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Paradoxical behavior effects of dopamine receptor inactivation in preweanling rats: Role of the dorsal striatum

Taleen Siran Der-Ghazarian

PARADOXICAL BEHAVIORAL EFFECTS OF DOPAMINE RECEPTOR INACTIVATION IN PREWEANLING RATS: ROLE OF THE DORSAL STRIATUM

A Thesis

Presented to the

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In Partial Fulfillment

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by

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ABSTRACT

The ontogeny of dopamine-mediated motor systems has not been thoroughly investigated in young animals. It has generally been shown that preweanling and adult rats respond similarly to dopaminergic compounds; however, some behaviors vary across ontogeny due to maturational changes in dopamine systems. N-ethoxycarbonyl-2-ethoxy-1, 2-dihydroquinoline (EEDQ), an irreversible receptor antagonist, has been used to study the ontogeny of dopamine receptor functioning. Systemic administration of EEDQ attenuates dopamine agonist-induced behaviors of adult rats, while leaving the behaviors of young rats unaffected. The purpose of this thesis was to: a) investigate the effects of intrastriatally administered EEDQ on NPA-induced locomotor activity in preweanling and adults rats; b) determine which dopamine receptor subtype (D1- or D2-like) is responsible for modulating EEDQ's paradoxical behavioral effects in preweanling rats; and c) examine the magnitude of EEDQ-induced D1- and D2-like receptor inactivation in both adult and preweanling rats. In Experiment 1, EEDQ or DMSO were bilaterally infused into the dorsal striatum on PD 84. After 24 hr, rats were given bilateral microinjections of the full dopamine agonist R(-)-propylnorapomorphine (NPA) or distilled water

and locomotor activity was assessed for 40 min. In Experiments 2 and 3, preweanling rats (PD 17) were treated in the same manner as adults, with the exception that D1- and D2-like receptors (either alone or in combination) were protected from EEDQ-induced alkylation by pretreating rats with a D1- and/or D2-like receptor antagonist. As expected, infusing EEDQ into the dorsal striatum attenuated the NPA-induced locomotor activity of adult rats. Conversely, preweanling rats given bilateral EEDQ infusions on PD 17 exhibited a potentiated locomotor response when treated with NPA. Experiments 2 and 3 showed that dopamine receptor inactivation was responsible for the exaggerated locomotor response to NPA. Specifically, NPA-induced locomotor potentiation was not evident if both D1- and D2-like receptors were protected from EEDQ-induced receptor alkylation. In Experiments 4 and 5, homogenate ligand binging and autoradiography assays showed that EEDQ significantly reduced D1- and D2-like receptor levels in both preweanling and adult rats. A plausible explanation for these results is that a receptor reserve, or the lack thereof, may account for the qualitatively different patterns of NPA-induced locomotor activity exhibited by EEDQ-treated preweanling and adult rats. Alternatively, the potentiated locomotor response exhibited by

preweanling rats may be caused by receptor supersensitivity resulting from EEDQ treatment. It is possible that adult rats do not show potentiated locomotor activity because EEDQ does not produce a similar change in receptor dynamics.

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I would like to extend my appreciation to Dr. Arturo Zavala for his advice and mentoring during my time in the graduate program. He continues to provide advice and support when I need it most. Additionally, I would like to thank Danut Mitroi for the time he devoted to my thesis. And finally, I would like to thank my family and close friends for their continued support throughout the last several years of my life (i.e., Cynthia, Tyler, Alena, etc.)! Love you mom and dad! This thesis is in memory of my father who loved me so dearly and wanted nothing more than success and happiness for his family.

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CHAPTER ONE

BASAL GANGLIA

Introduction

The basal ganglia is important for the initiation of motor movements and responses, although it is also implicated in drug-seeking behavior as well as cognition, emotion, and memory (Alcaro, Huber, & Panksepp, 2007; Brown, Schneider, & Lidsky, 1997; Cardinal, Parkinson, Hall, & Everitt, 2002; Nicola, Surmeier & Malenka, 2000; Phillips & Carr, 1987; Schultz, 1994; White, 1997; White & Salinas, 2003). The basal ganglia is involved in various aspects of motor control including the initiation and execution of movements (Denny-Brown & Yanagisawa, 1976; Evarts, Teravainen, & Calne, 1981; Flowers, 1976), sequencing of movements (Schwab, Chafetz, & Walker, 1954), automatic execution of routine movements (Marsden & Obeso, 1994), and inhibition of competing motor programs (Mink, 1996). In terms of motor control, the basal ganglia is composed of several interconnected nuclei located in the telencephalon, diencephalon, and midbrain (Hauber, 1998). Structures that comprise the basal ganglia include the striatum, globus pallidus, subthalamic nucleus, entopeduncular nucleus, and substantia nigra (Figure 1).

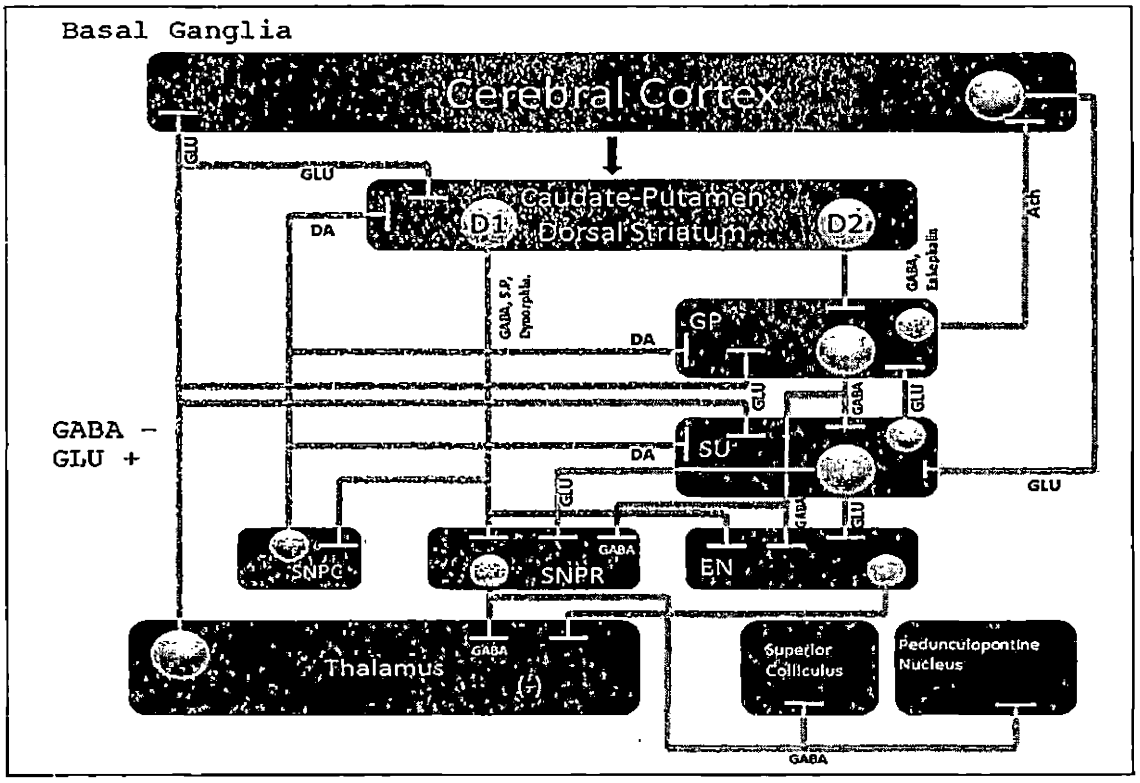


Figure 1. The Circuitry of the Basal Ganglia. GABAergic Fibers Descend from the Dorsal Striatum and Project to the Substantia Nigra pars Reticulata and Entopeduncular Nucleus via the Direct (striatonigral) Pathway and to the Globus Pallidus and, Subsequently, to the Substantia Nigra pars Reticulata and Entopeduncular Nucleus by way of the Subthalamic Nucleus via the Indirect (striatopallidal and striatoentopeduncular) Pathway. GP, globuspallidus; SU, subthalamic nucleus; EN, entopeduncular nucleus; SNPR, substantianigra pars reticulata, SNPC, substantianigra pars compacta; DA, dopamine; GLU, glutamate; SP, substance P; Ach, acetylcholine. Adapted and modified from Charntikov (2009).

Charntikov, S. (2009) *Role of dorsostriatal D1 and D2 receptors in in modulating the kappa opiood-mediated locomotor activity of preweanling rats: importance of synergistic activation* (Unpublished master's thesis). Available from California State Universty, San Bernaridino.

Two notable neurodegenerative diseases that arise from basal ganglia dysfunction are Parkinson's disease and Huntington's chorea (Albin, Young, & Penney, 1989).

Overview of Basal Ganglia Circuitry

The striatum is the major input structure of the basal ganglia. It receives afferents from the entire cerebral cortex and relays these signals to the entopeduncular nucleus and substantia nigra pars reticulata (i.e., the two major output nuclei of the basal ganglia) via the direct and indirect pathways (Hauber, 1998). The globus pallidus and subthalamic nucleus (integral parts of the indirect pathway) process and transfer signals from input structures to output structures (Carter & Fibiger, 1978; DeVito & Anderson, 1982). Efferents from output structures project to motor nuclei of the brainstem and to motor association cortex via the thalamus (Hauber, 1998).

Striatum

The caudate and putamen are collectively referred to as the dorsal striatum. The nucleus accumbens (also called the ventral striatum), located rostro-ventrally to the dorsal striatum, is the other major component of the striatum. The major input to the striatum is via

excitatory glutamatergic projections from the cerebral cortex. GABAergic medium spiny neurons, which constitute approximately 90-95% of all striatal neurons in rodents, are segregated in different compartments within the striatum (Gerfen, 1985; Herkenham, Edley, & Stuart, 1984; Izzo, Graybiel, & Bolam 1987; Penny, Afsharpour, & Kitai, 1984) and contain both D1- and D2-like receptors (Chang & Kitai, 1985; Chang, Wilson, & Kitai, 1982; Chevalier, Vacher, Deniau, & Desban, 1985; Kawaguchi, Wilson, & Emson, 1990; Kita & Kitai, 1988). Descending GABAergic fibers project to the substantia nigra pars reticulata and entopeduncular nucleus via the direct pathway; whereas, GABAergic neurons projecting to the globus pallidus (i.e., striatopallidal pathway) comprise the first segment of the indirect pathway.

Dopaminergic neurons provide the most important modulatory input to the striatum. Striatonigral and striatoentopeduncular neurons of the direct pathway prominently express D1-like receptors (Gerfen, 1992; Gerfen, Keefe, & Gauda, 1995; Hersch, Ciliax, Gutekunst, Rees, Heilman, Yung, Bolam, Ince, Yi, & Levey, 1995; Le Moine & Bloch, 1995; Le Moine, Normand, Guitteny, Teoule, & Bloch, 1990) and co-release GABA, substance P, and dynorphin (Beckstead, 1985; Kanazawa, Emson, & Emson,

1997; Vincent, Hökfelt, Christensson, & Terenius, 1982). In contrast, striatopallidal neurons, which are the initial neurons of the indirect pathway, prominently express D2-like receptors (Gerfen, 1992; Gerfen et al., 1995; Hersh et al., 1995; Le Moine & Bloch, 1995; Le Moine et al., 1990) and co-release GABA and enkephalin (Beckstead, 1985; Kanazawa et al., 1997; Vincent et al., 1982). Even though D1-like receptor stimulation activates the direct pathway and D2-like receptor stimulation inhibits the indirect pathway, there appears to be a small degree of receptor co-localization on these descending neurons (Aizman, Brismar, Uhlen, Zettergren, Levey, Forssberg, Greengard, & Aperia, 2000; Hersch et al., 1995; Surmeier, Eberwine, Wilson, Cao, Stefani, & Kitai, 1992; Surmeier, Reiner, Levine, & Ariano, 1993; Surmeier, Song, & Yan, 1996).

Striatal neurons are primarily modulated by dopaminergic input; however, these dopaminergic effects can be further modulated by other neurotransmitters. For example, adenosine regulates the functional properties of dopamine receptors by interacting with G protein-coupled adenosine receptors. Adenosine A_1 receptors are co-localized on neurons of the direct pathway containing D1-like receptors (Ferre, O'Connor, Svenningsson,

Bjorklund, Lindberg, Tinner, Stromberg, Goldstein, Ogren, Ungerstedt, Fredholm, & Fuxe, 1996), while adenosine A_{2A} receptors are co-localized on neurons of the indirect pathway containing D2-like receptors (Schiffmann, Jacobs, & Vanderhaeghen, 1991). The functional significance of this co-localization suggests an antagonistic interaction between striatal adenosine receptors and dopamine receptors (Ferre et al., 1996; Ferre, O'Connor, Fuxe, & Ungerstedt, 1993).

