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THE IMPORTANCE OF SEROTONERGIC AND ADRENERGIC RECEPTORS FOR THE INDUCTION AND EXPRESSION OF ONE-TRIAL COCAINE-INDUCED BEHAVIORAL SENSITIZATION

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THE IMPORTANCE OF SEROTONERGIC AND ADRENERGIC RECEPTORS

FOR THE INDUCTION AND EXPRESSION OF ONE-TRIAL COCAINE-

INDUCED BEHAVIORAL SENSITIZATION

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A Thesis

Presented to the

Faculty of

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by

Krista Nicole Rudberg

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ABSTRACT

Addiction is a complex process in which behavioral sensitization may be an important component. While the behavioral effects of sensitization are well established, the intricate neurobiology of the phenomenon is still largely unknown. Dopamine systems mediate the induction of behavioral sensitization in adult rats, but there is a large amount of evidence showing that other neurotransmitter systems also modulate the induction process. For example, the α1b-adrenergic and 5-HT$_{2A}$ receptor systems are known to modulate the sensitized responding of adult rats, but the roles that these receptor systems play in the induction and expression of behavioral sensitization during the preweanling period has yet to be investigated. Therefore, the purpose of this thesis was to determine whether the serotonergic and adrenergic receptor systems mediate the induction and/or expression of cocaine-induced one-trial behavioral sensitization in preweanling rats. I used a novel approach to address this question, as the receptors of interest were "protected" from the alkylating effects of EEDQ (an irreversible nonselective receptor antagonist) by prior treatment with selective antagonist drugs. More specifically, rats were given ritanserin (a serotonergic receptor antagonist), prazosin (an adrenergic receptor antagonist), or a combination of the two drugs prior to an injection of EEDQ. To study the induction of behavioral sensitization, this series of injections was administered on PD 18 (24 h before the pretreatment injection of cocaine). To study the expression of behavioral sensitization, the injections were administered on PD

20, which was the day between the drug pretreatment day and the test day. In all experiments, the test day (i.e., the day on which the challenge dose of cocaine was given) was on PD 21. Control experiments were performed for both the induction and expression paradigms in order to determine whether prazosin and ritanserin independently affected sensitization. Results showed that the receptor inactivation caused by EEDQ blocked both the induction and expression of cocaine-induced one-trial behavioral sensitization. Importantly, administering prazosin and ritanserin did not protect the induction of the sensitized locomotor response, which suggests that serotonergic and adrenergic receptors do not mediate cocaine-induced one-trial behavioral sensitization in preweanling rats. This conclusion should be tempered, however, because co-administration of prazosin and ritanserin affected the locomotor activity and sensitized responding of cocaine-treated rats independent of the actions of EEDQ. Considering both past and present results, the most harmonious conclusion is that multiple receptor systems (i.e., dopaminergic, serotonergic, adrenergic, etc.) work in unison to produce the complex phenomenon of behavioral sensitization.

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CHAPTER ONE

MODEL OF ADDICTION

Psychostimulants possess highly addictive qualities and can have many complex and detrimental health effects, thus constituting a serious public health concern. The acutely rewarding properties of psychostimulants, such as cocaine and the amphetamines, often lead to compulsive use (Hyman, 1996). In addition, drug users report many adverse effects such as anxiety, depression, mood swings, paranoia, and panic attacks, as well as sleep and appetite disturbances (Williamson, Gossop, Powis, Griffiths, Fountain, & Strang, 1997).

Addiction is a complex process in which behavioral sensitization may be an important component (Robinson & Berridge, 1993). In animals, behavioral sensitization is observed as a progressive increase in behavioral responsiveness after repeated treatment with a psychostimulant drug (Kalivas & Stewart 1991; Robinson & Becker, 1986). In animal models, sensitization is often described in terms of drug-induced changes in locomotor activity or stereotyped movement. For example, many studies have reported increased locomotor activity after a challenge dose of psychostimulant (Duke, O'Neil, & McDougall, 1997; Kalivas & Stewart, 1991; Kolta, Shreve, De Souza, & Uretsky, 1985; Leith & Kuczenski, 1982; McDougall, Duke, Bolanos, & Crawford, 1994; Robinson & Becker, 1986). In addition, rats given repeated

injections of cocaine exhibit increased stereotypy when challenged with a high dose of cocaine (Kuczenski & Segal, 1999; Kuczenski, Segal, & Aizenstein, 1991; Wood, Tirelli, Snyder, Heyser, LaRocca, & Spear, 1998).

Although many studies have used animal models to examine psychostimulant-induced behavioral sensitization, the number of studies investigating sensitization in humans is limited. The few studies that have been done in humans have produced generally positive findings. For instance, when participants were given two twice-daily doses of amphetamine one day apart, eye-blink rate, energy level, mood, and rate and amount of speech increased (Strakowski, Sax, Setters & Keck, 1996). In another study, after being given three doses of amphetamine (0.25 mg/kg) at 48 h intervals, a progressive increase in eye-blink, mood, energy level, motor activity, and speech was observed after each administration of the drug (Sax & Strakowski, 1998). Further investigation of this phenomenon lead to the finding that mood elevation was affected by characteristics of the participant personality, namely neophilia (Sax & Strakowski, 1998; Strakowski, Sax, Rosenberg, DelBello, & Adler, 2001). Boileau et al. (2006) concluded that the observed increases in mood, as well as changes in underlying neural mechanisms, were consistent with the behavioral and neurochemical effects occurring in animal sensitization.

While the behavioral effects of sensitization are well established, the complex neurobiology of the phenomenon is still largely unknown. It has long

been recognized that the dopamine system is critically involved in mediating reward (Bozarth, 1986). Therefore, it is not surprising that dopaminergic pathways are also implicated in the induction of psychostimulant-induced behavioral sensitization. This is perhaps best illustrated by studies using nonselective or selective dopamine receptor antagonists (Kuribara & Uchihashi, 1993; Vezina & Stewart, 1989). Specifically, pretreating rats or mice with dopamine antagonists often blocks the induction of methamphetamine and cocaine sensitization.

Compared to adults, adolescents are more vulnerable to developing a drug addiction (Schramm-Sapyta, Walker, Caster, Levin, & Kuhn, 2009; Spear, 2000), yet relatively few behavioral sensitization studies have been conducted in young rats (for reviews, see Laviola, Adriani, Terranova, & Gerra, 1999; Tirelli, Laviola, & Adriani, 2003). In fact, initial reports suggested that young rats do not exhibit behavioral sensitization (Fujiwara, Kazahaya, Nakashima, Sato, & Otsuki, 1987; Kolta, Scalzo, Ali, & Holson, 1990). Recent studies show that behavioral sensitization will occur in young rats, although the behavioral sensitization of young rats is often weaker and endures for a more limited period of time than in adults (McDougall et al., 1994; Wood et al., 1998; Zavala, Nazarian, Crawford, & McDougall, 2000). Importantly, these differences in the manifestation of behavioral sensitization are specific to the multi-trial paradigm. In one-trial sensitization, where testing occurs one or more days after a single administration of psychostimulant, the sensitized

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response is equally robust in young and adult rats (McDougall, Baella, Stuebner, Halladay, & Crawford, 2007; McDougall, Cortez, Palmer, Herbert, Martinez, Charntikov, & Amodeo, 2009).

In addition to ontogenetic differences in the behavioral manifestation of sensitization, there is evidence that the underlying neural mechanisms responsible for sensitization also change throughout development. For example, dopamine D1-like and D2-like receptor stimulation is necessary for the induction of one-trial cocaine-induced behavioral sensitization in adult rats (Fontana, Post, Weiss, & Pert, 1993; Valjent, Bertran-Gonzalez, Aubier, Greengard, Hervé, & Girault, 2010; Weiss, Post, Pert, Woodward, & Murman, 1989). In contrast, D1-like and D2-like receptor antagonists do not block the methamphetamine- or cocaine-induced behavioral sensitization of preweanling rats (Mohd-Yusof, Gonzalez, Veliz, & McDougall, 2014; Mohd-Yusof, Veliz, Rudberg, Stone, Gonzalez, & McDougall, 2016). The latter results suggest that the induction of behavioral sensitization in young rats is mediated by non-dopaminergic receptor systems.

Although dopamine systems are known to mediate the induction of behavioral sensitization in adult rats, there is a large amount of evidence showing that other neurotransmitter systems also modulate the induction process. For example, Auclair, Drouin, Cotecchia, Glowinski, and Tassin (2004) reported that blocking α1b-adrenergic and 5-HT$_{2A}$ receptors partially attenuated morphine-, cocaine-, and amphetamine-induced behavioral

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sensitization. Importantly, combined treatment with α1b-adrenergic and 5-HT$_{2A}$ receptor antagonists fully attenuated the induction of cocaine- and amphetamine-induced behavioral sensitization in adult mice (Auclair et al., 2004).

In young rats, the involvement of α1b-adrenergic and 5-HT$_{2A}$ receptor systems in the induction of behavioral sensitization had not been investigated. Because the dopamine system does not mediate the induction of behavioral sensitization in young rats, and antagonists at α1b-adrenergic and 5-HT$_{2A}$ receptors attenuate the induction process in adults, I hypothesized that the serotonergic and adrenergic systems mediate the induction of behavioral sensitization at earlier ages. In summary, the purpose of this thesis was to determine whether the serotonergic and adrenergic systems mediate the induction of cocaine-induced one-trial behavioral sensitization during the preweanling period. The results of this study provide additional knowledge about the relationship between neurotransmitter systems underlying behavioral sensitization, and increase our understanding of the addiction process.

CHAPTER TWO

THE DOPAMINE SYSTEM

Introduction

Catecholamines, such as epinephrine, norepinephrine and dopamine, are organic compounds that derive from the amino acid tyrosine (Kujar, Couceyro, & Lambert, 1999). Of the catecholamines in the central nervous system, dopamine is the most abundant. Dopamine is characterized by a single amine group, a central molecule of benzene, ethylamine, and hydroxyl groups named "catechol" (Feldman, Meyer, & Quenzer, 1997). Reward, movement, emotion, and neuroendocrine secretion are a few of the major functions regulated by dopamine (Jaber, Robinson, Missale, & Caron, 1996). Because of its important role in the function of the central nervous system, dopamine dysregulation leads to a number of neuropsychiatric disorders. For example, neurodegeneration of the dopamine system can lead to Parkinson's Disease, whereas imbalance and dysfunction within the dopamine system plays a role in addiction and schizophrenia.

George Barger and James Ewens first synthesized dopamine in 1910 at Wellcome Laboratories in London, England (Levite, 2012). However, dopamine was not discovered to be a neurotransmitter until 1958 by Arvid Carlsson and Nils-Ake Hillarp in Sweden (Carlsson, Lindqvist, Magnusson, &

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Waldeck, 1958). Until this time, dopamine was only thought to be a precursor for norepinephrine and epinephrine.

Dopamine Synthesis

The initial step in the synthesis of dopamine is the conversion of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) in a reaction catalyzed by tyrosine hydroxylase (Nagatsu, Levitt, & Udenfriend, 1964). The enzyme aromatic amino acid decarboxylase then catalyzes L-DOPA into dopamine (Roth, 1979; Sourkes, 1979). The rate limiting step in the production of dopamine is the availability of tyrosine hydroxylase, which is regulated by multiple feedback mechanisms (Binder, Kinkead, Owens, & Nemeroff, 2001). Dopamine production occurs in the presynaptic terminals of dopaminergic neurons. Following synthesis, dopamine is packaged into synaptic vesicles via a transporter protein generated proton gradient and released via calcium-dependent exocytosis (Binder et al., 2001). Dopamine can be released either tonically or phasically (Keeler, Pretsell, & Robbin, 2014).

Dopaminergic Pathways

There are four major pathways that make up the dopaminergic system; the nigrostriatal, mesolimbic, mesocortical, and the tuberoinfundibular pathways. As the name implies, the nigrostriatal pathway originates in the substantia nigra pars compacta, and terminates in the striatum. This pathway

is primarily involved in the regulation of motor movement (Geffen, Jessell, Cuello, & Iverrson, 1976; Huang, Zhou, Chase, Gusella, Aronin, & DiFiglia, 1992). The mesolimbic pathway, which is known as the "reward pathway", begins in the ventral tegmental area (VTA) and terminates in the nucleus accumbens (Chang & Kitai, 1985). The cell bodies of neurons in the mesocortical pathway, which is involved in motivation and emotion, are located in the VTA, and the axons project to the prefrontal cortex (Carr & Sesack, 2000; Lewis & O'Donell, 2000; Seamans, Floresco, & Phillips, 1998). Finally, the tuberoinfundibular pathway projects from the hypothalamus to the posterior pituitary. Because of its role in mediating pituitary function, this pathway modulates the secretion of hormones (Ben-Jonathan, 1985; Leong, Frawley, & Neill, 1983; Sawai, Iijima, Ozawa, & Matsuzaki, 2014). As is suggested by the involvement of dopamine in these major pathways, dopamine is an important neurotransmitter that is crucial to normal functioning of the brain.

