6-2016

IMPORTANCE OF THE D2 RECEPTOR FOR ONE- AND MULTI-TRIAL PSYCHOSTIMULANT-INDUCED BEHAVIORAL SENSITIZATION IN PREWEANLING RATS

Martha A. Mohd-Yusof
California State University - San Bernardino, alenayusof@hotmail.com

IMPORTANCE OF THE D2 RECEPTOR FOR ONE- AND MULTI-TRIAL PSYCHOSTIMULANT-INDUCED BEHAVIORAL SENSITIZATION IN PREWEANLING RATS

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

General-Experimental Psychology

---

by

Martha Alena Mohd-Yusof

June 2016

IMPORTANCE OF THE D2 RECEPTOR FOR ONE- AND MULTI-TRIAL PSYCHOSTIMULANT-INDUCED BEHAVIORAL SENSITIZATION IN PREWEANLING RATS

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

---

by

Martha Alena Mohd-Yusof

June 2016

Approved by:

Dr. Sanders McDougall, Committee Chair, Psychology

Dr. Cynthia Crawford, Committee Member

Dr. Andre Obenaus, Committee Member, Loma Linda University

ABSTRACT

The neural mechanisms mediating one-trial and multi-trial behavioral sensitization during early ontogeny are poorly understood. The purpose of this thesis was to assess the importance of D2-like receptors for the induction of cocaine- and methamphetamine-induced one-trial and multi-trial behavioral sensitization during the middle and late preweanling period. In a series of four experiments, rats were injected with saline or the selective dopamine D2-like receptor antagonist raclopride 15 min prior to treatment with the indirect dopamine agonists cocaine or methamphetamine. Acute control groups received two injections of saline. The pretreatment regimens occurred on either PND 16 or PND 20 (one-trial behavioral sensitization) or PND 13-16 or PND 17-20 (multi-trial behavioral sensitization). On PND 17 or PND 21, rats were challenged with either cocaine or methamphetamine and sensitized responding was assessed. With only a single exception, both one-trial and multi-trial cocaine- and methamphetamine-induced sensitization was evident on PND 17 and PND 21. Importantly, the D2-like receptor antagonist raclopride did not prevent the induction of cocaine- or methamphetamine-induced one-trial behavioral sensitization. In regards to multi-trial behavioral sensitization, raclopride failed to inhibit cocaine-induced sensitized responding on PND 17 and PND 21. Interestingly, higher doses of raclopride (0.5 and 1 mg/kg) were able to prevent the induction of multi-trial methamphetamine-induced sensitization on PND 17. Therefore, D2-like

receptor antagonism differentially affected methamphetamine-induced behavioral sensitization depending on whether a one-trial or multi-trial paradigm was employed. When considered together, these results suggest that the neural mechanisms underlying the methamphetamine-induced behavioral sensitization of preweanling rats differs depending on the type of experimental paradigm (one- vs multi-trial) being used. Other potential explanations (i.e., nonspecific antagonist effects, impact of contextual conditioning, etc.) for this interesting effect are presented in the Discussion.

ACKNOWLEDGMENTS

I would like express with great gratitude to my mentor and committee chair Dr. McDougall for his support throughout this thesis project. His persistent guidance and wisdom has enabled me to overcome the many obstacles that I have encountered during my graduate studies. His enthusiasm for research has allowed me to grow and prosper in the field of behavioral sciences.

I would like to thank Dr. Crawford for her mentorship that allowed me the opportunity to begin my path as a researcher. I would also like to thank Dr. Obenaus for his teachings and passion for research which has given me the insight to explore outside conventional thinking and to further develop my leadership skills.

To my research colleagues that contributed to this thesis, I greatly appreciated all of the help. With your assistance, I was able to manage and complete this thesis.

Thank you to my family who have supported me throughout this journey. Thank you for all of the sacrifices that have been made in order for me to succeed.

Last but not least, I would like thank my wonderful husband for his unconditional love and encouragement. Thank you for teaching me how to live life to the fullest.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

HUMAN MODELS OF ADDICTION

Psychostimulants have addictive properties that can induce complex effects on human behavior. Taking into account the increasing rate of psychostimulant use, psychostimulant addiction has become a profound public health concern (Sax & Strakowski, 2001). One of the most frequently used models to study the underlying mechanisms of drug addiction is behavioral sensitization. Behavioral sensitization is characterized by a progressive increase in behavioral responsiveness as a result of repeated exposures to a psychostimulant drug (Kalivas & Stewart, 1991; Robinson & Becker 1986; Sax & Strakowski, 2001; Strakowski & Sax, 1998). Figure 1 compares the typical pattern of drug-taking in human addicts with a model of behavioral sensitization in animals.

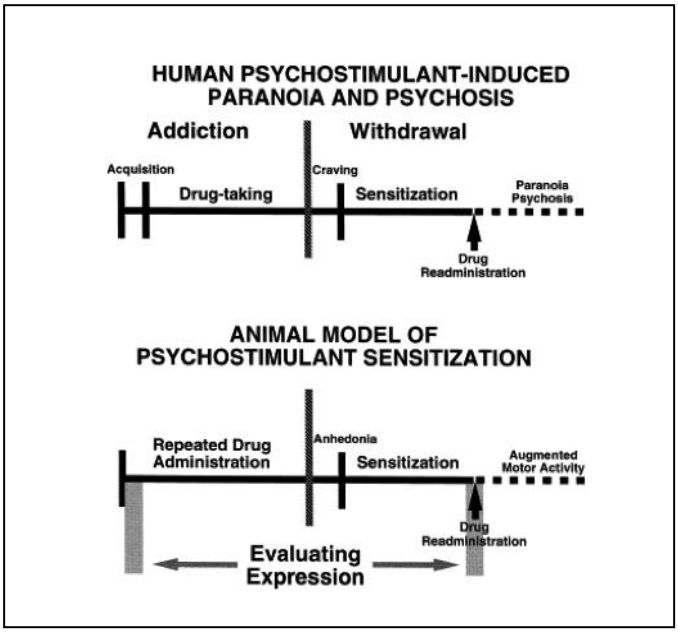

Figure 1. Comparison between Human Psychostimulant-induced Psychosis and an Experimental Model of Behavioral Sensitization in Rats. The Addiction Phase can Progressively Lead to Sensitization in Psychostimulant-induced Psychosis. In Rats, Repeated Exposure to Psychostimulants can Lead to Heightened Motor Activity. Adapted from Pierce and Kalivas (1997).

Although most behavioral sensitization studies are based on animal models, recent evidence in the clinical literature has shown a role for sensitization in human drug-seeking (Strakowski & Sax, 1998). For instance, Strakowski, Sax, Setters, and Keck (1996) reported that volunteers with no prior substance abuse history showed a progressive increase in energy, mood, speech, and eye-blink rates when repeatedly treated with amphetamine (0.25 mg/kg) twice daily over a span of four days. In another study, participants showed a progressive increase in eye-blink rates and

motor activity after receiving a single oral dose of amphetamine (0.25 mg/kg) for six days (Strakowski & Sax, 1998). These data indicate that behavioral sensitization occurs in humans and that the phenomenon may play a role in drug addiction.

While numerous studies have examined the sensitizing effects of psychostimulants in animal and human models, less focus has been paid to the underlying neurobiological processes responsible for behavioral sensitization. Dopamine plays a key role in mediating the stimulatory properties of psychostimulants. Therefore, it is not surprising that dopamine receptor systems have been linked to the induction (i.e., development) and expression of psychostimulant-induced behavioral sensitization (Kuribara & Uchihashi, 1993; Vezina & Stewart, 1989). The induction of behavioral sensitization appears to be due to transient changes in neural functioning, while the expression of sensitization is associated with enduring changes in cellular function during withdrawal (Kalivas & Stewart, 1991).

CHAPTER TWO

DOPAMINE PHARMACOLOGY

Introduction

Catecholamine neurotransmitters are notable for their core chemical structure referred to as the catehol (McTavish, Cowen, & Sharp, 1999). Dopamine is one of the predominant catecholamine neurotransmitters in the central nervous system (Ho & Loh, 1972). It is involved in a variety of functions, such as motor activity, cognition, and hormone secretion (Jaber, Robinson, Missale, & Caron, 1996).

In 1910, George Barger and James Ewens were the first to synthesize dopamine. With the progression of research, dopamine was discovered to be a neurotransmitter in the late 1950s. This was achieved when spectrophoto-fluorometric techniques revealed significantly higher concentrations of dopamine in the caudate nucleus than norepinephrine (Carlsson, Lindqvist, Magnusson, & Waldeck, 1958). The dopaminergic system was further explored by Dahlstrom and Fuxe (1965), who discovered dopamine-containing pathways and their associated projections to various areas of the forebrain.

Dopamine Projection Pathways

Dopaminergic neurons mediate gross and fine motor movements, as well as reinforcement and planning (Ando, Johanson, Seiden, & Schuster,

1985). Areas of the brain that have particularly high dopamine concentrations include the substantia nigra pars compacta, hypothalamus, and ventral tegmental area (Geffen, Jessell, Cuello & Iversen, 1976; Palkovits, Brownstein, Saavedra, & Axelrod, 1974). These specific brain regions give origin to three major dopamine projection pathways. The nigrostriatal pathway, which modulates motor activity, begins at the substantia nigra and projects to the basal ganglia (Geffen et al., 1976; Huang, Zhou, Chase, Gusella, Aronin, & DiFiglia, 1992). Interestingly, degeneration of nigral neurons, which make up the nigrostriatal pathway, contributes to the progression of Parkinson's disease (Damier, Hirsch, Agid, & Graybiel, 1999). The mesolimbic pathway originates in the ventral tegmental area and projects to the nucleus accumbens (i.e., ventral striatum). These brain areas mediate reward and play a key role in addictive behaviors (Chang & Kitai, 1985). Lastly, the mesocortical pathway originates in the ventral tegmental area and projects to the prefrontal cortex (Carr & Sesack, 2000; Lewis & O'Donell, 2000). This pathway contributes to higher-level cognitive functions and planning (Seamans, Floresco, & Phillips, 1998).

Synthesis of Dopamine

Synthesis of dopamine occurs in several biochemical steps (Nagatsu, Levitt, & Udenfriend, 1964). The process is initiated when tyrosine is catalyzed by tyrosine hydroxylase (TH) and produces dihydroxyphenylalanine (L-DOPA) (Nagatsu, Levitt, & Udenfriend, 1964; Smidt, Smits, & Burbach, 2003).

Dopamine is made when L-DOPA is further catalyzed by the enzyme aromatic amino acid decarboxylase (AADC) (Roth, 1979; Sourkes, 1979). The rate of dopamine synthesis is determined by the activity of tyrosine hyrdroxylase (i.e. the rate-limiting step). Production of dopamine occurs in the presynaptic terminals of dopaminergic neurons, after which the neurotransmitter is packaged into synaptic vesicles. Dopamine is then released via calcium-dependent exocytosis (Binder, Kinkead, Owens, & Nemeroff, 2001).

Dopamine Receptors: D1-Like and D2-Like

The classification of dopamine receptors is based on their interaction with G-coupled protein complexes. There are a total of five dopamine receptor subtypes that can be categorized into two populations of dopamine receptors, namely D1-like and D2-like receptors. Based on similarities in pharmacological actions and structure, the D1-like family is made up of D_1 and D_5 receptors; whereas, the D2-like family consists of the D_2, D_3, and D_4 receptors (Jaber et al., 1996).

In regards to pharmacological actions, D1-like receptors are coupled with G_s complexes. When activated, these G_s complexes stimulate the activity of adenylyl cyclase and increase the production of cyclic AMP. In contrast, D2-like receptors are coupled with G_i complexes and inhibit the activation of adenylyl cyclase (Kebabian, Beaulieu, & Itoh, 1984). The disparate actions of D1-like and D2-like receptors are described in pharmacological studies using agonists and antagonists. For example, Roberts-Lewis et al. (1986) provided

evidence that D1-like receptors are positively coupled with adenylyl cyclase activity by measuring dopamine release after SKF38393 or amphetamine treatment. The inhibitory relationship between adenylyl cyclase activity and D2-like receptors was discovered by applying vasoactive intestinal peptide in the anterior pituitary gland (Onali, Schwartz, & Costa, 1981).

Dopamine Receptor Distribution in the Brain

D1-Like Receptors

The D_1 receptor subtype is the most widespread and highly concentrated dopamine receptor in the brain (Boyson, McGonigle, & Molinoff, 1986). A technique called *in-situ* hybridization is commonly used to examine gene expression in individual cells (Langdale, 1994). In regards to distribution of dopamine receptors in the brain, *in-situ* hybridization studies have shown that dopamine D_1 receptors are primarily localized in the striatum, nucleus accumbens, and olfactory tuburcle, whereas cells expressing D_1 receptor mRNA are located in the thalamus, hypothalamus, and limbic system (Fremeau, Duncan, Fornaretto, Dearry, Gingrich, Breese, & Caron, 1991). Other techniques, such as autoradiography, have also been used to localize dopamine receptor sites. For example, Boyson et al. (1986) examined the distribution of dopamine receptors by using the D1-like radioligand SCH 23390. Results showed that D_1 receptors were present throughout the forebrain, with the highest densities occurring in the substantia nigra, nucleus accumbens, olfactory tubercle, and striatum (Boyson et al., 1986).

Although difficult to examine due to the lack of selective ligands, D_5 receptor mRNA is expressed in striatum, cerebral cortex, lateral thalamus, medial thalamus, and hippocampus (Choi, Machida, & Ronnekleiv, 1995; Meador-Woodruff, Mansour, Grandy, Damask, Civelli, & Watson, 1992).

D2-Like Receptors

D_2 receptors are found in lower quantities than D_1 receptors (Boyson et al., 1986). *In-situ* hybridization revealed that D_2 receptor mRNA was found in the ventral tegmental area, nucleus accumbens, olfactory tubercle, and substantia nigra as well as in other dopamine projection fields (Meador-Woodruff, Mansour, Bunzow, Van Tol, Watson, & Civelli, 1989). Autoradiography studies also show that D_2 receptors are found in high densities in the olfactory bulb and lateral septum (Charuchinda, Supavilai, Karobath, & Palacios, 1987).

The D_3 receptor is expressed preferentially in the mesolimbic system, but in lower quantities than D_1 and D_2 receptors (Richtand et al., 1995). While D_3 mRNA is found in the olfactory tubercle and nucleus accumbens, especially high densities of D_3 mRNA are expressed in the islands of Calleja (Diaz, Levesque, Lammers, Griffon, Martres, Schwartz, & Sokoloff, 1995).

Distribution of the D_4 receptor is unique, because D_4 mRNA expression is minuscule in striatal areas; whereas, D_1, D_2, D_3, and D_5 receptor mRNA is abundant in the striatum. The D_4 receptor subtype is mainly concentrated in the hippocampus, lateral septal nucleus, entorhinal cortex, and medial

preoptic area of the hypothalamus (Primus, Thurkauf, Xu, Yevich, McInerney, Shaw, Tallman, & Gallager, 1997).

CHAPTER THREE

ONTOGENY OF THE DOPAMINE SYSTEM

Previous studies examining the dopamine system during postnatal development have focused on age-dependent changes in dopamine levels and the distribution of dopamine receptors across brain. Techniques such as *in-situ* hybridization, autoradiography, as well as receptor binding are commonly used to visualize and quantify ontogenetic changes in the dopamine system.

As is true of adult rats, dopamine neurons in neonatal and preweanling rats are predominantly found in the substantia nigra, ventral tegmental area, and hypothalamus. These brain regions give rise to three main pathways (i.e. the nigrostriatal, mesolimbic, and mesocortical pathways) that are present at birth (Antonopoulos, Dori, Dinopoulos, Chiotelli, Parnavelas, 2002; Chang & Kitai, 1985; Geffen, Jessell, Cuello, & Iversen, 1976; Lewis & O'Donell, 2000; Olson & Seiger, 1972). Dopamine synthesis can be detected at birth, with dopamine levels increasing linearly until approximately puberty, when adult levels are reached (Olson & Seiger, 1972; Park, Kitahama, Geffard, & Maeda, 2000).

Postnatal Development: D1-Like and D2-Like Receptors

During the first three postnatal weeks, profound changes in the profile of dopamine receptors take place. As mentioned previously, dopamine

receptors are classified based on their interaction with G-coupled protein complexes that either stimulate or inhibit adenylyl cyclase activity. Autoradiography studies examining the development of dopamine receptors indicate that D1-like receptors steadily increase in density from postnatal day 1 (PND 1) to around PND 28, when dopamine receptors have reached adult levels (Murrin & Zeng, 1990; Rao, Molinoff, & Joyce, 1991; Zeng, Hyttel, & Murrin, 1988). Other studies demonstrate a gradual increase in D1-like receptors until approximately the onset of puberty (PND 40) when dopamine receptors are over-expressed. The number of dopamine receptors then decline (pruning), to levels that are maintained throughout adulthood (Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000; Giorgi, DeMontis, Porceddu, Mele, Calderini, Toffano, & Biggio, 1987).

D2-like receptors also progressively increase with age, and reach adult levels around PND 21 (Hartley & Seeman 1983; Murrin & Zeng, 1986; Schrambra, Duncan, Breese, Fornaretto, Caron, & Fremeau, 1994). Other studies report a linear increase in D2-like receptors up to adolescence when dopamine receptors are over-expressed, followed by a decline in receptors that are maintained throughout adulthood (Andersen et al., 2000).

Dopamine Receptor Distribution During Postnatal Development

D1-Like Receptors

The D_1 receptor subtype can be detected in the striatum and nucleus accumbens at birth (Leslie, Robertson, Cutler, & Bennett, 1991; Zeng, Hyttel,

& Murrin, 1988). In addition, D_1 receptors are highly concentrated in the frontal cortex as well as the entorhinal cortex during early postnatal development (Tarazi & Baldessarini, 2000). Normal development of dopamine D_1 receptors can be disrupted when neonatal pups are injected with the neurotoxin 6-hydroxydopamine, resulting in a decrease in D_1 binding sites (Neal & Joyce, 1992).

Postnatal development of the D_5 receptor can be detected in the striatum, globus pallidus, frontal cortex, and cingulate cortex (Araki, Sims, & Bhide, 2007). The developmental profile of D_5 mRNA expression shows a linear increase from PND 0 until PND 21, when they reach maximal levels (Araki et al., 2007).

D2-Like Receptors

During early stages of development, the D_2 receptor subtype is generally found in higher quantities than the D_1 receptor (Tarazi & Baldessarini, 2000). More specifically, autoradiographic results show that D_2 receptor densities in the striatum and nucleus accumbens are greater than other dopamine receptor subtypes (Tarazi & Baldessarini, 2000).

The ontogenetic profile of the D_3 receptor is characterized by an increase in the number of binding sites across early postnatal development. For example, Gurevich, Himes, and Joyce (1998) showed that D_3 mRNA expression and D_3 binding sites were detectable in low quantities at PND 7 in both the nucleus accumbens and islands of Caleja. In these brain regions, D_3

receptors reached maximal levels at PND 14 and PND 90, respectively. As with adults, the D_3 receptor subtype show that they are preferentially localized in the islands of Calleja (Diaz et al., 1995; Levesque, Diaz, Pilon, Martres, Giros, Souil, Schott, Morgat, Schwartz, & Sokoloff, 1992).

