 or 8 mg/kg amphetamine had more sniffing counts than saline controls; whereas, wild-type mice given 2 or 8 mg/kg amphetamine had more sniffing counts than saline-pretreated mice on day 7 (see upper graph, Figure 4) [pretreatment × day interaction, $F(4,49) = 2.87$, $p < .05$; and Tukey tests, $p < .05$]. D$_{1A}$-deficient mice exhibited a different pattern of effects, because only mice given 8 mg/kg amphetamine sniffed more than saline-pretreated mice (see lower graph, Figure 4) [pretreatment main effect, $F(4,33) = 3.36$, $p < .05$; and Tukey tests, $p < .05$].
Figure 4. Mean stereotyped sniffing counts (±SEM) of adult wild-type and D1A-deficient mice (n = 6 - 10 per group) administered saline (SAL) or amphetamine (AMPH; 1.0, 2.0, 4.0, or 8.0 mg/kg, i.p.) for seven consecutive pretreatment days. Behavioral assessment lasted for 60 min and occurred immediately after injection. ○ = SAL; ● = 1.0 mg/kg AMPH; ♦ = 2.0 mg/kg AMPH; ▲ = 4.0 mg/kg AMPH; ■ = 8.0 mg/kg AMPH. * Significantly different from the SAL group (p < .05).
Test Day Locomotor Activity: Three Abstinence Days. On time blocks 1-4, mice pretreated with 4 or 8 mg/kg amphetamine exhibited more locomotor activity than mice pretreated with saline (see Figure 5) [pretreatment x time interaction, $F(56,910) = 5.93, p < .001$; and Tukey tests, $p < .05$]. Analyses involving only the wild-type mice showed that pretreatment with the two highest doses of amphetamine (4 and 8 mg/kg) resulted in more locomotor activity on time blocks 1-4 than saline pretreatment (see upper graph, Figure 5) [pretreatment x time interaction, $F(56,546) = 5.77, p < .001$; and Tukey tests, $p < .05$]. D1A-deficient mice exhibited a more complex pattern of drug effects. For example, D1A-deficient mice pretreated with 8 mg/kg amphetamine exhibited more locomotor activity than saline pretreated mice on time blocks 1 and 2 (see lower graph, Figure 5) [pretreatment x time interaction, $F(56,364) = 1.70, p < .01$; and Tukey tests, $p < .05$]. On the other hand, D1A-deficient mice pretreated with 4 mg/kg amphetamine showed enhanced levels of locomotor activity across time blocks 1-8. It is important to realize, however, that the total amount of distance traveled did not vary between
Three Abstinence Days

Figure 5. Mean distance traveled (±SEM) of adult wild-type and D_{1A}-deficient mice (n = 7–8 per group) during testing (these are the same mice as in Figures 1 and 2). Mice had previously received seven consecutive injections of saline (SAL) or amphetamine (AMPH; 1.0, 2.0, 4.0, or 8.0 mg/kg, i.p.). After 3 abstinence days all mice received a challenge injection of AMPH (1.0 mg/kg, i.p.). Behavioral testing lasted for 150 min and occurred immediately after injection. * Significantly different from the SAL group (p < .05).
wild-type and D_{1A}-deficient mice [p > .05].

Test Day Stereotyped Sniffing: Three Abstinence Days. After a three day abstinence period, amphetamine-pretreated wild-type and D_{1A}-deficient mice exhibited enhanced stereotyped sniffing when compared to saline-pretreated mice (see Figure 6) [pretreatment main effect, F(4,76) = 8.18, p < .001; and Tukey tests, p < .05]. Specifically, mice pretreated with 2, 4, or 8 mg/kg amphetamine, and then challenged with 1 mg/kg amphetamine, had more stereotyped sniffing counts than mice given amphetamine for the first time on the test day. The stereotyped sniffing of wild-type and D_{1A}-deficient mice did not differ [p > .05].

Test Day Locomotor Activity: Seventeen Abstinence days. After 17 drug abstinence days, both wild-type and D_{1A}-deficient mice showed a sensitized locomotor response (see Figure 7) [pretreatment main effect, F(56,770) = 5.57, p < .001; and Tukey tests, p < .05]. More specifically, wild-type mice pretreated with 4 or 8 mg/kg amphetamine had more distance traveled on time blocks 1-3 than saline-pretreated mice (see upper graph, Figure 7) [pretreatment x time interaction, F(56,420) = 4.08, p < .001; and Tukey tests, p < .05]. An almost identical pattern of effects
Figure 6. Mean stereotyped sniffing (±SEM) of adult wild-type and D1A-deficient mice (n = 7 - 8 per group) during testing (these are the same mice as in Figures 1 and 2). Mice had previously received seven consecutive injections of amphetamine (AMPH; 1.0, 2.0, 4.0, or 8.0 mg/kg, i.p.) or saline (SAL). After 3 abstinence days all mice received a challenge injection of AMPH (1.0 mg/kg, i.p.). Behavioral testing lasted for 150 min and occurred immediately after injection. * Significantly different from the SAL group (p < .05).
Figure 7. Mean distance traveled (±SEM) of adult wild-type and D1A-deficient mice (n = 6 - 7 per group) during the second test day (these are the same mice as in Figures 1 and 2). Mice had previously received seven consecutive injections of saline (SAL) or amphetamine (AMPH; 1.0, 2.0, 4.0, or 8.0 mg/kg, i.p.). After 17 abstinence days all mice received a challenge injection of AMPH (1.0 mg/kg, i.p.). Behavioral testing lasted for 150 min and occurred immediately after injection. * Significantly different from the SAL group (p < .05).
was shown by D_{1A}-deficient mice, with the only exception being that the differences between the amphetamine (4 and 8 mg/kg) and saline-pretreated mice were observed on time blocks 1 and 2 (see lower graph, Figure 7) [pretreatment × time interaction, $F(56,350) = 2.59$, $p < .001$; and Tukey tests, $p < .05$]. The overall locomotor activity of wild-type and D_{1A}-deficient mice did not differ [$p > .05$].