<div align="center">Classification of Dopamine Receptors</div>

Dopamine receptors are categorized into two families: D1-like receptors and D2-like receptors. These families can be further subdivided into individual subtypes. Both D_1 and D_5 receptor subtypes are members of the D1-like family, whereas D_2, D_3, and D_4 receptors are part of the D2-like family. All dopamine receptors are coupled to G proteins (Keeler et al., 2014).

D1-Like Receptors

  D1-like receptors are generally excitatory (Keeler et al., 2014). These receptors are coupled to G_s complexes that, when stimulated, increase the activity of adenylyl cyclase. This action, in turn, increases the production of cyclic AMP (Kebabian et al., 1984; Roberts-Lewis, Roseboom, Iwaniec, & Gnergy, 1986). D_1 and D_5 receptors have a low affinity for dopamine, causing them to be more sensitive to changes in phasic dopamine release (Dreyer, Herrik, Berg, & Hounsgaard, 2010; Kebabian et al., 1984).

  Generally speaking, D1-like receptors are more abundant in brain than D2-like receptors (Boyson, McGonigle, & Molinoff, 1986). The D_1 subtype differs from the D_5 subtype in its distribution throughout the brain. *In-situ* hybridization, a technique used to detect gene expression in individual cells, shows that D_1 receptors are primarily found in the caudate-putamen, nucleus accumbens, thalamus, hypothalamus, and olfactory tubercle (Fremeau, Duncan, Fornaretto, Dearry, Gingrich, Breese, & Caron, 1991). Autoradiography shows that D_1 receptors are also found in the substantial nigra, as well as the caudate-putamen, nucleus accumbens, and olfactory tubercle (Boyson et al., 1986). Ribonuclease protein assays and *in-situ* hybridization suggest that D_5 receptors are found in low numbers in the hippocampus, cortex, substantia nigra, thalamus, nucleus accumbens and caudate-putamen (Choi, Machida, & Ronnekleiv, 1995; Meador-Woodruff, Mansour, Grandy, Damask, Civelli, & Watson, 1992).

<u>D2-Like Receptors</u>

D2-like receptors, in contrast to D1-like receptors, function in an inhibitory manner (Keeler, Pretsell, & Robbins, 2014). D2-like receptors are coupled with G_i complexes that, when stimulated, inhibit the activation of adenylyl cyclase (Kebabian, Beaulieu, & Itoh, 1984; Onali, Schwartz, & Costa, 1981). In contrast to D1-like receptors, D2-like receptors have a high affinity for dopamine, and are more sensitive to changes in tonic dopamine release (Dreyer et al., 2010; Kebabian et al., 1984).

D_2 receptors are distributed widely throughout the brain, but are found in lesser densities than D_1 receptors (Boyson et al., 1986). Using autoradiography, Boyson et al. (1986) found substantial numbers of D_2 receptors in the caudate-putamen, nucleus accumbens, olfactory tubercle, substantia nigra, and choroid plexus. *In-situ* hybridization studies support the presence of D_2 receptors in the nucleus accumbens, olfactory tubercle, and substantia nigra, as well as the ventral tegmental area (Meador-Woodruff, Mansour, Bunzow, Van Tol, Watson, & Civelli, 1989).

D_3 receptors are not as widely distributed as D_2 receptors. In a study employing both autoradiography and *in-situ* hybridization, D_3 receptors were expressed abundantly in the islands of Calleja, but were expressed more restrictedly in the nucleus accumbens, substantia nigra, ventral tegmental area, and cerebellum (Diaz, Lévesque, Lammers, Griffon, Martres, Schwartz, & Sokoloff, 1995). Finally, while D_2 and D_3 receptors are primarily expressed

in the basal ganglia, D_4 receptors are mainly found in the entorhinal cortex, lateral septal nucleus, hippocampus, and the medial preoptic area of the hypothalamus (Primus, Thurkauf, Xu, Yevich, McInerney, Shaw, Tallman, & Gallager, 1997).

<div align="center">Ontogeny of the Dopamine System</div>

It is well established that the dopamine system changes throughout ontogeny. Interestingly, D1-like and D2-like receptors develop on different schedules. Specifically, D1-like receptors are most abundant at postnatal day (PD) 40, whereas, D2-like receptor numbers peak between PD 25 and 40 (Teicher, Andersen, & Hostetter, 1995). After these time points, the receptors are pruned to adult levels (Teicher et al., 1995). In addition, the rate of proliferation of D1-like and D2-like receptors varies across ontogeny. Although rats are born with an approximately equal number of each receptor type, there are three times as many D1-like receptors than D2-like receptors when rats reach adulthood (Gelbard, Teicher, Faedda, & Baldessarini, 1989).

The concentration of dopamine in the central nervous system also changes across early development. Specifically, dopamine levels in rat brain increase steadily until adulthood (Agrawal, Glisson, & Himwich, 1966). In addition, the daily cyclicity of brain dopamine levels differs according to age. For example, radioenzymatic assays show that dopamine concentrations in 3- and 21-day-old rabbits peak during the early light phase; whereas, the

dopamine concentrations of adult animals peak during the early dark phase

(Gingras, Lawson, & McNamara, 1995). Further, different brainstem regions

develop unique patterns of dopamine cyclicity that change across ontogeny

(Gingras et al., 1995).

CHAPTER THREE

SEROTONIN AND NOREPINEPHRINE PHARMACOLOGY

Introduction: Serotonin

The monoamine neurotransmitter 5-hydroxytryptamine (5-HT), more commonly referred to as serotonin, is found diffusely across the central nervous system. Serotonin has a role in many cognitive processes, such as anxiety, memory, and aggression (Bear, Connors, & Paradiso, 2007). In addition, serotonin modulates the release of many other neurotransmitters, such as glutamate, GABA, epinephrine, norepinephrine, and dopamine (Ciranna, 2006). The modulatory action of serotonin is also important for the control of motor movement, as serotonin receptor antagonists attenuate hyperlocomotion and stereotypy (Carlsson, Martin, Nilsson, Sorenson, Carlsson, Waters, & Waters, 1999; Higgins, Enderlin, Haman, & Fletcher, 2003). Because of the varied roles of serotonin, it is no surprise that serotonergic dysfunction is implicated in several diseases, such as major depression, Alzheimer's Disease, and schizophrenia (Ciranna, 2006).

Serotonin Synthesis

Serotonin is synthesized in the central nervous system in two steps. First, tryptophan is hydroxylated by tryptophan hydroxylase (TPH) to form 5-hydroxytryptophan (5-HTP). Second, 5-HTP is decarboxylated by aromatic L-

amino acid decarboxylase (AADC) to create serotonin (Fitzpatrick, 1999; Li, Chalazonitis, Huang, Mann, Margolis, Yang, Kim, Côté, Mallet, & Gershon, 2011). This process primarily occurs in the raphe, where the majority of serotonergic cell bodies are located (Abrams, Johnson, Hollis, & Lowry, 2004).

The location of serotonergic cell clusters has largely been studied with immunocytochemical localization, and can be grouped into three pathways (Cooper, Bloom & Roth, 2003). The serotonergic cell bodies located more caudally project to the medulla and spinal cord, whereas the cell bodies located more rostrally project to the telencephalon and diencephalon. Finally, the clusters located in intermediate areas primarily innervate the cortex (Cooper et al., 2003).

Classification of Serotonin Receptors

Serotonin receptors are generally categorized into seven distinct families. The families, 5-HT$_{1-7}$, include subtypes that differ slightly in structure and function (for reviews, see Barnes & Sharp, 1999; Bradley, Engel, Feniuk, Fozard, Humphrey, Middlemiss, Mylecharane, Richardson, & Saxena, 1986; Glennon, 2003; Hoyer, Hannon, & Martin, 2002; Tecott & Julius, 1993). With the exception of 5-HT$_3$, which is ligand gated, most of the serotonin receptor types are coupled to G proteins (Ciranna, 2006). Typically, serotonin receptors are studied using radioligand binding assays, autoradiographic

mapping, and, more recently, *in-situ* hybridization (Hoyer et al., 2002). Using *in-situ* hybridization, Sumner, Rosie, and Fink (1992) reported that 5-HT$_{1a}$ mRNA was found in the suprachiasmatic nucleus, supraoptic nucleus, paraventricular nucleus, medial septum, medial preoptic area, ventromedial hypothalamic nucleus, and perikarya of the diagonal band of Broca, as well as the hippocampus. Autoradiographic analysis revealed that 5-HT$_{1a}$ binding sites were highly concentrated in the dentate gyrus, hippocampus, lateral septum, and frontal cortex, whereas 5-HT$_{1b}$ binding sites were highly concentrated in the caudate nucleus, globus pallidus, and substantia nigra (Vergé, Daval, Marcinkiewicz, Patey, el Mestikawy, Gozian, & Hamon, 1986). 5-HT$_{1c}$ mRNA, on the other hand, was found in the dorsal and median raphe nuclei, as well as the lateral septum and choroid plexus (Sumner et al., 1992).

Not surprisingly, *in-situ* hybridization and autoradiography have revealed that 5-HT$_2$ and 5-HT$_3$ receptors are found in similar locations as the 5-HT$_1$ family. For example, 5-HT$_2$ mRNA was found in the cingulate and frontal cortices, medial septum, medial preoptic area, ventromedial hypothalamic nucleus, perikarya of the diagonal band of Broca, and dorsal and median raphe nuclei (Sumner et al., 1992). In addition, autoradiographic analysis revealed a high density of 5-HT$_2$ receptor binding sites in the cortex and the caudate putamen (Schotte & Leysen, 1988). Further, calcium binding has shown that 5-HT$_3$ mRNA is located in the neocortex, olfactory cortex, hippocampus, and amygdala (Morales & Bloom, 1997). Using

autoradiography, high densities of 5-HT$_3$ receptor binding sites were also found in the amygdala, hippocampus, frontal cortex, and entorhinal cortex (Laporte, Koscielniak, Ponchant, Vergé, Hamon, & Gozlan, 1992).

Ontogeny of the Serotonin System

In rats, 5-HT neurotransmitter levels peak at PD 5, and then decline until PD 7 (Artigas, Suñol, Tussel, Martínez, & Gelpí, 1985; Bennett & Giarman, 1965; Nachmias, 1960). Following PD 7, 5-HT levels increase gradually until they reach adult levels at about PD 15 (Artigas et al., 1985). Importantly, the density of serotonergic receptors changes across ontogeny. In rats, the density of 5-HT$_1$ receptors increases after birth, with adult levels being reached around PD 9 to PD 14 (Zilles, Schleicher, Glaser, Traber, & Rath, 1985). In contrast, 5-HT$_2$ receptor densities increase rapidly during the first postnatal week, until asymptoting at PD 7 (Morilak & Ciaranello, 1993). At PD 28, 5-HT$_2$ receptor levels then decline to adult levels (Morilak & Ciaranello, 1993).

Introduction: Norepinephrine

Norepinephrine, like dopamine, is a catecholamine neurotransmitter that derives from tyrosine (Kujar et al., 1999). Norepinephrine was first discovered in 1946 by Ulf von Euler, and further investigated by Peter Holtz in 1957 (Shore & Olin, 1958). In general, norepinephrine is important for the

"fight or flight" response, but it is also critical for attention, cognition, learning, memory, stress, and mood regulation (Aston-Jones, Rajkowski, & Cohen, 1999; Ordway, Schwartz & Frazer, 2012). Because norepinephrine is necessary for many basic brain functions, it is not a surprise that dysfunction involving this system can lead to disorders such as major depression, anxiety, and schizophrenia (Anand & Charney, 2000; Biederman & Spencer, 1999; Goldstein, 1981; Redmond & Huang, 1979).

Norepinephrine Synthesis

Norepinephrine is a catecholamine that derives from phenylalanine. First, tyrosine hydroxylase converts tyrosine into L-DOPA. Second, L-DOPA is catalyzed by aromatic amino acid decarboxylase, resulting in dopamine (Roth, 1979; Sourkes, 1979). Finally, dopamine is transported into vesicles where it is converted by the enzyme dopamine β-hydroxylase into norepinephrine (Kaufman, 1974; Kujar et al., 1999). Cell bodies of noradrenergic neurons are primarily located in the pons, especially in the locus coeruleus (Samuels & Szabadi, 2008). From the locus coeruleus, noradrenergic projections form two major pathways (Noback, Strominger, Demarest, & Ruggiero, 2005). First, axons comprising the central tegmental tract project to the hypothalamus (Nurcombe & Gallagher, 1986). Second, the dorsal longitudinal fasciculus innervates the medulla, cortex, thalamus, and

hippocampus (Nurcombe et al., 1986). Rostrally, these pathways converge in the medial forebrain bundle (Segal, Yager, & Sullivan, 1976).