The serotonergic, noradrenergic, and cholinergic neurotransmitter systems also modulate striatal neurotransmission. Serotonergic inputs from the dorsal nucleus of the raphe and caudal linear nucleus project densely to the striatum, substantia nigra, and globus pallidus (Imai, Steindler, & Kitai, 1986; Van der Kooy & Hattori, 1980; Vertes, 1991; for a review, see Halliday, Harding, & Paxinos, 1995). Several serotonin receptor subtypes are co-localized with striatal enkephalin, substance P, and dynorphin receptors (Ward & Dorsa, 1996). Noradrenergic fibers originating in the locus coeruleus project to the striatum and alter signal processing (Marien, Letagan, & Colpaert, 1994), while cholinergic interneurons within the striatum modulate the activity of

the direct and indirect pathways (Di Chiara, Morelli, & Consolo, 1994).

Globus Pallidus

The globus pallidus receives substantial input from the dorsal striatum via striatopallidal neurons of the indirect pathway. These striatopallidal projections co-release GABA and enkephalin (Fonnum, Gottesfeld, & Grofova, 1978). The globus pallidus also receives glutamatergic input from the subthalamic nucleus. Axonal collaterals from the substantia nigra pars compacta provide dopaminergic innervation to the globus pallidus (Lindvall & Bjorklund, 1979). The globus pallidus expresses D1- and D2-like receptors (Richfield, Young, & Penny, 1987; Yung et al., 1995), as well as NMDA, AMPA, kainate, and metabotropic glutamate receptors (Albin, Mzrcowiec, Hollingsworth, Dure, Penny, & Young, 1992).

Pallidal projections send inhibitory GABAergic signals to the dorsal striatum, subthalamic nucleus, substantia nigra, pedunculopontine nucleus, and the reticular thalamic nucleus (Carter & Pycock, 1978; DeVito & Anderson, 1982; Fonnum et al., 1978; Grofová, 1975; Kincaid, Penney, Young, & Newman, 1991; Kita & Kitai, 1991; Parent & Hazrati, 1995; Staines & Fibiger, 1984; Van der Kooy & Carter, 1981), as well as sending cholinergic

and noncholinergic signals to the cortex (Heimer, Zahm, & Alheid, 1995). The globus pallidus controls basal ganglia output via multiple direct and indirect connections to various basal ganglia nuclei. Therefore, the globus pallidus is more than just a relay station transferring signals between the striatum (input structure) and the entopeduncular nucleus and substantia nigra pars reticulata (output structures) (Chesselet & Delfs, 1996).

Subthalamic Nucleus

The subthalamic nucleus receives prominent glutamatergic excitatory input from the frontal cortex (Canteras, Shammah-Lagnado, Silva, & Ricardo, 1990; Fujimoto & Kita, 1993) and GABAergic input from the globus pallidus (Feger, 1981; Vincent et al., 1982). Certain thalamic nuclei (centromedian and parafascicular) and brainstem nuclei (pedunculopontine tegmental and dorsal raphe) innervate the subthalamic nucleus (Feger, Bevan, & Crossman, 1994; Sugimoto, Hattori, Mizuno, Itoh, & Sato, 1983). Some evidence suggests the existence of dopaminergic inputs from the substantia nigra pars compacta (Fremeau, Duncan, Fornaretto, Dearry, Gingrich, Breese, & Caron, 1991; Hassani, Francois, Yelnik, & Feger, 1997).

The subthalamic nucleus sends excitatory (glutamatergic) signals to the globus pallidus, entopeduncular nucleus, and substantia nigra pars reticulata (Hammond, Deniau, Rizk, & Feger, 1978; Nakanishi, Kita, & Kitai, 1987). Subthalamic efferents also project to the dorsal striatum and the pedunculopontine tegmental nucleus (Moriizumi & Hattori, 1992; Takada, Nishihama, Nishihama, & Hattori, 1988; Van der Kooy & Hattori, 1980).

Substantia Nigra Pars Reticulata

The substantia nigra consists of two nuclei (pars compacta and pars reticulata) and is located in the tegmentum of the midbrain. Along with the entopeduncular nucleus, the substantia nigra pars reticulata (i.e., the two major output structures of the basal ganglia) integrates and conveys incoming signals to thalamic and midbrain nuclei. The substantia nigra pars reticulata receives inhibitory GABAergic input from the dorsal striatum, via the direct pathway (Chevalier & Deniau, 1990; Deniau, Hammond, Riszk, & Feger, 1978), and from the nucleus accumbens (Deniau, Menetrey, & Thierry, 1994). The substantia nigra pars reticulata receives excitatory glutamatergic input from the subthalamic nucleus via the

indirect pathway (Hammond et al., 1978; Nakanishi et al., 1987).

As just described, an important convergence occurs in the substantia nigra pars reticulata. Efferents projecting from the dorsal striatum (i.e., the direct pathway) and globus pallidus/subthalamic nucleus (i.e., the indirect pathway) converge onto single neurons of the substantia nigra pars reticulata and allow for an integration of neural processing (Bolam, Smith, Ingham, Von Krosigk, & Smith, 1993). The substantia nigra pars reticulata sends GABAergic output from the basal ganglia to the thalamus, superior colliculus, and pedunculopontine nuclei (Beckstead, Domesick, & Nauta, 1979; Deniau & Chevalier, 1992; Gerfen, Staines, Arbuthnott, & Fibiger, 1982; Kita & Kitai, 1987; Nakanishi et al., 1987). The thalamus then provides input to the frontal cortex and the dorsal striatum (Hauber, 1998).

Entopeduncular Nucleus

The entopeduncular nucleus is the smallest basal ganglia structure and, along with the substantia nigra pars reticulata, is a major output structure of the basal ganglia. Like the substantia nigra pars reticulata, the entopeduncular nucleus receives GABAergic inhibitory innervation from the striatum (Fonnum et al., 1978; Nagy,

Carter, & Fibiger, 1978; Smith & Parent, 1988) and excitatory innervation from the subthalamic nucleus (Kita & Kitai, 1987; Parent & Smith, 1987; Nakanishi et al., 1987; Smith & Parent, 1988). The substantia nigra pars compacta sends dopaminergic input to the entopeduncular nucleus (Lindvall & Bjorklund, 1979). GABAergic efferents project from the entopedundcular nucleus (Joel & Weiner, 1994) to the thalamus (Carter & Fibiger, 1978; Van der Kooy & Carter, 1981), pedunculopontine nucleus (Nauta, 1979), and superior colliculus (Takada, Tokuno, Ikai, & Mizuno, 1994).

Substantia Nigra Pars Compacta

The substantia nigra pars compacta is a dopamine-rich nucleus that sends projections to the dorsal striatum (nigrostriatal pathway), subthalamic nucleus (nigrosubthalamic pathway), and globus pallidus (nigropallidal pathway), and receives input from multiple brain areas. Dopamine cell bodies in an associated midbrain structure, the ventral tegmental area, project to the ventral striatum (also called the nucleus accumbens). Interestingly, dopaminergic neurons in the substantia nigra pars compacta have a distinguishing feature in that they are able to synthesize, store, and release dopamine at the somatodendritic level (Bustos, Abarca, Campusano,

Bustos, Noriega, & Aliaga, 2004). This feature endows dopamine neurons with exceptional communicating abilities, which might contribute to information processing within the substantia nigra pars compacta (Geffen, Jessell, Cuello, & Iversen, 1976; Jaffe, Marty, Schulte, & Chow, 1998; Korf, Zieleman, & Westerink, 1976; Nieoullon, Cheramy, & Glowinski, 1976).

Dopamine neurons projecting from the substantia nigra pars compacta synapse on GABAergic neurons of the dorsal striatum (Clarke, Dunnett, Isacson, Sirinathsinghji, & Bjorklund, 1988). Stimulation of striatal D2-like receptors inhibits the indirect pathway; whereas, stimulation of D1-like receptors activates the direct pathway (Gerfen, 1992). As mentioned previously, there is some degree of receptor co-localization on GABAergic neurons, because striatonigral neurons express some D2-like receptors, while striatopallidal and striatoentopeduncular neurons express some D1-like receptors (Aizman et al., 2000; Hersch et al., 1995; Surmeier et al., 1992, 1993, 1996).

Functioning of the Basal Ganglia

Overview

The basal ganglia is implicated in the initiation of motor movement as well as other behaviors. The striatum is

the major input structure, which conveys information to the substantia nigra pars reticulata and entopeduncular nucleus via the direct and indirect pathways. The striatum receives glutamatergic innervation from the cortex, and dopaminergic innervation from the substantia nigra pars compacta and ventral tegmental area. This dopaminergic innervation modulates the direct and indirect pathways and is necessary for intact motor initiation and execution.

Basic Basal Ganglia Functioning

The direct (striatonigral) pathway provides direct inhibitory input to the substantia nigra pars reticulata and entopeduncular nucleus, thereby inhibiting GABAergic neurons projecting to motor nuclei in the thalamus, superior colliculus, and pedunculopontine nucleus. Thus, the direct pathway, by disinhibiting motor nuclei, permits motor movement (Gerfen, Engber, Mahan, Susel, Chase, Monsma, & Sibley, 1990). Activation of the indirect pathway via glutamatergic input from the cortex stimulates striatopallidal neurons and disinhibits subthalamic input to the substantia nigra pars reticulata. In this way, the firing rate of GABAergic nigral output neurons is intensified and motor nuclei in the thalamus, superior colliculus, and pedunculopontine nucleus are further inhibited. In the absence of signal, the substantia nigra

pars reticulata is tonically active and provides inhibitory input to these motor nuclei, thus not allowing movement.

Impact of Dopamine on Basal Ganglia Functioning

Striatal D1- and D2-like receptor stimulation alters the firing rate of GABAergic neurons and indirectly modulates motor movement. Specifically, activation of the direct pathway, via D1-like receptor stimulation, decreases the firing rate of tonically active substantia nigra pars reticulata neurons (Chevalier et al., 1985) and further intensifies on-going motor movements. Inhibition of the indirect pathway, via D2-like receptor stimulation, softens (reduces) the inhibition of the globus pallidus. The globus pallidus, becomes progressively more active and inhibits the subthalamic nucleus. This inhibition reduces excitatory output from the subthalamic nucleus and, in turn, reduces the firing rate of GABAergic nigral output neurons. Therefore, through very different mechanisms, D1- and D2-like receptor stimulation inhibits GABAergic neurons of the substantia nigra pars reticulata and permits motor movement.

Behavioral Impact of Basal Ganglia Degeneration

Two very common motor disorders result from the degeneration of basal ganglia cells: Parkinson's disease

and Huntington's chorea. Parkinson's disease is characterized by difficulty in initiating desired motor movements. Damage to the substantia nigra pars compacta leads to the degeneration of the nigrostriatal dopamine pathway and, ultimately, a loss of striatal GABAergic neurotransmission (Albin et al., 1989; Barbeau, 1986). This causes striatal projections to the substantia nigra pars reticulata to become less active (i.e., due to the loss of D1-like receptor stimulation), while projections to the globus pallidus become more active (i.e., due to the loss of D2-like receptor stimulation). The end result is increased excitatory input from the subthalamic nucleus to the substantia nigra pars reticulata and an inhibition of motor movement.

Huntington's chorea is a disease characterized by an excess of motor movements and behavior (Albin et al., 1989; Martin & Gusella, 1986). Striatal projections to the globus pallidus and substantia nigra pars reticulata degenerate (Arregui, Iversen, Spokes, & Emson, 1979; Strittmatter, Lo, Javitch, & Snyder, 1984). This degeneration results in a disinhibition of the globus pallidus, which causes diminished activity in the subthalamic nucleus. The lack of excitatory input to the

substantia nigra pars reticulata allows an excess of motor movement to occur.

CHAPTER TWO

DOPAMINE PHARMACOLOGY

Introduction

Dopamine is a catecholamine neurotransmitter that controls a variety of functions in the mammalian brain and periphery (Missale, Nash, Robinson, Jaber, & Caron, 1998). Dopamine was first synthesized in 1910 by George Barger and James Ewens. In 1952, Arvid Carlsson and Nils-Ake Hillarp established that dopamine acts as a neurotransmitter in brain. Some of the functions mediated by dopamine include motor movement, reward, emotion, cognition, hormone secretion, and renal control (Dziedzicka-Wasylewska 1994; Gingrich & Caron, 1993). Dysregulation of the dopaminergic system has been extensively studied due to its impact on pathological disorders such as Parkinson's disease, schizophrenia, Tourette's syndrome, attention deficit hyperactivity disorder, dopamine dysregulation syndrome, bipolar discrder, and manic depression (Missale et al., 1998). Dopamine is also extensively investigated because of its involvement in the reward mechanisms associated with cocaine, amphetamine, and methamphetamine addiction (Le Foll, Gallo, Le Strat, Lu, & Gorwood, 2009; Missale et al., 1998).