**Test Day Stereotyped Sniffing: Seventeen Abstinence Days.** After the extended abstinence period, only mice pretreated with 2 mg/kg amphetamine exhibited a sensitized sniffing response on the test day (see Figure 8) [pretreatment main effect, $F(4,59) = 3.44$, $p < .01$; and Tukey tests, $p < .05$]. The stereotyped sniffing of wild-type and D_{1A}-deficient mice did not differ, since the main effect and interactions involving genotype as a variable did not reach statistical significance.

**Summary**

These results indicate that D_{1A}-deficient and wild-type mice exhibit both short- and long-term behavioral sensitization after repeated amphetamine treatment. This suggests that the DA D_{1A} receptor subtype is not necessary for amphetamine-induced behavioral sensitization.
Figure 8. Mean stereotyped sniffing (±SEM) of adult wild-type and D₁A-deficient mice (n = 6 - 7 per group) during the second test day (these are the same mice as in Figures 1 and 2). Mice had previously received seven consecutive injections of saline (SAL) or amphetamine (AMPH; 1.0, 2.0, 4.0, or 8.0 mg/kg, i.p.). After 17 abstinence days all mice received a challenge injection of AMPH (1.0 mg/kg, i.p.). Behavioral testing lasted for 150 min and occurred immediately after injection. * Significantly different from the SAL group (p < .05).
EXPERIMENT 2

Previous studies have shown that DA D1-like receptors are important for amphetamine-induced locomotor sensitization (Bjijou et al., 1996; Crawford et al., 1997; Vezina, 1996). Furthermore, several studies have emphasized the importance of environmental cues and conditioning factors for the development of behavioral sensitization (Badiani et al., 1997; Badiani, Anagnostaras, & Robinson, 1995; Badiani, Browman, & Robinson, 1995). The purpose of Experiment 2 was to determine whether the DA D1A receptor is necessary for the environmental conditioning effects typically observed in amphetamine-induced sensitization. I predicted that drug-paired environmental cues enhanced the amphetamine-induced behavioral sensitization of wild-type, but not D1A-deficient mice. This pattern of results would indicate that the D1A receptor is necessary for environmental conditioning effects.

Method

Subjects. Subjects were 67 male and female C57Bl/6 D1A-deficient and wild-type mice

Procedure. The apparatus and procedure described in the General Methods were used with the following exceptions. Animals received either amphetamine (8.0 mg/kg, i.p.) or
saline prior to being placed in the activity chamber. Behavior was assessed for 60 min. Prior to being returned to the home cage, mice were injected with either amphetamine (8.0 mg/kg, i.p.) or saline. Specifically, mice injected with amphetamine prior to being placed in the testing chamber were injected with saline prior to being returned to the home cage; whereas, mice injected with saline prior to being placed in the testing chamber were injected with amphetamine prior to being returned to the home cage. The pretreatment phase lasted for seven days. After three abstinence days, mice were challenged with 1.0 mg/kg amphetamine in the testing chamber. Locomotor activity and stereotyped sniffing were assessed for a total of 150 min.

To assess conditioned activity, all mice were injected with saline one day after the first test day. On the second test day, locomotor activity and stereotyped sniffing were assessed for 60 min. In summary, D1A-deficient and wild-type mice received one of the following three sequences (PRE-POST/TEST DAY 1/TEST DAY 2) of drugs during the pretreatment phase and on the first and second test day (injection location is in parentheses): SAL(chamber)-SAL(home)/AMPH/SAL, SAL(chamber)-AMPH(home)/AMPH/SAL, or AMPH(chamber)-SAL(home)/AMPH/SAL.
Results

Locomotor Activity On Test Day 1 (Amphetamine Challenge): Behavioral Sensitization. Pretreatment condition interacted with genotype to affect the locomotor activity of the mice (see Figure 9) [pretreatment condition \( \times \) genotype interaction, \( F(2,36) = 4.19, p < .05; \) and Tukey tests, \( p < .05 \)]. Wild-type mice exhibited a sensitized locomotor response, but only if amphetamine pretreatment was given in the test chamber (see upper graph, Figure 9) [pretreatment condition \( \times \) time interaction, \( F(28,280) = 4.94, p < .001; \) and Tukey tests, \( p < .05 \)]. More specifically, wild-type mice pretreated with 8 mg/kg amphetamine in the test chamber (filled squares), and challenged with 1 mg/kg amphetamine, exhibited more locomotor activity on time blocks 1 and 2 than did mice acutely challenged with amphetamine on the test day (open circles). Wild-type mice pretreated with amphetamine in the home cage (open squares) did not differ from saline-pretreated controls (open circles) \([p > .05]\).

D1A-deficient mice pretreated with amphetamine in the test chamber also exhibited more test day locomotor activity than saline-pretreated mice (see lower graph,
Test Day 1: Amphetamine Challenge

**Figure 9.** Mean distance traveled (±SEM) of adult wild-type and D_{1A}-deficient mice (n = 6 - 8 per group) during the first test day. Mice had previously received seven daily injections of saline (SAL) or amphetamine (AMPH; 8.0 mg/kg, i.p.) in either their home cage or in the test chamber. After 3 abstinence days all mice received a challenge injection of AMPH (1.0 mg/kg, i.p.) in the testing chamber. Behavioral testing lasted for 150 min and occurred immediately after injection.

* Significantly different from the SAL group (p < .05).
Figure 9) [pretreatment condition x time interaction, $F(28,244) = 1.93, p < .01$; and Tukey tests, $p < .05$]. This effect was more robust in $D_{1A}$-deficient mice than with wild-types, since significant differences were apparent on time blocks 1-8. Unlike wild-type mice, $D_{1A}$-deficient mice showed a sensitized locomotor response if amphetamine pretreatment was given in the home cage [pretreatment condition main effect, $F(2,16) = 7.58, p < .01$; and Tukey tests, $p < .05$]. Even so, $D_{1A}$-deficient mice pretreated with amphetamine in the test chamber (filled squares) had larger distance traveled scores than $D_{1A}$-deficient mice given amphetamine in the home cage (open squares).