Classification of Norepinephrine Receptors

There are two families of adrenergic receptors, alpha and beta (Ordway et al., 2012). Adrenergic receptors from both families are coupled to G proteins (Qin, Sethi, & Lambert, 2008). The alpha family has two subtypes (α_1 and α_2), and the beta family has three subtypes (β_1, β_2, and β_3). Recently, the α_1 and α_2 subtypes have each been further classified into three groups (α_{1a}, α_{1b}, and α_{1d}; α_{2a}, α_{2b}, and α_{2c}) (Taniguchi, Ukai, Tanaka, Yano, Kimura, Moriyama, & Kawabe, 1997). *In-situ* hybridization studies show that mRNA of the α_{1a} group was found mainly in the globus pallidus, olfactory bulb, and spinal cord (Chapple, Burt, Andersson, Greengrass, Wyllie, & Marshall, 1994), whereas α_{1b} and α_{1d} mRNA is located mainly in the cortex (Weinberg, Trivedi, Tan, Mitra, Perkins-Barrow, Borkowski, Strader, & Bayne, 1994). In contrast, receptors from the α_2 groups are more widely dispersed in the brain. For example, *in-situ* hybridization studies show that α_{2a} mRNA is located in the cortex, locus coeruleus, reticular formation, pontine nuclei, thoracic spinal cord, and the paraventricular nucleus of the hypothalamus (Dalman & Neubig, 1991; Nicholas, Pieribone, & Hökfelt, 1993). In the central nervous system, a small amount of α_{2b}-receptor mRNA is found in the hypothalamus; whereas,

α_{2c}-receptor mRNA is located in the olfactory bulb, cortex, striatum, dorsal root ganglion, and hippocampus (Nicholas et al., 1993).

The second family of norepinephrine receptors, the β-adrenergic, has three subtypes. The α_1 subtype is not found in the central nervous system, but rather in cardiac and stomach tissue (Zhao, Sakata, Li, Liang, Richardson, Brown, Goldstein, & Zigman, 2010). This is also the case for the β_3 subtype, which is found in the colon, gall bladder, and adipose tissue (Krief, Lönnqvist, Raimbault, Baude, Van Spronsen, Arner, Strosberg, Ricquier, & Emorine, 1993). In contrast, β_2-receptors are located in the amygdala, cerebellum, and cortex, as well as the heart, smooth muscle, liver, and kidneys (Beane & Marrocco, 2004; Elenkov, Wilder, Chrousos, & Vizi, 2000).

Ontogeny of the Norepinephrine System

The levels of norepinephrine in the developing rat brain follow a similar pattern as the levels of serotonin. Norepinephrine neurotransmitter levels increase dramatically from PD 1 to PD 2 (Dygalo, Iushkova, Kalinina, Surnina, Mel'nikova, & Shishkina, 2000). After the initial peak, there is a decline until PD 5, after which there is a gradual increase until PD 30 when adult levels are reached (Dygalo et al., 2000; Karki, Kuntzman, & Brodie, 1962).

In terms of receptor densities, β-adrenergic receptors sharply increase in density soon after birth, and at three weeks achieve adult levels (Dygalo et al., 2000). In contrast, adult-like levels of α_2-receptors are already present at

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PD 1 (Happe, Coulter, Gerety, Sanders, O'Rourke, Bylund & Murrin, 2004).

Interestingly, the density of α_1-receptors depends on neuroanatomical

location. For example, α_1-receptors in the globus pallidus increase in density

from PD 1 to PD 7, and then undergo pruning throughout the remainder of

life. In the olfactory bulb, α_1-receptors increase in number for the first two

weeks after birth and then remain at a constant level thereafter (Jones,

Gauger, Davis, Slotkin & Bartolome, 1985).

CHAPTER FOUR

ADULT BEHAVIORAL SENSITIZATION

Indirect Dopamine Agonists: Adult Multi-Trial
Behavioral Sensitization

Typically, multi-trial behavioral sensitization consists of 4-6 daily injections of an indirect dopamine agonist, followed by a withdrawal period, and then the administration of a challenge injection of the same agonist (Robinson & Becker, 1986). Sensitization is characterized by a heightened behavioral response following the challenge injection. Enhanced locomotor activity is the most commonly studied sensitized response in rats, although intense stereotypy can also occur. A multiphasic behavioral response is also possible. For example, repeatedly administering a high dose of amphetamine causes an initial increase in locomotor activity followed by intense stereotypy, and then a period of post-stereotypy locomotion (Leith & Kuczenski, 1982). These components of the sensitized response persist for different periods of time, with stereotypy lasting longer than locomotor activity (Leith & Kuczenski, 1982).

Although the persistance of sensitized stereotypy and locomotor activity differ, the intensity of each response is similarly affected by drug dose. Specifically, larger doses of a psychostimulant will produce more robust sensitized stereotypy and locomotor activity (Frantz, O'Dell, & Parsons, 2007; Post & Rose, 1976). For example, five administrations of a small dose (10

mg/kg) of cocaine lead to increased locomotion and stereotypy, whereas six administrations of a large dose (40 mg/kg) of cocaine generated more intense locomotion and stereotypy (Davidson, Lazarus, Lee, & Ellinwood, 2002; Frantz et al., 2007). Even at smaller doses of amphetamine (0.5, 1.0, or 1.5 mg/kg), a gradual strengthening of the sensitized response occurs as the drug dose increases (Hooks, Jones, Neill, & Justice, 1992). In sum, it is clear that in multi-trial behavioral sensitization the psychostimulant dose is positively correlated with the intensity of the sensitized response.

Importantly, the multi-trial procedure produces a sensitized locomotor response that persists for a long period of time in adult rats. In fact, behavioral sensitization can be observed for many months after drug administration is discontinued (Kalivas & Stewart, 1991; Kolta et al., 1985; Leith & Kuczenski, 1982; Robinson & Becker, 1986). In general, the robustness of the sensitized response to cocaine and amphetamine increases as the period of withdrawal increases (Heidbreder, Thompson, & Shippenberg, 1996; Kalivas & Duffy, 1993a, 1993b; Kolta et al., 1985; Segal & Kuczenski, 1992; Vanderschuren, Schmidt, De Vries, Van Moorsel, Tilders, & Schoffelmeer, 1999). Behavioral sensitization observed within a week of drug discontinuation is considered short-term sensitization, whereas a sensitized response observed weeks to months later is considered long-term sensitization. This distinction is important, since the neural mechanisms mediating short- and long-term behavioral sensitization differ. In fact, the neuroadaptations responsible for

short-term behavioral sensitization disappear over time, yet are a necessary precursor for neuroadaptations that support long-term sensitization (for reviews, see Pierce & Kalivas, 1997; White, Hu, Zhang, & Wolf, 1995; White & Kalivas, 1998; Wolf, 1998).

The robustness of the sensitized response can also be affected by the context in which the drug is administered. It is evident that a stronger sensitized response occurs when drug pretreatment and testing occur in the same environment (Anagnostaras & Robinson, 1996). Context independent sensitization is possible, but only when higher doses of cocaine are repeatedly administered to adult rats or mice (Badiani, Browman, & Robinson, 1995; Browman, Badiani, & Robinson, 1998; Crombag, Badiani, Chan, Dell'Orco, Dineen, & Robinson, 2001). Similarly, multi-trial amphetamine sensitization is more robust when the drug is administered in a previously novel environment (Crombag, Badiani, Maren, & Robinson, 2000). In addition, drug-environment associations are important for the persistence of the sensitized response (Anagnostaras & Robinson, 1996). Together, these results show that associative learning is an important part of the sensitization process in adult rats and mice.

Pavlovian conditioning is the primary associative process involved in behavioral sensitization. Specifically, the environmental context acts as the conditioned stimulus (CS), and the psychostimulant is the unconditioned stimulus (US). After repeated drug-environment pairings, the CS elicits a

potentiated locomotor response (i.e., a conditioned response, CR) if the animal is tested in the same environmental context (Franklin & Druhan, 2000; Johnson, Sediqzadah, & Erb, 2012; Michel & Tirelli, 2002). According to classical learning theory, the robustness of the CR should increase with the number of CS-US pairings (Mackintosh, 1974). Consistent with this tenet, Michel, Tambour, and Tirelli (2003) found that rats injected with cocaine for 12 days, as opposed to 3 or 6 days, exhibited a more robust sensitized response on the test day. As will be discussed shortly, Pavlovian contextual conditioning appears to be even more essential for the one-trial behavioral sensitization of adult rats and mice (Battisti, Chang, Uretsky, & Wallace, 1999; Jackson & Nutt, 1993; Weiss et al., 1989).

Indirect Dopamine Agonists: Adult One-Trial Behavioral Sensitization

Research regarding multi-trial behavioral sensitization in adults is extensive; however, studies examining one-trial behavioral sensitization are more limited. One-trial behavioral sensitization consists of two administrations of the same drug (i.e., a pretreatment dose and a challenge dose). This procedure is also known as a two-injection protocol of sensitization (TIPS; Valjent et al., 2010). Relative to the multi-trial procedure, the one-trial paradigm has some distinct advantages because it minimizes the possibility of dopamine receptor up-regulation and dopamine supersensitivity due to multiple agonist administrations (Mohd-Yusof et al., 2016; Robinson &

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Becker, 1986; Valjent et al., 2010; White, Joshi, Koeltzow, & Hu, 1998). The one-trial paradigm also provides an unbiased procedure for differentiating the induction and expression of behavioral sensitization (Valjent et al., 2010).

As with multi-trial behavioral sensitization, the one-trial procedure can result in a sensitized locomotor or stereotypic response. With high doses of amphetamine, intense stereotyped behaviors occur after a single conditioning trial (Battisti et al., 1999). In contrast, locomotor sensitization is evident when adult mice are pretreated with a single moderate dose of cocaine or morphine (Valjent et al., 2010). The one-trial behavioral sensitization of adult rats and mice shows great persistence, as a sensitized locomotor response is still detectable months after a single psychostimulant administration (Fontana et al., 1993; Robinson, Becker, & Presty, 1982; Valjent et al., 2010).

Although there is only one conditioning trial, associative learning is necessary for the induction of one-trial behavioral sensitization in adult animals (for a discussion, see White et al., 1998). While the induction of multi-trial behavioral sensitization is strengthened by contextual conditioning, the one-trial behavioral sensitization of adult rats and mice is completely context dependent (Battisti et al., 1999; Jackson & Nutt, 1993; Weiss et al., 1989). Several different methodologies have been employed to investigate whether context-independent one-trial sensitization is obtainable, but all attempts thus far have failed (Battisti et al., 1999; Weiss et al., 1989). For example, Battisti et al. (1999) administered amphetamine or apomorphine to adult mice and

then placed them in various environments (cages of different size, color, etc.).

When mice were tested in a distinctly different environment, there was no

evidence of a sensitized response. Context dependency can also be shown

when rats are pretreated with a psychostimulant in their home cage and then

tested in a novel environment (Badiani et al., 1995; Post, Lockfeld, Squillace,

& Contel, 1981). In addition, drug dose is an important constraint for one-trial

behavioral sensitization, because increasing the dose of the agonist

increases the intensity of the sensitized response (Battisti et al., 1999). This

finding is consistent with Pavlovian principles, as enhancing CS intensity (i.e.

the drug dose) should increase the robustness of the CR (i.e. the locomotor

response).

CHAPTER FIVE

ADULT BEHAVIORAL SENSITIZATION:

NEURAL MECHANISMS

Studies regarding the neural mechanisms underlying multi-trial behavioral sensitization in adult rats primarily focus on dopaminergic systems, although recent studies have also examined serotonergic and noradrenergic mediation. Interestingly, the neural mechanisms underlying behavioral sensitization differ depending on the type of psychostimulant used. For example, glutamatergic mechanisms involved in cocaine sensitization appear to be unimportant for amphetamine sensitization (for a review, see Vanderschuren & Kalivas, 2000).

Dopamine Receptor Systems Underlying Behavioral Sensitization

Multi-Trial Behavioral Sensitization

Perhaps not surprisingly, the dopamine receptor subtypes mediating the induction and expression of behavioral sensitization differ according to both the induction paradigm employed (one- vs. multi-trial) and the psychostimulant used. For example, neither D1-like nor D2-like receptor antagonists block the induction of multi-trial cocaine sensitization in adult rats (Mattingly, Hart, Lim & Perkins, 1994). In contrast, both D1-like and D2-like receptor antagonists block the induction of multi-trial amphetamine and

methamphetamine sensitization (Kelly, Low, Rubinstein & Phillips, 2008; Kuribara & Uchihashi, 1993, 1994; White et al., 1998). Dopamine receptors play a different role in the expression of multi-trial behavioral sensitization. More specifically, the expression of multi-trial cocaine sensitization is prevented when D1-like receptor antagonists are administered before cocaine on the test day (Sorg, Li, & Wu, 2001; White et al., 1998). In contrast, D1-like and D2-like receptor antagonists do not block the expression of amphetamine-induced multi-trial behavioral sensitization in adult rats (Moro, Sato, Ida, Oshima, Sakurai, Shihara, Horikawa, & Mukini, 2007).

One-Trial Behavioral Sensitization

Unlike multi-trial behavioral sensitization, a functioning dopamine system is necessary for the induction of one-trial cocaine sensitization in adult rats. It has been reported in more than one study that D1-like and D2-like receptor antagonists block the induction of one-trial cocaine sensitization in adult rats (Fontana et al., 1993; Weiss et al., 1989; see also Valjent et al., 2010). Interestingly, expression of one-trial cocaine sensitization is not affected by dopamine receptor antagonism, since administering D1-like and D2-like receptor antagonists before cocaine treatment on the test day does not prevent the occurrence of a sensitized response (Fontana et al., 1993). No studies have examined the effects of selective dopamine receptor antagonists on the induction and expression of one-trial amphetamine and methamphetamine behavioral sensitization.