During early postnatal development, the D_4 receptor subtype is present in lower quantities than D_1 and D_2 receptors in the nucleus accumbens, striatum, frontal cortex, and entorhinal cortex (Tarazi & Baldessarini, 2000). Interestingly, D_4 mRNA expression reaches maximal levels by PND 3. This contrasts with the D_2 receptor subtype, which does not reach maximal levels until PND 28 or later (Andersen et al., 2000; Nair & Mishra, 1995).

CHAPTER FOUR

MECHANISM OF ACTION: INDIRECT DOPAMINE AGONISTS

Indirect dopamine agonists, such as cocaine and amphetamine, increase extracellular monoamine concentrations. More specifically, cocaine preferentially increases dopamine, norepinephrine, and serotonin levels, whereas amphetamine increases dopamine and norepinephrine (Ritz, Lamb, Goldberg, & Kuhar, 1987; Seiden, Sabol, & Ricaurte, 1993). In terms of cocaine, monoamine concentrations are enhanced by blocking the reuptake of newly released neurotransmitter from the synaptic cleft (Meyer & Quenzer, 2005). For example, using an *in-vivo* microdialysis technique, Reith, Li, and Yan (1997) discovered that dialysate levels of all three amines were increased in the ventral tegmental area following administration of cocaine (20 mg/kg). Excitatory amino acids, such as glutamate, also play a role in the behavioral effects of cocaine. Cocaine indirectly enhances glutamatergic neurotransmission by activating these excitatory neurons, particularly in the nucleus accumbens and ventral tegmental area (Kalivas & Duffy, 1998; Smith, Mo, Guo, Kunko, & Robinson, 1995).

Amphetamine- and methamphetamine-like stimulants increase monoamine concentrations by blocking reuptake transporters; however, unlike cocaine, they also bind to monoamine transporters by acting as a false substrate. The end result is that amphetamine and methamphetamine promote reverse transport of cytosolic transmitter, thereby releasing

monoamines from non-vesicular stores (Reith, Li, & Yan, 1997). Fumagalli, Gainetdinov, Valenzano, and Caron (1998) reported an 18-fold increase in extracellular dopamine levels in wild type mice that were subcutaneously injected with methamphetamine (30 mg/kg). In the same study, DOPAC levels were decreased by roughly 60% in both wild type and DAT knockout mice. The latter finding shows that methamphetamine also increases dopamine levels by inhibiting monoamine oxidase (MAO), which is an enzyme that catabolizes dopamine (Fumagalli et al., 1998).

CHAPTER FIVE

ADULT SENSITIZATION: INDIRECT DOPAMINE AGONISTS

Indirect Dopamine Agonists: Adult Multi-Trial Sensitization

Behavioral sensitization occurs when an animal is repeatedly exposed to various indirect dopamine agonists (e.g. cocaine, amphetamine, or methamphetamine), and is then challenged with the same drug at a later time point. This procedure results in an augmented locomotor response that can be observed when the animal is tested one day to several months after discontinuation of the drug (Kalivas & Stewart, 1991; Leith & Kuczenski, 1982; McDougall et al., 2007; Robinson & Becker, 1986). Using this paradigm, adult rats usually exhibit an enhanced locomotor response when challenged later with an indirect dopamine agonist (Leith & Kuczenski, 1982).

Indirect dopamine agonists are able to produce short- and long-term behavioral sensitization in adult rats. For example, sensitization occurs when a short withdrawal interval is employed. In one case, rats receiving repeated treatments of methamphetamine, followed by a five-day withdrawal period, showed an enhanced behavioral response after a challenge injection of methamphetamine (Laviola, Pascucci, & Pieretti, 2001). Sensitization is also robust when a longer duration withdrawal period is used. Kolta, Shreve, De Souza, and Uretsky (1985) showed enhanced locomotor activity after rats were chronically treated with amphetamine and challenged 15 or 30 days

later. This enhanced locomotion occurs in parallel with increased levels of endogenous dopamine, which is evident after longer withdrawal periods.

Drug dose is a contributing factor to the sensitizing effects of psychostimulants. More specifically, repetitive treatment with large doses of a psychostimulant typically produces strong behavioral sensitization (Frantz, O'Dell, & Parsons, 2007; Post & Rose, 1976). For example, repetitive injections of a low dose of cocaine (10 mg/kg) across a five-day interval produces increased locomotion with minute stereotypic movements (Frantz et al., 2007). Davidson and colleagues (2002), however, observed a more robust sensitized response, as well as intense stereotypy, when rats were repeatedly given 40 mg/kg cocaine across a six-day interval (Davidson, Lazarus, Lee, & Ellinwood, 2002).

Repeated administration of various doses of amphetamine can also lead to sensitization. For instance, a low pretreatment dose of either 0.5 or 1 mg/kg amphetamine is enough to produce sensitization (Hall, Stanis, Avila, & Gulley, 2008; Hooks, Jones, Neil, & Justice Jr., 1992). Higher doses of amphetamine lead to focused stereotypy, such as sniffing and licking (Eichler, Antelman, & Black, 1980). In regards to locomotion, Leith and Kuczenski (1982) observed a multi-phasic response after repeated administration of moderate to high doses of amphetamine (2 or 3 mg/kg) for six days. This multi-phasic response is best represented by a U-shape curve, in which there is a rapid onset of locomotor activity, a decrease in locomotion due to intense

focused stereotypy, followed by a post increase in locomotor activity (Leith & Kuczenski, 1982).

Repeated administration of methamphetamine also causes a progressive increase in locomotor activity. For example, mice treated with 10 doses of 1, 2, or 4 mg/kg methamphetamine showed a progressive enhancement in locomotor activity. In general, mice receiving 1 mg/kg methamphetamine showed a slight increase in locomotor sensitization; whereas, 4 mg/kg methamphetamine produced robust stereotypic behavior (Hirabayashi & Alam, 1981).

During the conditioning phase, the nature of the drug-environment pairings can affect the magnitude of the sensitized response. More specifically, behavioral sensitization is more pronounced when the adult rat or mouse is pretreated and tested in the same environment (i.e. context-dependent sensitization) (Anagnostaras & Robinson, 1996; Battisti, Chang, Uretsky, & Wallace, 1999; Drew & Glick, 1989; McDougall et al., 2007). In contrast, behavioral sensitization is weaker in adult animals when drug pretreatment and drug challenge occur in separate environments (i.e. context-independent sensitization). (Laviola, Wood, Kuhn, Francis & Spear, 1995; McDougall, Cortez, Palmer, Herbert, Martinez, Charntikov, & Amodeo, 2009). For example, adult rats failed to express a sensitized response when drug challenge occurred in a context that was never paired with the pretreatment drug (Anagnostaras, Schallert, & Robinson, 2002).

Indirect Dopamine Agonists: Adult One-Trial Sensitization

Although most studies examine behavioral sensitization using multi-trial procedures, sensitization has also been observed in adult rats and mice after a single pretreatment injection of a psychostimulant (McDougall, Reichel, Cyr, Karper, Nazarian, & Crawford, 2005; Weiss, Post, Pert, Woodward, & Murman, 1989). When a one trial procedure is used, sensitization is typically measured soon after the pretreatment injection and within the same environmental context (i.e., pretreatment and test injections occur in the test chamber). For example, adult rats conditioned with 30 mg/kg cocaine showed robust locomotor sensitization when challenged a day later with 10 mg/kg cocaine (McDougall et al., 2007). Amphetamine-induced sensitization was also observed in wild type and D1-defiecient mice after a one-day pre-exposure phase (McDougall et al., 2005).

Environmental context is especially critical when adult rats and mice are provided only a single exposure to a psychostimulant. For example, adult mice displayed robust behavioral sensitization when conditioned with a high dose of cocaine (40 mg/kg) and challenged one day later with a lower dose of cocaine (10 mg/kg) in the same previously novel environment (Jackson & Nutt, 1993). In contrast, adult rats and mice do not exhibit behavioral sensitization when pretreatment and testing occur in different environments. For example, adult male and female rats pretreated with cocaine in the home

cage showed a lack of sensitized responding when injected with cocaine in the testing chamber 24 hours later (McDougall et al., 2009).

The ability of psychostimulants to enhance locomotion and stereotopy are dose-dependent. Increased locomotor activity is observed when the animal is pretreated with a high dose of psychostimulant and is then challenged with a lower dose (Battisti et al., 1999; Jackson et al., 1993; McDougall et al., 2007; 2009). Stereotypy is preferentially observed when high doses of psychostimulant are used. For example, Battisti and colleagues showed that mice pretreated with 10 mg/kg amphetamine and challenged 48 hours later with 7 mg/kg amphetamine displayed robust stereotypic behaviors (Battisti et al., 2009).

CHAPTER SIX

PREWEANLING SENSITIZATION: INDIRECT DOPAMINE AGONISTS

Indirect Dopamine Agonists in Preweanling Rats: Multi-Trial Behavioral Sensitization

As is true with adult animals, young rats exhibit behavioral sensitization when repeatedly exposed to psychostimulants (e.g. cocaine, amphetamine, or methamphetamine) (Duke, O'Neil, & McDougall, 1997; McDougall, Duke, Bolanos, & Crawford, 1994; Wood, Tirelli, Syder, Heyser, LaRocca, & Spear, 1998). Although qualitatively similar, behavioral sensitization differs between young and adult animals. Some of the factors that differentially affect behavioral sensitization in young and adult rats include duration of the withdrawal period, the number of drug exposures, as well as the importance of drug-environment pairings.

Indirect dopamine agonists produce long-term behavioral sensitization in adult rats that can be detected for months after the last drug exposure (Leith & Kuczenski, 1982; Robinson et al., 1982). In contrast, the longevity of multi-trial behavioral sensitization in preweanling rats is much shorter. For example, McDougall and colleagues (1994) examined the effects of amphetamine-induced behavioral sensitization in the early preweanling period and found that amphetamine produced short-term sensitization when using a 2-day interval, but long-term sensitization was not evident when an 8-day treatment-to-test interval was employed (McDougall et al., 1994). In another

case, however, long-term cocaine sensitization was evident during the late preweanling period when testing occurred after 21 days of drug abstinence (Snyder, Katovic, & Spear, 1998). Taken together, age-related neural changes appear to affect the magnitude of the sensitized response after short or long drug abstinence (Snyder et al., 1998).

In adult rats, the dose of psychostimulant used is a constraining influence on the robustness of the sensitized response (Jackson & Nutt, 1993; Weiss, Post, Pert, Woodward, & Muran, 1989). Similarly, drug dose impacts pharmacological responsiveness during early ontogeny. For example, cocaine-induced behavioral sensitization was more robust when medium (15 mg/kg) to large doses (30 mg/kg) of cocaine were administered at PND 21, with an adult-like pattern of locomotor activity being evident (Ujike, Tsuchida, Akiyama, Fujiwara, & Kuroda, 1995). In addition, repeated treatment with a moderate dose (2 mg/kg) of methamphetamine produces robust behavioral sensitization in late preweanling age groups (Fujiwara, Kazahaya, Nakashima, Sato, & Otsuki, 1987).

Environmental cues influence the sensitized responding of preweanling rats when a multi-trial procedure is used. Specifically, the sensitized response is more robust when pretreatment and testing occur in the same environment (i.e. context-dependent sensitization); however, the sensitized response is weaker or absent when pretreatment and testing occur in distinct environments (i.e. context-independent sensitization). For example,

preweanling rats repeatedly treated with cocaine (5, 15, or 30 mg/kg) in a novel context showed strong behavioral sensitization when tested in the same previously novel environment (Wood et al., 1998). In contrast, preweanling rats would only exhibit short-term behavioral sensitization, but not long-term sensitization, when cocaine was repeatedly administered in the home cage during the pretreatment phase (McDougall, Cortez, Palmer, Herbert, Martinez, Charntikov, & Amodeo, 2009; Zavala, Nazarian, Crawford, & McDougall, 2000).

Indirect Dopamine Agonists in Preweanling Rats: One-Trial Behavioral Sensitization

Like adults, preweanling rats exhibit a strong sensitized response after being given a single exposure to a variety of indirect agonists (e.g. cocaine, methamphetamine, and amphetamine) (Herbert, Der-Ghazarian, Palmer, & McDougall, 2010; Kozanian, Gutierrez, Mohd-Yusof, & McDougall, 2012). Adult rats only show one-trial behavioral sensitization when a context-dependent procedure is used (Battisti, Uretsky, & Wallace, 2000; McDougall, Baella, Stuebner, Halladay, & Crawford, 2007); whereas, young rats show strong sensitized responding when pretreatment and testing occur in distinct environments. For example, preweanling rats pretreated with 30 mg/kg cocaine showed robust context-independent sensitization when challenged with 20 mg/kg cocaine on the test day (McDougall, Kozanian, Greenfield, Horn, Gutierrez, & Mohd-Yusof, 2011). In another case, Kozanian

et al. (2012) showed that young rats conditioned with 4 mg/kg methamphetamine in the home cage exhibited strong context-independent behavioral sensitization when challenged 24 hours later with 2 mg/kg methamphetamine in the test chamber.

Previous research has also characterized the ontogenetic profile of psychostimulant-induced one-trial behavioral sensitization. More specifically, psychostimulants preferentially induce one-trial behavioral sensitization depending on the age of the animal. For example, McDougall et al. (2011) showed that cocaine produced robust one-trial behavioral sensitization when young rats were pretreated on PND 19 and tested on PND 21, while various dose combinations of methamphetamine and amphetamine did not produce one-trial behavioral sensitization in this age group. In contrast, one-trial cocaine-induced behavioral sensitization was not evident when younger (PND 16-17) and older (PND 24-25) rats were tested. Methamphetamine, on the other hand, was able to produce one-trial sensitization in younger age groups (PND 12-13 or PND 16-17) (Kozanian et al., 2012). These age-dependent differences in psychostimulant-induced effects could be due to ontogenetic changes in the dopamine system (see “Ontogeny of the Dopamine System” chapter).

CHAPTER SEVEN

NEURAL MECHANISMS UNDERLYING THE DEVELOPMENT AND EXPRESSION OF BEHAVIORAL SENSITIZATION

Previous studies have employed selective D1-like and D2-like receptor antagonists to assess the neural mechanisms responsible for the induction (i.e. development) and expression of behavioral sensitization. Current theory suggests that the induction of behavioral sensitization is due to transient changes in neural function caused by repeated injections of the drug, while expression of the sensitized response is associated with enduring changes in cellular function during withdrawal (Kalivas & Stewart, 1991). D1-like and D2-like receptors are thought to play an important role in these processes, however, the relationship of these receptors to sensitization is complex.

Induction of Behavioral Sensitization

Role of Dopamine D1-Like Receptors

Researchers have suggested that stimulation of D1-like receptors, particularly in the ventral tegmental area, is necessary for the induction of amphetamine-induced behavioral sensitization (Vezina, 1996). Curiously, the importance of D1-like receptors appears to vary depending on the psychostimulant used. Specifically, D1-like receptor antagonists block the induction of amphetamine-induced behavioral sensitization (Vezina & Stewart 1989). For example, rats repeatedly treated with SCH 23390 prior to

methamphetamine administration for 14 days did not show locomotor sensitization when tested after 3 months of drug abstinence (Hamamura, Akiyama, Akimoto, Kashihara, Okumura, Ujike, & Otsuki, 1991). Likewise, Ujike, Onoue, Akiyama, Hamamura, and Otsuki (1989) showed that rats receiving daily administrations of SCH 23390 (0.5 mg/kg) in combination with methamphetamine (4 mg/kg) for 14 days did not show elevated locomotor or stereotypic behavior on the test day, thus indicating that SCH 23390 blocked the induction of methamphetamine-induced sensitization.

In contrast, many studies have shown that SCH 23390 does not affect the induction of cocaine-induced behavioral sensitization when using a multi-trial paradigm (Kuribara & Uchihashi, 1993; Vezina & Stewart, 1989; White, Joshi, Koeltzow, & Hu, 1998). However, Fontana and colleagues (1993) reported that SCH 23390 was able to prevent the conditioned effects of one-trial cocaine-induced sensitization. Thus, it appears that D1-like receptor blockade differentially affects behavioral sensitization depending on the psychosimulant being used. It is possible that cocaine and amphetamine differentially affect compensatory mechanisms or redundant pathways (Karper, De La Rosa, Newman, Krall, Nazarian, McDougall, & Crawford, 2002; White et al., 1998). The discrepant findings reported by Fontana et al. (1993) suggest that D1-like receptors are associated with conditioning effects rather than reward; whether this factor may underlie the differences between cocaine and amphetamine is uncertain.

Role of Dopamine D2-Like Receptors

D2-like receptors also play a role in mediating behavioral sensitization. For example, mice repeatedly treated with the D2-like antagonist haloperidol (0.025, 0.1, 0.4 mg/kg), followed by methamphetamine, showed a dose-dependent decrease in the strength of the sensitized response (Kuribara, 1994). In the same manner, other studies found that haloperidol was able to block the induction of cocaine-induced behavioral sensitization (Mattingly, Rowlett, Ellison, & Rase, 1996; Weiss et al., 1989). Interestingly, the D2-like receptor antagonist sulpiride was unable to block the induction of cocaine sensitization (Mattingly, Hart, Lim, & Perkins, 1994). The disparate actions of haloperidol and sulpiride could be due to their respective mechanisms of action. For example, haloperidol not only binds to dopamine receptor sites but, at high doses, binds to serotonin receptor sites (O'Dell, La Hoste, Wildmark, Shapiro, Potkin, & Marshall, 1990). Hence, the ability of haloperidol to block cocaine-induced sensitization may be related to its combined actions on dopaminergic and non-dopaminergic systems (Mattingly et al., 1995). Sulpiride also has great difficulty in crossing the blood-brain barrier, so sulpiride's lack of efficacy could be due to pharmacokinetic factors.

Although it is generally accepted that D2-like receptors are important for the induction of behavioral sensitization, the brain areas where these receptors are located is largely unknown. In 2002, Beyer and Steketee reported that D2-like receptors in the medial prefrontal cortex are responsible

for mediating the induction of behavioral sensitization; whereas, Jung and colleagues (2013) showed that stimulation of D2-like receptors in the nucleus accumbens is not involved the inductive process.

In summary, the role of D1-like and D2-like receptors in mediating the induction of psychostimulant-induced behavioral sensitization is complex. For example, while D1-like receptors play a prominent role in the induction of amphetamine-induced sensitization, their importance for cocaine-induced sensitization is much less critical. Likewise, D2-like receptor functioning appears to be necessary for the induction of methamphetamine- and cocaine-induced multi-trial sensitization, although studies involving sulpiride provide inconsistent findings. Considering these disparate results, more research is needed to determine the importance of dopamine receptors for the induction of psychostimulant-induced behavioral sensitization.