Stereotyped Sniffing On Test Day 1 (Amphetamine Challenge): Behavioral Sensitization. $D_{1A}$-deficient mice had significantly more test day sniffing counts than wild-type controls (see Figure 10) [genotype main effect, $F(1,36) = 5.80, p < .05$]. The differences between $D_{1A}$-deficient and wild-type mice were only observed in those groups given amphetamine pretreatment (in either the test chamber or home cage). Overall, mice given 8 mg/kg amphetamine in the test chamber had significantly more test day sniffing counts than saline-pretreated mice, with mice
Figure 10. Mean stereotyped sniffing (±SEM) of adult wild-type and D₁₉-deficient mice (n = 6 - 8 per group) during the first test day. Mice had previously received seven daily injections of saline (SAL) or amphetamine (AMPH; 8.0 mg/kg, i.p.) in either their home cage or in the test chamber. After 3 abstinence days all mice received a challenge injection of AMPH (1.0 mg/kg, i.p.) in the testing chamber. Behavioral testing lasted for 150 min and occurred immediately after injection. * Significantly different from the SAL group (p < .05).
given amphetamine in the home cage being intermediate 
[pretreatment condition main effect, $F(2,36) = 68.34, p < .001$].

**Locomotor Activity On Test Day 2 (Saline Challenge):**

*Conditioned Activity.* After a challenge injection of saline, $D_{1A}$-deficient mice had larger distance traveled scores than wild-type mice (see Figure 11) [genotype main effect, $F(1,36) = 6.83, p < .05$]. The enhanced locomotor activity exhibited by $D_{1A}$-deficient mice only occurred in groups that had been pretreated with amphetamine. More specifically, $D_{1A}$-deficient mice pretreated with 8 mg/kg amphetamine (in either the home cage or test chamber) exhibited more locomotor activity than saline-pretreated mice (see lower graph, Figure 11) [pretreatment condition x time interaction, $F(10,80) = 2.14, p < .05$; and Tukey tests, $p < .05$]. The differences between amphetamine- and saline-pretreated $D_{1A}$-deficient mice reached statistical significance on time blocks 1-4. In contrast, wild-type mice pretreated with amphetamine did not show any conditioned activity (see upper graph, Figure 11).

**Stereotyped Sniffing On Test Day 2 (Saline Challenge):**

*Conditioned Sniffing.* After receiving a challenge
Figure 11. Mean distance traveled (±SEM) of adult wild-type and D₁₇A-deficient mice (n = 6 - 8, per group) during the second test day. Mice had previously received seven daily injections of saline (SAL) or amphetamine (AMPH; 8.0 mg/kg) in either their home cage or in the test chamber. After 4 abstinence days all mice received a challenge injection of SAL in the testing chamber to assess conditioned activity. Behavioral testing lasted for 60 min and occurred immediately after the second injection. * Significantly different from the SAL group (p < .05).
injection of saline, D₁A-deficient mice sniffed more than wild-type mice (see Figure 12) [genotype main effect, F(1, 36) = 5.42, p < .05]. The differences between the D₁A-deficient and wild-type mice were only apparent after amphetamine pretreatment. Mice pretreated with 8 mg/kg amphetamine in the test chamber, but not in the home cage, had significantly more stereotyped sniffing counts than saline-pretreated mice [pretreatment condition main effect, F(2, 36) = 14.71, p < .001]. Therefore, both D₁A-deficient and wild-type mice showed conditioned sniffing, but the effect was more robust in D₁A-deficient mice.

Summary

These results indicate that both wild-type and D₁A-deficient mice show more robust behavioral sensitization when amphetamine is given in the test chamber (i.e., Pavlovian associations are allowed to form) than in the home cage. Wild-type mice did not show conditioned activity, whereas D₁A-deficient mice showed an exaggerated locomotor response regardless of whether amphetamine pretreatment occurred in the test chamber or home cage. The latter result suggests that D₁A-deficient mice are not showing true 'conditioned activity', but may only be showing a generalized hyperactive response caused by
Figure 12. Mean stereotyped sniffing counts (±SEM) of adult wild-type and D_{1A}-deficient mice (n = 6 - 8 per group) during the second test day. Mice had previously received seven daily injections of saline (SAL) or amphetamine (AMPH; 8.0 mg/kg) in either their home cage or in the test chamber. After 4 abstinence days all mice received a challenge injection of SAL (1.0 mg/kg) in the testing chamber to assess conditioned sniffing. Behavioral testing lasted for 60 min and occurred immediately after the second injection. * Significantly different from the SAL group (p < .05).
amphetamine pretreatment.
EXPERIMENT 3

Research has shown that DA D₁-like receptors are necessary for amphetamine-induced sensitization (Bjijou et al., 1996; Vezina, 1996). By blocking DA D₁-like receptors with the putative DA D₁-like antagonist SCH-23390, locomotor activity was reduced to control levels (Bjijou et al., 1996; Vezina, 1996). However, because the D₁-like family of receptors is composed of two receptors, D₁A and D₅, it is uncertain whether the D₅ receptor subtype is necessary for amphetamine-induced sensitization. Therefore, in the present experiment I pretreated D₁A-deficient and wild-type mice with SCH-23390 prior to their daily amphetamine injections. I predicted that SCH-23390 pretreatment would block amphetamine-induced sensitization in D₁A-deficient mice. The latter result suggested that the D₅ receptor is essential for behavioral sensitization.

Method

Subjects. Subjects were 112 male and female C57BL-6 D₁A-deficient and wild-type mice.

Procedure. The apparatus and procedure described in the General Methods were used with the following exceptions. Subjects were given a preinjection of SCH-23390 (0.15, 0.5 or 1.5 mg/kg, i.p.) or saline 30 min prior to amphetamine
(8.0 mg/kg, i.p.) or saline injections. SCH-23390 injections were given in the home cage, whereas amphetamine was given prior to placement in the activity chamber. Behavioral assessment lasted for 60-min and occurred on seven consecutive days. After three abstinence days, mice were challenged with amphetamine (1.0 mg/kg, i.p.) or saline. Locomotor activity and stereotyped sniffing were assessed for a total of 150 min.