In summary, a number of interesting yet inconsistent findings have been reported concerning cocaine sensitization: first, neither D1-like nor D2-like receptor antagonists block the induction of multi-trial behavioral sensitization; second, both D1-like and D2-like receptor antagonists block the induction of one-trial behavioral sensitization; third, only D1-like receptor antagonists block the expression of multi-trial behavioral sensitization; and fourth, neither D1-like nor D2-like receptor antagonists block the expression of one-trial behavioral sensitization.

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Serotonin and Adrenergic Receptor Systems
Underlying Behavioral Sensitization
</div>

The inconsistent actions of D1-like and D2-like receptor antagonists on the induction and expression of cocaine sensitization strongly suggest that some other receptor system is more fundamentally involved in mediating the neural processes underlying behavioral sensitization. Because cocaine increases synaptic levels of both dopamine and serotonin (for reviews, see Meyer & Quenzer, 2005; Vanderschuren & Kalivas, 2000), White et al. (1998) proposed that redundant dopamine and serotonin pathways may mediate the induction of cocaine sensitization. In fact, recent evidence suggests that the serotonergic system, as well as the noradrenergic system, are important mediators of behavioral sensitization.

Serotonin Receptor Systems: Multi-Trial Behavioral Sensitization

In terms of the serotonin system, the involvement of specific receptor subtypes in behavioral sensitization appears to depend on the psychostimulant used. For example, 5-HT$_{2C}$ receptor antagonists block the induction of multi-trial cocaine sensitization (Craige & Unterwald, 2013); whereas, the induction of multi-trial methamphetamine sensitization is blocked by a 5-HT$_3$ receptor antagonist (Yoo, Nam, Lee, & Jang, 2006). The serotonergic receptors mediating the induction of multi-trial behavioral sensitization are often the same as those mediating expression. For instance, 5-HT$_2$ and 5-HT$_3$ receptor antagonists block the expression of multi-trial cocaine sensitization in adult rats (Davidson, Lazarus, Xiong, Lee, & Ellinwood, 2002; King, Xiong, Douglas, & Ellinwood, 2000; King, Xiong, & Ellinwood, 1998). In addition, the expression of methamphetamine-induced multi-trial behavioral sensitization is prevented by the non-selective 5-HT$_2$ receptor antagonist ritanserin (Ago, Nakamura, Baba, & Matsuda, 2007).

Serotonin Receptor Systems: One-Trial Behavioral Sensitization

The serotonergic receptors necessary for multi-trial behavioral sensitization are also important for one-trial sensitization. For example, administering 5-HT$_3$, 5-HT$_{2A}$, and 5-HT$_{2C}$ receptor antagonists prior to methamphetamine on the pretreatment day blocks the induction of one-trial behavioral sensitization (Steed, Jones, & McCreary, 2011; Yoo, Nam, Lee, & Jang, 2008). The induction of amphetamine and cocaine one-trial behavioral

sensitization is also prevented by 5-HT$_2$ receptor antagonism (O'Neill, Heron-Maxwell, & Shaw, 1999). Unfortunately, no studies have examined the effects of selective serotonin antagonists on the expression of one-trial cocaine and methamphetamine sensitization. In summary, it is clear that the 5-HT$_2$ and 5-HT$_3$ receptor families are important for the induction of multi- and one-trial behavioral sensitization of adult animals, but their role in expression is uncertain.

Adrenergic Receptor Systems: Multi-Trial Behavioral Sensitization

Studies examining the role of adrenergic receptor systems in behavioral sensitization are less abundant than those assessing serotonin and dopamine system involvement. In a comprehensive study, Auclair et al. (2004) found that concomitant administration of 5-HT$_2$ and α$_1$-adrenergic receptor antagonists inhibits the induction and expression of multi-trial behavioral sensitization to both amphetamine and cocaine (Auclair et al., 2004; see also Drouin, Blanc, Villégier, Glowinski, & Tassin, 2002). Consistent with these findings, amphetamine- and cocaine-induced locomotor activity is dramatically attenuated in mice lacking α$_1$-adrenergic receptors (Drouin et al., 2002). The locus of these effects may be the nucleus accumbens, since infusing an α$_1$-adrenergic antagonist into the accumbens prevents the induction of amphetamine-induced behavioral sensitization (Blanc, Trovero, Vezina, Hervé, Godeheu, Glowinski, & Tassin, 1994).

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Adrenergic Receptor Systems: One-Trial Behavioral Sensitization

Studies investigating the involvement of the adrenergic receptor system in one-trial behavioral sensitization are limited. Both α_2-adrenergic and α_1-adrenergic antagonists block the induction of amphetamine sensitization, but not cocaine sensitization (Vanderschuren, Beemster, & Schoffelmeer, 2003). As mentioned above, concurrent blockade of 5-HT$_2$ and α_1-adrenergic receptors prevents the induction of one-trial cocaine and amphetamine sensitization (Auclair et al., 2004). No studies have examined the effects of selective adrenergic antagonists on the expression of one-trial behavioral sensitization in adult rats.

In summary, receptor antagonist and knock-out studies provide strong evidence that serotonin and norepinephrine receptor systems mediate at least some components of behavioral sensitization. First, 5-HT$_2$ receptor antagonists block the induction of one- and multi-trial cocaine sensitization; second, 5-HT$_3$ receptor antagonists prevent the induction of one-trial methamphetamine sensitization; third, co-administration of 5-HT$_2$ and α_1-adrenergic receptor antagonists completely prevents the induction and expression of cocaine and methamphetamine multi-trial sensitization; and, fourth, "knocking out" α_1-adrenergic receptors attenuates psychostimulant-induced locomotor activity. Collectively, the available evidence suggests that the serotonergic and noradrenergic systems are critically involved in the mediation of behavioral sensitization in adult rats and mice.

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CHAPTER SIX

PREWEANLING BEHAVIORAL SENSITIZATION

Indirect Dopamine Agonists: Preweanling
Multi-Trial Behavioral Sensitization

As is true of adult rats, psychostimulant-induced behavioral

sensitization occurs in young rats (Duke et al., 1997; McDougall et al., 1994;

Wood et al., 1998). Importantly, the manifestation of behavioral sensitization

is different in young rats, as the sensitized response in younger animals is

much less robust than in adults (Smith & Morrell, 2008). Additionally, the

sensitization of adult rats persists for months after cessation of drug

administration (Kalivas & Stewart, 1991; Leith & Kuczenski, 1982; Robinson &

Becker, 1986); whereas, the sensitized response of young rats only lasts for a

week or two after cessation of drug administration (McDougall et al., 1994;

Wood et al., 1998; Zavala et al., 2000). The latter finding suggests that the

neural mechanisms involved in long-term behavioral sensitization are not yet

mature in young rats. As for short-term behavioral sensitization, the

mechanisms underlying induction and expression are functional by at least

PD 10 (Tirelli, 2001).

As with adult rats, associative learning modifies the multi-trial

behavioral sensitization of preweanling rats. For example, repeatedly

administering cocaine in a novel environment causes a sensitized stereotypic

response in young rats; whereas, administering the drug in the home cage

produces no sensitization (Wood et al., 1998). After 10 daily injections of cocaine and a short abstinence period (i.e. one day) context-independent behavioral sensitization occurs, although after a long abstinence period (i.e. one week) behavioral sensitization is context-dependent (Zavala et al., 2000). In addition, after only three pretreatment injections of cocaine and a 24 h withdrawal period, context-independent behavioral sensitization occurs in preweanling rats (McDougall et al., 2009). In agreement with classical learning theory, the longevity of the sensitized response increases as the number of pretreatment psychostimulant administrations increases (Zavala et al., 2000). In other words, increasing the number of CS-US pairings enhances the persistence of the CR.

Indirect Dopamine Agonists: Preweanling One-Trial Behavioral Sensitization

Preweanling rats show robust one-trial psychostimulant-induced behavioral sensitization (McDougall et al., 2007; McDougall, Kozanian, Greenfield, Horn, Gutierrez, Mohd-Yusof, & Castellanos, 2011). The sensitized response is strongest when testing occurs one to three days after drug pretreatment and disappears entirely after five days (McDougall et al., 2009). Interestingly, the different classes of dopamine agonists preferentially induce behavioral sensitization at different ontogenetic ages (Kozanian, Gutierrez, Mohd-Yusof, & McDougall, 2012; McDougall, Nuqui, Quiroz, & Martinez, 2013; McDougall et al., 2011). For example, one-trial amphetamine-

and methamphetamine-induced behavioral sensitization was observed in rats tested on postnatal day (PD) 13 and PD 17 (McDougall et al., 2011, 2013); whereas, cocaine preferentially induces behavioral sensitization at PD 21 (Kozanian et al., 2012). These data suggest that each drug activates the neural mechanisms underlying behavioral sensitization in slightly different ways.

Interestingly, the relative importance of contextual stimuli is the most striking age-dependent difference in the ontogeny of behavioral sensitization. In adult rats, contextual conditioning is necessary for robust one-trial behavioral sensitization (Battisti, Uretsky, & Wallace, 2000; Jackson & Nutt, 1993; Weiss et al., 1989). In contrast, preweanling rats show strong context-independent behavioral sensitization with the one-trial paradigm (Herbert, Der-Ghazarian, Palmer, & McDougall, 2010; McDougall et al., 2009). For example, preweanling rats pretreated with cocaine in the home cage or in a novel chamber show no difference in sensitized responding when tested in an activity chamber (Herbert et al., 2010). Furthermore, young rats anesthetized before receiving a pretreatment injection of cocaine still show a sensitized response on the test day (Herbert et al., 2010). Finally, electroconvulsive shock-induced retrograde amnesia administered a few h after a pretreatment dose of cocaine does not prevent the expression of a sensitized response (McDougall et al., 2011). These various results show that associative learning

processes are not necessary for the one-trial behavioral sensitization of preweanling rats.

Overall, it is clear that behavioral sensitization is manifested differently in preweanling and adult rats. Adult behavioral sensitization is more robust than in preweanling rats (Smith & Morrell, 2008). In addition, the effects of contextual conditioning are stronger in adults than pups, an effect that is especially evident in the one-trial paradigm (Battisti et al., 2000; Herbert et al., 2010; Jackson & Nutt, 1993; McDougall et al., 2009; Weiss et al., 1989). Finally, the behavioral sensitization exhibited by adult rats shows much greater persistence than in younger rats (Kalivas & Stewart, 1991; Kolta et al., 1985; Leith & Kuczenski, 1982; Robinson & Becker, 1986; Zavala et al., 2000). Taken together, the implications of these data are two-fold. First, the neural mechanisms underlying behavioral sensitization appear to differ in preweanling and adult rats. For example, young rats possess the non-associative neural mechanisms necessary for short-term behavioral sensitization, but evidence suggests that the processes mediating long-term behavioral sensitization are not functional. Second, associative processes may be necessary for robust long-term behavioral sensitization. Thus, the associative deficits exhibited by preweanling rats may be the critical factor responsible for both the weaker sensitized response and the striking lack of persistence.

CHAPTER SEVEN

PREWEANLING BEHAVIORAL SENSITIZATION:

NEURAL MECHANISMS

In contrast to studies using adult rats and mice, there are relatively few studies examining the neural mechanisms mediating behavioral sensitization in preweanling rats. According to the few existing ontogenetic studies, it appears that the neural mechanisms governing the behavioral sensitization of preweanling rats may differ depending on the induction paradigm employed (one- vs. multi-trial).

Dopamine Receptor Systems Underlying Behavioral Sensitization

Multi-Trial Behavioral Sensitization

Although few studies have assessed dopaminergic involvement in the induction of multi-trial methamphetamine sensitization, none have examined the role of dopamine receptors in the expression of multi-trial methamphetamine sensitization. In terms of induction, administering a D2-like receptor antagonist during the pretreatment phase attenuates the multi-trial methamphetamine-induced behavioral sensitization of preweanling rats (Mohd-Yusof et al., 2016). Interestingly, concurrent pretreatment with both D1-like and D2-like receptor antagonists completely blocks the induction of multi-trial methamphetamine sensitization (Mohd-Yusof et al., 2016). In other

words, antagonizing both dopaminergic receptor types produces a greater effect on the sensitized behavioral response than antagonizing the D2 system alone. Unfortunately, there are no studies examining the induction or expression of multi-trial cocaine sensitization during the preweanling period.

<u>One-Trial Behavioral Sensitization</u>

As mentioned above, the neural mechanisms underlying behavioral sensitization appear to differ depending on the paradigm used (one- vs. multi-trial). For example, Mohd-Yusof et al. (2014) reported that D1-like receptor antagonism does not affect the induction of one-trial cocaine- or methamphetamine-induced behavioral sensitization in young rats. Likewise, D2-like receptor antagonism does not prevent the induction of one-trial methamphetamine-induced behavioral sensitization (Mohd-Yusof et al., 2016). In contrast to the multi-trial paradigm, concurrent D1-like and D2-like receptor antagonism does not block the induction of one-trial methamphetamine sensitization (Mohd-Yusof et al., 2016).