Dopamine Synthesis

The enzyme tyrosine hydroxylase converts the amino acid tyrosine to dihydroxyphenylalanine (DOPA), which is then converted into dopamine by the enzyme DOPA decarboxylase (Roth, 1979; Smidt, Smits, & Burbach, 2003; Sourkes, 1979). Dopamine is also an intermediary step in the synthesis of norepinephrine and epinephrine. Tyrosine hydroxylase is the rate-limiting enzyme in dopamine synthesis. Dopamine is synthesized in the presynaptic terminals of dopaminergic neurons, packaged into synaptic vesicles, and released via calcium-dependent exocytosis (Binder, Kinkead, Owens, & Nemeroff, 2001).

Dopamine Receptor Structure and Subtypes

The dopamine receptor belongs to a class of receptors known as seven transmembrane G protein-coupled receptors (GPCRs) (Missale et al., 1998; Sealfon & Olanow, 2000). Dopamine receptor subtypes are categorized as being D1-like or D2-like (Civelli, Bunzow, & Grandy, 1993; Gingrich & Caron, 1993; Jackson & Westlind-Danielsson, 1994; O'Dowd, 1993). These two families of receptors differ structurally, pharmacologically, and biochemically. Included in the D1-like family are the D_1 and D_5 receptors, while the D2-like family contains the D_2, D_3, and D_4 receptors.

The D1- and D2-like receptor families differ based on their ability to stimulate or inhibit adenylyl cyclase (Dearry, Gingrich, Falardeau, Fremeau, Bates, & Caron, 1990; Kebabian, Petzgold, & Greengard, 1972; Monsma, Mahan, McVittie, Gerfen, Sibley, 1990; Nicola et al., 2000; Onali, Olianas, & Gessa, 1985; Spano, Govoni, & Trabucchi, 1978; Stoof & Kebabian, 1984; Zhou, Grandy, Thambi, Kushner, Van Tol, Cone, Pribnow, Salon, Bunzow, & Civelli, 1990). Dopamine receptors are typically coupled to G proteins (e.g., G_s, G_{olf}, G_i, G_o, G_q and possibly others)(Binder et al., 2001). When D1-like receptors are activated, the bound G protein (G_s or G_{olf}) detaches from the receptor and stimulates adenylyl cyclase. Adenylyl cyclase increases cyclic adenosine monophosphate (cAMP) levels and, in turn, activates protein kinase A (PKA). When D2-like receptors are stimulated, an inhibitory G protein (G_i) is activated, which increases phosphodiesterase levels. Phosphodiesterase breaks down cAMP, thus inhibiting PKA and causing an attenuation of cellular events and responsiveness (Missale et al., 1998).

Distribution of Dopamine Receptors

Dopamine cell bodies are primarily located in the olfactory bulb, ventral tegmental area, hypothalamus, and substantia nigra pars compacta (Snyder, Roberts, &

Sealfon, 1991) and these cells give rise to four major pathways: the nigrostriatal, mesolimbic, mesocortical, and tuberoinfundibular pathways. Not surprisingly, D1- and D2-like receptor mRNA is highly concentrated in the dorsal striatum, nucleus accumbens, and olfactory tubercle (Sealfon & Olanow, 2000). The majority of dopamine receptors are located postsynaptically, at the terminal regions of GABAergic, glutamatergic, serotonergic, cholinergic, and peptidergic neurons (Baldessarini & Tarazi, 1996; Jaber, Robinson, Missale, & Caron, 1996; Tarazi & Baldessarini, 1999; Tarazi, Campbell, Yeghiayan, & Baldessarini, 1998). Presynaptic autoreceptors are found at the terminal regions of dopamine neurons.

Dopamine receptor subtypes differ according to their anatomical distribution and relative abundance. The most abundant dopamine receptor subtype in the central nervous system is the D_1 receptor (Dearry et al., 1990; Weiner, Levey, Sunahara, Niznik, O'Dowd, Seeman, & Brann, 1991). There is no D_1 mRNA in the entopeduncular nucleus and substantia nigra pars reticulata (Dearry et al., 1990; Fremeau et al., 1991; Weiner et al., 1991) even though D_1 receptors are expressed (Gerfen et al., 1990; Le Moine, Normand, & Bloch, 1991). These receptors are mainly present in projections localized on striatal GABAergic

neurons co-expressing substance P (Gerfen et al., 1990; Le Moine et al., 1991). The GABAergic fibers projecting from the striatum to the substantia nigra pars reticulata represent the direct pathway.

The D_5 receptor subtype is far less abundant than the D_1 receptor, with D_5 receptor mRNA detectable in the cerebral cortex, lateral thalamus, diagonal band, dorsal striatum, substantia nigra, medial thalamus, and hippocampus (Choi, Machida, & Ronnekleiv, 1995; Huntley, Morrison, Prikhozhan, & Selfon, 1992; Jackson & Westlind-Danielsson, 1994).

The D_2 receptor subtype is present on medium spiny GABAergic neurons co-expressing enkephalin (Gerfen, 1992; Gerfen et al., 1995; Hersh et al., 1995; Le Moine & Bloch, 1995; Le Moine et al., 1990) and is found mainly in the dorsal striatum, olfactory tubercle, and nucleus accumbens core (Bouthenet, Souil, Martres, Sokoloff, Giros, & Schwartz, 1991). The medium spiny neurons that extend to the globus pallidus (striatopallidal neurons) make up the indirect pathway. D_2 receptors are also co-expressed on neurotensin-containing neurons in the nucleus accumbens shell (Diaz, Levesque, Griffon, Lammers, Martres, Sokoloff, & Schwartz, 1994). To a slightly lesser degree, D_2 receptors have been found in the prefrontal, cingulate,

temporal, and entorhinal cortices, as well as the granule cells of the hippocampal formation (Bouthenet et al., 1991). Interestingly, D_2 receptors are expressed by dopaminergic neurons located in the substantia nigra pars compacta and ventral tegmental area (Bouthenet et al., 1991; Meador-Woodruff, Mansour, Bunzom, Van Tol, Watson, & Civelli, 1989; Weiner et al., 1991). These dopaminergic neurons synthesize, store, and release dopamine at the somatodendritic level (Bjorklund & Lindvall, 1975; Bustos et al., 2004).

D_3 receptors are expressed by substance P and neurotensin-containing neurons that project to the ventral pallidum, olfactory tubercle, and islands of Calleja (Bouthenet et al., 1991; Diaz et al., 1994; Levesque et al., 1992). Few D_3 receptors are present in the dorsal striatum (Bouthenet et al., 1991; Lévesque, Diaz, Pilon, Martres, Giros, Souil, Schott, Morgat, Schwartz, & Sokoloff, 1992; Sokoloff, Giros, Martres, Barthenet, & Schwartz, 1990), while low levels of D_3 receptor mRNA are expressed in dopaminergic neurons located in the substantia nigra pars compacta and ventral tegmental area (Diaz et al., 1994).

In the basal ganglia, only low numbers of D_4 receptors are present in GABAergic neurons of the globus

pallidus and substantia nigra pars reticulata (Mrzljak, Bergson, Pappy, Huff, Levenson, & Goldman-Rakic, 1996). The D_4 receptor, however, is found in abundant quantities in the frontal cortex, amygdala, hippocampus, hypothalamus, mesencephalon, and retina (Cohen, Todd, Harmon, & O'Malley, 1992; O'Malley, Harsmon, Tang, & Todd, 1992; Tarazi, Kula, & Baldessarini, 1997; Van Tol, Bunzow, Guan, Sunahara, Seeman, Niznik, & Civelli, 1991). In the hippocampus and cerebral cortex, D_4 receptors are located on both pyramidal and nonpyramidal GABA interneurons and act to modulate GABAergic transmission (Mrzljak et al., 1996).

CHAPTER THREE

DOPAMINE PHARMACOLOGY: ONTOGENY

Overview

Research on the prenatal and postnatal development of dopamine receptors has mainly focused on the dorsal striatum and nucleus accumbens and has been investigated using multiple techniques (i.e., autoradiography, receptor binding, in situ hybridization, 6-hydroxy-dopamine [6-OHDA] lesions, irreversible receptor antagonism, etc.). Receptor binding and autoradiography use labeled radioactive ligands to determine the tissue distribution of dopamine receptors. Because the data are precise and quantifiable, receptor binding is preferred when a specific brain area is of interest. Autoradiography is the preferred technique when the goal is to visualize the distribution of dopamine receptors across brain.

Prenatal Development of the Dopamine System

Dopaminergic cell bodies are first detectable on embryonic day (E) 12 (Smidt & Burbach, 2007). On E 13, the axons begin to extend and by E 14 they are present in the striatum (Van den Heuvel & Pasterkamp, 2008; Voorn, Kalsbeek, Jorritsma-Byham, & Groenewegen, 1988). Also on E 14, dopamine and tyrosine hydroxylase activity can be

detected in the striatum (Olson & Seiger, 1972; Specht, Pickel, Joh, & Reis, 1981). Segregation of ascending axons is not fully mature until birth (E 21), at which time projections from the midbrain to the dorsal striatum can be differentiated from projections to the ventral striatum (Hu, Cooper, Crockett, & Zhou, 2004). During the late stages of embryonic development (after E 15), dopaminergic axons innervate other forebrain structures such as the prefrontal cortex, hypothalamus, hippocampus, and amygdala (van den Heuvel & Pasterkamp, 2008).

Dopamine projections from the substantia nigra pars compacta to the striatum are evident as early as E 14 (Olson & Seiger, 1972; Specht et al., 1981). Striatal D1-like binding sites are present in low numbers on E 14, E 15, and E 16 (Jung & Bennett, 1996). On E 18, as the striatum is becoming more differentiated, there is a significant increase in D1-like binding sites followed by a decline until birth (Jung & Bennett, 1996; Schambra, Duncan, Breese, Fornaretto, Caron, & Fremeau, 1994). Striatal D2-like binding sites appear on E 14 (Jung & Bennett, 1996; Sales, Martes, Bouthenet, & Schwartz, 1989), increase dramatically until E 18, and then decline from E 18 to E 20 (Jung & Bennett, 1996).

Postnatal Development of the Dopamine System

The dopamine system is not mature at birth and continues to develop across ontogeny. Findings regarding the postnatal development of D1-like receptors have been inconsistent. A linear increase in D1-like receptors has been reported from birth (postnatal day 0; PD 0) into adulthood (Leslie, Robertson, Cutler, & Bennett, 1991; Rao, Molinoff, & Joyce, 1991; Schambra et al., 1994; Zeng, Hyttel, & Murrin, 1988). Alternately, there are reports of a gradual increase in striatal D1-like receptors until approximately PD 35-40, followed by a decline in receptor numbers (pruning) to a level that is sustained into adulthood (Gelbard, Teicher, Faedda, & Baldessarini, 1989; Giorgi, DeMontis, Porceddu, Mele, Calderini, Toffano, & Biggio, 1987). Regardless, researchers agree that receptor stimulation during a critical postnatal period is necessary for the normal development of D1-like receptors (Kostrzewa & Saleh, 1989; Neal-Beliveau & Joyce, 1992; Saleh & Kostrzewa, 1988; Thomas, Neal-Beliveau, & Joyce, 1998). Not surprisingly, the normal development of D1-like receptors is dependent on the presence of endogenous dopamine (Gelbard, Teicher, Baldessarini, Gallitano, Marsh, Zorc, & Faedda, 1990; Neal & Joyce, 1992).

Research on the postnatal development of D2-like receptors has also yielded inconsistent findings. Some studies describe a progressive increase in D2-like binding sites that reach adult-like levels approximately two weeks after birth (Hartley & Seeman, 1983; Murrin & Zeng, 1986; Pardo, Creese, Burt, & Snyder, 1977; Rao et al., 1991; Schambra et al., 1994). D2-like binding sites have also been reported to increase linearly from birth until PD 40, followed by a decline (pruning) into adulthood (Gelbard et al., 1989; Teicher, Anderson, & Hostetter, 1995). Similar to D1-like receptors, normal development of D2-like receptors is dependent on receptor stimulation (Kostrzewa & Saleh, 1989; Neal-Beliveau & Joyce, 1992); however, the availability of endogenous dopamine is not important for D2-like receptor development (Breese, Duncan, Napier, Bondy, Iorio, & Mueller, 1987; Neal-Beliveau & Joyce, 1992).