In summary, D_{1A}-deficient and wild-type mice received one of the following nine sequences (ANTAGONIST DRUG-PRETREATMENT DRUG/TEST DAY DRUG) of SCH-23390, amphetamine or saline during the pretreatment phase and on the test day (doses are in parentheses): SAL-SAL/SAL, SAL-SAL/AMPH, SCH(0.15)-SAL/AMPH, SCH(0.5)-SAL/AMPH, SCH(1.5)-SAL/AMPH, SAL-AMPH/AMPH, SCH(0.15)-AMPH/AMPH, SCH(0.5)-AMPH/AMPH, or SCH(1.5)-AMPH/AMPH.

Results

Drug Pretreatment Phase: Locomotor Activity. During the drug pretreatment phase D_{1A}-deficient mice exhibited more locomotor activity than wild-type mice [genotype main effect, F(1,96) = 27.74, p < .001]. The effects of genotype interacted with pretreatment, since D_{1A}-deficient mice pretreated with amphetamine had substantially larger
distance traveled scores \( (M = 150,259 \text{ cm}) \) collapsed across the seven days) than amphetamine-pretreated wild-type mice \( (M = 41,507 \text{ cm}) \) \{genotype \times pretreatment interaction, \( F(1,96) = 12.52, p < .001; \) and Tukey tests, \( p < .05 \}. \) In addition, \( D_{1A} \)-deficient mice pretreated with saline traveled a greater distance \( (M = 28,834 \text{ cm}) \) than similarly treated wild-type mice \( (M = 7,492 \text{ cm}) \) \{Tukey tests, \( p < .05 \}. \) The effects of genotype and pretreatment condition varied according to antagonist \( (\text{i.e., SCH-23390}) \) treatment, so those effects will be described in the subsequent subsections.

**Amphetamine-pretreated mice.** Amphetamine-pretreated \( D_{1A} \)-deficient mice had larger distance traveled scores than wild-type mice \( (\text{see Figure 13}) \) \{genotype main effect, \( F(1,56) = 26.74, p < .001 \}. \) Not surprisingly, this effect varied according to antagonist treatment. Amphetamine-pretreated wild-type mice given 0.15 mg/kg SCH-23390 exhibited more locomotor activity than the 0.5 and 1.5 mg/kg SCH-23390 groups, with the 0.0 mg/kg group being intermediate \( (\text{see upper graph, Figure 13}) \) \{antagonist main effect, \( F(3,28) = 7.11, p < .001; \) and Tukey tests, \( p < .05 \}. \) The differences between the 0.0 mg/kg (open circles)
Figure 13. Mean distance traveled (±SEM) of adult wild-type and $D_{1A}$-deficient mice (n = 8 per group) pretreated with saline (SAL) or the $D_1$-like antagonist SCH-23390 (SCH; 0.15, 0.5, or 1.5 mg/kg i.p.) followed by an injection of amphetamine (8.0 mg/kg, i.p.) 30 min later. This injection regimen was administered for seven consecutive days. Behavioral assessment lasted for 60 min and occurred immediately after the second injection. * Significantly different from 0.00 mg/kg SCH group (p < .05).
and 0.5 mg/kg (filled diamonds) SCH-233390 groups reached statistical significance on pretreatment days 6 and 7 (see upper graph, Figure 13) [antagonist x day interaction, $F(18,168) = 2.79, p < .001$; and Tukey tests, $p < .05$].

SCH-23390 also affected the locomotor activity of D_{1A}-deficient mice injected with amphetamine. For example, amphetamine-pretreated D_{1A}-deficient mice given 0.5 mg/kg SCH-23390 (filled triangles) exhibited more locomotor activity than amphetamine-pretreated mice given 0.0 mg/kg SCH-23390 (open circles) (see lower graph, Figure 13) [genotype x antagonist interaction, $F(3,56) = 2.97, p < .001$; and Tukey tests, $p < .05$]. Amphetamine-pretreated D_{1A}-deficient mice showed a dose-dependent increase in locomotor activity as the pretreatment phase progressed [pretreatment day main effect, $F(6,168) = 10.06, p < .001$; and Tukey tests, $p < .05$].

Saline-pretreated mice. Saline-pretreated D_{1A}-deficient mice had larger distance traveled scores than wild-type mice (see Figure 14) [genotype main effect, $F(1,40) = 41.62, p < .001$]. This effect varied according to antagonist treatment. More specifically, saline-pretreated wild-type mice given SCH-23390 (0.15, 0.5, or 1.5 mg/kg)
Figure 14. Mean distance traveled (±SEM) of adult wild-type and D₁A⁻
deficient mice (n = 6 per group) pretreated with saline (SAL) or the D₁-like antagonist SCH-23390 (SCH; 0.15, 0.5, or 1.5 mg/kg, i.p.) followed
by an injection of SAL 30 min later. This injection regimen was
administered for seven consecutive days. Behavioral assessment lasted
for 60 min and occurred immediately after the second injection.
* Significantly different from 0.00 mg/kg SCH group (p < .05).
had smaller distance traveled scores than mice given 0.0 mg/kg SCH-23390 (open circles) (upper graph, Figure 14) [antagonist main effect, $F(3,20) = 10.12, p < .001$; and Tukey tests, $p < .05$]. Thus, SCH-23390 depressed the locomotor activity of saline-pretreated wild-type mice.

$D_{1A}$-deficient mice exhibited a different pattern of drug effects, since $D_{1A}$-deficient mice given both saline and 0.15 mg/kg SCH-23390 (filled diamonds) exhibited more locomotor activity than $D_{1A}$-deficient mice given saline and 0.0 mg/kg SCH-23390 (open circles) (see bottom graph, Figure 14). Due to the large amount of variance, the latter effect only reached statistical significance on pretreatment day 7 [antagonist x day interaction, $F(18,120) = 1.70, p < .05$; and Tukey tests, $p < .05$].