Although little is known concerning the neural mechanisms underlying the expression of one-trial methamphetamine-induced behavioral sensitization, there is a limited amount of information about the expression of cocaine sensitization. Specifically, D1-like, but not D2-like, receptor antagonists attenuate the expression of cocaine-induced one-trial behavioral sensitization (McDougall, Rudberg, Veliz, Romero, Mohd-Yusof & Gonzalez, 2016). Administering a combination of D1-like and D2-like receptor

antagonists on the test day causes a decline in the locomotor activity of preweanling rats, but it is uncertain whether this effect is due to the disruption of the sensitization process or a general motoric disturbance (McDougall et al., 2016). No studies have examined the expression of one-trial methamphetamine-induced behavioral sensitization. Overall, there is an unfortunate paucity of studies examining the involvement of the dopaminergic system in one- and multi-trial behavioral sensitization.

Serotonin and Adrenergic Receptor Systems

Currently, there are no published studies that have examined whether serotonergic and adrenergic receptor systems mediate the induction and expression of behavioral sensitization during the preweanling period. The lack of such studies leaves an important void in our understanding of the ontogeny of behavioral sensitization.

CHAPTER EIGHT

EEDQ

The drug N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) is a nonselective irreversible receptor antagonist (Meller, Goldstein, Friedhoff, & Schweitzer, 1988). Evidence suggests that EEDQ affects dopaminergic, serotonergic, and adrenergic receptors. First, Belleau, Martel, Lacasse, Ménard, Weinberg, and Perron (1968) discovered that EEDQ was an irreversible α-adrenergic receptor antagonist in smooth muscle. Later, Kalsner (1973) found that EEDQ was also effective in inactivating serotonergic receptors in rabbit aortic tissue. Finally, Hamblin and Creese (1983) reported that EEDQ was a potent and irreversible dopamine receptor antagonist.

More recently, studies have primarily focused on the effects of EEDQ in the central nervous system. For instance, homogenate binding studies revealed that 7.5 mg/kg EEDQ causes a 61-86% reduction in D1 and D2 receptor densities in the caudate-putamen of adult rats (Crawford, McDougall, Rowlett, & Bardo, 1992). Importantly, Meller Bohmaker, Goldstein and Friedhoff (1985) reported that dopaminergic receptors can be protected from inactivation by injecting rats with selective dopamine receptor antagonists prior to EEDQ administration. In addition to its dopaminergic actions, EEDQ reduces $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, $5\text{-}HT_2$, $5\text{-}HT_{1C}$, α_2-adrenergic, GABA, and muscarinic receptor densities in various brain regions of rats (Adler, Meller, & Goldstein,

1985; Alper & Nelson, 2000; Miller, Lumpkin, Galpern, Greenblatt, & Shader, 1991; Norman, Eubanks, & Creese, 1989; Pinto & Battaglia, 1993).

EEDQ can also be useful for investigating the neural mechanisms underlying behavior. For example, systemic or bilateral administration of EEDQ attenuates the dopamine agonist-induced locomotor activity of adult rats (Der-Ghazarian, Gutierrez, Varela, Herbert, Amodeo, Charntikov, Crawford, & McDougall, 2012; McDougall, Crawford, & Nonneman, 1992); whereas, unilateral administration of EEDQ in the caudate-putamen causes ipsilateral circling (Giorgi & Biggio, 1990a, 1990b). Additionally, EEDQ attenuates or completely blocks apomorphine- and NPA-induced stereotypy (Cameron & Crocker, 1989; Meller, Bordi, & Bohmaker, 1989). Therefore, it is clear that EEDQ-induced receptor inactivation attenuates the dopamine-mediated behaviors of adult rats.

Because EEDQ is a nonselective irreversible antagonist, additional techniques are required to specify which receptor types mediate particular behaviors. As mentioned above, it is possible to protect one or more receptor types from the effects of EEDQ, thus allowing the actions of these receptors to be individually studied. For example, selectively inactivating D2-like receptors, but not D1-like receptors, inhibits apomorphine-induced stereotypy (Arnt, Hyttel, & Meier, 1988; Cameron & Crocker, 1988). Additionally, Arnt and Hyttel (1989) reported that selectively inactivating D1-like or D2-like

receptors in the caudate-putamen of adult rats attenuates the circling

behavior produced by selective dopamine agonists.

Ineterestingly, few EEDQ studies have focused on learned behaviors,

as opposed to unlearned behaviors (Arnt et al., 1988; Arnt & Hyttel, 1989;

Der-Ghazarian, Widarma, Gutierrez, Amodeo, Valentine, Humphrey,

Gonzalez, Crawford, & McDougall, 2014). Although, McDougall et al. (2016)

have used EEDQ as a tool to assess whether D1-like and/or D2-like receptors

mediate behavioral sensitization in preweanling rats. Based on the results of

this study, it was reported that neither D1-like nor D2-like receptors mediate

the induction of cocaine sensitization during the preweanling period.

Unfortunately, the same study did not shed light on which receptor systems

do mediate the induction of cocaine sensitization in preweanling rats

(McDougall et al., 2016).

CHAPTER NINE

SUMMARY AND THESIS STATEMENT

Behavioral sensitization is an important component of the addiction process (Robinson & Berridge, 1993). Several factors, such as age, induction paradigm (one- vs. multi-trial), and environmental context, influence the manifestation of behavioral sensitization. In terms of age, the behavioral sensitization of preweanling rats is less robust and persists for a much shorter period of time than it does in adults (Smith & Morrell, 2008; Tirelli et al., 2003).

The neural mechanisms underlying behavioral sensitization also differ depending on the psychostimulant used, the induction paradigm and, perhaps, age. For example, D1-like and D2-like receptor antagonists prevent the induction of one-trial cocaine-induced behavioral sensitization during adulthood (Fontana et al., 1993; Weiss et al., 1989), but not during the preweanling period (Mohd-Yusof et al., 2014). The latter result suggests that some other receptor type mediates the induction of behavioral sensitization in preweanling rats. Suggestively, Auclair et al. (2004) reported that serotonin and adrenergic receptor antagonists inhibit the induction and expression of behavioral sensitization in adult mice. Unfortunately, no studies have examined the importance of serotonergic and adrenergic systems for the induction or expression of behavioral sensitization during the preweanling period.

The purpose of this thesis was to assess the involvement of the serotonergic and adrenergic systems in the induction and expression of one-trial cocaine-induced behavioral sensitization. Rats were assessed during the late preweanling period (PD 18-21), when cocaine sensitization is most robust (Kozanian et al., 2012). The aims of this thesis were two-fold: first, to determine whether EEDQ prevents the induction or expression of one-trial cocaine sensitization. It was hypothesized that EEDQ would prevent both the induction and expression of one-trial cocaine sensitization. This hypothesis was based on combined evidence that the dopaminergic, serotonergic, and adrenergic receptor systems mediate behavioral sensitization.

The second goal of this thesis was to differentiate among the receptor types and determine whether 5-HT and/or α_1-adrenergic receptors mediate the induction and/or expression of cocaine sensitization during the preweanling period. It was hypothesized that both 5-HT and α_1-adrenergic receptor stimulation will be necessary for the induction and expression of behavioral sensitization. This hypothesis was based on evidence from the adult mouse literature showing that each of these receptor types is involved in the induction and expression of multi-trial psychostimulant-induced behavioral sensitization (Auclair et al., 2004).

CHAPTER TEN

MATERIALS AND METHODS

Subjects

Subjects were 320 (n=8 per group) 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 PD 3. Rats were housed in large polycarbonate maternity cages (30.5 × 43 × 19 cm) on a ventilated rack. Food and water was freely available. The colony room was maintained at 22–23 °C and kept under a 12:12 light/dark cycle. Except during testing, rats were kept with the dam and littermates. Testing was done in a separate experimental room and was conducted during the light phase of the cycle. 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 done in activity monitoring chambers (25.5 × 25.5 × 41 cm) that consist of acrylic walls, a plastic floor, and an open top (Coulbourn Instruments, Whitehall, PA). Each chamber includes an X–Y photobeam array,

with 16 photocells and detectors, that was used to determine distance traveled (a measure of locomotor activity).

Drugs

EEDQ was dissolved in a 50% DMSO solution (1:1 (v/v) in distilled water), while (-)-cocaine hydrochloride and prazosin hydrochloride were dissolved in saline. Ritanserin was dissolved in a minimal amount of glacial acetic acid (15 µl/ml) and diluted in saline. All 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 EEDQ on the Induction of One-Trial Cocaine-Induced Behavioral Sensitization

On the preinjection day (PD 18), rats were injected with EEDQ (0, 7.5, or 15 mg/kg) and immediately returned to their home cage. On the pretreatment day, which occurred 24 h later (PD 19), half of the rats in each group were injected with saline and the other half received 30 mg/kg cocaine. Immediately afterwards, rats were placed in activity chambers for 30 min and distance traveled was recorded. On the test day (PD 21), all rats (n=8 per group) were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min. The design of Experiment 1 is shown in Table 1.

Experiment 2a: The Use of 5-HT and/or α_1-Adrenergic Receptor
Protection to Assess the Effects of EEDQ on the Induction of
One-Trial Cocaine-Induced Behavioral Sensitization

On the preinjection day (PD 18), rats first received a protection injection of
prazosin (25 mg/kg), ritanserin (3 mg/kg), prazosin+ritanserin, or saline in the
home cage. After 30 min, rats received a preinjection of EEDQ (15 mg/kg). A
separate control group received a protection injection of saline and a preinjection
of vehicle. On the pretreatment day, which occurred 24 h later (PD 19), half of
the rats in each group received saline and the other half received 30 mg/kg
cocaine. Immediately afterwards, rats were placed in activity chambers for 30
min and distance traveled was recorded.

Table 1. Design of Experiment 1

Group	Treatment (Age)		
	Preinjection	Pretreatment Day	Test Day
	(PD 18)	(PD 19)	(PD 21)
Acute Control Group	Vehicle	Saline	Cocaine
Sensitization Control Group	Vehicle	Cocaine	Cocaine
7.5 mg/kg EEDQ Acute Control	7.5 mg/kg EEDQ	Saline	Cocaine
7.5 mg/kg EEDQ Sensitization	7.5 mg/kg EEDQ	Cocaine	Cocaine
15 mg/kg EEDQ Acute Control	15 mg/kg EEDQ	Saline	Cocaine
15 mg/kg EEDQ Sensitization	15 mg/kg EEDQ	Cocaine	Cocaine

On the test day (PD 21), all rats (n=8 per group) were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min. The design of Experiment 2a is shown in Table 2.

Experiment 2b: Effects of Ritanserin and Prazosin on the Induction of One-Trial Cocaine Sensitization

A separate four group experiment was conducted to determine whether the protection treatments affected the locomotor activity of nonEEDQ-treated rats. On the preinjection day (PD 18), rats first received an injection of saline or prazosin+ritanserin in the home cage. After 30 min, all rats received a preinjection of vehicle. On the pretreatment day, which occurred 24 h later (PD 19), half of the rats in each group received saline and the other half received 30 mg/kg cocaine. Immediately afterwards, rats were placed in activity chambers for 30 min and distance traveled was recorded. On the test day (PD 21), all rats (n=6 per group) were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min. The design of Experiment 2b is shown in Table 3.

Experiment 3: Effects of EEDQ on the Expression of One-Trial Cocaine-Induced Behavioral Sensitization

On the pretreatment day (PD 19), rats were injected with saline or 30 mg/kg cocaine and placed in activity chambers for 30 min. On the preinjection day, which occurred 24 h later (PD 20), an equal number of saline- and cocaine-pretreated rats were injected with 0, 7.5, or 15 mg/kg EEDQ and immediately returned to their home cage. On the test day (PD 21), all rats (n=8 per group)

Table 2. Design of Experiment 2a

Group	Treatment (Age)			
	Protection	Preinjection	Pretreatment Day	Test Day
	(PD 18)	(PD 18)	(PD 19)	(PD 21)
Acute Control Group	Saline	Vehicle	Saline	Cocaine
Nonprotected-EEDQ Acute Control	Saline	EEDQ	Saline	Cocaine
Adrenergic Protected-EEDQ Acute Control	Prazosin	EEDQ	Saline	Cocaine
5-HT Protected-EEDQ Acute Control	Ritanserin	EEDQ	Saline	Cocaine
Adrenergic/5-HT Protected-EEDQ Acute Control	Prazosin+ Ritanserin	EEDQ	Saline	Cocaine
Sensitization Control Group	Saline	Vehicle	Cocaine	Cocaine
Nonprotected-EEDQ Sensitization	Saline	EEDQ	Cocaine	Coaine
Adrenergic Protected-EEDQ Sensitization	Prazosin	EEDQ	Cocaine	Cocaine
5-HT Protected-EEDQ Sensitization	Ritanserin	EEDQ	Cocaine	Cocaine
Adrenergic/5-HT Protected-EEDQ Sensitization	Prazosin+ Ritanserin	EEDQ	Cocaine	Cocaine

Table 3. Design of Experiment 2b

Group	Treatment (Age)			
	Preinjection (1)	Preinjection (2)	Pretreatment Day	Test Day
	(PD 18)	(PD 18)	(PD 19)	(PD 21)
Acute Control Group	Saline	Vehicle	Saline	Cocaine
Sensitization Control Group	Saline	Vehicle	Cocaine	Cocaine
Adrenergic/5-HT Antagonist Acute Control	Prazosin+ Ritanserin	Vehicle	Saline	Cocaine
Adrenergic/5-HT Antagonist Sensitization	Prazosin+ Ritanserin	Vehicle	Cocaine	Cocaine

were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min.