At birth, D1-like receptor mRNA levels are 75% of adult levels (Schambra et al., 1994). Regardless of these high mRNA levels, [^{3}H]SCH23390 binding is very low (Schambra et al., 1994). The density of D1-like binding sites increases until PD 14 to PD 21, where it remains relatively stable throughout adulthood (Schambra et al., 1994). At birth, D2-like receptor mRNA levels are also

high, but as observed for D1-like receptors, D2-like receptor binding sites are low. Maximum levels of D2-like binding sites are not observed until PD 30 (Schambra et al., 1994).

Ontogeny of Dopamine Systems

Across ontogeny, dopamine systems undergo numerous functional changes. For example, there are age-dependent alterations in dopamine levels and the functioning of release-modulating autoreceptors, there are changes in receptor-mediated adenylyl cyclase activity, and both quantitative and qualitative alterations in drug-induced behaviors. In the first case, striatal dopamine levels increase linearly from birth until approximately PD 35, when adult levels are reached (Walters, Chapman, & Howard, 1990). In terms of release-modulating autoreceptors, SKF38393 (a selective D1-like agonist) evokes significantly less dopamine release in the striatum of preweanling rats than adults, with adult-like functioning not occurring until approximately PD 35 (Walters & Howard, 1990). Indirect dopamine agonists also differentially affect release depending on age, because a low dose of amphetamine increases dopamine release in adult rats, while preweanling rats show an initial increase in dopamine release followed by a persistent decline

(Gazzara, Fisher, & Howard, 1986). Also, methamphetamine causes less dopamine release on PD 14 than PD 21 (Tsuchida, Akiyama, Sakai, Ujike, Li, & Kuroda, 1996). Research shows that synthesis-modulating dopamine autoreceptors are functional during the preweanling period (Andersen, Dumont, & Teicher, 1997; Der-Ghazarian, Charntikov, Varela, Crawford, & McDougall, 2010), with adult-like functioning being achieved by approximately PD 40 (Anderson, 2003; Booth, Baldessarini, Marsh, & Owens, 1994).

Dopamine receptors, as mentioned earlier, belong to a class of G protein coupled receptors that either activate or inhibit adenylyl cyclase. Specifically, D1-like receptor stimulation increases adenylyl cyclase activity, while D2-like receptor stimulation inhibits adenylyl cyclase. When considering striatal structures, D1-like receptors are functional and coupled to adenylyl cyclase by E 17 (De Vries, Mulder, & Schoffelmeer, 1992). D1 mediated adenylyl cyclase activity increases from E 20 to PD 21, at which time activity declines until adult levels are reached at PD 35 (Sakagami, Sawamura, & Kondo, 1995). The functional development of D2-like receptors is much slower, as evidenced by the inability of D2-like receptors to inhibit adenylyl cyclase activity until PD 14 (De Vries

et al., 1992). D2-like mediated adenylyl cyclase activity does not reach adult levels until PD 21 (De Vries et al., 1992).

Dopamine system functioning at the pre- and postsynaptic levels can also be assessed by examining drug-induced changes in behavior. Interestingly, stimulating postsynaptic dopamine receptors can induce different behavioral effects in preweanling and adult rats. For example, the D_3 agonist (+)-PD128,907 increases locomotor activity in 14-day-old rat pups, while attenuating locomotion in adult rats (Heijtz, Ogren, & Fuxe, 2000). (+)-PD128,907 does not induce adult-like behaviors until PD 21, thus indicating that the D_3 receptor is not functionally mature until the second to third postnatal week (Heijtz et al., 2000). Also, administering the nonselective dopamine agonist R-propylnorapomorphine (NPA) into the dorsal striatum of adult animals causes minimal locomotion (Bordi, Carr, & Meller, 1989; Bordi & Meller, 1989); whereas, NPA produces robust locomotor activity in preweanling rats (Charntikov, Halladay, Herbert, Marquez, & McDougall, 2008). This finding suggests that adult-like responsiveness to NPA is not observed until after the preweanling period. Similarly, the D1-like agonist SKF38393 induces grooming

and sniffing throughout the preweanling period (McDougall, Arnold, & Nonneman, 1990; Moody & Spear, 1992), even though SKF38393 does not produce adult-like stereotyped responding until approximately PD 21 (Moody & Spear, 1992).

The ontogeny of presynaptic dopamine receptors has also been investigated by assessing drug-induced behavior. Administering a low dose of a direct dopamine agonist (e.g., quinpirole) decreases the locomotor activity of adult rats (Eilam & Szechtman, 1989; Montanaro, Vaccheri, Dall'Olio, & Gandolfi, 1983), presumably by stimulating release- and synthesis-modulating autoreceptors (for a review. see Starke, Gothert, & Kilbinger, 1989). In contrast, low doses of quinpirole (a selective D_2/D_3 dopamine agonist), apomorphine (a non-selective dopamine agonist), and (+) 3-PPP ([3-(3-hydroxyphenyl)-N-n-propylpiperidine]; a dopamine partial agonist) increase the locomotor activity of preweanling rats (Arnt, 1983; Camp & Rudy, 1987; Kellogg & Lundborg, 1972; Lal & Sourkes, 1973; McDougall et al., 1990; McDougall & Nonneman, 1989; Moody & Spear, 1992; Shalaby & Spear, 1980; Sobrian, Jones, Varghese, & Holson, 2003). In young animals, the ability of apomorphine, (+) 3-PPP, and quinpirole to suppress locomotion is not apparent until

approximately PD 28 or PD 30 (Arnt, 1983; Hedner & Lundborg, 1985; Shalaby & Spear, 1980; Van Hartesveldt, Meyer, & Potter, 1994). The latter finding suggests that dopamine autoreceptors are not functionally mature until after the preweanling period.

CHAPTER FOUR

ADULT DOPAMINE-MEDIATED BEHAVIOR

Introduction

Dopamine agonists and antagonists have been used to elucidate the role played by certain basal ganglia structures (e.g., the dorsal striatum and nucleus accumbens) in mediating the locomotor activity and stereotyped behaviors of animals. Administration of such compounds is achieved by systemic injections or precisely targeted intracranial infusions. Techniques such as brain lesioning and irreversible dopamine receptor antagonism have also been employed to determine the involvement of basal ganglia structures in motoric function. Early behavioral and anatomical studies indicated that the dorsal striatum was important for the expression of stereotyped behaviors, while the nucleus accumbens was important for mediating locomotion (Bordi et al., 1989; Carr & White, 1984; Delfs, Schreiber, & Kelley, 1990; Kelley, Lang, & Gauthier, 1988; Plaznik, Stefanski, & Kostowski, 1989). Further research has shown that there is less behavioral specificity to these structures than originally reported (Canales & Iversen, 1998; Carrera, Brunhara, Schwarting, & Tomaz, 1998; Dias, Carey, & Carrera, 2006; Dickson, Lang, Hinton, & Kelley, 1994;

Koene, Prinssen, & Cools, 1993; Neisewander, Fuchs, O'Dell, & Khroyan, 1998).

Systemic Administration of Dopamine Receptor Agonists

Selective D1- and D2-like receptor agonists differentially affect the unlearned behaviors of adult rats. Selective D2-like agonists, such as quinpirole and RU24213, cause an increase in locomotor activity, sniffing, yawning, and rearing (Arnt, 1987; Clark & White, 1987). In contrast, systemically administering the D1-like partial agonist SKF38393 or the D1-like full agonist LU24-040 does not cause locomotion but preferentially increases grooming behavior; although low levels of locomotion are sometimes observed after SKF38393 (Arnt, 1985, 1987; Dall'Olio, Gandolfi, Vaccheri, Roncada, & Montanaro, 1988; Molloy & Waddington, 1984; Murray & Waddington, 1989; Neisewander, Lucki, & McGonigle, 1991; Serra, Collu, & Gessa, 1987; Starr & Starr, 1987). Interestingly, monoamine depleted and 6-OHDA-treated rats show increased locomotor activity when challenged with SKF38393 (Arnt, 1985; Breese, Baumeister, Napier, Frye, & Mueller, 1985; Breese et al., 1987; Neisewander et al., 1991).

Systemic administration of selective D2-like receptor agonists (i.e., quinpirole or pergolide) affects the locomotor activity of adult rats in a biphasic manner (Bradbury, Cannon, Costall, & Naylor, 1984; Costall, Lim, & Naylor, 1981; Eilam & Szechtman, 1989; Frantz & Van Hartesveldt, 1995; Koller & Herbster, 1988). Specifically, low doses of quinpirole attenuate locomotor activity, while high doses augment locomotion (Eilam & Szechtman, 1989; Frantz & Van Hartesveldt, 1995; Van Hartesveldt et al., 1994). The biphasic behavioral effects of quinpirole are explained by its relative affinity for pre- and postsynaptic D2-like receptors. Specifically, quinpirole has a higher affinity for presynaptic D2-like receptors than for postsynaptic receptors (Eilam & Szechtman, 1989; Van Hartesveldt, Cottrell, Potter, & Meyer, 1992). Therefore, when administered at high doses, quinpirole stimulates postsynaptic receptors and locomotion is increased. When administered at low doses, quinpirole preferentially stimulates presynaptic receptors thereby decreasing dopamine levels and locomotion. High doses of quinpirole induce mild stereotypies, such as sniffing and rearing, even though selective D2-like agonists typically do not produce intense stereotyped behaviors (Arnt,

Hyttel, & Perregaard, 1987; Christensen, Arnt, & Svendsen, 1985; Meller, Bordi, & Bohmaker, 1988).

A full range of stereotyped behaviors are also observed when selective D1- and D2-like agonists are co-administered (Arnt, 1987; Braun & Chase, 1986). When given in combination, the selective D1- and D2-like agonists SKF38393 and quinpirole elicit a dose-dependent increase in locomotor activity, contralateral circling after a unilateral lesion, as well as stereotypies like repetitive grooming and sniffing (Braun & Chase, 1986; Clark & White, 1987; Kashihara, Akiyama, Ishihara, Shiro, & Shohmori, 1996). Co-administration of quinpirole with either the D1-like agonist SKF75670 or LU24-040 causes a dose-dependent increase in licking and occasional biting behavior (Arnt et al., 1987).

Systemic Administration of Nonselective Dopamine Agonists

Nonselective dopamine agonists stimulate both D1- and D2-like receptors. Not surprisingly, co-stimulation of D1- and D2-like receptors induces a pattern of behavior that is similar to, but more intense than, what is observed after D2-like agonist administration (Bradbury et al., 1984; Ljungberg, 1986). At low doses, apomorphine or (+) 3-PPP, like quinpirole, increases the locomotor activity

of adult animals (Bradbury et al., 1984; Costall et al., 1981). At high doses, nonselective dopamine receptor agonists induce more intense behavioral responses. For example, adult rats receiving a high dose of apomorphine exhibit intense stereotyped behaviors, such as sniffing, rearing, gnawing, biting, and licking (Costall & Naylor, 1973; Lepekhina & Tsitsurina, 2007; Ljungberg, 1986; Schiørring, 1971; Szechtman, Ornstein, Teitelbaum, & Golani, 1985). Systemic treatment with either a selective D1- or D2-like receptor antagonist attenuates these stereotyped behaviors (Arnt, 1987; Braun & Chase, 1986).

Intracranial Microinjection of Dopamine Agonists in the Dorsal Striatum

Early studies investigating the neural substrates of unlearned motor behavior indicated that the dorsal striatum is necessary for the expression of stereotyped behaviors (Allen & Winn, 1995; Bordi et al., 1989; Canales, Gilmour, & Iversen, 2000; Carr & White 1984; Kelley et al., 1988; Waszczak, Martin, Finlay, Zahr, & Stellar, 2002). Infusing quinpirole into the dorsal striatum results in moderate stereotyped behaviors, such as head-down sniffing and licking (Canales & Iversen, 1988; Delfs & Kelley, 1990). Intense oral stereotypies, such as biting or gnawing, are not observed when

quinpirole is administered alone. Conversely, infusing SKF38393 (a partial D1-like agonist) into the dorsal striatum does not elicit stereotyped behaviors; whereas SKF82958 (a full D1-like agonist) infusions produce mild stereotypy (Gower & Marriott, 1982; Kreipke & Walker, 2004; Krolewski, Bishop, & Walker, 2005).