Drug Pretreatment Phase: Stereotyped Sniffing. Overall, $D_{1A}$-deficient mice had more stereotyped sniffing counts than wild-type mice during the drug pretreatment phase [genotype main effect, $F(1,96) = 15.11, p < .001$]. Not surprisingly, stereotyped sniffing was most prominent in amphetamine-pretreated mice [pretreatment main effect, $F(1,96) = 71.08, p < .001$; and Tukey tests, $p < .05$].

Amphetamine-pretreated mice. Overall, amphetamine-
pretreated D₁A-deficient mice sniffed more than wild-type controls [genotype main effect, $F(1,56) = 14.84$, $p < .001$]. It is apparent, however, that the latter effect was entirely due to the actions of SCH-23390 (see Figure 15) [genotype x antagonist interaction, $F(3,56) = 3.98$, $p < .05$; and Tukey tests, $p < .05$]. Specifically, all doses of SCH-23390 (0.15, 0.5, and 1.5 mg/kg) caused a significant reduction in the stereotyped sniffing of amphetamine-pretreated wild-type mice (see upper graph, Figure 15) [antagonist main effect, $F(3,28) = 38.09$, $p < .001$; and Tukey tests, $p < .05$]. In contrast, only 1.5 mg/kg SCH-23390 decreased the stereotyped sniffing of amphetamine-pretreated D₁A-deficient mice (see lower graph, Figure 15) [antagonist main effect, $F(3,28) = 5.38$, $p < .01$; and Tukey tests, $p < .05$].

Saline-pretreated mice. The stereotyped sniffing of saline-pretreated D₁A-deficient and wild-type mice did not differ (see Figure 16). Nor was the stereotyped sniffing of saline-pretreated D₁A-deficient and wild-type mice affected significantly by SCH-23390 pretreatment.

Test Day Locomotor Activity: Amphetamine Pretreatment Groups. Genotype interacted with antagonist treatment to
Figure 15. Mean stereotyped sniffing counts (±SEM) of adult wild-type and D1A-deficient mice (n = 8 per group) pretreated with saline (SAL) or the D1-like antagonist SCH-23390 (SCH; 0.15, 0.5, or 1.5 mg/kg, i.p.) followed by an injection of amphetamine (8.0 mg/kg, i.p.) 30 min later. This injection regimen was administered for seven consecutive days. Behavioral assessment lasted for 60 min and occurred immediately after the second injection. * Significantly different from 0.00 mg/kg SCH group (p < .05).
Figure 16. Mean stereotyped sniffing counts (±SEM) of adult wild-type and D₁A-deficient mice (n = 6 per group) pretreated with saline (SAL) or the D₁-like antagonist SCH-23390 (SCH; 0.15, 0.5, or 1.5 mg/kg, i.p.) followed by an injection of SAL 30 min later. This injection regimen was administered for seven consecutive days. Behavioral assessment lasted for 60 min and occurred immediately after the second injection.
affect the locomotor activity of amphetamine-pretreated mice (see Figure 17) [genotype × antagonist × time interaction, \( F(42,784) = 2.35, p < .001 \); and Tukey tests, \( p < .05 \)]. The locomotor activity of amphetamine-pretreated wild-type mice was affected by SCH-23390 (see upper graph, Figure 17) [antagonist × time interaction, \( F(42,392) = 2.45, p < .001 \); and Tukey tests, \( p < .05 \)]. More specifically, wild-type mice pretreated with both 0.5 mg/kg SCH-23390 and amphetamine (filled triangles) exhibited significantly less locomotor activity on time blocks 1-3 than wild-type mice given 0.0 mg/kg SCH-23390 and amphetamine (open circles). Thus, a moderate dose of SCH-23390 (0.5 mg/kg) was able to attenuate the development of amphetamine-induced sensitization in wild-type mice. The higher dose of SCH-23390 (1.5 mg/kg) produced a decline in amphetamine-induced activity, but this did not reach statistical significance.

The locomotor activity of amphetamine-pretreated D_{1A}-deficient mice was not significantly affected by SCH-23390 (see lower graph, Figure 17). Thus, there is no evidence that SCH-23390 pretreatment blocked the development of amphetamine-induced locomotor sensitization in D_{1A}-deficient mice.
Figure 17. Mean distance traveled (±SEM) of adult wild-type and D_{1A}-deficient mice (n = 8 per group) during testing (these are the same mice as in Figures 11 and 13). Mice had previously received seven consecutive injections of either saline (SAL) or the D_{1}-like antagonist SCH-23390 (SCH; 0.15, 0.50, or 1.5 mg/kg, i.p.) followed by a second injection of amphetamine (0.0 mg/kg, i.p.) 30 min later. After 3 abstinence days all mice received a challenge injection of AMPH (1.0 mg/kg, i.p.). Behavioral testing lasted for 150 min and occurred immediately after second injection. * Significantly different from the 0.0 mg/kg SCH group (p < .05).
Test Day Locomotor Activity: Saline Pretreatment Groups. Genotype and antagonist treatment interacted to affect the distance traveled scores of saline-pretreated mice (see Figure 18) [genotype x antagonist interaction, \( F(3,40) = 5.17, p < .01; \) and Tukey tests, \( p < .05 \)]. In wild-type mice, pretreatment with SCH-23390 alone (i.e., no amphetamine was given during the pretreatment phase) resulted in enhanced locomotor responding after acute injection of 1 mg/kg amphetamine on the test day (see upper graph, Figure 18). More specifically, wild-type mice pretreated with both saline and SCH-23390 (0.5 or 1.5 mg/kg) exhibited more test day locomotor activity than mice pretreated with only saline [antagonist main effect, \( F(3,20) = 5.73, p < .001; \) and Tukey tests, \( p < .05 \)]. SCH-23390-pretreated D1A-deficient mice showed a similar pattern of effects, but the results did not reach statistical significance (see lower graph, Figure 18).