The design of Experiment 3 is shown in Table 4.

Experiment 4a: The Use of 5-HT and/or α_1-Adrenergic Receptor
Protection to Assess the Effects of EEDQ on the Expression of
One-Trial Cocaine-Induced Behavioral Sensitization

On the pretreatment day (PD 19), rats were injected with saline or 30

mg/kg cocaine and placed in activity chambers for 30 min. On the preinjection

day, which occurred 24 h later (PD 20), rats received a protection injection of

prazosin (25 mg/kg), ritanserin (3 mg/kg), prazosin+ritanserin, or saline in the

home cage. After 30 min, rats received a preinjection of EEDQ (15 mg/kg). A

separate control group received a protection injection of saline and a preinjection

Table 4. Design of Experiment 3

Group	Treatment (Age)		
	Pretreatment Day (PD 19)	Preinjection (PD 20)	Test Day (PD 21)
Acute Control Group	Saline	Vehicle	Cocaine
Sensitization Control Group	Cocaine	Vehicle	Cocaine
7.5 mg/kg EEDQ Acute Control	Saline	7.5 mg/kg EEDQ	Cocaine
7.5 mg/kg EEDQ Sensitization	Cocaine	7.5 mg/kg EEDQ	Cocaine
15 mg/kg EEDQ Acute Control	Saline	15 mg/kg EEDQ	Cocaine
15 mg/kg EEDQ Sensitization	Cocaine	15 mg/kg EEDQ	Cocaine

of vehicle. On the test day (PD 21), all rats (n=8 per group) were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min. The design of Experiment 4a is shown in Table 5.

Experiment 4b: Effects of Ritanserin and Prazosin on the
Expression of One-Trial Cocaine Sensitization

Another four group experiment was conducted to determine whether the protection treatments affected the locomotor activity of nonEEDQ-treated rats. On the pretreatment day, which occurred 24 h later (PD 19), half of the rats in each group received saline and the other half received 30 mg/kg cocaine. Immediately afterwards, rats were placed in activity chambers for 30 min and distance traveled was recorded. On the preinjection day (PD 20), rats first

received an injection of saline or prazosin+ritanserin in the home cage. After 30 min, all rats received a preinjection of vehicle. On the test day (PD 21), all rats (n=8 per group) were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min. The design of Experiment 4b is shown in Table 6.

Table 5. Design of Experiment 4a

Group	Treatment (Age)			
	Pretreatment Day	Protection	Preinjection	Test Day
	(PD 19)	(PD 20)	(PD 20)	(PD 21)
Acute Control Group	Saline	Saline	Vehicle	Cocaine
Nonprotected-EEDQ Acute Control	Saline	Saline	EEDQ	Cocaine
Adrenergic Protected-EEDQ Acute Control	Saline	Prazosin	EEDQ	Cocaine
5-HT Protected-EEDQ Acute Control	Saline	Ritanserin	EEDQ	Cocaine
Adrenergic/5-HT Protected-EEDQ Acute Control	Saline	Prazosin+ Ritanserin	EEDQ	Cocaine
Sensitization Control Group	Cocaine	Saline	Vehicle	Cocaine
Nonprotected-EEDQ Sensitization	Cocaine	Saline	EEDQ	Coaine
Adrenergic Protected-EEDQ Sensitization	Cocaine	Prazosin	EEDQ	Cocaine
5-HT Protected-EEDQ Sensitization	Cocaine	Ritanserin	EEDQ	Cocaine
Adrenergic/5-HT Protected-EEDQ Sensitization	Cocaine	Prazosin+ Ritanserin	EEDQ	Cocaine

Table 6. Design of Experiment 4b

Group	Treatment (Age)			
	Pretreatment Day	Preinjection (1)	Preinjection (2)	Test Day
	(PD 19)	(PD 20)	(PD 20)	(PD 21)
Acute Control Group	Saline	Saline	Vehicle	Cocaine
Sensitization Control Group	Cocaine	Saline	Vehicle	Cocaine
Adrenergic/5-HT Antagonist Acute Control	Saline	Prazosin+ Ritanserin	Vehicle	Cocaine
Adrenergic/5-HT Antagonist Sensitization	Cocaine	Prazosin+ Ritanserin	Vehicle	Cocaine

Data Analysis

To statistically analyze data from both the pretreatment and test days, distance traveled data was analyzed using one-way or factorial analyses of variance (ANOVA) depending on experiment. For example, the test day of Experiment 2a was analyzed using a $5 \times 2 \times 12$ (preinjection $\times$ pretreatment $\times$ time block) mixed factorial ANOVA, with the preinjection and pretreatment

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variables being between-subject factors and time block being a within-subject

repeated measures factor. The Huynh-Feldt epsilon statistic was used to adjust

degrees of freedom when the assumption of sphericity was violated (Huynh &

Feldt, 1976), as determined by Mauchly's test of sphericity. Corrected degrees of

freedom were rounded to the nearest whole number. When further analyzing

statistically significant higher order interactions, the mean square error terms

(i.e., MS_{error}) used for the Tukey calculations was based on separate one-way

ANOVAs at each time block.

Litter effects were minimized by assigning no more than one subject from

each litter to a particular group (for a discussion of litter effects, see Zorrilla,

1997). Young rats do not typically exhibit sex differences after psychostimulant

treatment (Bowman, Blatt, & Kuhn, 1997; Frantz, Babcock, & Van Hartesveldt,

1996; McDougall et al., 2013; Snyder, Katovic, & Spear, 1998), so sex was not

included as a factor in the statistical analysis.

CHAPTER ELEVEN

RESULTS

Experiment 1: Effects of EEDQ on the Induction of One-Trial
Cocaine-Induced Behavioral Sensitization

Pretreatment Day

On the pretreatment day, rats given cocaine (M = 7439.16 cm, SEM =

716.08) had greater locomotor activity than rats given saline (M = 2639.40 cm,

SEM = 392.53) (Figure 1, upper graph) [Drug effect, $F_{(1,47)}=36.76$, $p<0.001$].

EEDQ treatment did not significantly affect locomotion, although a nonsignificant

decline in locomotor activity was observed in rats treated with the higher dose of

EEDQ (15 mg/kg).

Test Day

On the test day, locomotor sensitization was evident (Figure 1, lower graph)

since rats in the 0 mg/kg EEDQ-sensitization group had significantly greater

locomotor activity than rats in the 0 mg/kg EEDQ-acute control group

[Preinjection × Drug Interaction, $F_{(2,47)}=5.90$, $p<0.01$; and Tukey tests,

$p<0.001$]. Both the low (7.5 mg/kg) and high doses (15 mg/kg) of EEDQ

significantly attenuated sensitized locomotor activity [Tukey tests, $p<0.05$]. A

separate Preinjection × Time Block ANOVA comparing only the sensitized

groups showed that EEDQ significantly reduced locomotor activity during time

blocks 1-9 [Preinjection × Time Block interaction, $F_{(7,76)}=2.84$, $p<0.05$; and

Tukey tests, $p<0.05$].

Experiment 2a: The Use of 5-HT and/or α_1-Adrenergic Receptor
Protection to Assess the Effects of EEDQ on the
Induction of One-Trial Cocaine-Induced
Behavioral Sensitization

Pretreatment Day

On the pretreatment day, rats that received cocaine (M = 6361.51 cm, SEM

= 466.18) showed significantly more locomotor activity than rats given saline (M

= 2763.78 cm, SEM = 345.09) (Figure 2, upper graph) [Drug main effect,

$F(1,70)= 42.28$, $p<0.001$]. Treatment with prazosin, ritanserin, and EEDQ did not

significantly affect locomotor activity on the pretreatment day.

Test Day

On the test day, it is clear that sensitization occurred since cocaine-

pretreated rats in the Sal-DMSO group (i.e., the Sensitization Control group) had

significantly more locomotor activity counts than saline-pretreated rats in the Sal-

DMSO group (i.e., the Acute Control group) (right panels, Figure 2) [Preinjection

$\times$ Drug interaction, $F(4,70)=3.62$, $p<0.05$; and Tukey tests, $p<0.05$]. Among the

saline-pretreated groups, EEDQ did not affect locomotion. Among the cocaine-

pretreated groups, EEDQ significantly reduced locomotor activity, with the

decline being most evident in the Praz-EEDQ group [Tukey tests, $p<0.05$].

Overall, both the Preinjection and Drug variables interacted with time block to

affect behavior (left panels, Figure 2) [Preinjection $\times$ Time Block interaction,

$F(19,333)=3.35$, $p<0.001$; Drug $\times$ Time Block interaction, $F(5,333)=3.08$, $p<0.05$].

A separate analysis of only the cocaine-pretreated groups (i.e., the Sensitization

groups) showed that EEDQ significantly reduced locomotor activity on time

blocks 1–7 relative to the DMSO controls (lower graph, left panel, Figure 2) [EEDQ × Time Block interaction, $F(4,162)=8.49$, $p<0.001$; and Tukey tests, $p<0.05$].

Experiment 2b: Effects of Ritanserin and Prazosin on the Induction of One-Trial Cocaine Sensitization

Pretreatment Day

Rats that received cocaine (M = 6573.22 cm, SEM = 540.71) showed significantly more locomotor activity than rats given saline (M = 1964.36 cm, SEM = 318.22) (Figure 3, upper graph) [Drug main effect, $F(1,23)= 76.48$, $p<0.001$]. Locomotor activity was not significantly affected by combined treatment with prazosin and ritanserin.

Test Day

On the test day, sensitization was not observed because cocaine-pretreated rats in the saline group (i.e., the Sensitization Control group; M = 45110.70 cm, SEM = 7419.72) exhibited only marginally more locomotor activity than saline-pretreated rats in the saline group (i.e., the Acute Control group; M = 26541.95 cm, SEM = 6093.54) [Preinjection × Drug interaction, $F(1,20)= 3.56$, $p=0.074$] (lower graph, right panels, Figure 3). Combined treatment with the two antagonists did not affect locomotor activity; however, there was a trend for