Expression of Behavioral Sensitization

Role of D1-Like Receptors

Stimulation of D1-like receptors in the nucleus accumbens is required for the expression of behavioral sensitization (Pierce & Kalivas, 1997). For example, rats and mice treated with SCH 23390 on the test day displayed an absence of cocaine or methamphetamine sensitization (Kuribara & Uchihashi, 1994; White et al., 1981). In another case, administering SCH 23390 prior to cocaine at various intervals (Day 1, 17, 14, and 21) blocked the expression of cocaine-induced behavioral sensitization (McCreary & Marsden, 1993).

Role of D2-Like Receptors

While dopamine D2-like receptors are involved in the induction of behavioral sensitization, these receptors are not necessary for the expression of sensitization. For example, the D2-like antagonist pimozide is unable to block the expression of amphetamine-induced sensitization (Beninger & Hahn, 1983). Similarly, Beninger and Herz (1986) found that pimozide (0.4 mg/kg) pretreatment failed to prevent the expression of cocaine-induced behavioral sensitization in a context-specific environment.

In summary, repeated treatment with a psychostimulant can lead to neurochemical alterations that are associated with the long-term expression of behavioral sensitization. Although D1-like and D2-like receptors are intimately involved in the induction of amphetamine-induced sensitization, only D1-like receptors are necessary for expression.

Development of Sensitization in Young Rats

While numerous studies have focused on the role of dopamine receptors for the induction and expression of behavioral sensitization in adult rats and mice (Hamamura et al., 1991; Kuribara, 1994; Kuribara & Uchihashi, 1994; Vezina & Stewart, 1989), the role of dopamine receptors for the ontogeny of behavioral sensitization has not been studied in detail. Recently, however, one study did examine the importance of D1-like receptors for the induction of psychostimulant-induced behavioral sensitization in preweanling rats. Using a one-trial procedure, rat pups were pretreated with SCH 23390 at

0, 15, 30, or 60 min before methamphetamine (2 mg/kg) or cocaine (20 mg/kg) administration. At none of these time points did D1-like receptor blockade disrupt sensitized responding. Thus, the inability of SCH 23390 to impact methamphetamine- or cocaine-induced behavioral sensitization suggests that D1-like receptor stimulation is unnecessary for behavioral sensitization during the preweanling period (Mohd-Yusof, Gonzalez, Veliz, & McDougall, 2014).

CHAPTER EIGHT

SUMMARY

Thesis Statement

Behavioral sensitization occurs when rats are repeatedly exposed to various indirect dopamine agonists (e.g. cocaine, methamphetamine, or amphetamine). In multi-trial behavioral sensitization paradigms, adult rats and mice typically show robust “context-dependent” sensitization when drug pretreatment and testing occur in the same novel environment. “Context-independent” behavioral sensitization can also be observed in adults when pretreatment and testing occurs in two separate and distinct environments. Different patterns of results are observed in adult rats and mice when using a one-trial procedure; adults only exhibit one-trial behavioral sensitization if drug pretreatment and testing occur in the same previously novel environment.

Preweanling rats also exhibit behavioral sensitization after repeated exposures to an indirect dopamine agonist; however, the sensitized responding appears to be weaker and less persistent than in adult rats (Kolta et al., 1990; Smith & Morell, 2008). Similar to adults, the multi-trial sensitized responding of preweanling rats is more robust when pretreatment and testing occur in the same environmental context (Zavala et al., 2000). Unlike adults however, preweanling rats exhibit robust context-independent behavioral sensitization when using a one-trial paradigm (Kozanian et al., 2012). When

these results are considered together, it appears that the characteristics of behavioral sensitization (e.g. the strength and longevity of the sensitized response, the role of associative processes, etc.) differ dramatically across ontogeny.

In adult rats, the neural mechanisms underlying behavioral sensitization differ depending on the psychostimulant being used. Previous studies have shown that the selective D1-like receptor antagonist SCH 23390 blocks the induction of amphetamine- and methamphetamine-, but not cocaine-induced sensitization (Hamamura et al., 1991; Ujike et al., 1989). The only exception was reported by Fontana et al. (1993), who demonstrated that SCH 23390 prevented the conditioned effects of cocaine-induced sensitization when using a one-trial procedure. In terms of D2-like receptors, haloperidol blocks the induction of methamphetamine- and cocaine-induced behavioral sensitization in adult rats, while sulpiride is ineffective. Considering the inconsistent findings, more research is needed to assess the importance of dopamine receptors for the induction of behavioral sensitization.

In contrast to the large number of studies examining the induction of psychostimulant-induced behavioral sensitization in adult rats and mice, the role dopamine receptors play in the ontogeny of behavioral sensitization has been largely ignored. In the only study to examine the importance of D1-like receptors during early ontogeny, Mohd-Yusof et al. (2014) showed that SCH 23390 was unable to block methamphetamine- and cocaine-induced

behavioral sensitization of young rats. These results indicate that the mechanism mediating behavioral sensitization differ across ontogeny.

The purpose of this thesis was to assess the importance of D2-like receptors for the induction of psychostimulant-induced behavioral sensitization during early ontogeny. Rats were assessed during both the middle (PND 13-17) and late (PND 17-21) preweanling periods because rats of these ages often show different patterns of psychostimulant-induced behavioral sensitization (Kozanian et al., 2012; McDougall et al., 2013). Likewise, both methamphetamine and cocaine were tested, since adult rat sensitization studies indicate that these two psychostimulants are differentially affected by D2-like receptor blockade (Mattingly et al., 1994; Weiss et al., 1989).

The specific goals of this thesis were two-fold. First, to investigate the importance of the D2-like receptor for cocaine- and methamphetamine-induced one-trial behavioral sensitization during early ontogeny. It was predicted that the D2-like antagonist raclopride would prevent the induction of methamphetamine- and cocaine-induced behavioral sensitization at PND 17 and PND 21. The basis for this prediction is that raclopride prevents the induction of one-trial cocaine-induced sensitization in adult rats (Fontana et al., 1993). The second goal of this thesis was to assess the importance of the D2-like receptor for cocaine- and methamphetamine-induced multi-trial behavioral sensitization during the middle and late preweanling periods. It was predicted that raclopride would

block both methamphetamine- and cocaine-induced multi-trial behavioral sensitization at PND 17 and PND 21. Again, the basis for these predictions are adult rat studies showing that raclopride and pimozide attenuate sensitized responding when using multi-trial procedures (Beninger & Hahn, 1983; Beninger & Herz, 1986; Ushijima, Carino, & Horita, 1995).

CHAPTER NINE

MATERIALS AND METHODS

Subjects were young male and female rats of Sprague-Dawley descent (Charles River, Hollister, CA) that were born and raised at California State University, San Bernardino (CSUSB). Litters were culled to ten pups on PND 3. All rats were housed on racks in large polycarbonate maternity cages (56 × 34 × 22 cm) with wire lids and Tek-Fresh® bedding (Harlan, Indianapolis, IN). Food and water was freely available. The colony room was maintained at 22-23˚C, and kept under a 12L:12D cycle, with behavioral testing occurring during the light phase of the cycle. Except during testing, rats were kept with the dam and littermates. Testing was done in a separate experimental room, maintained at 24-25˚C. Subjects were cared for according to the “Guide for the Care and Use of Laboratory Animals” (National Research Council, 2010) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

Apparatus

Behavioral testing was performed in commercially available (Coulbourn Instruments, Allentown, PA, USA) activity monitoring chambers (25 × 25 × 41 cm) housed in a testing room separate from the animal colony. The activity chambers had acrylic walls, a gray plastic floor, and an open top. Each chamber included an X-Y photobeam array, with 16 photocells and

detectors, that was used to measure horizontal locomotor activity (distance traveled). Photobeam resolution is 0.76 cm. The position of each rat was determined every 100 ms (i.e., the sampling interval).

Drugs

(+)-Methamphetamine hydrochloride, (–)-cocaine hydrochloride, and S(-)-raclopride (+)-tartrate were dissolved in saline. Drugs were purchased from Sigma-Aldrich (St. Louis, MO) and injected intraperitoneally (IP) at a volume of 5 ml/kg.

Procedure

Experiment 1: Effects of D2 Receptor Blockade on Cocaine-Induced One-Trial Behavioral Sensitization

One-trial cocaine-induced behavioral sensitization was assessed on PND 20–21. On the pretreatment day, PND 20 rats were injected with raclopride (0, 0.1, 0.5, 1, or 5 mg/kg) followed, 15 min later, by an injection of 30 mg/kg cocaine (see Figure 2). Rats in the acute control group were given two injections of saline. After the second injection, rats were placed in activity chambers and distance traveled was measured for 30 min. On the test day, all rats were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min.

Group	PND 20 Pretreatment Drugs		PND 21 Test Day Drug
Control Group	Saline	Saline	Cocaine (20 mg/kg)
Sensitization Group	Saline	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)
Antagonist Group	0.1 mg/kg Raclopride	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)
Antagonist Group	0.5 mg/kg Raclopride	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)
Antagonist Group	1 mg/kg Raclopride	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)
Antagonist Group	5 mg/kg Raclopride	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)

Figure 2. Schematic Showing Drug Treatments for the Various Groups in Experiment 1

Experiment 2: Effects of D2 Receptor Blockade on Methamphetamine-Induced One-Trial Behavioral Sensitization

Since indirect dopamine agonists preferentially induce one-trial sensitization at different ages (Kozanian et al., 2012), one-trial methamphetamine-induced sensitization was assessed on PND 16-17. On the pretreatment day, PND 16 rats were injected with raclopride (0, 0.1, 0.5, 1, or 5 mg/kg) followed, 15 min later, by an injection of 4 mg/kg methamphetamine (see Figure 3). The acute control group was given two injections of saline. After the second injection, locomotor activity was assessed for 30 min. On the test day, all rats were injected with 2 mg/kg methamphetamine and locomotor activity was assessed for 120 min. Doses of cocaine and methamphetamine were based on previous studies using preweanling rats (Herbert et al., 2010; McDougall et al., 2007, 2011; Kozanian et al., 2012).

Group	PND 16 Pretreatment Drugs		PND 17 Test Day Drug
Control Group	Saline	Saline	METH (2 mg/kg)
Sensitization Group	Saline	METH (4 mg/kg)	METH (2 mg/kg)
Antagonist Group	0.1 mg/kg Raclopride	METH (4 mg/kg)	METH (2 mg/kg)
Antagonist Group	0.5 mg/kg Raclopride	METH (4 mg/kg)	METH (2 mg/kg)
Antagonist Group	1 mg/kg Raclopride	METH (4 mg/kg)	METH (2 mg/kg)
Antagonist Group	5 mg/kg Raclopride	METH (4 mg/kg)	METH (2 mg/kg)

Figure 3. Schematic Showing Drug Treatments for the Various Groups in Experiment 2

Experiment 3a: Effects of D2 Receptor Blockade on Cocaine-Induced Multi-Trial Behavioral Sensitization During the Late Preweanling Period

On PND 17-20, rats were injected with raclopride (0, 0.5, or 1 mg/kg) followed, 15 min later, by an injection of 30 mg/kg cocaine (see Figure 4). Rats in the acute control group were given two injections of saline. After the second injection, rats were placed in activity chambers and distance traveled was measured for 30 min. On PND 21, all rats were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min.

Group	PND 17-20 Pretreatment Drugs		PND 21 Test Day Drug
Control Group	Saline	Saline	Cocaine (20 mg/kg)
Sensitization Group	Saline	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)
Antagonist Group	0.1 mg/kg Raclopride	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)
Antagonist Group	0.5 mg/kg Raclopride	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)
Antagonist Group	1 mg/kg Raclopride	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)

Figure 4. Schematic Showing Drug Treatments for the Various Groups in Experiment 3a

Experiment 3b: Effects of D2 Receptor Blockade on Methamphetamine-Induced Multi-Trial Behavioral Sensitization During the Late Preweanling Period

To examine the effects of D2 receptor antagonism on methamphetamine, separate groups of rats were treated as in Experiment 3a, except that rats were pretreated with 4 mg/kg methamphetamine and tested with 2 mg/kg methamphetamine (see Figure 5).

Group	PND 17-20 Pretreatment Drugs		PND 21 Test Day Drug
Control Group	Saline	Saline	METH (2 mg/kg)
Sensitization Group	Saline	METH (4 mg/kg)	METH (2 mg/kg)
Antagonist Group	0.1 mg/kg Raclopride	METH (4 mg/kg)	METH (2 mg/kg)
Antagonist Group	0.5 mg/kg Raclopride	METH (4 mg/kg)	METH (2 mg/kg)
Antagonist Group	1 mg/kg Raclopride	METH (4 mg/kg)	METH (2 mg/kg)

Figure 5. Schematic Showing Drug Treatments for the Various Groups in Experiment 3b

Experiment 4a: Effects of D2 Receptor Blockade on Cocaine-Induced Multi-Trial Behavioral Sensitization During the Middle Preweanling Period

On PND 13-16, rats were injected with raclopride (0,0.5, or 1 mg/kg) followed, 15 min later, by an injection of 30 mg/kg cocaine (see Figure 6). Rats in the acute control group were given two injections of saline. After the second injection, rats were placed in activity chambers and distance traveled was measured for 30 min. On PND 17, all rats were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min.

	PND 13-16		PND 17
Group	Pretreatment Drugs		Test Day Drug
Control Group	Saline	Saline	Cocaine (20 mg/kg)
Sensitization Group	Saline	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)
Antagonist Group	0.1 mg/kg Raclopride	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)
Antagonist Group	0.5 mg/kg Raclopride	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)
Antagonist Group	1 mg/kg Raclopride	Cocaine (30 mg/kg)	Cocaine (20 mg/kg)

Figure 6. Schematic Showing Drug Treatments for the Various Groups in Experiment 4a

Experiment 4b: Effects of D2 Receptor Blockade on Methamphetamine-Induced Multi-Trial Behavioral Sensitization During the Middle Preweanling Period

To examine the effects of D2-like receptor antagonism on methamphetamine during the middle preweanling period, separate groups of rats were treated as in Experiment 4a, except that rats were pretreated with

4 mg/kg methamphetamine and tested with 2 mg/kg methamphetamine (see Figure 7).

Group	PND 13-16 Pretreatment Drugs		PND 17 Test Day Drug
Control Group	Saline	Saline	METH (2 mg/kg)
Sensitization Group	Saline	METH (4 mg/kg)	METH (2 mg/kg)
Antagonist Group	0.1 mg/kg Raclopride	METH (4 mg/kg)	METH (2 mg/kg)
Antagonist Group	0.5 mg/kg Raclopride	METH (4 mg/kg)	METH (2 mg/kg)
Antagonist Group	1 mg/kg Raclopride	METH (4 mg/kg)	METH (2 mg/kg)

Figure 7. Schematic Showing Drug Treatments for the Various Groups in Experiment 4b

Data Analysis

For all experiments, omnibus repeated-measures analyses of variance (ANOVAs) were used for statistical analysis of distance traveled data. More specifically, pretreatment data for Experiments 1 and 2 were analyzed using 6 × 6 (Group × 5-min time block) mixed repeated measures ANOVAs, while Experiments 3a, 3b, 4a, and 4b were analyzed using 5 × 4 × 6 (Group × Day × 5-min time block) mixed repeated measures ANOVAs. Test day data for Experiments 1 and 2 were analyzed using 6 × 12 (condition × 10-min time block) repeated measures ANOVAs, whereas Experiments 3a, 3b, 4a, and 4b were analyzed using 5 × 12 (condition × 10-min time block) mixed repeated measures ANOVAs. Post hoc analysis of distance traveled data was done using Tukey tests ($P < 0.05$). The

Huynh-Feldt epsilon statistic was used to adjust degrees of freedom (Huynh & Feldt, 1976) when the assumption of sphericity was violated, as determined by Mauchly's test of sphericity. Corrected degrees of freedom was represented by a superscripted "a" and rounded to the nearest whole number.

Litter effects were controlled through both experimental design and statistical procedures. In most experiments, no more than one subject per litter was assigned to a particular group. In cases where this procedure is not possible (e.g., analysis of the pretreatment day), a single litter mean was calculated from multiple littermates assigned to the same group (Holson & Pearce 1992; Zorrilla, 1997). When possible, litter was used as the unit of analysis for statistical purposes (Zorrilla, 1997). With this statistical model each litter, rather than each rat, was treated as an independent observation (i.e., a within analysis using one value/condition/litter).

CHAPTER TEN

RESULTS

Synopsis

In general, both cocaine and methamphetamine were able to induce one-trial and multi-trial behavioral sensitization on PND 17 and PND 21 (see Table 1). The only exception was that repeated methamphetamine treatment did not cause multi-trial behavioral sensitization when testing occurred on PND 21. The dopamine D2-like receptor antagonist raclopride had no effect on cocaine- or methamphetamine-induced one-trial behavioral sensitization. Furthermore, raclopride did not block the induction of cocaine-induced multi-trial sensitization on PND 17 or PND 21. Interestingly, the D2-like receptor antagonist was able to prevent the induction of multi-trial methamphetamine sensitization when testing occurred on PND 17. Detailed coverage of the various experimental results now follows.

Table 1. Summary of the Test Day Results for the Various Experiments

Experiment	Pretreatment Age	Test Age	Design	Agonist	Sensitization	Raclopride's Actions	Figure
1	PD 20	PD 21	One-Trial	Cocaine	Yes	No Effect	9
2	PD 16	PD 17	One-Trial	Methamphetamine	Yes	No Effect	11
3a	PD 17-PD 20	PD 21	Multi-Trial	Cocaine	Yes	No Effect	13
3b	PD 17-PD 20	PD 21	Multi-Trial	Methamphetamine	No	No Effect	15
4a	PD 13-PD 16	PD 17	Multi-Trial	Cocaine	Yes	No Effect	17
4b	PD 13-PD 16	PD 17	Multi-Trial	Methamphetamine	Yes	Blockade	19

Experiment 1: Effects of D2 Receptor Blockade on Cocaine-Induced One-Trial Behavioral Sensitization

Pretreatment Day

When collapsed across the pretreatment day, rats pretreated with cocaine alone or cocaine plus raclopride (0.1 or 0.5 mg/kg) had greater distance traveled scores than saline controls [group main effect, $F(3, 18) = 7.99$, $p < 0.01$] (see Figure 8A). The effect was not evident with the two higher doses of raclopride (1 or 5 mg/kg) as locomotor activity was reduced to the level of the saline controls. The effects of the D2 receptor antagonist varied across the session, because rats treated with cocaine plus raclopride (0.5, 1, or 5 mg/kg) had smaller distance traveled scores than the cocaine-alone group on time blocks 1 and 2 [group × time block interaction, $F(13,88) = 3.41$, $p < 0.001$; Tukey tests, $p < 0.05$] (see Figure 8B).