Test Day Stereotyped Sniffing. Amphetamine pretreatment produced a robust sensitized sniffing response (see Figure 19). More specifically, mice pretreated with 8 mg/kg amphetamine, and challenged with 1 mg/kg amphetamine, exhibited more stereotyped sniffing than mice acutely
Figure 18. Mean distance traveled (±SEM) of adult wild-type and D_{1A}-deficient mice (n = 6 per group) during testing (these are the same mice as in Figures 12 and 14). Mice had previously received seven consecutive injections of either saline (SAL) or the D_{1}-like antagonist SCH-23390 (0.15, 0.50, or 1.5 mg/kg, i.p.) followed by an injection of SAL 30 min later. After 3 abstinence days all mice received a challenge injection of AMPH (1.0 mg/kg, i.p.). Behavioral testing lasted for 150 min and occurred immediately after injection. * Significantly different from the 0.0 mg/kg SCH group (p < .05).
Figure 19. Mean stereotyped sniffing counts (±SEM) of adult wild-type and D1A-deficient mice (n = 6 - 8 per group) during testing (these are the same mice as in Figures 11, 12, 13, and 14). Mice had previously received seven consecutive injections of either saline (SAL) or the D1-like antagonist SCH-23390 (SCH; 0.15, 0.50, or 1.5 mg/kg, i.p.) followed by an injection of SAL or amphetamine (8.0 mg/kg, i.p.) 30 min later. After 3 abstinence days all mice received a challenge injection of AMPH (1.0 mg/kg, i.p.). Behavioral testing lasted for 150 min and occurred immediately after injection.
challenged with amphetamine on the test day [pretreatment main effect, $F(1,96) = 23.26, p < .001$]. Neither genotype nor SCH-23390 pretreatment affected the overall pattern of these results [$p > .05$].

Summary

These results indicate that D$_{1A}$-deficient mice do not require stimulation of either member of the D$_1$-like family of receptors to exhibit amphetamine-induced behavioral sensitization. In contrast, wild-type only exhibit amphetamine-induced behavioral sensitization if the D$_1$-like receptor system is functional.
DISCUSSION

Previous research has indicated that the DA D₁-like family of receptors is important for behavioral sensitization (Bjijou et al., 1996; Crawford et al., 1997; Vezina, 1996). The purpose of the present study was to determine whether a specific member of the D₁-like receptor family, the DA D₁A receptor subtype, is necessary for amphetamine-induced behavioral sensitization. As predicted, chronic amphetamine treatment produced a sensitized behavioral response in wild-type mice. In fact, wild-type mice given repeated injections of amphetamine showed both sensitized locomotor activity and stereotyped sniffing when tested after 3 and 17 abstinence days (see Figures 3, 4, 5 and 6). Importantly, chronic amphetamine treatment also produced a sensitized locomotor response in D₁A-deficient mice (see lower graphs, Figures 3, 5 and 7).

Although contrary to my original hypotheses, the latter results are not surprising because Crawford et al. (1997) have reported that D₁A-deficient mice exhibit amphetamine-induced locomotor sensitization after three abstinence days. The present results extend the findings of the Crawford et al. (1997) study in two important ways. First, the present study is the first to show that D₁A-
deficient mice will exhibit sensitization of a stereotyped behavior (i.e., stereotyped sniffing). Second, this is the first study to show that the sensitized locomotion and sniffing exhibited by D_{1A}-deficient mice will persist across an extended drug abstinence period (i.e., 17 days). Therefore, when these results are considered together, it is apparent that the D_{1A} receptor is not necessary for either the short- or long-term expression of amphetamine-induced behavioral sensitization.

An additional purpose of this study was to determine whether the associative learning processes involved in behavioral sensitization require a functioning D_{1A} receptor system. As predicted, wild-type mice pretreated with amphetamine in the testing chamber exhibited sensitized locomotor activity and sniffing (see upper graphs, Figures 9 and 10). Wild-type mice did not exhibit behavioral sensitization when amphetamine pretreatment occurred in the home cage. As with wild-type mice, D_{1A}-deficient mice showed more robust behavioral sensitization when amphetamine-pretreatment occurred in the testing chamber; however, only D_{1A}-deficient mice exhibited sensitized locomotor activity and sniffing when amphetamine pretreatment occurred in the home cage (see lower graphs,
Figures 9 and 10). Therefore, these data suggest that conditioning factors (i.e., Pavlovian associations) are less important for D_{IA}-deficient mice than for wild-type controls (i.e., only D_{IA}-deficient mice exhibited behavioral sensitization when amphetamine was not paired with the test chamber).

This conclusion is only tentative, since D_{IA}-deficient mice showed more robust conditioned activity than wild-type controls. Specifically, wild-type mice did not exhibit conditioned activity when saline was administered on the second test day (see upper graph, Figure 11), and only exhibited conditioned sniffing when amphetamine pretreatment was given in the test chamber, but not in the home cage (see Figure 12). In contrast, amphetamine-pretreated D_{IA}-deficient mice showed pronounced conditioned locomotion and sniffing (see Figures 11 and 12). This effect was very complex, however, since conditioned effects were observed when D_{IA}-deficient mice received amphetamine in either the home cage or test chamber. Thus, amphetamine-pretreated D_{IA}-deficient mice showed "conditioned" activity and sniffing regardless of whether amphetamine was actually paired with the testing chamber. Hence, rather than producing true conditioned activity,
amphetamine pretreatment in the home cage might simply have caused an exaggerated responsiveness in D_{1A}-deficient mice (i.e., Pavlovian associations may have only been partly responsible for the increased locomotor activity exhibited by saline-challenged D_{1A}-deficient mice).