Figure 1. Experiment 1. Mean distance traveled (±SEM) on the pretreatment and test day. On the preinjection day (PD 18), rats were injected with EEDQ (0, 7.5, or 15 mg/kg). On pretreatment day (PD 19), rats were injected with saline or 30 mg/kg cocaine, immediately followed by 30 min of behavioral assessment. On the test day (PD 21), all rats were injected with 20 mg/kg cocaine, immediately followed by 120 min of behavioral assessment. * Significantly different from the 0 mg/kg EEDQ-Saline group (acute control group; open circles and open bars). † Significantly different from the 0 mg/kg EEDQ-cocaine group (cocaine alone group; filled circles and black bars).
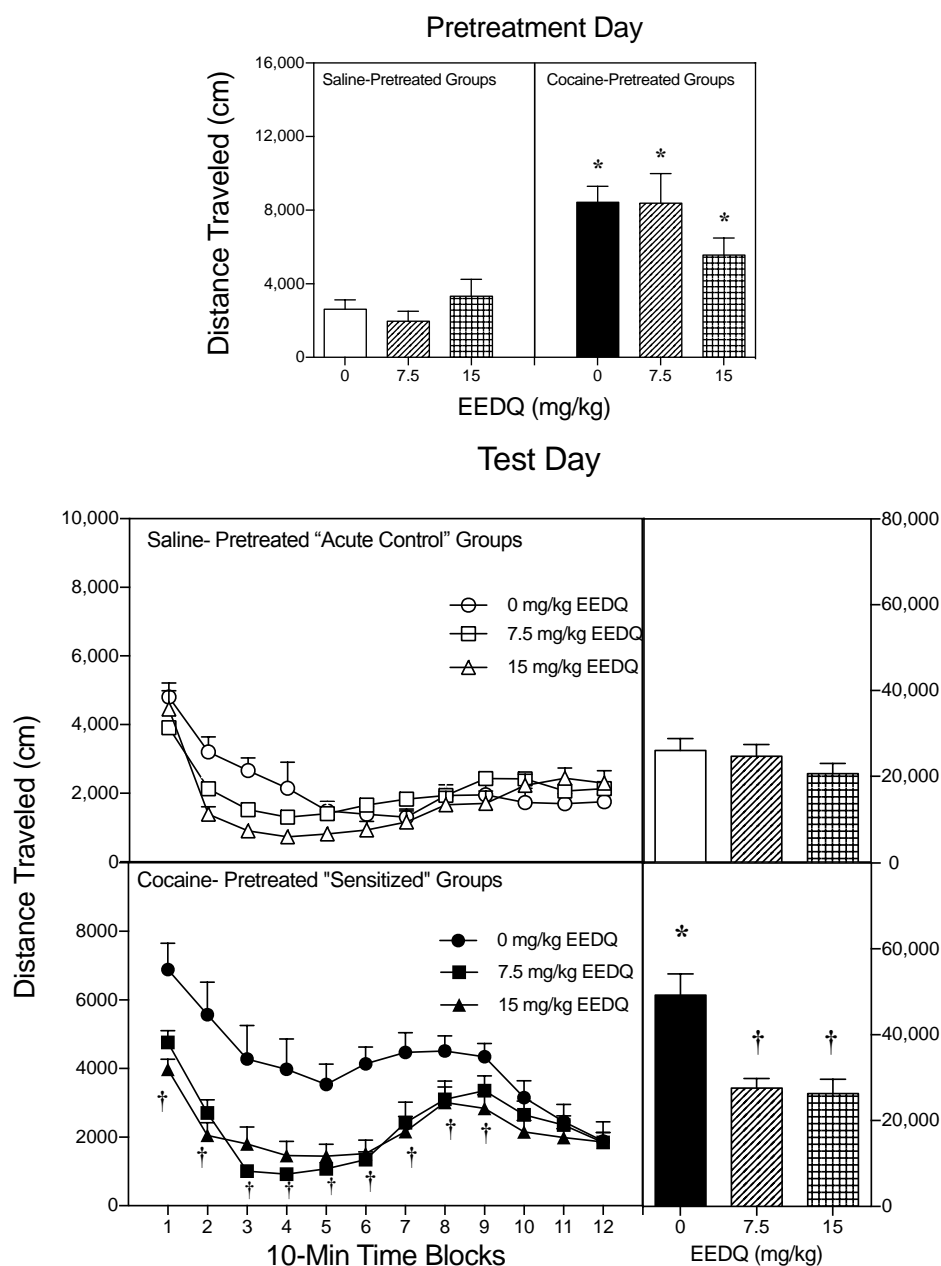
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Figure 2. Experiment 2a. Mean distance traveled (±SEM) on the pretreatment
and test day. On the preinjection day (PD 18), rats were injected with prazosin (5
mg/kg), ritanserin (5 mg/kg), prazosin+ritanserin, or saline in the home cage.
After 30 min, rats received a preinjection of EEDQ (15 mg/kg). On the
pretreatment day (PD 19), rats were injected with saline or 30 mg/kg cocaine,
immediately followed by 30 min of behavioral assessment. On the test day (PD
21), all rats were injected with 20 mg/kg cocaine, immediately followed by 120
min of behavioral assessment. * Significantly different from the Saline-DMSO
Acute Control group (open bar). † Significantly different from the Saline-DMSO
Sensitization group (filled bar, filled circles).

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prazosin+ritanserin to enhance the locomotor activity of saline-pretreated rats and depress the locomotion of cocaine-pretreated rats.

Experiment 3: Effects of EEDQ on the Expression of One-Trial Cocaine-Induced Behavioral Sensitization

On the pretreatment day, rats given cocaine (M = 8054.65 cm, SEM = 343.22) had greater locomotor activity than rats given saline (M = 2118.44 cm, SEM = 217.77) [$t(46)$= 14.60, $p<0.001$]. On the test day, locomotor sensitization was apparent since the 0 mg/kg EEDQ-sensitization group exhibited significantly more locomotor activity than the 0 mg/kg EEDQ-acute control group [Preinjection × Drug Interaction, $F(2,47)$= 5.74, $p<0.01$; and Tukey tests, $p<0.05$] (Figure 4). Both the low (7.5 mg/kg) and high (15 mg/kg) doses of EEDQ significantly attenuated locomotor activity, because the 7.5 mg/kg EEDQ-sensitization group and the 15 mg/kg EEDQ-sensitization group had significantly less locomotor activity than the 0 mg/kg EEDQ-sensitization group [Preinjection × Drug Interaction]. Moreover, the EEDQ-sensitization groups were not different from the acute control group.

Experiment 4a: The Use of 5-HT and/or α₁-Adrenergic Receptor Protection to Assess the Effects of EEDQ on the Expression of One-Trial Cocaine-Induced Behavioral Sensitization

On the pretreatment day, rats that received cocaine (M = 7691.17 cm, SEM = 331.28) had significantly more locomotor activity than rats given saline (M = 2593.23 cm, SEM = 219.26) [$t(78)$= 12.832, $p<0.001$]. On the test day, locomotor sensitization was not apparent since cocaine-pretreated rats (M = 28456.66 cm,

SEM = 1707.44) exhibited only marginally more locomotor activity than saline-pretreated rats (M = 24026.85 cm, SEM = 1702.95) [Preinjection main effect, $F_{(1,70)}$= 3.74, p=0.057] (right panels, Figure 5). A separate statistical analysis comparing only the saline-pretreated and cocaine-pretreated Sal-DMSO groups also indicated an absence of behavioral sensitization. Neither EEDQ or the protection treatments significantly affected performance on the test day [Preinjection main effect, $F_{(4,70)}$= 2.49, p=0.052].

<div align="center">

Experiment 4b: Effects of Ritanserin and Prazosin on the
Expression of One-Trial Cocaine Sensitization

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On the pretreatment day, rats given cocaine (M = 8214.45 cm, SEM = 548.58) had significantly more locomotor activity than rats that received saline (M = 2788.30 cm, SEM = 383.31) [$t_{(22)}$= 8.11, p<0.001]. On the test day, locomotor sensitization was evident since rats in the saline-sensitization group had significantly greater locomotor activity than rats in the saline-acute control group [Preinjection × Drug interaction, $F_{(1,20)}$= 6.53, p<0.05] (right panels, Figure 6). In addition, sensitized rats preinjected with prazosin and ritanserin had significantly less locomotor activity than those that received a preinjection of saline [Preinjection × Drug interaction]. None of the interactions involving the time block variable were statistically significant.

Figure 3. Experiment 2b. Mean distance traveled (±SEM) on the pretreatment and test day. On the preinjection day (PD 18), rats were injected with prazosin+ritanserin or saline in the home cage. After 30 min, rats received an injection of vehicle. On the pretreatment day (PD 19), rats were injected with saline or 30 mg/kg cocaine, immediately followed by 30 min of behavioral assessment. On the test day (PD 21), all rats were injected with 20 mg/kg cocaine, immediately followed by 120 min of behavioral assessment. * Significantly different from the Saline control group (open bar).

Test Day

Figure 4. Experiment 3. Mean Distance traveled (±SEM) on the test day. On the pretreatment day (PD19), rats were injected with saline or 30 mg/kg cocaine, immediately followed by 30 min of behavioral assessment. On the preinjection day (PD 20), rats were injected with EEDQ (0, 7.5, or 15 mg/kg) in the home cage. On the test day (PD 21), all rats were challenged with 20 mg/kg cocaine followed immediately by 120 min of behavioral assessment. * Significantly different from 0 mg/kg EEDQ-Saline group (acute control group; open circles and open bars). † Significantly different from the 0 mg/kg EEDQ-cocaine group (cocaine alone group; filled circles and black bars).

Figure 5. Experiment 4a. Mean distance traveled (±SEM) on the test day. On the pretreatment day (PD 19), rats were injected with saline or 30 mg/kg cocaine, immediately followed by 30 min of behavioral assessment. On the preinjection day (PD 20), rats were injected with prazosin (5 mg/kg), ritanserin (5 mg/kg), prazosin+ritanserin, or saline in the home cage. After 30 min, rats received a preinjection of EEDQ (15 mg/kg). On the test day (PD 21), all rats were injected with 20 mg/kg cocaine, immediately followed by 120 min of behavioral assessment.

Figure 6. Experiment 4b. Mean distance traveled (±SEM) on the test day. On the pretreatment day (PD 19), rats were injected with saline or 30 mg/kg cocaine, immediately followed by 30 min of behavioral assessment. On the preinjection day (PD 20), rats were injected with prazosin+ritanserin or saline in the home cage. After 30 min, rats received an injection of vehicle. On the test day (PD 21), all rats were injected with 20 mg/kg cocaine, immediately followed by 120 min of behavioral assessment. * Signi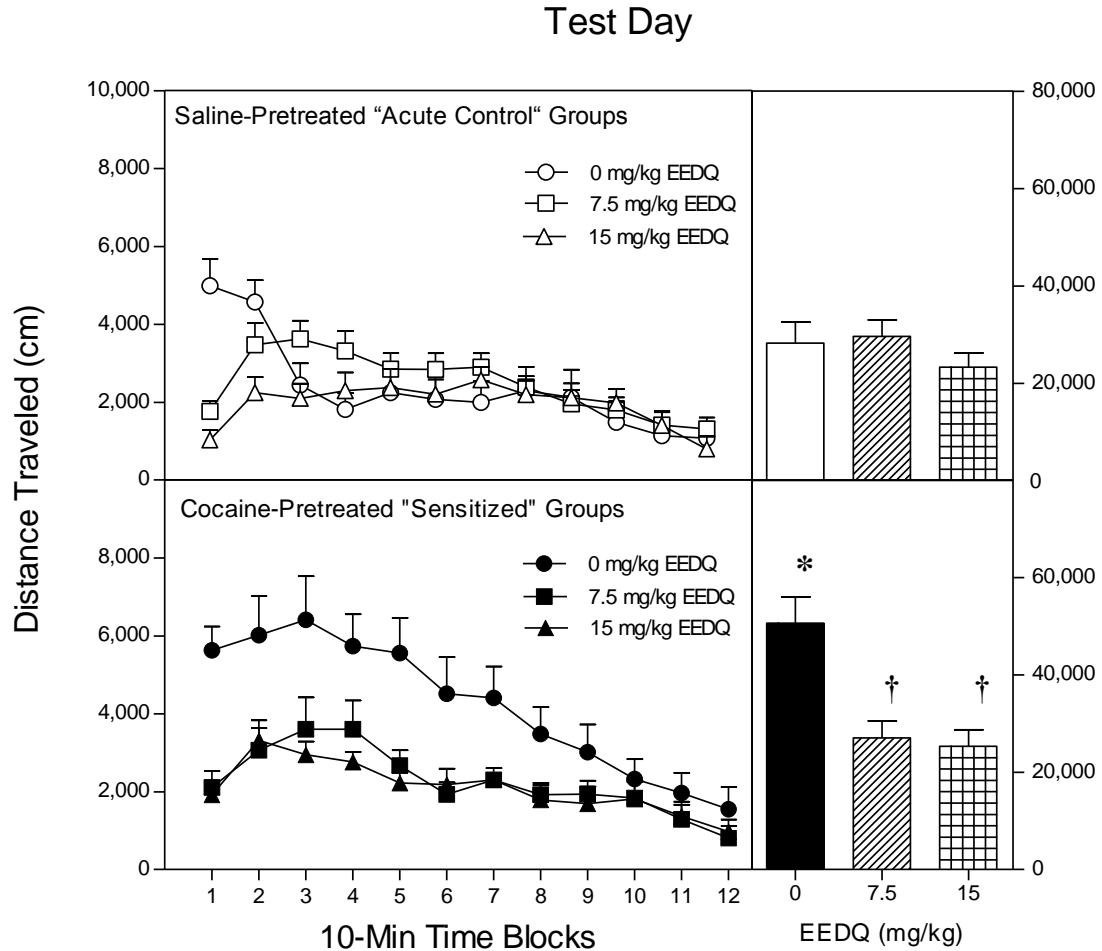ficantly different from the Saline control group (open bar). † Significantly different from the Saline Sensitization group (filled bar).