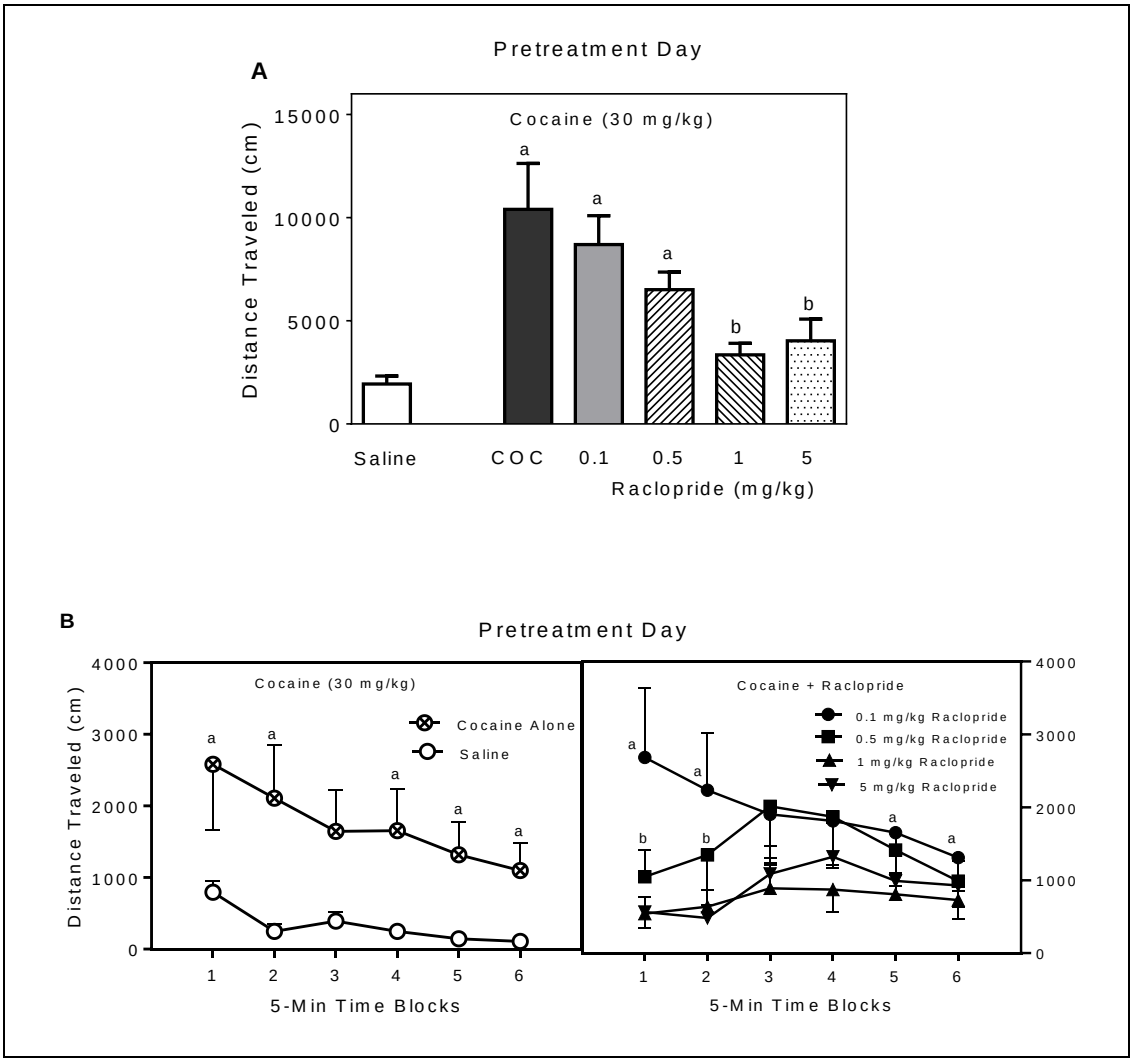

Figure 8. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per group) on the Pretreatment Day (PND 20). A. Mean Distance Traveled Scores Collapsed Across Time Blocks 1-6. B. Mean Distance Traveled Scores on Time Blocks 1-6. Rats were Injected with Saline or 30 mg/kg Cocaine Immediately before a 30-min Placement in activity Chambers (Left Panel). In Addition, Separate Group of Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Cocaine Treatment (Right Panel). *a* Significantly Different from the Saline Control. *b* Significantly Different from the Cocaine Alone Group.

Test Day

Cocaine-induced behavioral sensitization was evident in nonraclopride-treated rats, because rats pretreated and tested with cocaine had greater distance traveled scores than rats treated with only cocaine on the test day (i.e., the acute control group; Figure 9) [group main effect, $F(5,35) = 3.83$, $p < 0.01$, and Tukey tests, $p < 0.05$]. Interestingly, raclopride (0.1, 0.5, 1, or 5 mg/kg) pretreatment did not reduce locomotor activity when compared to rats treated with cocaine alone (i.e., raclopride did not block the development of behavioral sensitization; see right panel Figure 9). Overall, distance traveled scores showed a progressive decline across the testing session, with the effect beginning on time block 3 [time main effect, $F(11,31) = 30.97$, $P < 0.001$] (see Figure 9).

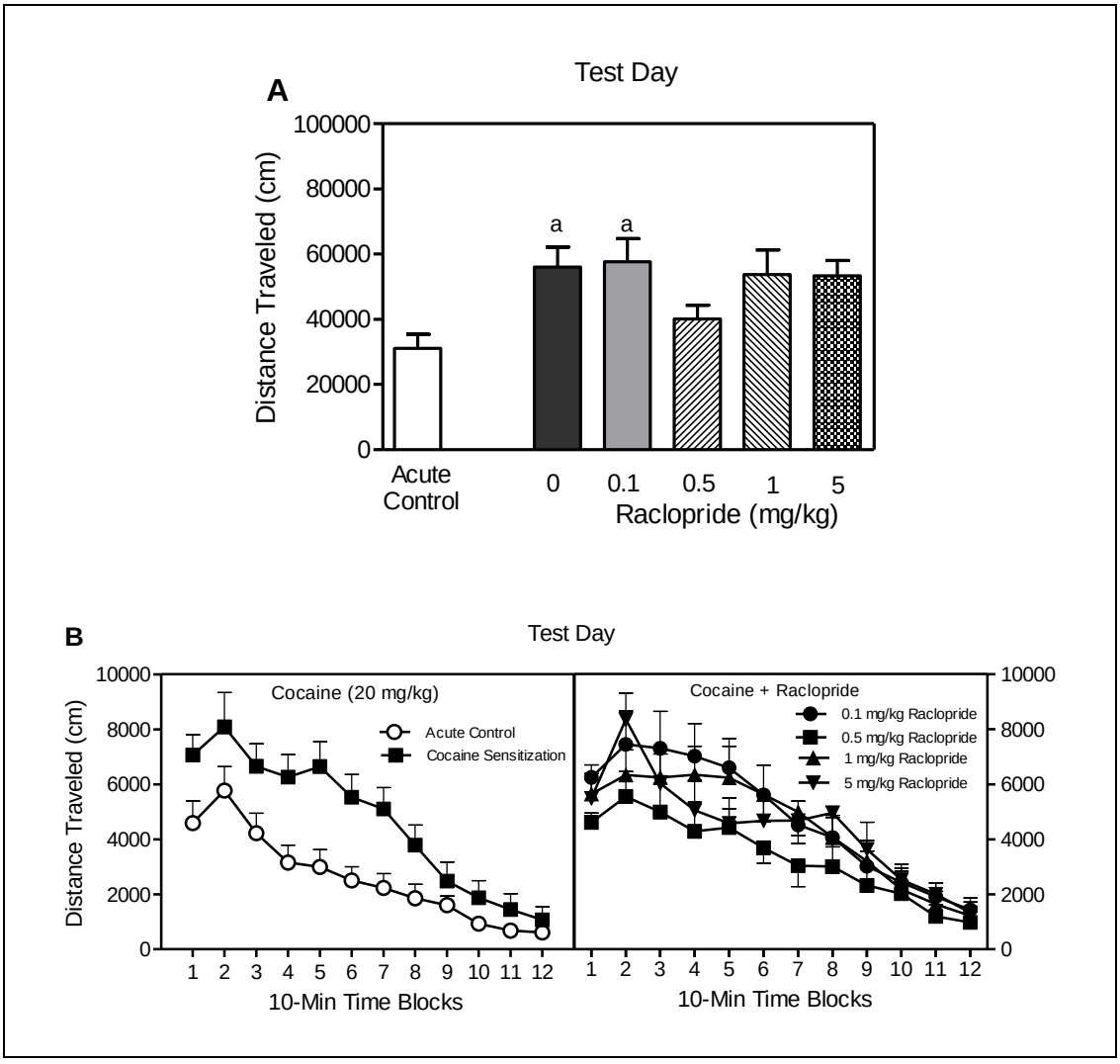

Figure 9. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per Group) on the Test Day (PND 21). A. Mean Distance Traveled Scores Collapsed across Time Blocks 1-12. B. Mean Distance Traveled Scores on Time Blocks 1-12. Rats were Challenged with Cocaine (20 mg/kg) Immediately before Behavioral Testing. On the Pretreatment Day, Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Cocaine Treatment (Right Panel). The Acute Control Group was Injected with Saline on the Pretreatment Day and Injected with Cocaine on the Test Day (Left Panel). Locomotor Activity was Assessed for 120 min. a Significantly different from the Acute Control Group.

Experiment 2: Effects of D2 Receptor Blockade on Methamphetamine-Induced One-Trial Behavioral Sensitization

Pretreatment Day

Rats injected with methamphetamine alone or methamphetamine plus raclopride (0.1 or 0.5 mg/kg) had greater distance traveled scores than saline-treated rats [group main effect, $F(5,35) = 7.67$, $p < 0.001$] (see Figure 10A). These effects varied across the testing session, because rats treated with methamphetamine alone had significantly more locomotor activity on time blocks 1 and 2 than saline controls [group × time block, $F(25,175) = 6.22$, $p < 0.001$] (see Figure 10B; left panel). The three higher doses of raclopride (0.5, 1, and 5 mg/kg) attenuated locomotor activity on time blocks 1, 2 and 4 when compared to the methamphetamine alone group (Tukey tests, $p < 0.05$) (see Figure 10B; right panel).

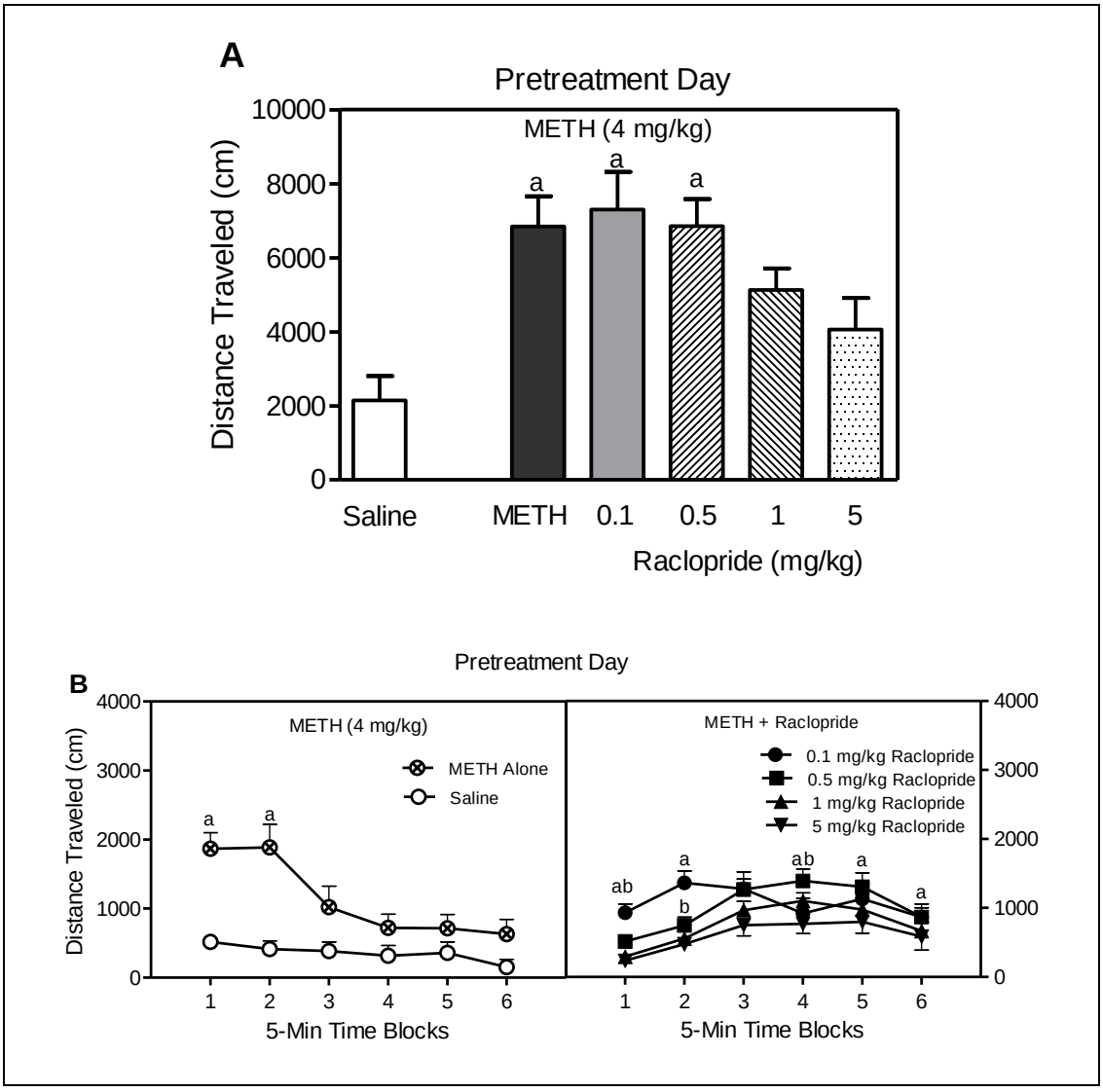

Figure 10. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per Group) on the Pretreatment Day (PND 16). A. Mean Distance Traveled Scores Collapsed across Time Blocks 1-6. B. Mean Distance Traveled Scores on Time Blocks 1-6. Rats were Injected with Saline or 4 mg/kg Methamphetamine Immediately before a 30-min Placement in Activity Chambers (Left Panel). In addition, Separate Group of Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Methamphetamine Treatment (Right Panel). a Significantly Different from the Saline Control Group. b Significantly Different from the Methamphetamine Alone Group.

Test Day

Overall, rats pretreated and tested with methamphetamine and rats pretreated with 1 mg/kg raclopride had greater distance traveled scores than the acute control group [group main effect, $F(5,35) = 5.54$, $P < 0.001$] (see left panel, Figure 11). Rats pretreated and tested with methamphetamine had greater distance travel scores than the acute control group on time blocks 3-11 [group × time block, $F(55,385) = 2.68$, $p < 0.001$]. Raclopride pretreatment, regardless of dose, did not attenuate locomotor activity on the test day (see Figure 11).

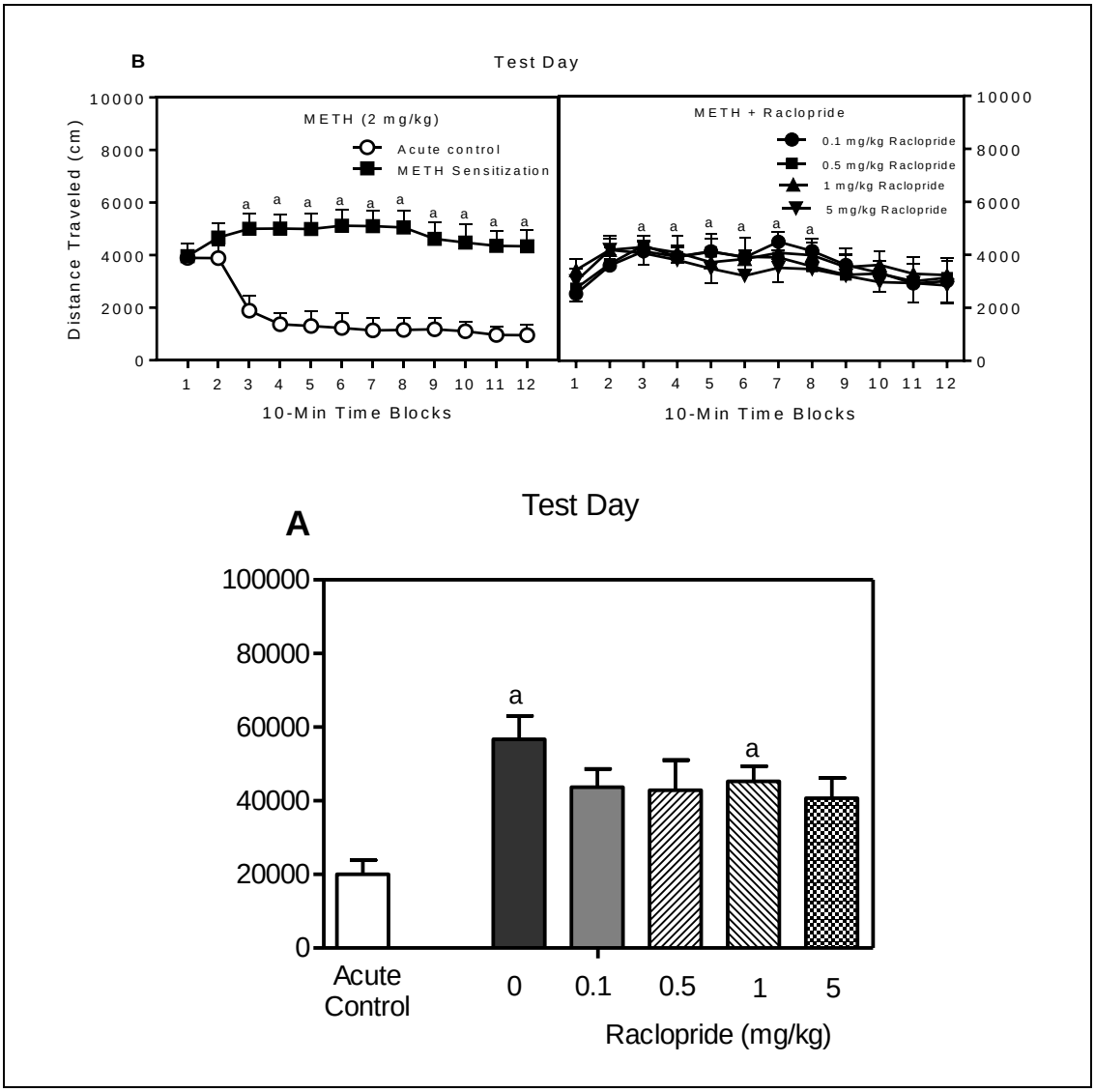

Figure 11. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per Group) on the Test Day (PND 17). A. Mean distance Traveled Scores Collapsed across Time Blocks 1-12. B. Mean Distance Traveled Scores on Time Blocks 1-12. Rats were Challenged with Methamphetamine (2 mg/kg) Immediately before Behavioral Testing. On the Pretreatment Day, Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Methamphetamine Treatment (Right Panel). The Acute Control Group was Injected with Saline on the Pretreatment Days and Injected with

Methamphetamine on the Test Day (Left Panel). Locomotor was Assessed for 120 min. a Significantly different from the Acute Control Group

Experiment 3a: Effects of D2 Receptor Blockade on Cocaine-Induced Multi-Trial Behavioral Sensitization During the Late Preweanling Period

Pretreatment Day

PND 17. Rats injected with cocaine alone or cocaine plus raclopride (0.1 mg/kg) had greater distance traveled scores than rats treated with saline or moderate to high doses of raclopride (0.5 and 1 mg/kg) [group main effect, $F(4,28) = 12.17$, $P < 0.001$](see left top panel; Figure 12). Rats treated with cocaine alone had greater distance traveled scores on time blocks 1-4 and 6 when compared to the saline group [group × time block interaction, $F(20,140) = 3.89$, $P < 0.001$; Tukey tests, $P < 0.05$]. The effects of the D2 antagonist varied across the session, because rats treated with cocaine plus raclopride (0.5 and 1 mg/kg) had smaller distance traveled scores than the cocaine-alone group on time blocks 1 and 2 (Tukey tests, $P < 0.05$) (see left top panel; Figure 12).