Co-stimulation of D1- and D2-like receptors in the dorsal striatum causes more intense behaviors to be expressed. At low doses, the nonselective agonists apomorphine and NPA cause both locomotion and rearing (Bordi et al. 1989; Dickson et al., 1994; Carrera et al., 1998; Dias et al., 2006); whereas, infusing higher doses of apomorphine or a "cocktail" of SKF38393 and quinpirole produces robust stereotypy (Bordi & Meller, 1989; Delfs & Kelley, 1990; Gower & Marriott, 1982; Waszczak et al., 2002). Similarly, microinjecting dopamine or NPA into the dorsal striatum causes minimal locomotor activity, but moderately intense oral and sniffing stereotypies as well as chewing and gnawing (Bordi et al., 1989; Costall, Marsden, Naylor, & Pycock, 1976; Jackson, Anden, & Dahlstrom, 1975; Pijnenburg, Honig, Van der Heyden, & Van Rossum, 1976). This robust stereotypy is similar to what is observed when indirect dopamine agonists, such as amphetamine or cocaine, are infused into the dorsal

striatum (Staton & Solomon, 1984; Waszczak et al., 2002). Therefore, concurrent D1- and D2-like receptor stimulation is necessary for the expression of intense stereotyped behaviors in adult rats (Waszczak et al., 2002).

Intracranial Microinjection of Dopamine Agonists in the Nucleus Accumbens

Research has shown that the nucleus accumbens is at least partially responsible for mediating the locomotor activity of adult rats (Canales & Iversen, 2000; Delfs et al., 1990; Hauber & Münkle, 1997; Neisewander, O'Dell, & Redmond, 1995; Plaznik, et al., 1989; Schildein, Ågmo, Huston, & Schwarting, 1998). Specifically, microinjecting the D1-like agonists SKF38393 or SKF82598 into the nucleus accumbens induces moderate locomotor activity in adult rats (Meyer, 1993; Meyer, Van Hartesveldt, & Potter, 1993; Swanson, Heath, Stratford, & Kelley, 1997). Also, intraaccumbal infusions of the D_2/D_3 receptor agonist quinpirole causes a modest increase in locomotion (Dreher & Jackson, 1989; Gong, Neill, Lynn, & Justice, 1999; Van Hartesveldt et al., 1992; but see Canales & Iversen, 2000; Mogenson & Wu, 1991). Bilateral co-administration of D1- and D2-like receptor agonists into the nucleus accumbens also produces a dose-dependent increase in locomotor activity (Canales & Iversen, 1998; Colle & Wise, 1991;

Dreher & Jackson, 1989; Fog, 1972; Ikemoto, 2002; Kelly, Seviour, & Iversen, 1975; Mrabet, Messier, & Destrade, 1989; Staton & Solomon, 1984).

Dopamine agonists, when given in combination or alone, cause other classes of behavior to be expressed as well. For example, selective D1-like agonists (i.e., SKF38393 and CY208243) increase wall climbing in addition to locomotor activity (Dreher & Jackson, 1989). More importantly, orofacial stereotypies and head-down sniffing are evident when cocaine or quinpirole is microinjected into the nucleus accumbens of adult rats (Canales & Iversen, 1998; Koene et al., 1993; Neisewander et al., 1998). Thus, targeted injections into the nucleus accumbens that stimulate D1-like receptors, D2-like receptors, or both receptor types increases locomotor activity as well as some stereotypies.

Synergistic Interaction between D1- and D2-Like Receptors

Overall, D1-like receptor stimulation in the dorsal striatum causes mild stereotypies, whereas D2-like receptor stimulation causes moderately intense stereotypied behaviors. High intensity stereotyped behaviors are only evident when both D1- and D2-like receptors are co-stimulated (Arnt, 1987; Braun & Chase,

1986). When considered together, these results suggest that both the D1- and D2-like receptor systems must be functional for the full expression of certain dopamine-mediated behaviors (i.e., high-intensity stereotypies). Consistent with this idea, SCH23390 (a selective D1 antagonist) attenuates D2-like agonist-induced behaviors (Arnt, 1985; Breese & Mueller, 1985; Pugh, O'Boyle, Molloy, & Waddington, 1985). Moreover, the behavioral effects produced by co-administering D1- and D2-like agonists can be attenuated by either a D1- or D2-like receptor antagonist (Arnt, 1987; Dreher & Jackson, 1989). Taken together, this evidence is consistent with the hypothesis that the D1 receptor system provides the necessary tonic background activation required for the full manifestation of D2-like receptor-mediated behaviors (Murray & Waddington, 1989).

Summary

In adult rats and mice, the dorsal striatum is largely responsible for mediating the stereotypy-inducing effects of dopamine agonists, while the nucleus accumbens is important for modulating locomotor activity. This dichotomy is not absolute, however, because infusing D1- or D2-like agonists into the nucleus accumbens produces some stereotypy. Conversely, infusing apomorphine, NPA, or

amphetamine into the dorsal striatum induces locomotor activity as well as stereotypy. In sum, the function of the dorsal striatum and nucleus accumbens is not mutually exclusive; multiple neural networks in the basal ganglia are responsible for the behavioral responses induced by dopamine receptor agonists.

CHAPTER FIVE

ONTOGENY OF DOPAMINE-MEDIATED BEHAVIOR

Introduction

The ontogeny of basal ganglia motor systems has not been thoroughly investigated, although dopamine-mediated behaviors often show maturational changes across development (Broaddus & Bennett, 1990; Hedner & Lundborg, 1985; Lin & Walters, 1994). As eveidence of this fact, the behavioral responses elicited by dopaminergic compounds can vary both qualitatively and quantitatively across ontogeny.

Systemic Administration of Selective and Nonselective Dopamine Agonists

From an early age, dopamine systems are responsive to direct and indirect dopamine agonists (for a review, see Spear, 1979). In some cases, dopamine agonists affect the behaviors of young and adult rats in a similar manner. For example, a high dose of apomorphine induces forward crawling and stereotyped tongue protrusions as early as PD 2 (Kellogg & Lundborg, 1972) and causes adult-typical increases in locomotor activity by PD 7 (Shalaby & Spear, 1980). Similarly, administering the indirect dopamine agonist amphetamine on PD 2 causes an initial period of locomotion (lasting 1-2 hr), followed by a phase of tongue

protrusions (Lal & Sourkes, 1973). Amphetamine-induced sniffing, locomotion, grooming, and licking is clearly observable from PD 12 to PD 16, and becomes more continuous and adult-like from PD 18 to PD 30 (Lal & Sourkes, 1973). By PD 35, these behaviors are indistinguishable from those of adults. Consistent with the latter studies, McDougall and colleagues have shown that systemically administered NPA, cocaine, and amphetamine increases both locomotor activity and head-down sniffing on PD 17 (McDougall & Bolaños, 1995; McDougall, Crawford, Nonneman, 1993; McDougall, Rodarte-Freeman, & Nazarian 1999; Nazarian, Rodarte-Freeman, & McDougall 1999).

Consistent with these results using nonselective dopamine agonists, young and adult rats show similar behavioral responses when treated with selective D1- and D2-like agonists. Specifically, high doses of the D1-like partial agonist SKF38393 elicits adult-typical grooming on PD 10 (McDougall, Arnold, & Nonneman, 1990; Moody & Spear, 1992), as well as head-down sniffing throughout the preweanling period (Byrnes & Bruno, 1994; Moody & Spear, 1992; Sobrian et al., 2003). The D2-like agonist quinpirole produces forward locomotion and stereotyped behaviors, such as licking, sniffing, and mouthing, from

at least PD 3 through PD 22 (Brynes & Bruno, 1994; McDougall et al., 1990, Moody & Spear, 1992; Sobrian et al., 2003; Van Hartesveldt et al., 1994). There are, however, some minor discrepancies in the literature because Moody and Spear (1992) reported that quinpirole decreases the incidence of grooming and licking on PD 21, although they did observe increased vertical movements. Quinpirole causes a biphasic locomotor response in both young and adult rats, with high doses attenuating locomotion and low doses augmenting locomotion (Moody & Spear, 1992; Van Hartesveldt et al., 1994). Co-administration of D1- and D2-like agonists on PD 10 causes an increase in forward locomotion, circling, sniffing, and vertical movements (Moody & Spear, 1992). Interestingly, co-stimulation of D1- and D2-like receptors does not induce adult-typical stereotyped licking and biting until PD 21 (Moody & Spear, 1992).

Although dopaminergic drugs typically produce similar effects in young and adult rats, some qualitative and quantitative age-dependent differences have occasionally been observed (for reviews, see Andersen, 2003; Shalaby & Spear, 1980; Spear, 1979; Spear & Brake, 1983). For example, apomorphine-induced stereotyped sniffing is not evident in animals younger than PD 21 (Shalaby & Spear,

1980) and a low dose of apomorphine is unable to suppress locomotor activity until after PD 35 (Shalaby & Spear, 1980). In a similar vein, greater doses of SKF38393 are needed to evoke a grooming response on PD 10 than at older ages (McDougall et al., 1990; Moody & Spear, 1992). Conversely, SKF38393 elicits a more pronounced locomotor response in preweanling rats than adults (Byrnes & Bruno, 1994; McDevitt & Setler, 1981; McDougall et al., 1990; Moody & Spear, 1992; Shieh & Walters, 1996).

Intracranial Microinjection of Selective and Nonselective Dopamine Agonists

Only a few microinjection experiments have been conducted during early ontogeny. Nonetheless, available research shows that dopamine agonists differentially affect the behavior of preweanling and adult rats. On PD 18, microinjecting NPA into the dorsal striatum causes a biphasic increase in locomotor activity (Charntikov et al., 2008). Specifically, a low dose of NPA (5 ug) dramatically increases forward locomotion, whereas higher doses (10 or 20 ug) have a lesser effect. Although not assessed, it is likely that 10 and 20 ug NPA preferentially induced stereotypy, rather than locomotor activity. Consistent with this interpretation, infusing moderate doses of NPA or a "cocktail" of SKF82958 and

quinpirole into the dorsal striatum, enhances both locomotor activity and stereotypy in young rats (Charntikov, Der-Ghazarian, Herbert, Horn, Widarma, Gutierrez, Varela, & McDougall, 2011). In contrast, infusing NPA into the same brain region of adult rats causes minimal locomotor activity and intense oral and sniffing stereotypies (Bordi, Carr, & Meller, 1989).

Ontogenetic behavioral differences are also observed after selective stimulation of D1- and D2-like receptors in the dorsal striatum. In terms of preweanling rats, bilaterally infusing a low dose of SKF82958 (3 ug) into the dorsal striatum preferentially increases locomotor activity, while a high dose of SKF82958 (10 ug) increases stereotypies, such as repetitive motor movements, head-down sniffing, and behavioral intensity scores (Charntikov et al., 2011). Infusing quinpirole into the dorsal striatum also induces both locomotor activity and stereotypy on PD 18 (Charntikov et al., 2011). More specifically, a low dose of quinpirole (10 ug) stimulates greater locomotion than higher doses (20 or 30 ug), whereas high doses of quinpirole induces more stereotypy than lower doses (Charntikov et al., 2011). In comparison, adult rats only exhibit mild stereotypies when a D1 agonist is infused into the dorsal striatum (Kreipke &

Walker, 2004; Krolewski et al., 2005), whereas a more intense stereotypic response results from D2 receptor stimulation (Delfs & Kelley, 1990).

Ontogeny of D1- and D2-Like Receptor Synergism

Ontogenetic studies have consistently shown that coupling between D1- and D2-like receptors is evident from a young age. For example, co-administration of D1- and D2-like agonists elicits synergistic increases in vertical movements, rolling, and curling at PD 3; enhanced forward locomotion by PD 10; and adult-like stereotyped licking at PD 21 (Moody & Spear, 1992).

Consistent with a D1/D2 synergism hypothesis, the D1-like antagonist SCH23390 attenuates quinpirole-induced locomotor activity in both adult and preweanling (PD 11 and PD 17) rats (Arnt et al., 1987; Dall'Olio et al., 1988; McDougall et al., 1990). In terms of microinjection studies, infusing SCH23390 into the dorsal striatum partially attenuates quinpirole-induced locomotor activity on PD 18, thus indicating that tonic D1-like receptor activation is necessary for the full expression of D2-mediated behaviors (Charntikov et al., 2011). Conversely, infusing the D2-like antagonist raclopride into the dorsal striatum completely attenuates SKF82958-induced locomotor activity on PD 18. Taken

together, these data indicate that the D1- and D2-like receptors systems are functionally coupled in an adult-like manner during the neonatal and preweanling periods.