CHAPTER TWELVE

DISCUSSION

Prior to this thesis, research examining the mechanisms underlying cocaine-induced one-trial behavioral sensitization in preweanling rats was scarce (e.g., see McDougall et al., 2016; Mohd-Yusof et al., 2014; 2016); however, multiple studies using adult rats indicated that dopaminergic, serotonergic, and adrenergic receptor systems are involved in the mediation of behavioral sensitization (Auclair et al., 2004; O'Neill et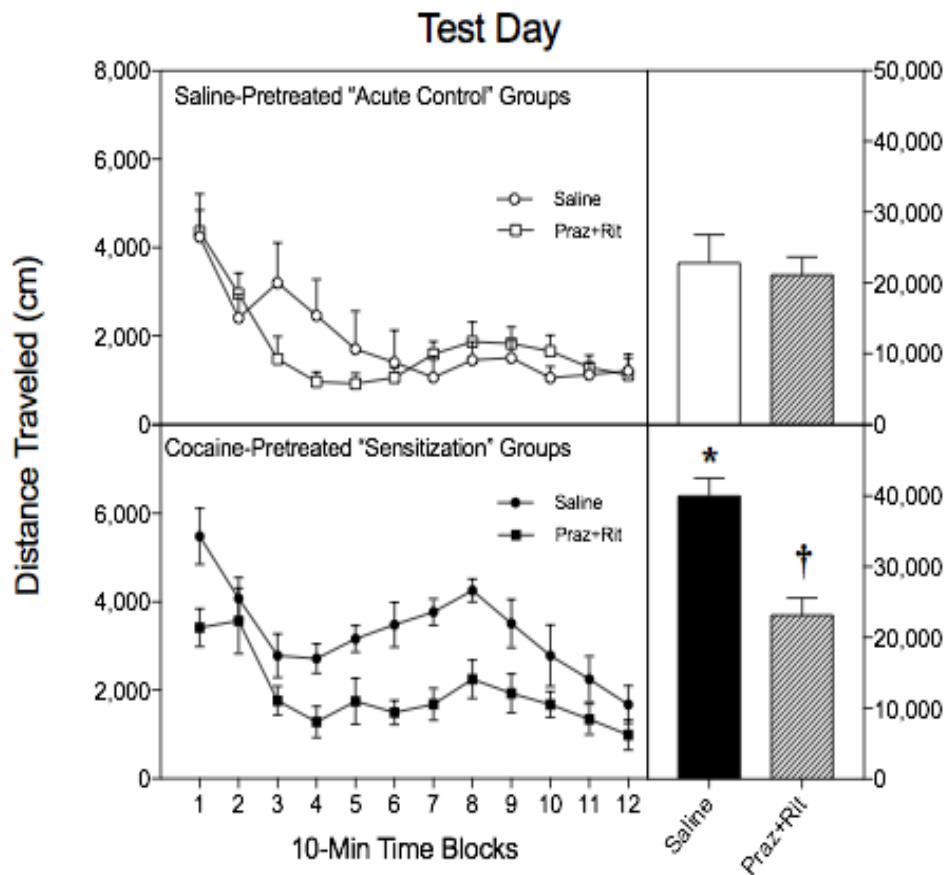 al., 1999; Vanderschuren et al., 2003). Behavioral and neurochemical data using preweanling rats suggest that selective protection from the nonspecific irreversible antagonist EEDQ could be useful for investigating the role of individual receptor types in behavioral sensitization (McDougall et al., 2016). Therefore, in the present thesis I used EEDQ in conjunction with selective protection experiments to examine whether the serotonergic and adrenergic receptor systems mediate the induction and expression of cocaine-induced one-trial behavioral sensitization in preweanling rats.

Results from this thesis showed that cocaine was able to induce one-trial behavioral sensitization in preweanling rats. By administering EEDQ prior to either the pretreatment day or the test day, it was apparent that general receptor inactivation blocked both the induction and expression of cocaine-induced one-trial behavioral sensitization. Importantly, administering

prazosin and ritanserin prior to EEDQ treatment did not protect the induction or expression of behavioral sensitization, which suggests that serotonergic and adrenergic receptors do not mediate cocaine-induced sensitized responding in preweanling rats. This negative result indicates that some other receptor type, or a combination of redundant receptor systems, mediates the induction and expression of behavioral sensitization (see also White et al., 1998).

Some findings from this study were unexpected. For example, it was concerning that cocaine did not induce a statistically significant sensitized response in Experiments 2b and 4a. In the case of Experiment 2b, there were only six subjects per group, as opposed to the eight subjects per group that we normally use. This lack of power may have been responsible for the inability to detect behavioral sensitization. Interestingly, the magnitude of the effect size between the Acute Control group and the Sensitization group of Experiment 2b is similar to, if not greater than, experiments in which statistically significant behavioral sensitization was observed. For example, the effect size magnitude was 18568.75 cm in Experiment 2b, which resulted in a nonsignificant difference; whereas, the effect size magnitude between the Acute Control and Sensitization group of Experiment 4b was 17137.88 cm, which was sufficient for a statistically significant effect. In the case of Experiment 4a, the reason for the marginal sensitized responding is more unclear. Perhaps the fact that this experiment examined the expression

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(rather than the induction) of behavioral sensitization was responsible for the weakened sensitization effect. More specifically, the nature of the expression paradigm may have led to increased stress on the control animals (i.e., the induction paradigm requires fewer injections on PD 20 and 21 than the expression paradigm), which may have affected our ability to detect cocaine-induced behavioral sensitization. Indeed, stress-induced behavioral sensitization is a well known phenomenon that can be initiated by the injection protocol itself (for a review, see Robinson & Becker, 1986). That being said, Experiment 4b was also an expression experiment, with the same number and timing of injections, and statistically significant behavioral sensitization was achieved. Therefore, it is uncertain why cocaine-induced behavioral sensitization was not evident in Experiment 4a. In the same experiment, EEDQ did not block behavioral sensitization; however, since a statistically significant sensitized response did not occur, it is reasonable to argue that EEDQ could not block a phenomenon that was not present. Even so, close examination of the data reveals that EEDQ did not appear to cause a robust decline in the locomotor activity of the "sensitization" groups.

Finally, it is concerning that prazosin and ritanserin affected the locomotor activity and sensitized responding of cocaine-treated rats independent of the actions of EEDQ (see Experiments 2b and 4b). The results of Experiment 2a suggest that neither the serotonergic or adrenergic receptor systems are responsible for one-trial cocaine-induced behavioral

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sensitization, because protecting these specific receptors from EEDQ failed to keep behavioral sensitization intact. The results of Experiment 2b and 4b challenge this conclusion, as the absence of behavioral sensitization may have been due to the drugs used to protect the serotonergic and adrenergic receptors. It is unclear whether it was prazosin or ritanserin that weakened sensitized responding, since these drugs were co-administered in the control experiments (2b and 4b). Testing the effects of prazosin or ritanserin alone on cocaine-induced one-trial behavioral sensitization would resolve this issue. Additional improvements for future studies would include using different serotonergic and adrenergic antagonists, as well as novel methods of receptor protection.

The finding that prazosin and ritanserin caused prolonged changes in cocaine-induced behavioral sensitization is interesting in its own right and deserves consideration. The most obvious possibility is that these serotonergic and adrenergic compounds altered the functioning of the dopaminergic system, which resulted in the observed changes in locomotor activity. For example, 5-HT$_2$ receptor antagonism by ritanserin potentiates amphetamine-induced dopamine release in adult rats (Pehek & Bi, 1997). In addition, prazosin decreases dopamine transmission in the reward pathway (Zhang & Kosten, 2005). Therefore, ritanserin and prazosin may impact both cocaine-induced locomotor activity and behavioral sensitization by altering normal dopaminergic functioning.

In addition to the uncertainty regarding the effects of prazosin and ritanserin on behavioral sensitization, we did not demonstrate that these drugs protected adrenergic and serotonergic receptors from the alkylating effects of EEDQ. Although previous receptor binding experiments show that 5 mg/kg prazosin and 1 mg/kg ritanserin protect adrenergic and serotonergic receptors, respectively, from alkylation by EEDQ, the animals used in these studies were adult rats (Giorgi & Biggio, 1990; Kettle, Cheetham, Martin, Prow, & Heal, 1999). Given the many ontogenetic differences involving the serotonergic and adrenergic receptor systems (e.g., Auclair et al., 2004; Drouin et al., 2002), there is the possibility that these compounds may not have the same protective effects in preweanling rats. In the future, it would be advantageous to conduct a receptor binding study to confirm the protective effects of prazosin and ritanserin in preweanling rats. In addition, the combined use of these drugs, in conjunction with EEDQ, may be producing an effect that interferes with behavioral sensitization. For example, in Experiments 2b and 4b, it is clear that antagonizing serotonergic and adrenergic receptors reduces locomotor activity. This evidence indicates that serotonergic and adrenergic receptor stimulation is necessary for the induction and expression of behavioral sensitization; however, the results of Experiments 2a and 4a do not support this conclusion. Therefore, the combined effects of ritanserin, prazosin, and EEDQ should be further

examined, and in future experiments, different drugs that do not have complex interactions should be considered.

It is well established that the neural mechanisms underlying behavioral sensitization differ depending on the psychostimulant drug being used (e.g., cocaine vs. amphetamine; see Vanderschuren & Kalivas, 2000; White et al., 1998). For example, Auclair et al. (2004) reported that serotonergic and adrenergic receptor antagonists prevent the induction and expression of amphetamine-induced behavioral sensitization in adult mice; whereas, I found that the same drugs did not affect cocaine-induced behavioral sensitization in preweanling rats. The reason for such psychostimulant-specific effects may lie in the mechanism of action for each drug. In general, amphetamine-like compounds affect brain function by enhancing the transmission of monamines like serotonin, norepinephrine, and dopamine (Shi, Pun, Zhang, Jones, & Bunney, 2000). Specifically, amphetamine blocks the reuptake of monoamines, while also increasing cytoplasmic dopamine concentrations via reverse receptor transport (Fleckenstein & Hanson, 2003). In contrast, cocaine increases extracellular monoamine levels through the sole mechanism of blocking monoamine reuptake transporters (Meyer & Quenzer, 2005; Vanderschuren & Kalivas, 2000).

Auclair et al. (2004) also reported that serotonergic and adrenergic receptor antagonists block the induction and expression of cocaine-induced behavioral sensitization. In the Auclair et al. (2004) experiment, adult mice

were used as opposed to preweanling rats. These discrepant results leave open the possibility that there are ontogenetic or species-based differences in the mechanisms mediating behavioral sensitization. Although species differences in behavioral sensitization are seldom reported, pronounced age-dependent differences in sensitized responding are well-established (for a review, see Tirelli et al., 2003). For example, one-trial behavioral sensitization persists for months in adult rats, while only lasting a few days in preweanling rats (McDougall et al., 2009; Robinson et al., 1982; Valjent et al., 2010). In addition, drug-environment associations are necessary for the one-trial behavioral sensitization of adult rats (Weiss et al., 1989), while environmental context does not influence the one-trial sensitized responding of preweanling rats (McDougall et al., 2009). Finally, D1-like antagonists block the induction of one-trial behavioral sensitization in adult rats, but not in preweanling rats (Mattingly et al., 1991; Mohd-Yusof et al., 2014; Kuribara, 1995; Valjent et al., 2010).

In addition to ontogenetic and species-based differences in the mechanisms underlying behavioral sensitization, there also appear to be ontogenetic differences in the actions of EEDQ. For example, EEDQ blocks behavioral sensitization in preweanling rats and locomotor activity in adults, but this alkylating agent does not reduce locomotor activity in the younger age group (Der-Ghazarian et al., 2014; McDougall, Valentine, Gonzalez, Humphrey, Widarma, & Crawford, 2014). These findings suggest that the

mechanisms mediating locomotor activity in preweanling rats are resistant to EEDQ, while the mechanisms mediating behavioral sensitization are not. The inability of EEDQ to block the locomotor activity of preweanling rats is interesting, and may be due to a compensatory mechanism involving an excess of high affinity D2-like receptors that is absent in older animals (McDougall et al., 2014). Consistent with this explanation, preweanling rats have a higher percentage of high affinity striatal D2-like receptors (i.e., D2^{High} receptors) than adolescent or adult animals (McDougall et al., 2015).

Although thousands of studies have examined the neural bases of behavioral sensitization, it remains unclear which receptor systems are important for the induction and expression of cocaine-induced behavioral sensitization. Despite contradictory evidence, it is still possible that dopaminergic, serotonergic, and adrenergic receptor systems all play a role in behavioral sensitization. In the typical study, investigation of these receptor systems has been restricted to antagonism or stimulation of individual receptor types. Instead, it is probable that many neurotransmitter systems work simultaneously, and perhaps redundantly, to mediate the complex process that is behavioral sensitization. Since it is likely that there are many neurotransmitter systems involved in the mediation of behavioral sensitization, antagonizing only a single receptor type may not have a great effect on the overall sensitization process. In other words, when one particular neurotransmitter system is antagonized, another neurotransmitter

system may compensate in order to keep the behavior intact. For this reason, it will be necessary to study the combined actions of multiple receptor systems on behavioral sensitization, instead of assessing each system independently.

Although the dopamine, serotonin, and noradrenergic neurotransmitter systems may interact to mediate behavioral sensitization, it remains possible that other receptor types are also involved with this complex behavior. In terms of the present study, EEDQ may have blocked behavioral sensitization by affecting a receptor type that was not protected from alkylation. In addition to irreversibly antagonizing dopaminergic, serotonergic, and adrenergic receptors, EEDQ inhibits the release and high-affinity uptake of acetylcholine in the hippocampus (Vickroy & Malphurs, 1994). Therefore, besides examining the combined effects of monoamine neurotransmitter systems on behavioral sensitization, future experiments should also consider the roles played by other neural mechanisms.

In conclusion, EEDQ blocks the induction and expression of one-trial cocaine-induced behavioral sensitization. The protection experiments using ritanserin and prazosin indicate, but do not conclusively show, that the serotonergic and adrenergic receptor systems do not mediate the induction and expression of one-trial cocaine-induced behavioral sensitization in preweanling rats. Considering both past and present results, the most harmonious conclusion is that multiple receptor systems (i.e., dopaminergic,

serotonergic, adrenergic, etc.) work in unison to produce the complex

phenomenon of behavioral sensitization.

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