A final goal of this study was to determine whether the other member of the D_{1}-like receptor family, the DA D_{5} receptor subtype, was necessary for amphetamine-induced behavioral sensitization. To that end, wild-type and D_{1A}-deficient mice were given daily pretreatment injections of the D_{1}-like receptor antagonist SCH-23390. As predicted, SCH-23390 (0.5 mg/kg) blocked the development of locomotor sensitization in wild-type mice (see upper graph, Figure 17). This suggests that the D_{1} family of receptors (either the D_{1A} or D_{5} receptor subtype) is important for behavioral sensitization. Importantly, SCH-23390 did not attenuate the locomotor sensitization exhibited by D_{1A}-deficient mice (see lower graph, Figure 17). The most parsimonious explanation is that neither the D_{1A} nor D_{5} receptor subtypes are necessary for the amphetamine-induced locomotor sensitization of D_{1A}-deficient mice; whereas, D_{1}-like receptors are necessary for the amphetamine-induced locomotor sensitization of wild-type controls.
These finding are novel and suggest many ideas: first, D_1A-receptors may not be important for long-term changes in behavior (i.e., plasticity); second, Pavlovian associations may not be as important to D_1A-deficient mice as they are for wild-type mice; third, it is possible that neither D_1A nor D_5 receptors are important for amphetamine-induced behavioral sensitization in D_1A-deficient mice; fourth, compensatory mechanisms may be responsible for the amphetamine-induced behavioral sensitization exhibited by D_1A-deficient mice; and, fifth, the behaviors (i.e., sensitized locomotor activity, sensitized stereotyped sniffing, exaggerated conditioned activity etc...) exhibited by D_1A-deficient mice may have been influenced by a lack of dynorphin.

**DA D_1A Receptor Involvement in Amphetamine Sensitization**

As previously reported, D_1-like receptor antagonists block amphetamine- and cocaine-induced behavioral sensitization (Bjijou et al., 1996; Kuribara, 1995; Vezina, 1996; Vezina & Stewart, 1989). Therefore, available evidence suggests that stimulation of D_1-like receptors is necessary for the occurrence of amphetamine-induced behavioral sensitization. Yet when D_1A-deficient mice were challenged with amphetamine, a sensitized response persisted for up to 17 abstinence days. That D_1A-deficient
mice also showed a sensitized stereotyped sniffing response is interesting, since D₁-like receptor stimulation is necessary for D₂-like mediated behaviors (stereotyped sniffing often requires a combination of D₁-like and D₂-like receptor stimulation) (see Clark & White, 1987, for a review). At the very least, it seems clear that the D₁A receptor is not required to produce stereotyped sniffing in D₁A receptor-deficient mice.

DA D₅ Involvement in Amphetamine-Induced Locomotor Sensitization

Both pretreatment and challenge day data present a similar picture. That is, SCH-23390 pretreatment blocked locomotor sensitization in wild-type mice, but not D₁A-deficient mice (see upper graph, Figure 17). During the pretreatment phase, the higher doses of SCH-23390 (0.5 and 1.5 mg/kg) completely blocked amphetamine-induced locomotor activity of wild-type mice (see upper graph, Figure 13). This is consistent with the challenge day data, because 0.5 mg/kg SCH-23390 blocked the expression of locomotor sensitization in amphetamine-challenged wild-type mice (see upper graph, Figure 17).

In contrast, SCH-23390 pretreatment did not block the amphetamine-induced locomotor activity of D₁A-deficient
mice. This pattern of results is also consistent with challenge day data, in that SCH-23390 pretreatment was unable to block the expression of amphetamine-induced locomotor sensitization in D_{1A}-deficient mice (see lower graph, Figure 17). So, D_{1A}-deficient and wild-type mice exhibit distinctly different behavior patterns after chronic amphetamine treatment. That is, D_{1A}-deficient mice do not need D_{1A} or D_{5} receptor stimulation to exhibit amphetamine-induced locomotor sensitization, whereas wild-type mice require a functioning D_{1}-like receptor system.

Originally, I hypothesized that if D_{1A}-deficient mice showed behavioral sensitization, the D_{5} receptor would play a necessary role in mediating this effect. The data do not support this hypothesis. Instead, it is apparent that D_{1}-like receptors are necessary for the locomotor sensitization of wild-type mice, but that neither D_{1A} nor D_{5} receptors are necessary for the locomotor sensitization of D_{1A}-deficient mice. Several explanations may account for these results. First, SCH-23390 may have a greater affinity for D_{1A}, as opposed to D_{5} receptors. Thus, SCH-23390 may not have fully antagonized D_{5} receptors, perhaps allowing them to mediate sensitization in D_{1A}-deficient mice. Second, inherent in recombinant technology is the issue of
compensation. In this case, compensatory mechanisms may have allowed amphetamine-induced behavioral sensitization to occur in the absence of D₁-like receptors.

The Influence of Compensatory Mechanisms in D₁A-Deficient Mice

The possibility that compensation may allow D₁A-deficient mice to exhibit locomotor sensitization is particularly interesting, because reports have suggested a role for compensation in several different knockout mice. For instance, the C57BL-6 mouse strain includes several knockout mice, some of which lack the DA transporter, or the D₁A, D₂, or D₄ receptors (Drago, Padungchaichot, Domenico, & Fuchs, 1998). The possibility of compensatory mechanisms is plausible since D₁-like receptors have been identified early in gestation in normal mice (i.e., during embryogenesis), leaving the strong possibility that some compensatory mechanism might have developed by adulthood in these mice (Clifford et al., 1998; Drago et al., 1998).

Nonetheless, although behavioral data from the present study strongly suggest the presence of compensatory mechanisms in D₁A-deficient mice, no compensatory mechanisms (e.g., upregulated D₅ receptors, dynorphin levels, changes in D₂-like receptor levels, etc.) have been discovered.
D2-Like Receptor Involvement in Plasticity

It is interesting that SCH-23390 pretreatment blocked the amphetamine-induced sniffing of wild-type mice during the pretreatment phase, but was ineffective at attenuating sniffing on the challenge day (see Figures 15 and 19). These results suggest that the D1-like receptor is necessary for the occurrence of stereotyped sniffing, but is not necessary for the eventual expression of a sensitized sniffing response. The pretreatment data are consistent with the idea that D1-like receptor activation is needed for the occurrence of D2-like receptor-mediated sniffing (Clark & White, 1987), but the challenge day data make a powerful statement that D1-like receptor activation may not be necessary to produce the underlying neurobiological changes (i.e., plasticity) required for a sensitized sniffing response.