CHAPTER SIX

IRREVERSIBLE DOPAMINE RECEPTOR ALKYLATION

Overview

N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) is an alkylating agent that non-competitively binds to and inactivates various receptors, including D1- and D2-like dopamine receptors, as well as α-adrenergic, serotonin, and GABA receptors (Adler, Meller, & Goldstein, 1985; Arnt & Hyttel, 1988; Hamblin & Creese, 1983; Meller, Bohmaker, Goldstein, & Friedhoff, 1985; Miller, Lumpkin, Galpern, Greenblatt, & Shader, 1991; Nowak, Arnt, & Hyttel, 1988; Saller, Kreamer, Adamovage, & Salama, 1989). Importantly, EEDQ inactivates only receptors, without damaging the neuron as a whole (Hamblin & Creese, 1983; Crawford, McDougall, & Bardo, 1994a, 1994b; Giorgi & Biggio, 1990a). To increase receptor specificity, antagonist pretreatment can be used to selectively protect the receptor of interest. Because of these properties, EEDQ is a useful tool for investigating neural pathways and age-dependent differences in dopamine receptor turnover and recovery (Battaglia et al., 1988; Crawford et al, 1994a, 1994b; Fuxe, Agnati, Merlo Pich, Meller, & Goldstein, 1987; Leff, Gariano, & Creese, 1984; McDougall et al., 1993; Nowak et al., 1988). Irreversible dopamine receptor antagonism has

also been used to study behavior. EEDQ is useful because it allows the effects of dopamine receptor blockade to be assessed without using acute treatment with reversible antagonists (Arnt, Hyttel, & Meier, 1988; McDougall, Crawford, & Nonneman, 1992; McDougall et al., 1993).

Behavioral Effects of EEDQ in Adult Animals

In adult rats, EEDQ has been administered both systemically and intracranially. When EEDQ is administered systemically, it causes catalepsy and attenuates amphetamine-induced stereotypy and locomotor activity (Belleau, Martel, Lacasse, Menard, Weinberg, & Perron, 1986; Hamblin & Creese, 1983; Henry, Joseph, Kochman, & Roth, 1987). EEDQ treatment also disrupts the ability of direct dopamine agonists to increase locomotor activity and decrease grooming (McDougall et al., 1992). For example, adult animals treated with EEDQ show decreased levels of apomorphine-, quinpirole- and NPA-induced sniffing and oral stereotypies, presumably because of reductions in D1- and D2-like receptors (Arnt et al., 1988; Arnt & Hyttel, 1989; Bordi et al., 1989; Cameron & Crocker, 1989; Hamblin & Creese, 1983; Keller & Molina, 1993; Meller, Hizami, & Kreuter, 1989). This attenuated behavioral response persists for 4-8 days, after which normal responsiveness to NPA and other agonists resumes.

The functional properties of dopamine receptors have also been studied after intracranial EEDQ administration. Bilateral infusions of EEDQ (0.5-1.5 ug/side) into the dorsal striatum causes a significant decrease in SKF38393-induced grooming and oral movements (Neisewander, Ong, & McGonigle, 1995). Attenuation of these SKF38393-induced stereotypies does not occur if the D1-like antagonist SCH23390 is administered (IP) prior to EEDQ infusions. In other words, pretreating rats with SCH23390 selectively protects D1-like receptors from the alkylating effects of EEDQ. This pattern of results suggests that SKF38393-induced grooming and oral behaviors are partially mediated by the D1-like receptor (Neisewander et al., 1995).

Consistent with the previous results, infusing EEDQ unilaterally into the dorsal striatum diminishes NPA-induced behaviors such as sniffing, licking, and biting (Bordi et al., 1989). Apomorphine-induced head-down sniffing was also attenuated after bilateral infusions of EEDQ into the anterior/dorsal portion of the dorsal striatum, but not the dorsal/posterior portion (Cameron & Crocker, 1989). In terms of selective D2-like agonists, systemic administration of quinpirole produces ipsilateral circling in adult rats given unilateral EEDQ infusions

into the dorsal striatum (Giorgi & Biggio, 1990b). The latter effect is mediated exclusively by D2-like receptors, because quinpirole-induced circling was only evident when D2-like receptors, but not D1-like receptors, were inactivated on a given side (Giorgi & Biggio, 1990a). More specifically, quinpirole-induced circling was not apparent if D2-like receptors were protected from EEDQ; whereas, protecting D1-like receptors did not impact circling (Giorgi & Biggio, 1990a).

Behavioral Effects of EEDQ in Preweanling Animals

EEDQ produces dramatically different behavioral effects during the preweanling period. Unlike what is observed in adult rats, NPA-induced locomotor activity is not blocked by EEDQ administration on PD 11 or PD 17 (McDougall et al., 1992). Likewise, EEDQ does not attenuate NPA-induced reductions in grooming behavior (McDougall et al., 1992). These paradoxical effects are not unique to NPA, because the SKF38393- and quinpirole-induced behaviors of preweanling rats are also not attenuated by EEDQ (McDougall et al., 1992, 1993; Mestlin & McDougall, 1993). Specifically, preweanling rats continue to exhibit normal NPA- and quinpirole-induced

locomotor activity and SKF38393-induced grooming after EEDQ pretreatment.

Interestingly, EEDQ does block amphetamine-induced locomotor activity in both preweanling and adult rats (Crawford et al., 1994b). In preweanling rats, this amphetamine-induced effect is not dopamine mediated, because behavior was not preserved if D1- and D2-like receptors were protected with SCH23390 and sulpiride. In contrast, amphetamine-induced increases in locomotion were still evident in EEDQ-treated adult rats that had been pretreated with SCH23390 and sulpiride (Crawford et al., 1994b). Thus, EEDQ depresses amphetamine-induced behaviors in both age groups, but only in adult rats is this effect mediated by dopamine receptors. When these results are considered together, it appears that EEDQ produces qualitatively different behavioral effects in preweanling and adult rats. In preweanling animals, EEDQ does not attenuate behaviors stimulated by direct D1- and D2-like agonists, whereas EEDQ blocks SKF38393- and quinpirole-induced behaviors in adult rats.

As just mentioned, various studies have examined the effects of systemically administering EEDQ during the preweanling period, however only a single experiment has assessed the behavioral impact of microinjecting EEDQ into

specific brain regions of the preweanling rat. In that study, Charntikov et al. (2011) bilaterally infused EEDQ (100 µg) into the dorsal striatum of PD 17 rats and then measured NPA-induced behaviors 24 hr later (i.e., on PD 18). Unexpectedly, EEDQ-pretreated rats showed a potentiated locomotor response when 5, 10, or 20 µg NPA was infused into the dorsal striatum. This result differs importantly from earlier ontogenetic work, because systemically administered EEDQ was reported to leave the dopamine-mediated behaviors of preweanling rats unaffected (Crawford et al., 1994b; McDougall et al., 1992, 1993; Mestlin & McDougall, 1993). In contrast, microinjecting EEDQ into the dorsal striatum produced receptor changes that permitted an exaggerated behavioral response after NPA treatment (Charntikov et al., 2011). It is unknown whether NPA's behavioral effects are caused by EEDQ-induced changes in dopamine receptors or some other receptor type.

Neurochemical Effects of EEDQ in Adult and Preweanling Animals

In adult animals, systemic administration studies have shown that EEDQ inactivates a substantial proportion of dopamine receptors. On PD 90, systemic treatment with 7.5, 15, or 25 mg/kg EEDQ reduced dorsal striatal D1-like

binding sites by 86%, 86%, and 93%, while D2-like binding sites were reduced by 80%, 82%, and 92%, respectively (Crawford, McDougall, Rowlett, & Bardo, 1992). Similarly, 6 mg/kg EEDQ (IP) reduced D1- and D2-like binding sites in the dorsal striatum of adult rats by 70%-82% (Meller et al., 1985). In the latter study, pretreatment with sulpiride and SCH23390 preferentially protected D1- and D2-like binding sites from EEDQ-induced receptor inactivation (Meller et al., 1985). More recent studies have also shown that peripheral administration of EEDQ causes substantial reductions of both D1- and D2-like receptors on the order of 70-80% and 53-75%, respectively (Kula, George, & Baldessarini, 1992; Riddall, 1992; Rosengarten, Schweitzer, & Friedhoff, 1993; Undie, Berki, & Beardsley, 2000; Zou, Cai, & Jin, 1996). In terms of receptor recovery, D1-like receptor binding sites in the dorsal striatum were reduced by more than 90% when measured 6 hr after EEDQ treatment, however D1-like receptors returned to basal levels after 8 days (Giorgi, Pibiri, & Biggio, 1991; Giorgi, Pibiri, Dal Toso, & Ragatzu, 1992).

Microinjecting EEDQ into the dorsal striatum causes a less robust reduction of D1- and D2-like receptors than when EEDQ is systemically administered. For example,

bilateral administration of EEDQ into the dorsal striatum of adult rats reduces D1-like receptors by as much as 44% at the injection site and up to 32% when measured 1 mm away (Neisewander et al., 1995). In a similar vein, unilateral EEDQ treatment decreases D1- and D2-like receptors by 48% and 51%, respectively, with receptors returning to basal levels after 7 days (Giorgi & Biggio, 1990a). Autoradiographic studies have also shown that both peripheral and central administration of EEDQ causes a significant loss of striatal D1- and D2-like receptors (Cameron & Crocker, 1989; Cox & Waszczak, 1993; Zhang, Tarazi, Kula, Baldessarini, & Neumeyer, 1996; Zhang, Weiss, Tarazi, Kula, & Baldessarini, 1999).

In young animals, systemic administration of EEDQ causes a potent inactivation of dorsal striatal dopamine receptors. Specifically, when measured on PD 17 (24 hr after EEDQ treatment), D1-like binding sites in the dorsal striatum were reduced by 69-79% depending on EEDQ dose (7.5-25 mg/kg) (Crawford et al., 1992). Likewise, 7.5, 15, and 25 mg/kg EEDQ caused a 61%, 64%, and 65%, respectively, reduction in dorsal striatal D2-like binding sites (Crawford et al., 1992). Similarly, administering 10 mg/kg EEDQ to PD 24 rats reduced the number of D1- and D2-like receptors by approximately 74% (Kula et al.,

1992). To date, no microinjection studies have assessed EEDQ-induced dopamine receptor inactivation in preweanling rats.

CHAPTER SEVEN

THESIS STATEMENT

Conclusion

The ontogeny of dopamine-mediated motor systems has not been thoroughly investigated in young animals. Generally, research has shown that young and adult animals respond similarly to D1- and D2-like agonists. However, some behaviors vary across ontogeny due to maturational changes in dopamine systems (Broaddus & Bennett, 1990; Hedner & Lundborg, 1985; Lin & Walters, 1994). Although much is known about the effects of irreversible dopamine receptor inactivation in adult animals, the effects of EEDQ on dopamine-mediated locomotor activity during early ontogeny is not fully understood.

Systemic administration of EEDQ attenuates dopamine agonist-induced behaviors of adult rats, while leaving the behaviors of young rats unaffected. Research from our laboratory has shown that dorsal striatal infusions of NPA (5, 10, or 20 µg) cause a robust increase in the locomotor activity of EEDQ-treated preweanling rats (Charntikov et al., 2011). This effect is opposite to what is observed in adult rats and suggests that the receptors mediating this paradoxical behavioral effect are differentially affected by EEDQ.

The overall goals of this thesis were three-fold. First, to investigate the effects of intrastriatally administered EEDQ on NPA-mediated behaviors in both adult and preweanling rats. Second, to use receptor protection experiments to determine which receptor type is responsible for EEDQ's paradoxical behavioral effects in preweanling animals. Third, to assess the magnitude of EEDQ-induced D1- and D2-like receptor inactivation in both adult and preweanling rats through autoradiography and receptor binding experiments.

Proposed Hypotheses

In Experiment 1, I tested whether infusing EEDQ into the dorsal striatum of adult rats blocks NPA-induced locomotor activity. Since previous research has shown that systemic EEDQ treatment blocks NPA-induced locomotion in adult rats, it was hypothesized that microinjecting EEDQ into the dorsal striatum would block NPA-induced locomotor activity (Bordi et al., 1989; Hamblin & Creese, 1983; Meller et al., 1989). Experiment 2 tested whether EEDQ paradoxically increases the NPA-induced locomotor activity of preweanling rats. In addition, I examined whether dopamine receptors were responsible for EEDQ-induced changes in the NPA-induced locomotor activity of preweanling rats. It was hypothesized that D1- and D2-like

receptors in the dorsal striatum are responsible for mediating NPA-induced locomotor activity. Because dopamine receptors were responsible for mediating EEDQ's effects in preweanling rats I conducted a third experiment. In Experiment 3, I tested whether alterations in D1-like, D2-like, or both D1- and D2-like receptors underlie the EEDQ-induced potentiated locomotor response to NPA. It was hypothesized that both D1- and D2-like receptors are responsible for the EEDQ-induced locomotor activity of NPA-treated rats.