PND 18. Rats injected with cocaine alone or cocaine plus 0.1 mg/kg raclopride had greater distance traveled scores than saline-treated rats [group main effect, $F(4,28) = 9.61$, $P < 0.001$] (see right top panel; Figure 12). More specifically, rats treated with cocaine alone had greater locomotor activity on time blocks 1 and 2 than saline controls [group × time block interaction,

F(20,140) = 5.11, *P* < 0.001; Tukey tests, *P* < 0.05]. Rats treated with 0.5 or 1 mg/kg raclopride had smaller distance traveled scores on time blocks 1 and 2 than the cocaine alone group (see right top panel; Figure 12).

PND 19. Rats injected with cocaine alone or cocaine plus a low dose of raclopride (0.1 mg/kg) had greater distance traveled scores than saline-treated rats [group main effect, *F*(4,28) = 12.21, *P* < 0.001] (see left bottom panel; Figure 12). Moreover, rats treated with cocaine alone had significantly greater locomotor activity on time blocks 1-4 than the saline control group. Rats pretreated with raclopride (0.5 or 1 mg/kg) had smaller distance traveled scores on time blocks 1-5 than the cocaine alone group [group × time block interaction, *F*(20,140) = 7.17, *P* < 0.001; Tukey tests, *P* < 0.05] (see left bottom panel; Figure 12).

PND 20. Rats injected with cocaine alone or cocaine plus 0.1 mg/kg raclopride had greater distance traveled scores than saline-treated rats [group main effect, *F*(4,28) = 7.16, *P* < 0.001] (see right bottom panel; Figure 12). More specifically, rats treated with cocaine alone had greater locomotion on time blocks 1 and 2 than saline controls, while rats treated with 1 mg/kg raclopride had smaller distance traveled scores on time blocks 1 and 2 when compared to the cocaine alone group [group × time block interaction, *F*(20,140) = 3.76, *P* < 0.001; Tukey tests, *P* < 0.05] (see right bottom panel; Figure 12). Starting on time block 3, locomotor activity decreased across time [time main effect, *F*(5,35) = 41.74, *P* < 0.001].

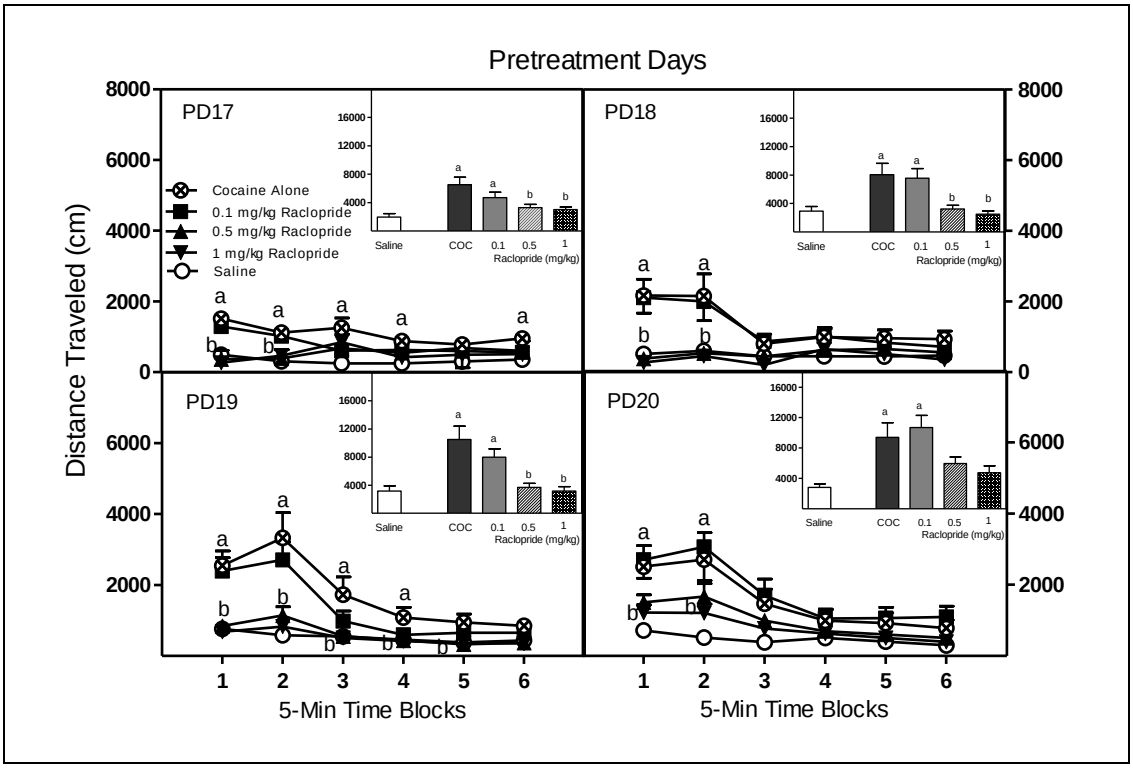

Figure 12. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per Group) on the Four Pretreatment Days (PD 17, PD 18, PD 19, and PD 20). The Insets Show Mean Distance Traveled Collapsed across the Conditioning Session. Rats were Injected with Saline or 30 mg/kg Cocaine Immediately before a 30-min Placement in Activity Chambers. In Addition, Separate Group of Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Cocaine Treatment. a Significantly Different from the Saline Control Group. b Significantly different from the Cocaine Alone Group.

Test Day

PND 21. Rats treated with cocaine alone had greater distance traveled scores than the acute control group on the test day [group main effect, $F(4,28) = 6.37$, $P < 0.001$] (see Figure 13). Raclopride pretreatment,

regardless of dose, did not attenuate locomotor activity on the test day (see Figure 13).

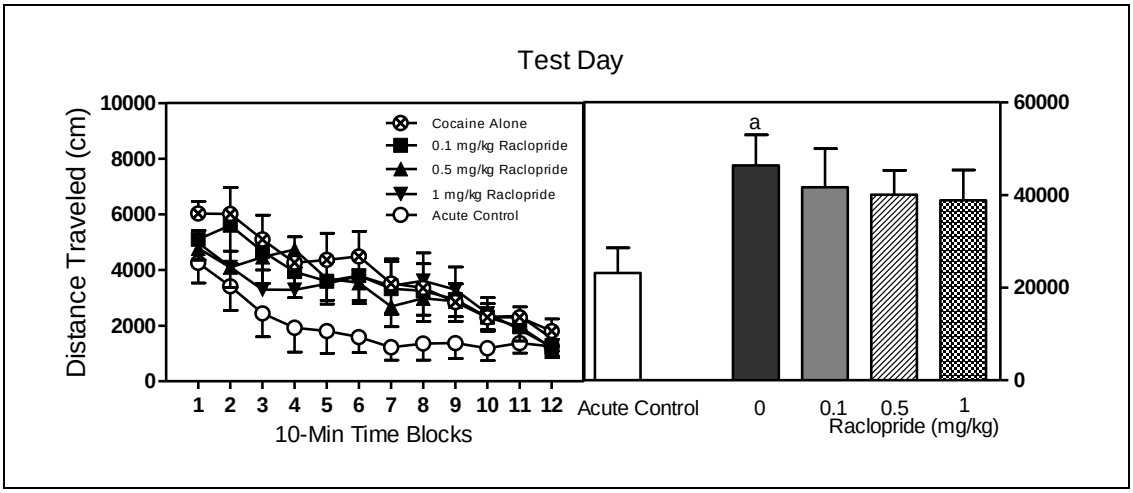

Figure 13. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per group) on the Test Day (PND 21). Rats were Challenged with Cocaine (20 mg/kg) Immediately before Behavioral Testing. On the Pretreatment Day, Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Cocaine Treatment. The Acute Control Group was Injected with Saline on the Pretreatment Days and Injected with Cocaine on the Test Day. Locomotor Activity was Assessed for 120 min. The Right Panel Shows Mean Distance Traveled Collapsed across the Testing Session.
a Significantly Different from the Acute Control Group.

Experiment 3b: Effects of D2 Receptor Blockade on Methamphetamine-Induced Multi-Trial Behavioral Sensitization During the Late Preweanling Period

Pretreatment Day

PND 17. Rats injected with methamphetamine-alone or methamphetamine plus raclopride (0.1, 0.5, or 1 mg/kg) had greater distance

traveled scores than the saline controls [group main effect, $F(4,28) = 7.57$, $P < 0.001$](see top left panel; Figure 14). The effects of the D2 antagonist varied across the session, because rats treated with methamphetamine alone had greater distance traveled scores on time blocks 1-3 than saline controls [group × time block interaction, $F(20,140) = 3.89$, $P < 0.001$; Tukey tests, $P < 0.05$](see top left panel; Figure 14). Groups receiving methamphetamine plus a moderate or high dose of raclopride (0.5 and 1 mg/kg) had smaller distance traveled scores than the methamphetamine-alone group on time blocks 1 and 2 (Tukey tests, $P < 0.05$).

PND 18. Rats injected with methamphetamine-alone or methamphetamine plus raclopride (0.1 or 0.5 mg/kg) had greater distance traveled scores than saline controls [group main effect, $F(4,28) = 21.93$, $P < 0.001$] (see top right panel; Figure 14). More specifically, rats treated with methamphetamine-alone had greater distance traveled scores on time blocks 1-6 when compared to saline controls [group × time block interaction, $F(20, 140) = 11.07$, $P < 0.001$; Tukey tests, $P < 0.05$]. The three doses of raclopride (0.1, 0.5, or 1 mg/kg) caused smaller distance traveled scores on time blocks 1, 2, and 3 when compared to the methamphetamine-alone group (Tukey tests, $P < 0.05$). In general, rats treated with raclopride (0.1, 0.5, or 1 mg/kg) exhibited increased locomotor activity as the session progressed [time main effect, $F(2,11) = 22.99$, $P < 0.001$; Tukey tests, $P < 0.05$) (see top right panel; Figure 14).

PND 19. Rats injected with methamphetamine alone or methamphetamine plus raclopride (0.1 or 0.5 mg/kg) had greater distance traveled scores than the saline controls [group main effect, $F(4,28) = 20.01$, $P < 0.001$] (see bottom left panel; Figure 14). More specifically, rats treated with methamphetamine alone had greater distance traveled scores than saline controls on time blocks 1-6 [group × time block interaction, $F(20, 140) = 11.07$, $P < 0.001$; Tukey tests, $P < 0.05$]. The three doses of raclopride (0.1, 0.5, or 1 mg/kg) caused smaller distance traveled scores on time blocks 1 and 2 when compared to the methamphetamine-alone group, while 0.5 or 1 mg/kg attenuated locomotor activity on time block 3 when compared to the methamphetamine-alone group (Tukey tests, $P < 0.05$) (see bottom left panel; Figure 14).

PND 20. Rats treated with methamphetamine alone or methamphetamine plus raclopride (0.1 or 0.5 mg/kg) had greater distance traveled scores were compared to saline controls, $F(4,28) = 19.99$, $P < 0.001$] (see bottom right panel; Figure 14). Rats treated with methamphetamine alone had greater distance traveled scores than saline controls on time blocks 1-6, while raclopride (0.1, 0.5, and 1 mg/kg) attenuated locomotor activity on time blocks 1 and 2 when compared to the methamphetamine alone group. Raclopride (0.5 and 1 mg/kg) reduced locomotor activity on time block 3 relative to the methamphetamine-alone group [group × time block interaction,

F(20, 140) = 9.04, *P* < 0.001; Tukey tests, *P* < 0.05](see bottom right panel; Figure 14).

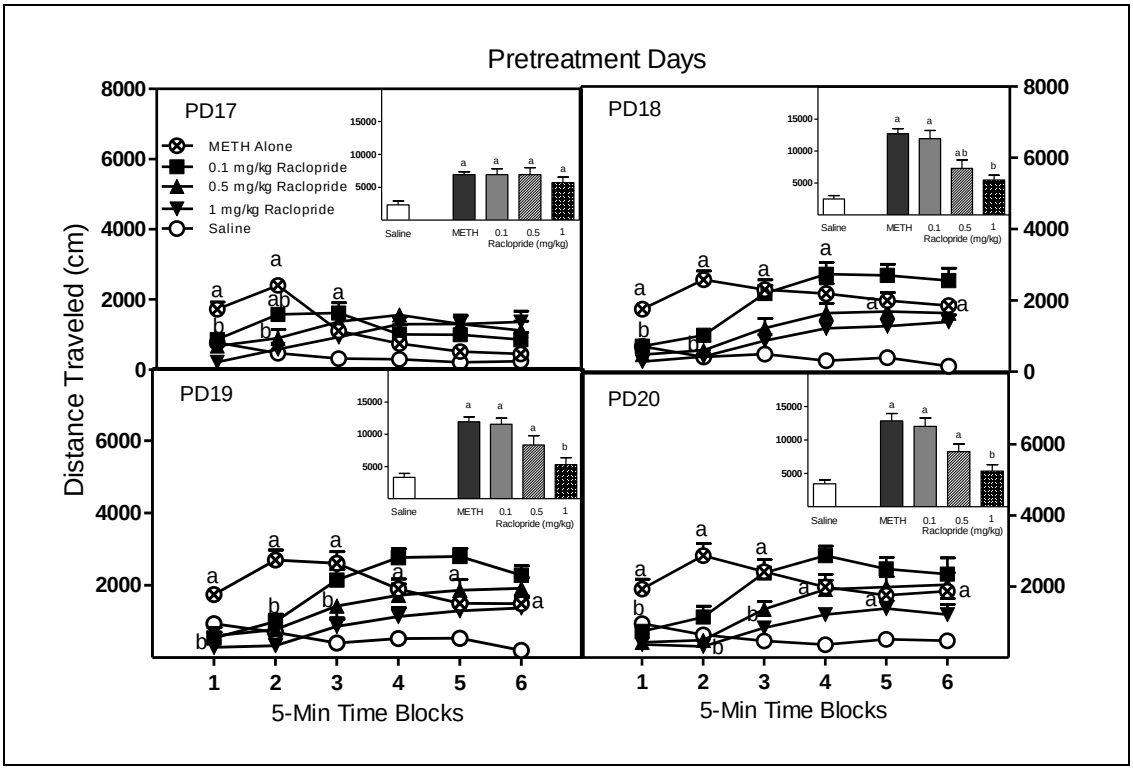

Figure 14. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per Group) on the Four Pretreatment Days (PND 17, PND 18, PND 19, and PND 20). The Insets Show Mean Distance Traveled Collapsed across the Conditioning Session. Rats were Injected with Saline or 4 mg/kg Methamphetamine Immediately before a 30-min Placement in Activity Chambers. In Addition, Separate Group of Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Cocaine Treatment. a Significantly Different from the Saline Control Group. b Significantly Different from the Methamphetamine Alone Group.

Test Day

PND 21. Rats pretreated with methamphetamine did not differ from the acute control group on the test day. Raclopride pretreatment, regardless of dose, did not attenuate locomotor activity on the test day (see right panel; Figure 15).

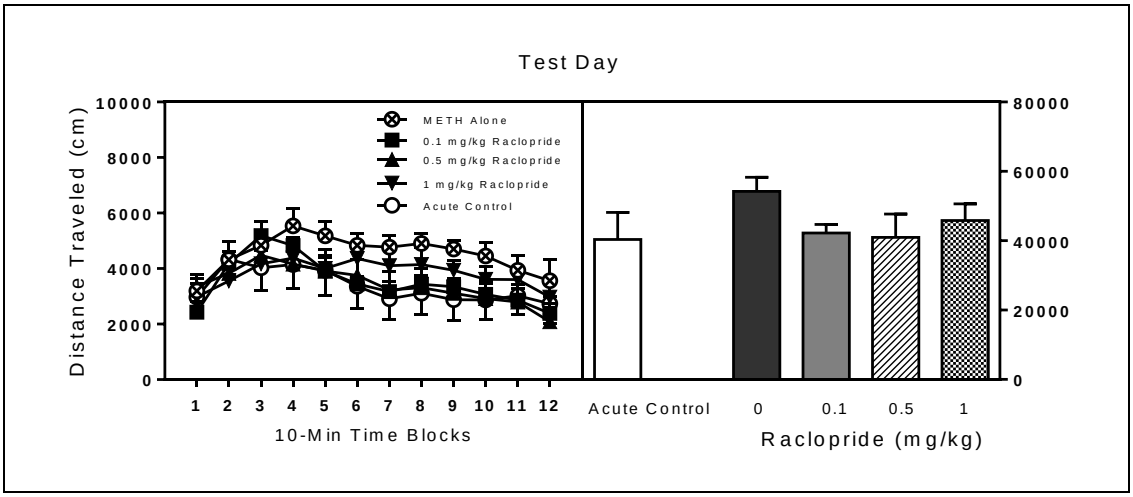

Figure 15. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per Group) on the Test Day (PND 21). Rats were Challenged with Methamphetamine (2 mg/kg) Immediately before Behavioral Testing. On the Pretreatment Days, Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Methamphetamine Treatment. The Acute Control Group was Injected with Saline on the Pretreatment Days and Injected with Methamphetamine on the Test Day. Locomotor Activity was Assessed for 120 min. The Right Panel Shows Mean Distance Traveled Collapsed across the Testing Session.

Experiment 4a: Effects of D2 Receptor Blockade on Cocaine-Induced Multi-Trial Behavioral Sensitization During the Middle Preweanling Period.

Pretreatment Day

PND 13. Rats injected with cocaine alone or cocaine plus 0.5 mg/kg raclopride had greater distance traveled scores than saline-treated rats [group main effect, $F(1,10) = 6.54$, $P < 0.05$] (see top left panel; Figure 16). Overall, rats treated with cocaine-alone had greater distance traveled scores on time blocks 1-3 and 5-6 than saline controls [group × time block interaction, $F(20, 140) = 2.62$, $P < 0.001$; Tukey tests, $P < 0.05$]. Rats treated with raclopride (0.1, 0.5, or 1 mg/kg) exhibited less locomotor activity on time blocks 1 and 6 than the cocaine alone group (Tukey tests, $P < 0.05$) (see top left panel; Figure 16).

PND 14. When collapsed across the pretreatment day, rats pretreated with cocaine alone or cocaine plus raclopride (0.1 or 0.5 mg/kg) had greater distance traveled scores than saline treated rats [group main effect, $F(2,14) = 12.30$, $P < 0.001$] (see top right panel inset; Figure 16). Rats treated with cocaine alone had greater locomotor activity than saline controls on time blocks 1, 2, 4, 5, and 6, while raclopride (0.5 or 1 mg/kg) treated rats exhibited less locomotor activity than the cocaine-alone group on time blocks 1 and 2 [group × time block interaction, $F(20, 140) = 2.36$, $P < 0.001$; Tukey tests, $P < 0.05$] (see top right panel; Figure 16).