Conditioned Activity in Wild-Type and D1A-Deficient Mice

One of the more interesting findings of this study was the robust locomotor activity exhibited by saline-challenged D1A-deficient mice (see lower graph, Figure 11). Specifically, D1A-deficient mice pretreated with amphetamine in either the testing chamber or home cage showed...
substantial amounts of locomotor activity after saline challenge (i.e., more than saline-pretreated rats). This effect was not observed in wild-type mice (see upper graph, Figure 11). Because robust locomotor activity was apparent in D1A-deficient mice pretreated with amphetamine in the home cage, it appears that non-associative mechanisms, other than Pavlovian processes, were responsible for this increased locomotor activity. One possibility is that a generalized heightened responsiveness could have caused the increased locomotion in saline-challenged D1A-deficient mice. Thus, rather than showing increased locomotor activity due to the presence of drug-paired environmental cues, amphetamine-pretreated D1A-deficient mice may have only been exhibiting a generalized heightened responsiveness that would have been expressed in any environment (see Tirelli & Terry, 1998, for a relevant discussion). Consistent with this idea, D1A-deficient mice have been described as 'hyperactive' in other reports (Clifford et al., 1998).

**D1-Like Receptor Antagonism: Behavioral Evidence For Receptor Upregulation or Supersensitivity**

Wild-type mice pretreated with 0.5 or 1.5 mg/kg SCH-23390 alone (i.e., no amphetamine) showed enhanced
locomotor activity when given an acute injection of amphetamine on the test day (see upper graph, Figure 18). The only plausible explanation for this effect was that chronic SCH-23390 pretreatment caused an upregulation of D₁-like receptors in wild-type mice (see Giorgi, Pibiri, Loi, & Corda, 1993; O’Boyle, Gavin, & Harrison, 1993). According to this idea, a test day injection of amphetamine had enhanced behavioral impact in SCH-23390-pretreated wild-type mice because of the increased number of D₁-like receptors. Conversely, SCH-23390-pretreated D₁A-deficient mice did not show a significant increase in amphetamine-induced locomotor activity on the test day (see lower graph, Figure 18), although a nonsignificant trend was apparent. Because D₁A-deficient mice lack D₁A receptors, only a drug-induced upregulation (or supersensitivity) of D₅ receptors could have occurred. If SCH-23390 pretreatment did result in an excess number of D₅ receptors (or supersensitive D₅ receptors) it was insufficient to significantly alter the amphetamine-induced locomotor activity of D₁A-deficient mice.

The Role of Dynorphin in D₁A-Deficient Mice

κ-Opioid receptors are located on the presynaptic
processes of DA neurons comprising the nigrostriatal and mesolimbic pathways (see Figure 20) (Hyman, 1996; Steiner & Gerfen, 1998). Dynorphin (or the endogenous ligand of \( \kappa \)-opioid receptors) acts on these presynaptic receptors to inhibit DA release in both the striatum and nucleus accumbens (Di Chiara & Imperato, 1988; Spanagel, Herz, & Shippenberg, 1992; Zaratin & Clarke, 1994). Importantly, dynorphin levels are dramatically reduced in \( D_{1\alpha} \)-deficient mice (Xu, Moratalla, Gold, Hiroi, Koob, Graybiel, & Tonegawa, 1994), thus \( D_{1\alpha} \)-deficient mice may have elevated basal levels of striatal and accumbal DA. If true, this excess DA may be responsible for the heightened responsiveness exhibited by \( D_{1\alpha} \)-deficient mice (see lower graph, Figure 11). This heightened responsiveness may have produced a behavior pattern that mimics behavioral sensitization and/or conditioned activity. Thus, it is interesting whether amphetamine sensitization would disappear in \( D_{1\alpha} \)-deficient mice if dynorphin was replaced and/or \( \kappa \)-opioid receptors were stimulated.

Since an upregulation of \( D_{5} \) receptors have been eliminated as a possible form of compensation in \( D_{1\alpha} \)-deficient mice (see Figure 18), questions arise whether
Figure 20. K-Opioid receptors are located on the presynaptic processes of DA neurons comprising the nigrostriatal and mesolimbic pathways. Dynorphin (the endogenous ligand of k-opioid receptors) acts on these presynaptic receptors to inhibit DA release in both the striatum and nucleus accumbens. Importantly, dynorphin levels are dramatically reduced in D₁A-deficient mice, thus D₁A-deficient mice may have elevated basal levels of striatal and accumbal DA. If true, this excess DA may be responsible for the heightened responsiveness exhibited by D₁A-deficient mice. DYN = Dynorphin; DA = Dopamine; k = K-Opioid receptors; GABA = γ-Amino Butyric Acid; EAA = Excitatory Amino Acid.
changes in dynorphin levels may be responsible for this compensation. As mentioned above, it is possible that the lack of dynorphin may act as a compensatory mechanism in D₁A-deficient mice, allowing these mice to express amphetamine-induced behavioral sensitization without a functional D₁-like receptor system. This is important because D₁-like receptors have been shown in several reports to have a central role in amphetamine-induced behaviors, as well as amphetamine sensitization. In addition, this proposed compensatory mechanism could be responsible for the hyperactivity evident in these mice, as well as the heightened display of conditioned activity. Further experimentation may reveal how these behaviors were manifest and what role dynorphin plays in D₁A-deficient mice.

Summary

When taken together, the results of the present study provide important evidence about the role of the D₁-like family of receptors in behavioral sensitization. For example, neither the D₁A nor the D₅ receptor is necessary for amphetamine-induced locomotor sensitization in D₁A-deficient mice, but the DA D₁-like receptor system is needed for locomotor sensitization in wild-type mice. D₁A-deficient
mice apparently have a compensatory mechanism, not involving D₅ receptors, that allows the development and expression of behavioral sensitization. The sensitization exhibited by D₁A-deficient and wild-type mice is more robust when drug-paired environmental cues are available (i.e., when drug pretreatment and drug challenge occur in the test chamber). Interestingly, amphetamine-pretreated D₁A-deficient mice, unlike wild-type controls, show a heightened behavioral responsiveness that is not due to Pavlovian associations. Therefore, while both D₁A-deficient and wild-type mice are capable of showing amphetamine-induced behavioral sensitization, these mice exhibit interesting behavioral differences that are presumably due to the lack of D₁A receptors and/or compensatory mechanisms.
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