In Experiments 4 and 5, receptor binding and autoradiography assays were used to quantify D1- and D2-like receptor loss after EEDQ infusions in preweanling and adult animals. It was hypothesized that intrastriatal infusions of EEDQ would cause a substantial reduction of D1- and D2-like receptor binding sites in preweanling and adult animals. It has already been established that systemic injections of EEDQ cause robust declines in both D1- and D2-like receptors in preweanling and adult rats (Crawford et al., 1992; Giorgi et al., 1991, 1992; Meller et al., 1985).

CHAPTER EIGHT

GENERAL METHODS

Subjects

Subjects were 252 male and female rat pups of Sprague-Dawley descent (Charles River Laboratories, Hollister, CA, USA), born and raised at California State, San Bernardino (CSUSB). Litters were culled to 10 rat pups by postnatal day (PD) 3 (day of parturition is PD 0). Rats tested on PD 18 were kept with the dam. Adult male rats (n=92) were obtained from Charles River Laboratories and arrived on PD 59. All rats were group housed until time of surgery. After surgery, adult rats were housed singly, whereas preweanling rats were returned to the dam and littermates. An approximately equal number of male and female preweanling rats were randomly assigned to each group. Only male rats were used for experiments conducted during adulthood.

The colony room was maintained at 22-24°C and kept under a 12 hr dark/light cycle, with behavioral testing occurring during the light phase of the cycle. Food and water was freely available. Subjects were treated according to the "Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research

Council, 2003) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

Apparatus

Behavioral testing was performed in commercially available activity monitoring chambers (Coulbourn Instruments, Allentown, PA, USA) housed in a testing room separate from the animal colony. The activity chambers have acrylic walls, a gray plastic floor, and an open top. Each chamber included an X-Y photobeam array, with 16 photocells and detectors, that was used to measure horizontal locomotor activity (distance traveled). Photobeam resolution was 0.76 cm. The position of each rat was determined every 100 ms (i.e., the sampling interval). To somewhat control for differences in body size, preweanling rats were placed in smaller chambers (25 × 25 × 41 cm) than adult rats (41 × 41 × 41 cm). In all other regards, the different sized chambers were identical.

Drugs

Sulpiride and SCH23390 were dissolved in saline and administered intraperitonealy (IP) to preweanling rats at a volume of 5 ml/kg. R(-)-propylnorapomorphine hydrochloride (NPA) was dissolved in distilled water containing 0.1% metabisulfite (an antioxidant) and

administered intracranially at a volume of 0.5 µl per side. N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was dissolved in DMSO and injected intracranially at a volume of 0.75 µl per side (Giorgi & Biggio, 1990b). All drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Statistical Analysis

Analysis of variance (ANOVA) were used for the statistical analysis of locomotor activity data and to quantify receptor loss. Statistically significant higher order interactions were further analyzed by one- or two-way ANOVAs and followed, when necessary, by Tukey tests ($P<.05$). The Huynh-Feldt epsilon statistic was used to adjust the degrees of freedom (Huynh and Feldt, 1976) when the assumption of sphericity was violated (determined by Mauchly's test of sphericity). Corrected degrees of freedom are represented by a superscripted "a" and rounded to the nearest whole number.

Litter effects were minimized by assigning precisely one subject from each litter to a particular group (for a discussion of litter effects, see Zorrilla, 1997). Unlike adults, prepubescent rats do not typically exhibit sex differences after treatment with dopamine agonists (see also Bowman et al., 1997; McDougall, Garmsen, Meier, &

Crawford, 2007; Scalzo & Holson, 1992; Snyder et al., 1998). Therefore, both males and females were tested at PD 18 and statistically significant sex effects were not evident. Male rats will be exclusively used at PD 85.

CHAPTER NINE

BEHAVIORAL PROCEDURES

Intracranial Cannulation Surgery

Cannulae implantation surgeries were performed on PD 16 and PD 83 (two days prior to behavioral testing). At both ages, anesthesia was induced by administering isoflurane (5%/5min in 100% oxygen during the induction phase, 2-3% in 100% oxygen during the maintenance phase) via a nose mask. Prior to surgeries, all rats were given a topical lidocaine solution (1%) and ketoprofen (2 mg/kg, IP) for pain management. A Kopf stereotaxic apparatus was used, with preweanling rats requiring a Cunningham Neonatal Rat Adapter for proper positioning. Stereotaxic coordinates for the dorsal striatum have been obtained from the developing rat brain atlas of Sherwood and Timiras (1970) for preweanling animals and from the rat brain atlas of Paxinos and Watson (1998) for adult rats.

Two craniotomies were performed and either a stainless steel double guide cannula (preweanling rats) or two single guide cannulae (adult rats) were implanted in the dorsal striatum of preweanling rats (A +6.5, L ±2.4, V -5.6 from interaural line) and adult rats (A +0.20, L ±3.1, V -5.7 from bregma). Both sets of guide cannulae (22-gauge; Plastics One, Roanoke, VA) were implanted 1 mm

above the target location. In preweanling rats, bilateral guide cannulae were anchored to the scull using cyanoacrylate gel followed by dental cement (Lang Dental, Wheeling, IL, USA). For adult rats, individual guide cannulae were fixed in place using two stainless steel anchor screws and cyanoacrylate gel followed by dental cement. In both age groups, stainless steel stylets (Plastics One) were used to seal the guide cannula until time of testing. Following cranioplasty, all rats were sutured and placed in a heated incubation chamber for 2-4 hr. Post-operative monitoring was performed in order to assess subject responsiveness. All rats underwent behavioral assessment 48 hr after surgery.

Microinjection Procedure

Stainless steel stylets were replaced by infusion cannulae (28-gauge; Plastics One), which extend 1 mm below the tip of the guide cannula. Infusion cannulae were attached via polyethylene tubing (28 mm; Becton Dickinson, Sparks, MD, USA) to Hamilton microsyringes (10 µl) controlled by dual infusion pumps (World Precision Instruments, Sarasota, FL, USA). Cannulae were left in place for 1-2 min and then the drug was delivered at a constant rate over a 60 s period. Following microinjection, cannulae were left in place for an

additional 1-2 min after which rats were returned to the activity monitoring chambers. Distance traveled (a measure of horizontal locomotor activity) was assessed continuously across the 80-min session (i.e., 40 min of habituation and 40 min of drug testing).

Procedures

Experiment 1. Effects of EEDQ on NPA-Mediated Behaviors of Adult Rats

Overview. The first experiment was conducted to determine whether intrastriatal infusions of EEDQ are capable of blocking NPA-induced locomotor activity in adult rats. This was a between-subject design with two independent variables: condition (EEDQ or DMSO) and post-drug (0 or 20 µg NPA). The dependent variable was distance traveled scores, which was assessed in automated activity chambers. A total of 33 rats (n = 8-9 per group) were used in Experiment 1 (Figure 2, Top Panel).

Methodology. On PD 84 (24 hr after surgery), rats received bilateral infusions of DMSO or EEDQ (100 µg; 0.75 µl per side). After an additional 24 hr (i.e., on PD 85), EEDQ- and DMSO-treated rats were placed in the automated locomotor activity chambers for 40 min. Immediately

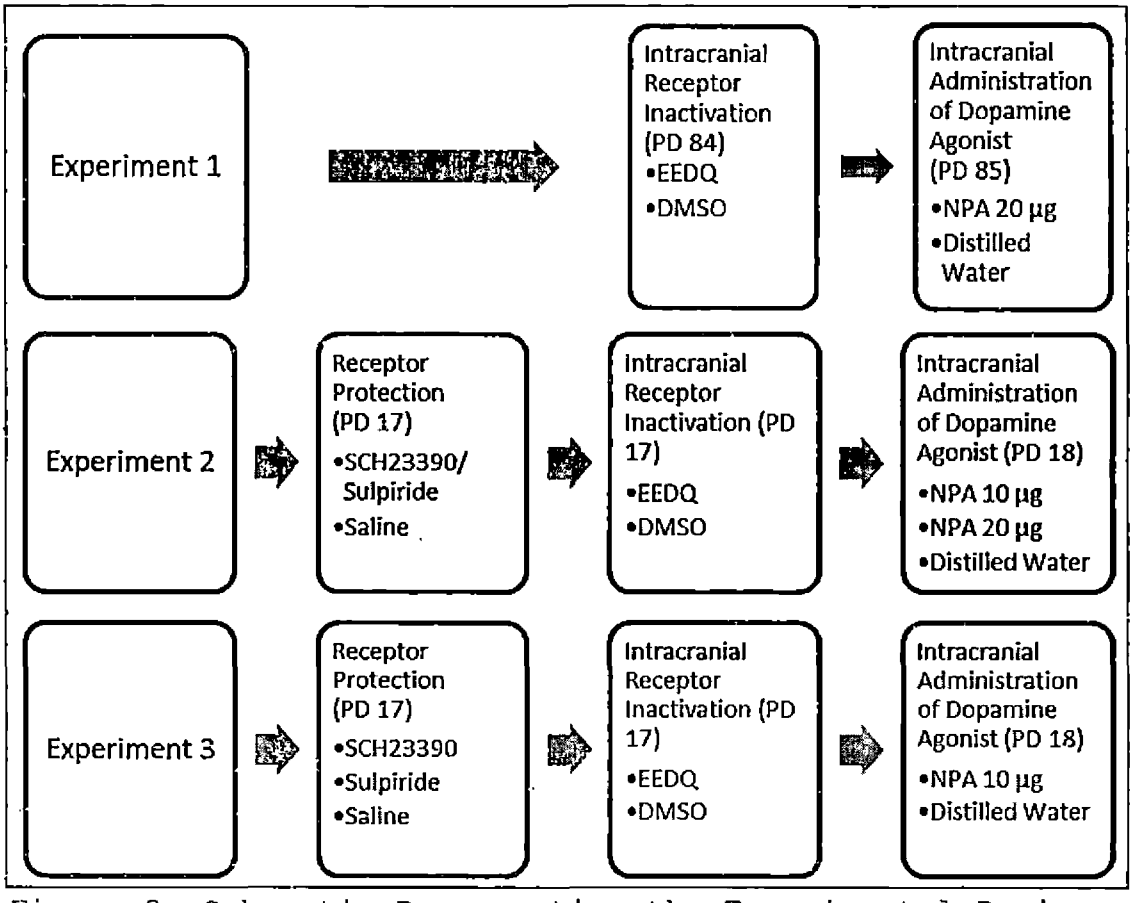


Figure 2. Schematic Representing the Experimental Designs Used in Experiment 1 (2 × 2 design), Experiment 2 (2 × 2 × 3 design), and Experiment 3 (3 × 2 × 2 Design). SCH23390, Selective D1 Antagonist; SULPIRIDE, Selective D2 Antagonist; EEDQ, Irreversible Monoamine Receptor Alkylating Agent; NPA, Nonselective Dopamine Agonist

following this 40 min baseline period, rats were microinjected with either distilled water or NPA (20 µg; 0.50 µl per side) into the dorsal striatum. Rats were then returned to the activity chambers for another 40 min, where locomotor activity was recorded. After behavioral testing, all subjects underwent histological examination.

Experiment 2: Role of D1- and D2-Like Striatal Receptors in EEDQ-Mediated Behaviors of Preweanling Rats

Overview. The second experiment was conducted to determine whether intrastriatal infusions of EEDQ block NPA-induced locomotor activity in preweanling rats. This experiment was also done to determine whether dopamine receptors located in the dorsal striatum mediate EEDQ-induced alterations in locomotor activity. This was a between-subject design with three independent variables: pretreatment group (combination of SCH23390/sulpiride or saline), condition (EEDQ or DMSO), and post-drug (e.g., 0, 10, or 20 µg NPA). The dependent variable was distance traveled scores. A total of 96 rats (n = 8 per group) were used in Experiment 2 (Figure 2, Middle Panel).

Methodology. On PD 17 (24 hr after cannulae implantation), different groups of rats were systemically treated (IP) with saline or a combination of SCH23390 (1 mg/kg) and sulpiride (100 mg/kg) 30 and 60 min,

respectively, before receiving intracranial drug infusions (Cameron & Crocker, 1989; Crawford et al., 1992; Giorgi & Biggio, 1990b). Groups were further subdivided with saline- or SCH23390/sulpiride-protected rats receiving bilateral infusions of EEDQ (100 µg; 0.75 µl per side) or DMSO into the dorsal striatum.