PND 15. Overall, rats injected with cocaine alone had greater distance traveled scores than rats treated with saline [group main effect, $F(4,28) = 4.69$, $P < 0.05$] (see bottom left panel inset; Figure 16). This effect varied across the testing session, because rats treated with cocaine alone had greater distance traveled scores on time blocks 1 and 2 than saline controls; the group given cocaine plus 1 mg/kg raclopride exhibited significantly less locomotor activity on time blocks 1 and 2 than the cocaine alone group [group × time block interaction, $F(20, 140) = 3.30$, $P < 0.001$; Tukey tests, $P < 0.05$] (see bottom left panel; Figure 16).

PND 16. Rats injected with cocaine alone or cocaine plus 0.1 mg/kg raclopride had greater distance traveled scores than rats treated with higher doses of raclopride (0.5 and 1 mg/kg) [group main effect, $F(2,17) = 9.72$, $P < 0.001$]. Rats treated with cocaine alone exhibited greater locomotor activity on time blocks 1 and 2 than saline controls, while rats treated with 1 mg/kg raclopride had significantly less locomotion than the cocaine alone group on time blocks 1 and 2 [group × time block interaction, $F(20, 140) = 5.98$, $P < 0.001$; Tukey tests, $P < 0.05$] (see bottom right panel inset; Figure 16). Overall, locomotor activity decreased across the first three time blocks for all groups [time main effect, $F(2,16) = 12.94$, $P < 0.001$] (see bottom right panel; Figure 16).

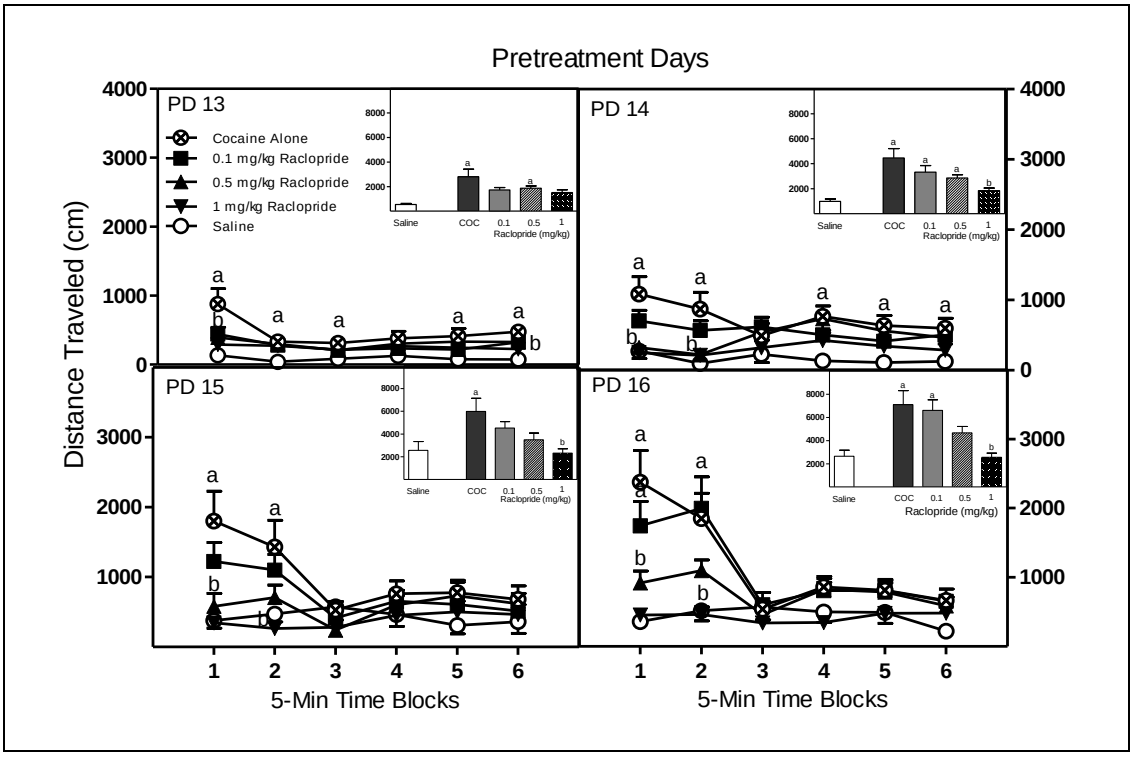

Figure 16. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per Group) on the Four Pretreatment Days (PND 13, PND 14, PND 15, and PND 16). The Insets Show Mean Distance Traveled Collapsed across the Conditioning Session. Rats were Injected with Saline or 30 mg/kg Cocaine Immediately before a 30-min Placement in Activity Chambers. In Addition, Separate Group of Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Cocaine Treatment. *a* Significantly Different from the Saline Control Group. *b* Significantly Different from the Cocaine Alone Group.

Test Day

PND 17. Rats pretreated and tested with cocaine had greater distance traveled scores than the acute control group [group main effect, $F(4,28) = 8.30$, $P < 0.001$] (see right panel; Figure 17). Moreover, rats

challenged with cocaine had greater locomotor activity than the acute control group on time blocks 1, 10, 11, and 12 [group × time block interaction, $F(44, 308) = 5.43$, $P < 0.001$; Tukey tests, $P < 0.05$]. Raclopride pretreatment, regardless of dose, did not attenuate locomotor activity on the test day.

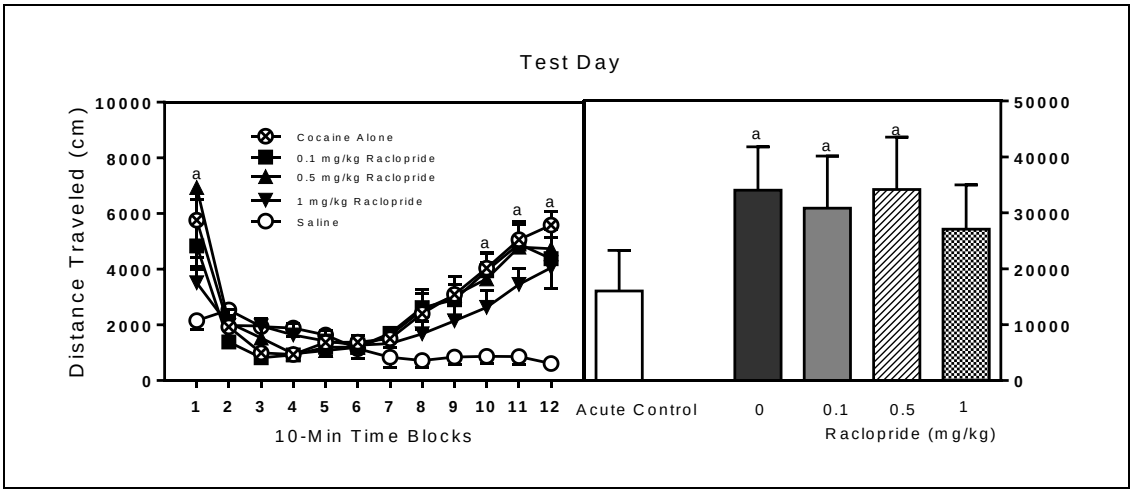

Figure 17. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per Group) on the Test Day (PND 17). Rats were Challenged with Cocaine (20 mg/kg) Immediately before Behavioral Testing. On the Pretreatment Days, Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Cocaine Treatment. The Acute Control Group was Injected with Saline on the Pretreatment Days and Injected with Cocaine on the Test Day. Locomotor Activity was Assessed for 120 min. The Right Panel Shows Mean Distance Traveled Collapsed across the Testing Session. *a* Significantly Different from the Acute Control Group.

Experiment 4b: Effects of D2 Receptor Blockade on Methamphetamine-Induced Multi-Trial Behavioral Sensitization During the Middle Preweanling Period

Pretreatment Day

PND 13. Rats injected with methamphetamine alone or methamphetamine plus raclopride (0.1, 0.5, or 1 mg/kg) had greater distance traveled scores than saline controls [group main effect, $F(4,28) = 9.47$, $P < 0.001$](see top left panel inset; Figure 18). Moreover, rats treated with cocaine alone exhibited greater locomotor activity on time blocks 1-3 than saline controls, while rats pretreated with raclopride (0.1, 0.5, or 1 mg/kg) had smaller distance traveled scores on time block 1 than the methamphetamine-alone group [group × time block interaction, $F(20, 140) = 6.60$, $P < 0.001$; Tukey tests, $p < 0.001$] (see top left panel; Figure 18).

PND 14. Rats injected with methamphetamine-alone or methamphetamine plus raclopride (0.1, 0.5, or 1 mg/kg) had greater distance traveled scores when compared to the saline controls [group main effect, $F(4,28) = 19.66$, $P < 0.001$] (see top right panel inset; Figure 18). Moreover, rats treated with cocaine alone showed greater locomotion on time blocks 2-5 than saline controls [group × time block interaction, $F(20, 140) = 17.33$, $P < 0.05$; Tukey tests, $p < 0.001$] (see top right panel; Figure 18). Rats pretreated with 1 mg/kg raclopride had smaller distance traveled scores on time block 1, 2, and 3 (Tukey tests, $P < 0.05$).

PND 15. Rats injected with methamphetamine alone or methamphetamine plus raclopride (0.1, 0.5, or 1 mg/kg) had greater distance traveled scores than saline controls [group main effect, $F(4,28) = 26.13$, $P < 0.001$] (see bottom left panel inset; Figure 18). More specifically, rats treated with methamphetamine alone had greater locomotor activity on time blocks 1 and 2 than saline controls [group × time block interaction, $F(20, 140) = 41.54$, $P < 0.001$; Tukey tests, $P < 0.05$]. Rats pretreated with raclopride (1 mg/kg) had smaller distance traveled scores on time block 1 and 2 when compared to the methamphetamine alone group (see bottom left panel; Figure 18).

PND 16. Rats injected with methamphetamine alone or methamphetamine plus raclopride (0.1, 0.5, or 1 mg/kg) had greater distance traveled scores than the saline controls [group main effect, $F(4,28) = 26.82$, $P < 0.001$] (see bottom right panel inset; Figure 18). Moreover, rats treated with methamphetamine alone exhibited greater locomotion than saline controls on time blocks 1 and 2 [group × time block interaction, $F(20, 140) = 17.33$, $P < 0.001$; Tukey tests, $p < 0.05$]. Rats pretreated with raclopride (1 mg/kg) had smaller distance traveled scores on time blocks 1 and 2 when compared to the methamphetamine-alone group, but they had greater distance traveled scores than the saline group later in the testing session (Tukey tests, $P < 0.05$) (see bottom right panel; Figure 18).

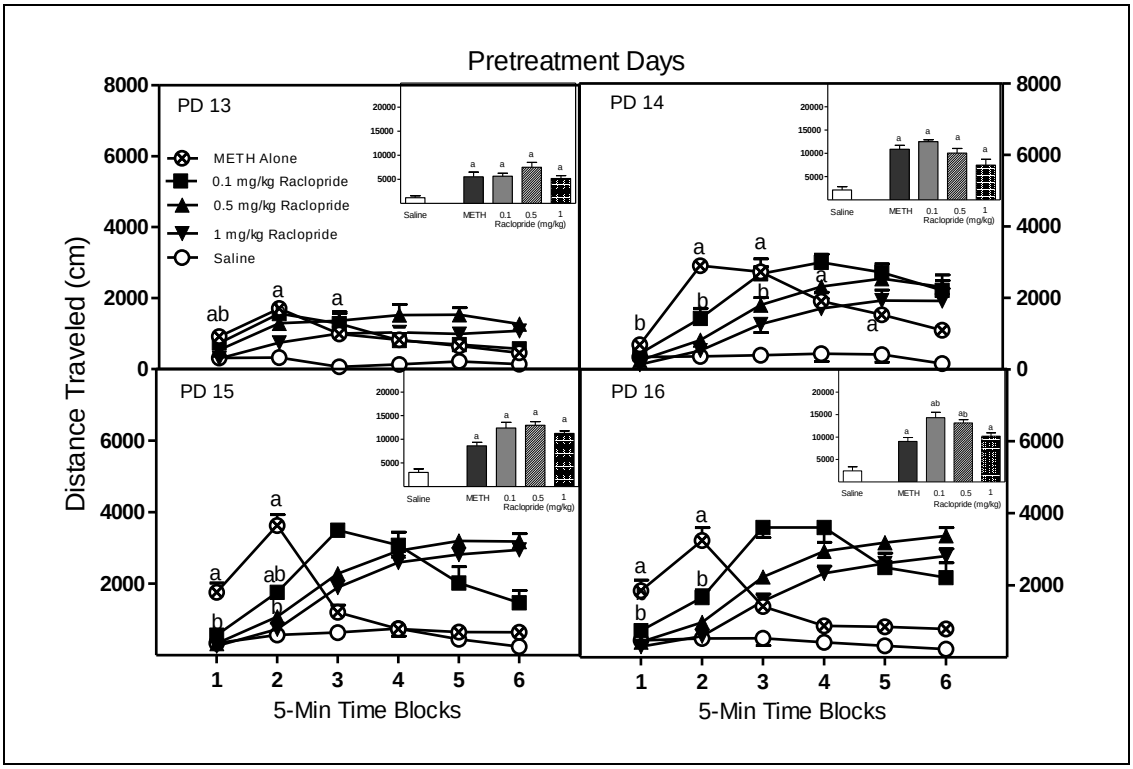

Figure 18. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per Group) on the Four Pretreatment Days (PND 13, PND 14, PND 15, and PND 16). The Insets Show Mean Distance Traveled Collapsed across the Conditioning Session. Rats were Injected with Saline or 4 mg/kg Methamphetamine Immediately before a 30-min Placement in Activity Chambers. In Addition, Separate Group of Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Methamphetamine Treatment. *a* Significantly Different from the Saline Control Group. *b* Significantly Different from the Methamphetamine Alone Group.

Test Day

PND 17. Overall, rats pretreated and tested with methamphetamine had greater distance traveled scores than the acute control group, while pretreatment with (0.5 or 1 mg/kg) raclopride attenuated locomotor activity on

the test day [group main effect, $F(4,28) = 18.24$, $P < 0.001$] (see right panel, Figure 19). Furthermore, rats pretreated and tested with methamphetamine exhibited greater locomotion on time blocks 2-12 than the acute control group (see left panel Figure 19). Raclopride pretreated rats had smaller locomotor activity scores than the methamphetamine alone group (i.e. the sensitized group) on time blocks 6-9 [group × time block interaction, $F(44, 308) = 5.43$, $P < 0.001$; Tukey tests, $p < 0.05$].

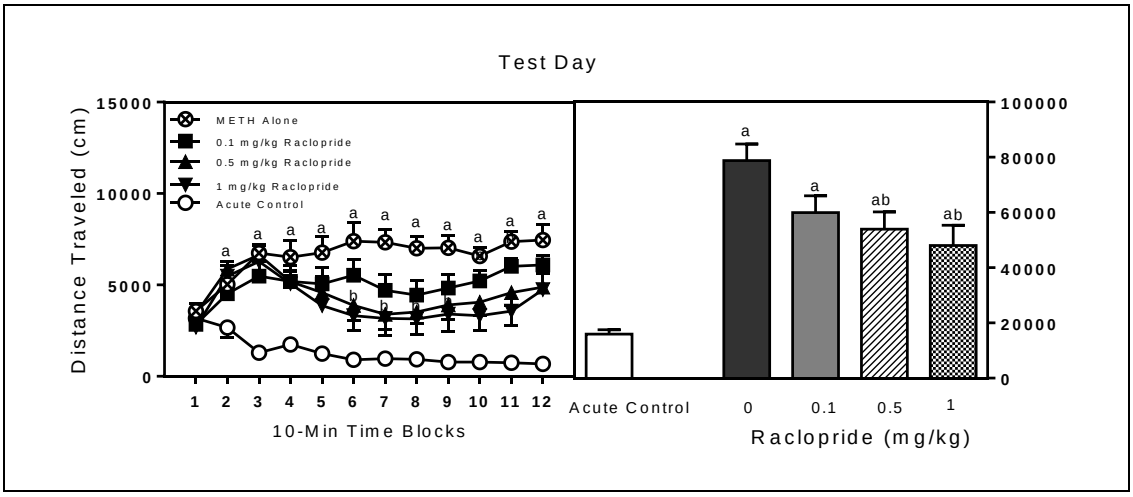

Figure 19. Mean Distance Traveled Scores (±SEM) of Rats (n = 8 per Group) on the Test Day (PND 17). Rats were Challenged with Methamphetamine (2 mg/kg) Immediately before Behavioral Testing. On the Pretreatment Days, Rats were Injected with Raclopride (0.1, 0.5, 1, or 5 mg/kg) 15 min before Methamphetamine Treatment. The Acute Control Group was Injected with Saline on the Pretreatment Days and Injected with Methamphetamine on the Test Day. Locomotor Activity was Assessed for 120 min. Right Panel Show Mean Distance Traveled Collapsed Across the Testing Session. *a* Significantly Different from the Acute Control Group. *b* Significantly Different from the Methamphetamine Alone Group.

CHAPTER ELEVEN

DISCUSSION

Summary of Results and Hypotheses

The purpose of this thesis was to investigate the importance of D2-like receptors for the induction of psychostimulant-induced behavioral sensitization during early ontogeny. More specifically, the goals of this thesis were to determine the role of D2-like receptors for cocaine- and methamphetamine-induced one-trial and multi-trial behavioral sensitization during the preweanling period. It was predicted that the D2-like receptor antagonist raclopride would prevent the induction of one-trial methamphetamine- and cocaine-induced behavioral sensitization at PND 17 and PND 21, respectively. Furthermore, it was predicted that raclopride would block both methamphetamine- and cocaine-induced multi-trial behavioral sensitization at PND 17 and PND 21.

Contrary to predictions, the D2-like receptor antagonist raclopride did not attenuate cocaine-induced sensitized responding on PND 21 when a one-trial procedure was employed. Furthermore, raclopride did not prevent the induction of methamphetamine-induced one-trial behavioral sensitization on PND 17. In regards to cocaine-induced multi-trial behavioral sensitization, sensitized responding was evident on both PND 17 and PND 21. However, the D2-like antagonist raclopride, regardless of dose, was unable to block the induction of cocaine-induced behavioral sensitization when assessed on the

test day. In contrast to all of the previously mentioned results, higher doses of raclopride (0.5 and 1 mg/kg) *blocked* the induction of methamphetamine-induced sensitization on PND 17. Methamphetamine did not produce behavioral sensitization during the late preweanling period (i.e. on PND 21), nor did raclopride inhibit methamphetamine-induced locomotor activity on PND 21.

Comparing the Present Results to Adult Studies

Multi-trial Behavioral Sensitization

Dopamine D2-like receptor antagonists attenuate multi-trial methamphetamine-induced behavioral sensitization in adult rats (Kuribara, 1994). A similar effect was observed in the present study since raclopride (0.5 and 1 mg/kg) attenuated the multi-trial sensitized responding of preweanling rats. The ability of raclopride to block behavioral sensitization is consistent with results using other reward-related paradigms, such as sucrose intake and conditioned place preference (Mizoguchi, Yamada, Mizuno, Mizuno Nitta, Noda, & Nabeshima, 2004; Tyrka, Gayle, & Smith, 1992). The ability of raclopride to block the induction of one-trial methamphetamine-induced behavioral sensitization in adult rats has not been assessed, so ontogenetic comparisons cannot be made.

According to some earlier studies using adult rodents, dopamine D2-like receptor antagonists prevent the induction of multi-trial cocaine-induced behavioral sensitization (Mattingly et al., 1996). Based on

these studies, it was predicted that raclopride would also attenuate the multi-trial cocaine-induced sensitized responding of preweanling rats; however, these results were not obtained. Despite using a broad dose range of raclopride (0.1-5 mg/kg), the dopamine D2-like receptor antagonist did not attenuate the cocaine-induced sensitized responding of preweanling rats. This finding implies that dopamine receptors are not involved in the induction of multi-trial cocaine-induced behavioral sensitization during the preweanling period. Whether these results represent a true ontogenetic difference is uncertain, since White et al. (1998) reported that the D2 receptor antagonist eticlopride failed to prevent the induction of multi-trial cocaine-induced sensitization in adult rats. These authors suggest that earlier studies showing D2 receptor involvement in the multi-trial behavioral sensitization of adult rats were confounded due to the use of an excessive dose of haloperidol causing nonspecific behavioral effects (Mattingly et al., 1996; White et al., 1998).

One-trial Behavioral Sensitization

Despite these contradictory findings involving multi-trial behavioral sensitization, it does appear that D2 receptor antagonists prevent the induction of one-trial behavioral sensitization in adult rats (Fontana et al., 1993; Weiss et al., 1989). In contrast, dopamine antagonists do not block the one-trial behavioral sensitization of preweanling rats (present study; Mohd-Yusof et al., 2014). This age-dependent difference may be due to environmental conditioning factors. In adult rats and mice, one-trial behavioral

sensitization is exclusively context-dependent, while multi-trial sensitization is not (Anagnostaras et al., 2002; Battisti et al., 1999; Drew & Glick, 1989). If the role of dopamine receptors is to mediate the contextual conditioning aspects of behavioral sensitization, then only the one-trial behavioral sensitization of adult rats should be sensitive to dopamine receptor blockade. Consistent with this suggestion, D1-like and D2-like receptor antagonists only prevent the induction of one-trial and not multi-trial cocaine sensitization in adult rats (White et al., 1998). Since the one-trial behavioral sensitization of preweanling rats is context-independent (Kozanian et al., 2012), then dopamine antagonists should not be effective at blocking induction. Consistent with this idea, raclopride did not block the cocaine-induced one-trial behavioral sensitization of preweanling rats.

Role of Non-Dopaminergic Receptor Systems

When considered together, results from adult and preweanling rat studies suggest that dopamine receptors either play no role or only a minor role in the induction of psychostimulant-induced behavioral sensitization. This begs the question as to which neurotransmitter systems are responsible for mediating the induction of behavioral sensitization. Cocaine and methamphetamine do not exclusively affect dopamine neurons. For example, cocaine increases serotonin and norepinephrine levels (Seiden et al., 1993), while amphetamine- and methamphetamine-like compounds increase norepinephrine levels as well as dopamine (Ritz et al., 1987; Seiden et al.,

1993). This lack of specificity leaves open the possibility that cocaine and methamphetamine may also affect behavioral sensitization by modulating serotonergic and/or noradrenergic processes.

Consistent with this idea, previous studies have shown that serotonin 5-HT_2 antagonists, such as ritanserin, partially block the induction of methamphetamine sensitization (Tanaka, Ishigooka, Watanabe, Nagata, & Miura, 1998). Cocaine sensitization is also inhibited by the serotonin 5-HT_3 antagonist ondansetron (King, Xiong, & Ellinwood, 1997). The adrenergic system may also mediate the induction of behavioral sensitization. In fact, Auclair et al. (2004) states that the induction of psychostimulant-induced behavioral sensitization is exclusively mediated by 5-HT_{2A} and α-$_{1B}$ adrenergic receptors. Thus, the inability of raclopride to block the one-trial behavioral sensitization of young rats may indicate that non-dopaminergic systems mediate the induction process during the preweanling period.

Comparing One-Trial and Multi-Trial Behavioral Sensitization in Preweanling Rats

According to Valjent and colleagues (2010), a single exposure protocol provides a simple paradigm that can measure the induction of behavioral sensitization, while avoiding the problems of tolerance and dependence. These authors also suggest that the neural mechanisms mediating one-trial and multi-trial methamphetamine-induced behavioral sensitization differ (Valjent et al., 2010). In the present study, a dopamine D2-like antagonist was

unable to block one-trial methamphetamine-induced behavioral sensitization on PND 17. In contrast, repeated treatment with high doses of raclopride (0.5 and 1 mg/kg) attenuated the induction of multi-trial methamphetamine-induced behavioral sensitization on PND 17.

Several explanations may account for the different pattern of results provided by the one- and multi-trial procedures. One possibility is that the underlying neural mechanisms mediating one-trial and multi-trial sensitization differ. Alternatively, it is possible that repeatedly exposing rats to dopamine antagonists may cause nonspecific neural changes (i.e., changes unrelated to modifying the acute effects of psychostimulant drugs) that weaken the sensitized response. Thus, the impaired sensitized responding that is evident when using a multi-trial procedure may be an artifact of repeated antagonist administration (Mohd-Yusof et al., 2014). Finally, the multi-trial behavioral sensitization of preweanling rats is context-dependent (Wood et al., 1998; Zavala et al., 2000), while one-trial behavioral sensitization is context-independent (Kozanian et al., 2012). This dichotomy leaves open the possibility that D2-like receptor antagonism was interfering with contextual conditioning and, thus, only multi-trial behavioral sensitization should be disrupted by D2 receptor antagonism.

Ontogeny of Dopamine Receptors

Past studies have shown that the dopamine system exhibits age-dependent changes across postnatal development. For example, firing

rates of neurons in the nigrostriatal pathway increase gradually with age (Pitts & Chiodo, 1990). Furthermore, the number of D1-like receptors gradually increases until approximately the onset of puberty (PND 40), when dopamine receptors are over-expressed. The number of D1-like and D2-like receptors then declines (i.e. pruning) to levels that are maintained throughout adulthood (Andersen et al., 2000). These changes in synaptic plasticity during development, when coupled with early drug exposure, could possibly explain the age-dependent differences in pharmacological sensitivity to psychostimulants.

Effects of Raclopride During the Pretreatment Phase

Data collected on the pretreatment days are also informative. When rats were given raclopride (0.1-1 mg/kg) during the pretreatment phase (one- or multi-trial), the D2-like antagonist was unable to block the acute locomotor activating effects of methamphetamine and cocaine. Importantly, the ability or inability of raclopride to block agonist-induced effects on the pretreatment day did not determine whether a sensitized response was expressed on the test day. For example, raclopride attenuated the acute effects of cocaine during the pretreatment phase; however, raclopride did not prevent sensitization from being expressed on the test day (see Fig. 16). In contrast, raclopride (0.1 and 0.5 mg/kg) actually potentiated methamphetamine-induced locomotion by the end of the pretreatment phase. Even so, the sensitized response was reduced on the test day (see Fig. 18).

Other lines of research also suggest that the occurrence or non-occurrence of agonist-induced locomotor activity on the pretreatment day does not determine whether a sensitized response will be evident on the test day. For example, preweanling and adult rats anesthetized during the pretreatment phase (i.e., no locomotor activity was possible) exhibited behavioral sensitization on the test day (Herbert et al., 2010; Wang & Hsiao, 2003), while adult mice injected with a D2-like antagonist up to 5 hours after methamphetamine pretreatment (i.e., A full locomotor response was evident on the pretreatment day) did not exhibit behavioral sensitization (Kuribara, 1995). Therefore, the induction of behavioral sensitization is independent of the overt manifestation of drug-induced locomotor activity during the pretreatment phase.

Summary

In summary, both cocaine and methamphetamine were able to produce behavioral sensitization when a one-trial or a multi-trial procedure was used. The dopamine D2-like antagonist raclopride failed to prevent the induction of one-trial cocaine- and methamphetamine-induced behavioral sensitization, thus D2-like receptor stimulation is unnecessary when a one-trial procedure is used. The ability of raclopride to prevent the induction of multi-trial methamphetamine-induced sensitization suggests that the neural mechanisms underlying behavioral sensitization in young rats differs depending on the type of paradigm being employed. Lastly, age-dependent

differences in the importance of contextual conditioning may explain why D1-like and D2-like receptor antagonists prevent the induction of one-trial behavioral sensitization in adult rats, but not rat pups (see also Mohd-Yusof et al., 2014).

REFERENCES

Anagnostaras, S. G., & Robinson, T. E. (1996). Sensitization to the psychomotor stimulant effects of amphetamine: modulation by associative learning. *Behavioral Neuroscience, 110*, 1397–1414.

Anagnostaras, S. G., Schallert, T., & Robinson, T. E. (2002). Memory processes governing amphetamine-induced psychomotor sensitization. *Neuropsychopharmacology*, *26*, 703–715.

Andersen, S. L., Thompson, A. T., Rustein, M., Hostetter, J. C., Jr., & Teicher, M. H. (2000). Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse, 37,* 167-169.

Ando, K., Johanson, C. E., Seiden, L. S., & Schuster, C. R. (1985). Sensitivity changes to dopaminergic agents in fine motor control of rhesus monkeys after repeated methamphetamine administration. *Pharmacology, Biochemistry, and Behavior, 22,* 737-743.

Antonopoulos, J., Dori, I., Dinopoulos, A., Chiotelli, M., & Parnavelas, J. G. (2002). Postnatal development of the dopaminergic system of the striatum in the rat. *Neuroscience, 110,* 245-256.

Araki, K. Y., Sims, J. R., & Bhide, P. G. (2007). Dopamine receptor mRNA and protein expression in the mouse corpus striatum and cerebral cortex during pre- and post-natal development. *Brain Research, 1156,* 31-45.

Auclair, A., Drouin, C., Cotecchia, S., Glowisnki, J., & Tassin, J. P. (2004). 5HT-$_{2A}$ and α-$_{1B}$ adrenergic receptors entirely mediate dopamine release, locomotor response and behavioral sensitization to opiates and pscyhostimulants. *European Journal of Neuroscience, 20,* 3073-3084.

Battisti, J. J., Chang, C. H., Uretsky, N. J., & Wallace, L. J. (1999). Sensitization of stereotyped behavior to amphetamine is context and response dependent. *Pharmacology, Biochemistry, and Behavior, 63,* 263-269.

Battisti, J. J., Uretsky, N. J., & Wallace, L. J. (2000). Importance of environmental context in the development of amphetamine- or apomorphine-induced stereotyped behavior after single and multiple doses. *Pharmacology, Biochemistry, and Behavior, 66*, 671–677.

Beninger, R. J., & Hahn, B. L. (1983). Pimozide blocks the establishment but not expression of amphetamine produced environment specific conditioning. *Science, 220,* 1304-1306.

Beninger, R. J., & Herz, R. S. (1986). Pimozide blocks establishment but not expression of cocaine-induced environment-specific conditioning. *Life Science, 38,* 1425–1431.

Beyer, C. E., & Steketee, J. D. (2002). Cocaine sensitization: modulation by dopamine D2 receptors. *Cereberal Cortex, 12,* 526–535.

Binder, E. B., Kinkead, B., Owens, M. J., & Nemeroff, C. B. (2001). Neurotensin and dopamine interactions. *Pharmacological Review, 53*, 453-486.

Boyson, S. J., McGonigle, P., & Molinoff, P. B. (1986). Quantitative autoradiographic localization of the D_1 and D_2 subtypes of dopamine receptors in rat brain. *Journal of Neuroscience, 11,* 3177-3188.

Carlsson, A., Lindqvist, M., Magnusson, T., & Waldneck, B. (1958). On the presence of 3-hydroxytyramine in brain. *Science, 127,* 471.

Carr, D. B., & Sesack, S. R. (2000). Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *Journal of Neuroscience, 20,* 3864-3873.

Chang, H. T., & Kitai, S. T. (1985). Projection neurons of the nucleus accumbens: an intracellular labeling study. *Brain Research, 347,* 112-116.

Charuchinda, C., Supavilai, P., Karobath, M., & Palacios, J. M. (1987). Dopamine D2 receptors in the rat brain: autoradiographic visualization using a high-affinity selective agonist ligand. *Journal of Neuroscience, 7,* 1352-1360.

Choi, W. S., Machida, C. A., & Ronnekleiv, O. K. (1995). Distribution of dopamine D1, D2, and D5 receptor mRNAs in the monkey brain: ribonuclease protection assay. *Molecular Brain Research, 31,* 86-94.

Dahlstrom, A., & Fuxe, K. (1965). Evidence for the existence of monoamine neurons in the central nervous system. *Acta Physiologica Scandinavica, 62,* 1-55.

Damier, P., Hirsch, E. C., Agid, Y., & Graybriel, A. Y. (1999). The substantia nigra of the human brain II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain, 122*, 1437-1448.

Davidson, C., Lazarus, C., Lee, T. H., & Ellinwood, E. H. (2002). Behavioral sensitization is greater after repeated versus single chronic cocaine dosing regimens. *European Journal of Pharmacology, 44,* 75-78.

Diaz, J., Levesque, D., Lammers, C. H., Griffon, N., Martres, M. P., Schwartz, J. C., & Sokoloff, P. (1995) Phenotypical characterization of neurons expressing the dopamine D_3 receptor. *Neuroscience, 65,* 731–745.

Drew, K. L., & Glick, S. D. (1989). Environmental-dependent sensitization to amphetamine-induced circling behavior. *Pharmacology, Biochemistry, and Behavior, 31*, 705-708.

Duke, M. A., O'Neal, J., & McDougall, S. A. (1997). Ontogeny of dopamine agonist-induced sensitization: role of NMDA receptors. *Psychopharmacology, 129*, 153-160.

Eichler, A. J., Antelman, S. M., & Black, C. A. (1980). Amphetamine stereotypy is not a homogenous phenomenon: sniffing and licking show distinct profiles of sensitization and tolerance. *Psychopharmacology, 68*, 287-290.

Fontana, D., Post, R. M., Weiss, S. R. B., & Pert, A. (1993). The role of D1 and D2 dopamine receptors in the acquisition and expression of cocaine-induced conditioned increases in locomotor behavior. *Behavioural Pharmacology, 4,* 375-387.

Frantz, K. J., O'Dell, L. E., & Parsons, L. H. (2007). Behavioral and neurochemical responses to cocaine in periadolescent and adult rats. *Neuropsychopharmacology, 32,* 625-637.

Fremeau, R. T.,Jr., Duncan, G. E., Fornaretto, M. G., Dearry, A., Gingrich, J. A., Breese, G. R., & Caron, M. G. (1991). Localization of D_1 dopamine receptor mRNA in brain supports role in cognitive, affective, and neuroendocrine aspects of dopaminergic neurotransmission. *Proceedings of the National Academy of Science of the United States of America, 88,* 3772-3776.

Fujiwara, Y., Kazahaya, Y., Nakashima, M., Sato, M., & Otsuki, S. (1987). Behavioral sensitization in the rat: an ontogenic study. *Psychopharmacology, 91*, 316-319.

Fumagalli, F., Gainetdinov, R. R., Valenzano, K. J., & Caron, M. G. (1998). Role of dopamine transporter in methamphetamine-induced neurotoxicity: evidence from mice lacking the transporter. *Journal of Neuroscience, 13,* 4861-4869.

Geffen, L. B., Jessell, T. M., Cuello, A. C., & Iversen, L. L. (1976). Release of dopamine from dendrites in the rat substantia nigra. *Nature, 260*, 258-260.

Giorgi, O., De Montis, G., Porceddu, M. L., Mele, S., Calderini, G., Toffano, G., & Biggio, G. (1987). Developmental and age related changes in D_1-dopamine receptors and dopamine content in the rat striatum. *Brain Research, 432,* 283-290.

Gurevich, E. V., Himes, J. W., & Joyce, J. N. (1998). Developmental regulation of expression of the D_3 dopamine receptor in rat nucleus accumbens and islands of Calleja. *Journal of Pharmacology and Experimental Therapeutics, 289,* 587-598.

Hall, D. A., Stanis, J. J., Avila, H. M., & Gulley, J. M. (2008). A comparison of amphetamine- and methamphetamine-induced locomotor activity in rats: evidence for qualitative differences in behavior. *Psychopharmacology, 195*, 469-478.

Hamamura, T., Akiyama, K., Akimoto, K., Kashihara, K., Okumura, K., Ujike, H., & Otsuki, S. (1991). Co-administration of either a selective D_1 or D_2 dopamine antagonist with methamphetamine prevents methamphetamine-induced behavioral sensitization and neurochemical change, studied by in vivo intracerebral dialysis. *Brain Research, 546,* 40-46.

Hartley, E. J., & Seeman, P. (1983). Development of receptors for dopamine and noradrenaline in rat brain. *European Journal of Pharmacology, 91,* 391-397.

Herbert, M. S., Der-Ghazarian, T., Palmer, A. G., & McDougall, S. A. (2010). One-trial cocaine-induced behavioral sensitization in preweanling rats: role of contextual stimuli. *Experimental and Clinical Psychopharmacology*, *18*, 284–295.

Hirabayashi, M., & Alam, M. R. (1981). Enhancing effect of methamphetamine on ambulatory activity produced by repeated administration in mice. *Pharmacology, Biochemistry, and Behavior, 15,* 925-932.

Ho, A. K. S., & Loh, H. H. (1972). Evidence of adrenergic-cholinergic interaction in the central nervous system II. Dopamine and its analogues. *European Journal of Pharmacology, 19*, 145-150.

Hooks, M. S., Jones, G. H., Neill, D. B., & Justice, J. B., Jr.(1992). Individual differences in amphetamine sensitization: dose-dependent effects. *Pharmacology, Biochemistry, and Behavior, 41,* 203-210.

Huang, Q., Zhou, D., Chase, K., Gusella, J. F., Aronin, N., & DiFiglia, M. (1992). Immunohistochemical localization of the D_1 dopamine receptor in rat brain reveals its axonal transport, pre- and postsynaptic localization, and prevalence in the basal ganglia, limbic system, and thalamic reticular nucleus. *Proceedings of the National Academy of Sciences of the United States of America, 89,* 11988-11992.

Huynh, H., & Feldt, L. S. (1976). Estimation of the Box correction for degrees of freedom from sample data in randomized block and split-plot designs. *Journal of Educational Statistics, 1*, 69–82.

Jaber, M., Robinson, S. W., Missale, C., & Caron, M. G. (1996). Dopamine receptors and brain function. *Neuropharmacology, 35,* 1503-1519.

Jackson, H. C., & Nutt, D. J. (1993). A single preexposure produces sensitization to the locomotor effects of cocaine in mice. *Pharmacology, Biochemistry, and Behavior, 45,* 733-735.

Jung, E. S., Lee, H. J., Sim, H. R., & Baik, J. H. (2013). Cocaine-induced behavioral sensitization in mice: effects of microinjection of dopamine D_2 receptor antagonist into the nucleus accumbens. *Experimental Neurobiology, 22,* 224-231.

Kalivas, P. W., & Stewart, J. (1991). Dopamine transmission in the initiation and expression of drug- and stress- induced sensitization of motor activity. *Brain Research Reviews, 16*, 223-224.

Kalivas, P. W., & Duffy P. (1998). Repeated cocaine administration alters extracellular glutamate in the ventral tegmental area. *Journal of Neurochemistry, 70,* 1497–1502.

Karper, P. E., De La Rosa, H., Newman, E. R., Krall, C. M., Nazarian, A., McDougall, S. A., & Crawford, C. A. (2002). Role of D_1-like receptors in amphetamine-induced behavioral sensitization: a study using D_{1A} receptor knockout mice. *Psychopharmacology, 159,* 407–414.

Kebabian, J. W., Beaulieu, M., & Itoh, Y. (1984). Pharmacological and biochemical evidence for the existence of two categories of dopamine receptor. *Canadian Journal of Neurological Sciences, 11*, 114-117.

King, G. R., Xiong, Z., & Ellinwood, E. J., (1997). Blockade of cocaine sensitization and tolerance by the co-administration of odansetron, a 5-HT_3 receptor antagonist, and cocaine. *Psychopharmacology, 30,* 159-165.

Kolta, M. B., Shreve, P., De Souza, V., & Uretsky, N. J. (1985). Time course of the development of the enhanced behavioral and biochemical responses to amphetamine after pretreatment with amphetamine. *Neuropharmacology, 24,* 823-829.

Kozanian, O. O., Gutierrez, A., Mohd-Yusof, A., & McDougall S. A. (2012). Ontogeny of methamphetamine-induced and cocaine-induced one-trial behavioral sensitization in preweanling and adolescent rats. *Behavioural Pharmacology, 23,* 367-379.

Kuribara, H. (1994). Early post-treatment with haloperidol retards induction of methamphetamine sensitization in mice. *European Journal of Pharmacology, 256,* 295-299.

Kuribara, H., & Uchihashi, Y. (1994). Effects of dopamine antagonism on methamphetamine sensitization: evaluation by ambulatory activity in mice. *Pharmacology, Biochemistry, and Behavior, 47,* 101-106.

Kuribara, H., & Uchihashi, Y. (1993). Dopamine antagonists can inhibit methamphetamine sensitization, but not cocaine sensitization, when assessed by ambulatory activity in mice. *Journal of Pharmacy and Pharmacology, 45,* 1042–1045.

Langdale, J. A. (1994). In Situ Hybridization. The Maze Handbook. Springer Lab Manuals. Springer New York.

Laviola, G., Pascucci, T., & Pieretti, S. (2001). Striatal dopamine sensitization to D-amphetamine in periadolescent but not in adult rats. *Pharmacology, Biochemistry, and Behavior, 68,* 115-124.

Laviola, G., Wood, R. D., Kuhn, C., Francis, R., & Spear, L. P. (1995). Cocaine sensitization in periadolescent and adult rats. *Journal of Pharmacology and Experimental Therapeutics, 275*, 345-357.

Leith, N. J., & Kuczenski, R. (1982). Two dissociable components of behavioral sensitization following repeated amphetamine administration. *Psychopharmacology, 76*, 310-315.

Leslie, C. A., Robertson, M. W., Cutler, A. J., & Bennett, J.P., Jr. (1991). Postnatal development of D_1 dopamine receptors in the medial prefrontal cortex, striatum and nucleus accumbens of normal and neonatal 6-hydroxydopamine treated rats: a quantitative autoradiographic analysis. *Developmental Brain Research, 62*, 109-114.

Lévesque, D., Diaz, J., Pilon, C., Martres, M. P., Giros, B., Souil, E., Schott, D., Morgat, J. L., Schwartz, J. C., & Sokoloff, P. (1992). Identification, characterization, and localization of the dopamine D_3 receptor in rat brain using 7-[^{3}H]hydroxy-*N*, *N*-di-*n*-propyl-2-aminotetralin. *Proceedings of the National Academy of the Sciences of United States of America, 89*, 8155-8159.

Lewis, B. L., & O'Donnell, P. (2000). Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential 'up' states in pyramidal neurons via D_1 dopamine receptors. *Cerebral Cortex, 10,* 1168-1175.

Mattingly, B. A., Hart, T. C., Lim, K., & Perkins, C. (1994). Selective antagonism of dopamine D_1 and D_2 receptors does not block the development of behavioral sensitization to cocaine. *Psychopharmacology, 114,* 239–242.

Mattingly, B. A., Rowlett, J. K., Ellison, T., & Rase, K. (1996). Cocaine-induced behavioral sensitization: effects of haloperidol and SCH 23390 treatments. *Pharmacology, Biochemistry, and Behavior, 53,* 481–486.

McCreary, A. C., & Marsden, C. A. (1993). Cocaine-induced behavior: dopamine D_1 receptor antagonism by SCH 23390 prevents expression of conditioned sensitization following repeated administration of cocaine. *Neuropharmacology, 32,* 387–391.

McDougall, S. A., Baella, S. A., Stuebner, N. M., Halladay, L. M., & Crawford, C. A. (2007). Cocaine-induced behavioral sensitization in preweanling and adult rats: effects of a single drug-environment pairing. *Psychopharmacology, 193,* 323-332.

McDougall, S. A., Cortez, A. M., Palmer, A. G., Herbert, M. S., Martinez, C.E., Charntikov, S., & Amodeo, D. A. (2009). Importance of environmental context for one-and three-trial cocaine-induced behavioral sensitization in preweanling rats. *Psychopharmacology, 206,* 377-388.

McDougall, S. A., Duke, M. A., Bolanos, C. A., & Crawford, C. A. (1994). Ontogeny of behavioral sensitization in the rat: effects of direct and indirect dopamine agonists. *Psychopharmacology, 116*, 483-490.

McDougall, S. A., Kozanian, O. O., Greenfield, V. Y., Horn, L. R., Gutierrez, A., Mohd-Yusof, A., & Castellanos, K. A., (2011). One-trial behavioral sensitization in preweanling rats: differential effects of cocaine, methamphetamine, methylphenidate, and D-amphetamine. *Psychopharmacology, 217,* 559-571.

McDougall, S. A., Nuqui, C. M., Quiroz, A. T., & Martinez, C. M. (2013). Early ontogeny of $_{D}$-amphetamine-induced one-trial behavioral sensitization. *Pharmacology, Biochemistry, and Behavior, 104,* 154-162.

McDougall, S. A., Reichel, C. M., Cyr, M. C., Karper, P. E., Nazarian, A., & Crawford, C. A. (2005). Importance of D_1 receptors for associative components of methamphetamine-induced behavioral sensitization and conditioned activity: a study using D_1 receptor knockout mice. *Psychopharmacology, 183,* 20-23.

McTavish, S. F., Cowen, P. J., & Sharp, T. (1999). Effect of a tyrosine-free amino acid mixture on regional brain catecholamine synthesis and release. *Psychopharmacology, 141,* 182-188.

Meador-Woodruff, J. H., Mansour, A., Bunzow, J. R., Van Tol, H. H., Watson, S. J., Jr., & Civelli, O. (1989). Distribution of D_2 dopamine receptor mRNA in rat brain. *Proceedings National Academy of Science of the United States of America, 86,* 7625–7628.

Meador-Woodruff, J. H., Mansour, A., Grandy, D. K., Damask, S. P., Civelli, O., & Watson, S. J., Jr. (1992). Distribution of D_5 dopamine receptor mRNA in rat brain. *Neuroscience Letters, 145,* 209-212.

Meyer, J. S., & Quenzer, L. F. (2005). Psychopharmacology: drugs, the brain, and behavior. Sunderland, Massachsetts, USA: Sinauer Associates Inc.

Mizoguchi, H., Yamada, K., Mizuno, M., Mizuno, T., Nitta, A., Noda, Y., & Nabeshima, T. (2004). Regulations of methamphetamine reward by extracellular-signal regulated kinase ½ ets-like gene-1 signal pathway via the activation of dopamine receptors. *Molecular Pharmacology, 65,* 1293-1301.

Mohd-Yusof, A., Gonzalez, A. E., Veliz, A., & McDougall, S. A. (2014). Role of the D_1 receptor for the dopamine agonist-induced one-trial behavioral sensitization of preweanling rats. *Psychopharmacology, 231,* 4167-4177.

Murrin, L. C., & Zeng, W. Y. (1986). Postnatal ontogeny of dopamine D2 receptors in rat striatum. *Biochemical Pharmacology, 35,* 1159 1162.

Murrin, L. C., & Zeng, W. Y. (1990). Ontogeny of dopamine D_1 receptors in rat forebrain: a quantitative autoradiographic study. *Developmental Brain Research 57,* 7-13.

Nagatsu, T., Levitt, M., & Udenfriend, S. (1964). Tyrosine hydroxylase: the initial step in norepinephrine biosynthesis. *Journal of Biological Chemistry, 239,* 2910-2917.

Nair, V. D., & Mishra, R. K. (1995). Ontogenic development of dopamine D_4 receptor in rat brain. *Developmental Brain Research, 90,* 180-183.

Neal, B. S., & Joyce, J. N. (1992). Neonatal 6-OHDA Lesions differentially affect striatal D1 and D2 receptors. *Synapse, 11,* 35-46.

O'Dell, S. J., La Hoste, G. J., Widmark, C. B., Shapiro, R. M., Potkin, S. G., & Marshall, J. F. (1990). Chronic treatment with clozapine or haloperidol differentially regulates dopamine and serotonin receptors in rat brain. *Synapse, 6,* 146-153.

Onali, P., Schwartz, J. P., & Costa, E. (1981). Dopamine modulation of adenylate cyclase stimulation by vasoactive intestinal peptide in anterior pituitary. *Neurobiology, 78*, 6531-6534.

Olson, L., & Seiger, A. (1972). Early prenatal ontogeny of central monoamine neurons in the rat: fluorescence histochemical observations. *Zeitschrift für Anatomie und Entwicklungsgeschichte, 137*, 301-316.

Palkovits, M., Brownstein, M., Saavedra, J. M., & Axelrod, J. (1974). Norepinephrine and dopamine content of hypothalamic nuclei of the rat. *Brain Research, 77,* 137-149.

Park, M., Kitahama, K., Geffard, M., & Maeda, T. (2000). Postnatal development of the dopaminergic neurons in the rat mesencephalon. *Brain Development,* 22*,* 38-44.

Pierce, R. C., & Kalivas, P. W. (1997). A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Research Reviews, 25*, 192-216.

Pitts, D. K., Freeman, A. S., & Chiodo, L. A. (1990). Dopamine neuron ontogeny: electrophysiological studies. *Synapse, 6,* 309-320

Post, R. M., & Rose, H. (1976). Increasing effects of repetitive cocaine administration in the rat. *Nature, 260,* 731-732.

Primus, R. J., Thurkauf, A., Xu, J., Yevich, E., McInerney, S., Shaw, K., Tallman, J. F., & Gallagher, D. W. (1997). Localization and characterization of dopamine D4 binding sites in rat and human brain by use of the novel, D_4 receptor-selective ligand [^{3}H]NGD 94-1. *Journal of Pharmacology and Experimental Therapy, 282,* 1020-1027.

Rao, P. A., Molinoff, P. B., & Joyce, J. N. (1991). Ontogeny of dopamine D_1 and D_2 receptor subtypes in rat basal ganglia: a quantitative autoradiographic study. *Developmental Brain Research, 60,* 161-177.

Reith, M. E. A., Li, M. Y., & Yan, Q. S. (1997). Extracellular dopamine, norepinephrine, and serotonin in the ventral tegmental area and nucleus accumbens of freely moving rats during intracerebral dialysis following systemic administration of cocaine and other uptake blockers. *Psychopharmacology, 134*, 309-317.

Richtand, N. M., Kelsoe, J. R., Segal, D. S., & Kuczenski, R. (1995). Regional quantification of D1, D2, and D3 dopamine receptor mRNA in rat brain using a ribonuclease protection assay. *Molecular Brain Research, 33,* 97–103.

Ritz, M. C., Lamb, R. J., Goldberg, S. R., & Kuhar, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science, 237,* 1219-1223.

Robinson, T. E., & Becker, J. B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Research Reviews*, *11*, 157–198.

Robinson, T. E., Becker, J. B., & Presty, S. K. (1982). Long-term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by a single exposure to amphetamine: sex differences. *Brain Research, 253,* 231-241.

Roberts-Lewis, J. M., Roseboom, P. H., Iwaniec, L. M., & Gnergy, M. E. (1986). Differential down-regulation of D1-stimulated adenylate cyclase activity in rat forebrain after *in vivo* amphetamine treatments. *Journal of Neuroscience, 6*, 2245-2251.

Roth, R. (1979). Tyrosine hydroxylase. In Horn, A. S., Korf, J., Westerink, B. H. C. (Eds.), *The neurobiology of dopamine*. London: Academic Press.

Sax, K. W., & Strakowski, S. M. (2001). Behavioral sensitization in humans. *Journal of Addictive Diseases*, *20*, 55—65.

Schambra, U. B., Duncan, G. E., Breese, G. R., Fornaretto, M. G., Caron, M. G., & Fremeau, R. T., Jr., (1994). Ontogeny of D1A and D2 dopamine receptor subtypes in rat brain using in situ hybridization and receptor binding. *Neuroscience, 62,* 65-85.

Seamans, J. K., Floresco, S. B., & Phillips, A. G. (1998). D_1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *Journal of Neuroscience, 18,* 1613-1621.

Seiden, L. S., Sabol, K. E., & Ricaurte, G. A. (1993). Amphetamine: effects on catecholamine systems and behavior. *Annual Review of Pharmacology and Toxicology, 33,* 639-676.

Smidt, M. P., Smits, S. M., & Burbach, J. P. (2003). Molecular mechanisms underlying midbrain dopamine neuron development and function. *European Journal of Pharmacology, 480,* 75-88.

Smith, J. A., Mo, Q., Guo, H., Kunko, P. M., & Robinson, S. E. (1995). Cocaine increases extraneuronal levels of aspartate and glutamate in the nucleus accumbens. *Brain Research, 683*, 264-269.

Snyder, K. J., Katovic, N. M., & Spear, L. P. (1998). Longevity of the expression of behavioral sensitization to cocaine in preweanling rats. *Pharmacology, Biochemistry, and Behavior, 60,* 909-914.

Sourkes, T. L. (1979). DOPA decarboxylase (aromatic amino acid decarboxylase). In Horn, A. S., Korf, J., Westerink, B. H. C. (Eds.), *The neurobiology of dopamine* (pp. 123-132). London: Academic Press.

Strakowski, S. M., & Sax, K. W. (1998). Progressive behavioral response to repeated d-amphetamine challenge: further evidence for sensitization in humans. *Biological Psychiatry*, *44*, 1171-1177.

Strakowski, S. M., Sax, K. W., Setters, M. J., & Keck, P. E., Jr. (1996). Enhanced response to d-amphetamine challenge: evidence for behavioral sensitization in humans. *Biological Psychiatry, 40*, 872-880.

Tanaka, T., Ishigooka, J., Watanabe, S., Nagata, E., & Miura, S. (1998). Partial inhibition of reverse tolerance by a high dose of ritanserin or low dose of haloperidol in methamphetamine-sensitized rat. *Psychopharmacology*, 18, 1-7

Tarazi, F. I., & Baldessarini, R. J. (2000). Comparative postnatal development of dopamine D_1, D_2, and D_4 receptors in rat forebrain. *International Journal of Developmental Neuroscience, 18,* 29-37.

Tyrka, A., Gayle, C., Smith, G.P. (1992). Raclopride decreases sucrose intake of rat pups in independent digestion tests. *Pharmacology, Biochemistry, Behavior, 43,* 863-869.

Ujike, H., Onoue, T., Akiyama, K., Hamamura, T., & Otsuki, S. (1989). Effects of selective D-1 and D-2 dopamine antagonists on development of methamphetamine-induced behavioral sensitization. *Psychopharmacology, 98,* 89–92.

Ujike, H., Tsuchida, K., Akiyama, K., Fujiwara, Y., & Kuroda, S. (1995). Ontogeny of behavioral sensitization to cocaine. *Pharmacology, Biochemistry, and Behavior, 50*, 613-617.

Ushijima, I., Carino, M. A., Horita, A. (1995). Involvement of D_1 and D_2 dopamine systems in the behavioral effects of cocaine in rats. *Pharmacology, Biochemistry, and Behavior, 52,* 737-741.

Valjent, E., Bertran-Gonzalez, J., Aubier, B., Greengard, P., Herve, D., & Girault, J. A. (2010). Mechanisms of locomotor sensitization to drugs of abuse in a two-injection protocol. *Neuropsychopharmacology, 35,* 401-415

Vezina, P. (1996). D_1 dopamine receptor activation is necessary for the induction of sensitization by amphetamine in the ventral tegmental area. *Journal of Neuroscience, 16,* 2411-2420.

Vezina, P., & Stewart, J. (1984). Conditioning and place-specific sensitization of increases in activity induced by morphine in the VTA. *Pharmacology, Biochemistry, and Behavior, 20*, 925-934.

Vezina, P., & Stewart, J. (1989). The effect of dopamine receptor blockade on the development of sensitization to the locomotor activating effects of amphetamine and morphine. *Brain Research, 499*, 108-120.

Wang, Y. C., & Hsiao, S. (2003). Amphetamine sensitization: nonassociative and associative components. *Behavioral Neuroscience, 117,* 961-969

Weiss, S. R. B., Post, R. M., Pert, A., Woodward, R., & Muran, D. (1989). Context-dependent cocaine sensitization: differential effect of haloperidol on development versus expression. *Pharmacology, Biochemistry, and Behavior, 34*, 655-661.

White, F. J., Joshi, A., Koeltzow, T. E., & Hu, X. T. (1998). Dopamine receptor antagonists fail to prevent induction of cocaine sensitization. *Neuropsychopharmacology, 18,* 26–40.

Wood, R. D., Tirelli, E., Snyder, K. J., Heyser, C. J., LaRocca, T. M., & Spear, L. P. (1998). Evidence for behavioral sensitization to cocaine in preweanling rat pups. *Psychopharmacology, 138,* 114-123.

Zavala, A. R., Nazarian, A., Crawford, C. A., & McDougall, S. A. (2000). Cocaine-induced behavioral sensitization in the young rat. *Psychopharmacology*, *151*, 291–298.

Zeng W. Y., Hyttel J., & Murrin L. C. (1988). Ontogeny of dopamine D1 receptors in rat striatum. *Journal of Neurochemistry, 50,* 862-867.

Zorrilla, E. P. (1997) Multiparous species present problems (and possibilities) to developmentalists. *Developmental Psychobiology, 30*, 141–150.