On PD 18 (i.e., 24 hr after EEDQ or DMSO treatment), preweanling rats were habituated to the automated testing chambers for 40 min. Rats then received dorsal striatal infusions of either distilled water or NPA (10 or 20 µg; 0.50 µl per side) and locomotor activity was assessed for an additional 40 min. All rats underwent histological examination upon completion of behavioral testing.

Experiment 3. Effects of D1- or D2-Like Striatal Receptor Inactivation on EEDQ-Induced Behaviors of Preweanling Rats

Overview. Experiment 2 showed that EEDQ's actions are mediated by dopamine receptors and therefore a third experiment was conducted. Experiment 3 was done to determine whether D1-like, D2-like, or both D1- and D2-like receptors located in the dorsal striatum mediate the EEDQ-induced behavioral effects of preweanling rats. This was a between-subject design with three independent variables: pretreatment group (SCH23390, sulpiride, or saline), condition (EEDQ or DMSO), and post-drug

(distilled water or NPA). The dependent variable was distance traveled scores. A total of 104 rats (n = 8 per group) were used in Experiment 3 (Figure 2, Bottom Panel).

Methodology. On PD 17, all rats received a systemic injection of SCH23390 (1 mg/kg), sulpiride (100 mg/kg) or saline. SCH23390 was administered 30 min prior to EEDQ (100 µg; 0.75 µl per side) or DMSO infusions, whereas sulpiride was injected 60 min before (Cameron & Crocker, 1989; Crawford et al., 1992; Giorgi & Biggio, 1990b). On PD 18 (i.e., 24 hr after EEDQ or DMSO treatment), preweanling rats were habituated to the automated testing chambers for 40 min. Rats then received dorsal striatal infusions of either distilled water or NPA (10 µg; 0.50 µl per side) and locomotor activity was assessed for an additional 40 min. All rats underwent histological examination upon completion of behavioral testing.

Histology

After behavioral testing, rats were given an overdose of sodium pentobarbital and brains were fixed in a 4% paraformaldehyde solution for 48-72 hr. Brains were then cryoprotected in a 20% sucrose solution (24-48 hr), sectioned coronally (70 µm) using a cryostat, and stained with thionin. Histological assessment of cannulae placement was performed by an observer blind to

experimental conditions. Cannulae placement was graphically mapped on coronal sections taken from Paxinos and Watson's *The rat brain: in stereotaxic coordinates* (1998). Only subjects with accurate cannulae placements were included in the statistical analysis. Cannula placements of rats included in the statistical analyses are shown in Appendix A.

Statistical Analysis

For Experiment 1, there were two independent variables: a) condition (EEDQ or DMSO) and b) post-drug (distilled water or 20 µg NPA) (Figure 2). Because of ongoing experimental manipulations, separate ANOVAs were used to analyze time blocks 1-8 (habituation) and time blocks 9-16 (agonist testing). Specifically, the first eight time blocks (0-40 min) were analyzed using a 2 × 8 (condition × time block) repeated measures ANOVA; whereas, time blocks 9-16 (40-80 min) were analyzed using a 2 × 2 × 8 (condition × post-drug × time block) repeated measures ANOVA.

For Experiments 2 and 3, there were three independent variables: a) receptor protection (pretreatment groups), b) condition (EEDQ and DMSO), and c) post-drug (distilled water and NPA). Time blocks 1-8 were analyzed using a 2 × 2 × 8 (Experiment 2) and 3 × 2 × 8 (Experiment 3)

(pretreatment × condition × time block) repeated measures ANOVA. Time blocks 9-16 were analyzed using a 2 × 2 × 3 × 8 (Experiment 2) and 3 × 2 × 2 × 8 (Experiment 3) (pretreatment × condition × post-drug × time block) repeated measures ANOVA.

CHAPTER TEN

NEUROCHEMICAL PROCEDURES

Intracranial Infusion Surgery

The procedures for this surgery were similar to those of the intracranial cannulation surgeries, but with the following exceptions. Bilateral burr holes were made above the dorsal striatum on PD 17 or PD 84 using the following coordinates: A +6.5, L ±2.4, V -6.6 from the interaural line (preweanling rats) and A +0.20, L ±3.1, V -6.7 from bregma (adult rats). Needles were slowly lowered into the dorsal striatum and bilateral injections of EEDQ or DMSO (0.75 ul) were made over 2 min. The syringe was left in place for 2 min prior to and after the infusions. The burr holes were sealed using Gelfoam (Upjohn, Kalamazoo, MI, USA). All rats were sutured, given ketoprofen (2 mg/kg IP), and placed in incubators for 2-4 hr prior to being returned to their home cages.

Experiment 4. Quantitative Autoradiography

A total of 20 adult and 20 preweanling rats (n = 5 per group) were used. Rats of both age groups received bilateral infusions of EEDQ (100 µg; 0.75 µl) or DMSO (Figure 3). Animals were decapitated and brains were rapidly removed 24 hr after surgery (i.e., on PD 18 and

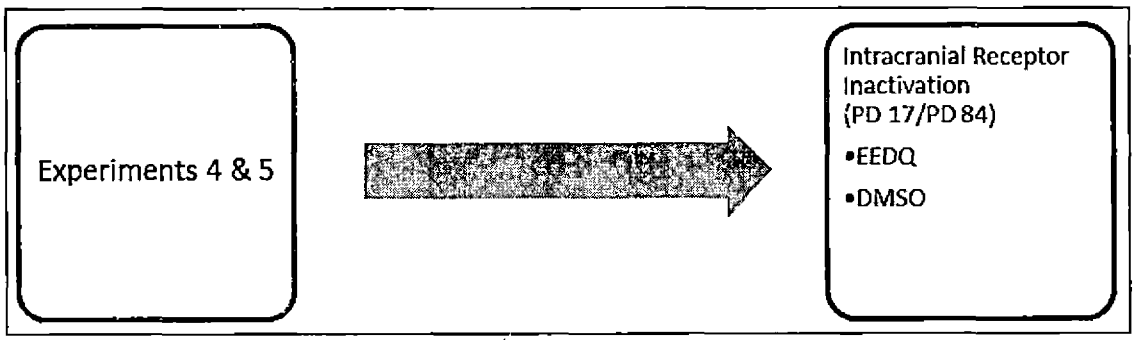


Figure 3. Schematic Representing the Experimental Design Used in Experiment 4 and Experiment 5 (EEDQ vs. DMSO). EEDQ, Irreversible Monoamine Receptor Alkylating Agent

PD 85). Once extracted, brains were immediately immersed in isopentane at -30°C for 2 min to promote uniform freezing, and then stored at -80°C until time of assay.

Brains were sectioned in the coronal plane at a thickness of 20 µm at -18°C. Sections were thaw-mounted onto electrostatically coated slides (Superfrost Plus; Fisher Scientific, Pittsburgh, PA), air-dried under vacuum, and stored at -80°C until assayed for radioligand binding. At time of assay, sections were thawed at room temperature for 5 min and preincubated in 50 mM Tris HCl (pH 7.4) for 30 min. Slides were incubated in 50 mM Tris buffer containing either 2 nM [^{3}H]SCH23390 (to label D1-like receptors) or 0.15 nM [^{3}H]spiperone (to label D2-like receptors) for 30 min at room temperature. Nonspecific binding was determined in the presence of 10 µM (+)butaclamol (for the D1 assay) or 10 µM (-)-sulpiride (for the D2 assay). After labeling, sections were washed in ice cold Tris buffer (3 washes for 20 s) and then dried under a stream of cold air. The sections were apposed to [^{3}H]-sensitive along with calibrated standards ([^{3}H]microscales; GE Healthcare, Piscataway, New Jersey, USA) for 10 weeks at -20°C. Following the exposure period, autoradiograms were analyzed using a computer-assisted image analyzer (MCID, InterFocus

Imaging, Cambridge, England). Optical density was converted into nCi/mg of radioligand bound using a standard curve as a reference. Cannula placements were determined during tissue sectioning by drawing the cannula tracts at each plate of the atlas of Paxinos and Watson (1986) for adult rats and Sherwood and Timiras (1970) for preweanling rats. The lowest point of the tract was estimated as the site of infusion.

Experiment 5. D1- and D2-Like Homogenate Ligand Binding Conditions

All rats received bilateral infusions of either EEDQ or DMSO on PD 17 or PD 84 (Figure 3). On PD 18 or PD 85 (24 hr after surgery), 71 rats (n = 5-6 per group; 23 adult and 48 preweanling rats) were killed by rapid decapitation and dorsal striatal sections were dissected bilaterally and stored at -80°C until time of assay. Homogenates from two PD 18 rats were combined to serve as a single subject for both the D1 and D2 assay. On assay day, tissue was thawed on ice and crude membrane homogenates were made using the following protocol. Striatal sections from each rat were homogenized in 100 volumes of 50 mM Tris-HCl buffer (pH 7.4) for approximately 20 s using a Brinkmann Polytron. The homogenates were centrifuged at 20,000 × g for 20 min. The

pellet was resuspended in 100 volumes of the same buffer and centrifuged again at 20,000 × g for 20 min.

The final pellet was suspended in approximately 30 volumes of buffer (pH 7.4). Protein concentrations for the final pellet were determined using the Bio-Rad Protein Assay, with BSA as the standard. For both D1- and D2-like receptor binding, tissue suspensions (15-30 µg/protein) were added to duplicate tubes containing 50 mM Tris, 2 mM $NaCl_2$, 5 mM KCl, 1 mM $MgSO_4$, and 2 mM $CaCl_2$ (pH 7.4) at a final volume of 1 ml. Concentrations ranging from 0.1-5.0 nM of [^{3}H]SCH23390 (for the D1 assay) or 0.05-0.9 nM [^{3}H]spiperone (for the D2 assay) were added to the tubes. For the D1 assay, 100 nM of mianserin was also added to the tubes to prevent binding of [^{3}H]SCH23390 to serotonin receptors. Due to the specificity of sulpiride, mianserin was not used in the D2 assay (Boyson, McGonigle, & Molinoff, 1986). The incubation time was 30 min at 37°C. Incubation was terminated by vacuum filtration over glass fiber filters (Whatman GF/B, pretreated with 0.1% polyethylenimine). Filters were washed twice with ice-cold Tris-HCl buffer and radioactivity was measured by liquid scintillation spectrometry. Non-specific binding was determined in the presence of 10 µM (+)-butaclamol (for D1 assay) or 10 µM (-)-sulpiride (for D2 assay). Specific

binding was defined as the difference in [^{3}H]SCH23390 bound in the presence and absence of 10 µM (+)-butaclamol or as the difference in [^{3}H]spiperone bound in the presence and absence of 10 µM (-)-sulpiride.

Statistical Analysis

For Experiment 4, autoradiograms were analyzed using t-tests (condition: EEDQ and DMSO) at each age to assess D1- and D2-like receptor densities. Additionally, separate 2 × 2 (condition × age) ANOVAs were conducted to analyze receptor densities across ontogeny. For the homogenate binding experiments (Experiment 5), D1- and D2-like receptor binding sites (B_{max}) and affinity (K_D) data from the homogenate ligand binding assays was determined using nonlinear regression with Prism (GraphPad Software, San Diego, CA, USA). B_{max} and K_D values were analyzed using *t*-tests (condition: EEDQ and DMSO) at each age. Additionally, separate 2 × 2 (drug × age) ANOVAs were used to analyze D1- and D2-like receptor B_{max} and K_D across ontogeny.

CHAPTER ELEVEN

RESULTS

Experiment 1

Effects of EEDQ on the NPA-Induced Behaviors of Adult Rats

Habituation Phase. During the chamber habituation phases (i.e., time blocks 1-4 and 5-8), EEDQ-treated adult rats had significantly lower distance traveled scores than controls (Figure 4, left panel) [Condition main effects, $F(1,31) = 20.02$, $P < 0.001$; $F(1,31) = 19.90$, $P < 0.001$, respectively]. Distance traveled scores were elevated immediately after rats were placed in the activity chambers, but scores then declined to a stable baseline on time block 7 [[a]Time Block main effects, $F(3,79) = 116.41$ $P < 0.001$; $F(3,93) = 38.68$, $P < 0.001$, respectively, and Tukey tests].

Testing Phase. During the testing phase (i.e., time blocks 9-16), bilateral infusions of NPA (20 µg) caused a significant increase in distance traveled scores for only the DMSO-treated rats (Figure 4, right panel and see inset) [Condition × Post-Drug interaction, $F(1,29)=5.73$, $P<0.05$]. In contrast, microinjecting NPA (20 µg) into the dorsal striatum of EEDQ-treated rats had minimal effect on locomotor activity because distance traveled scores

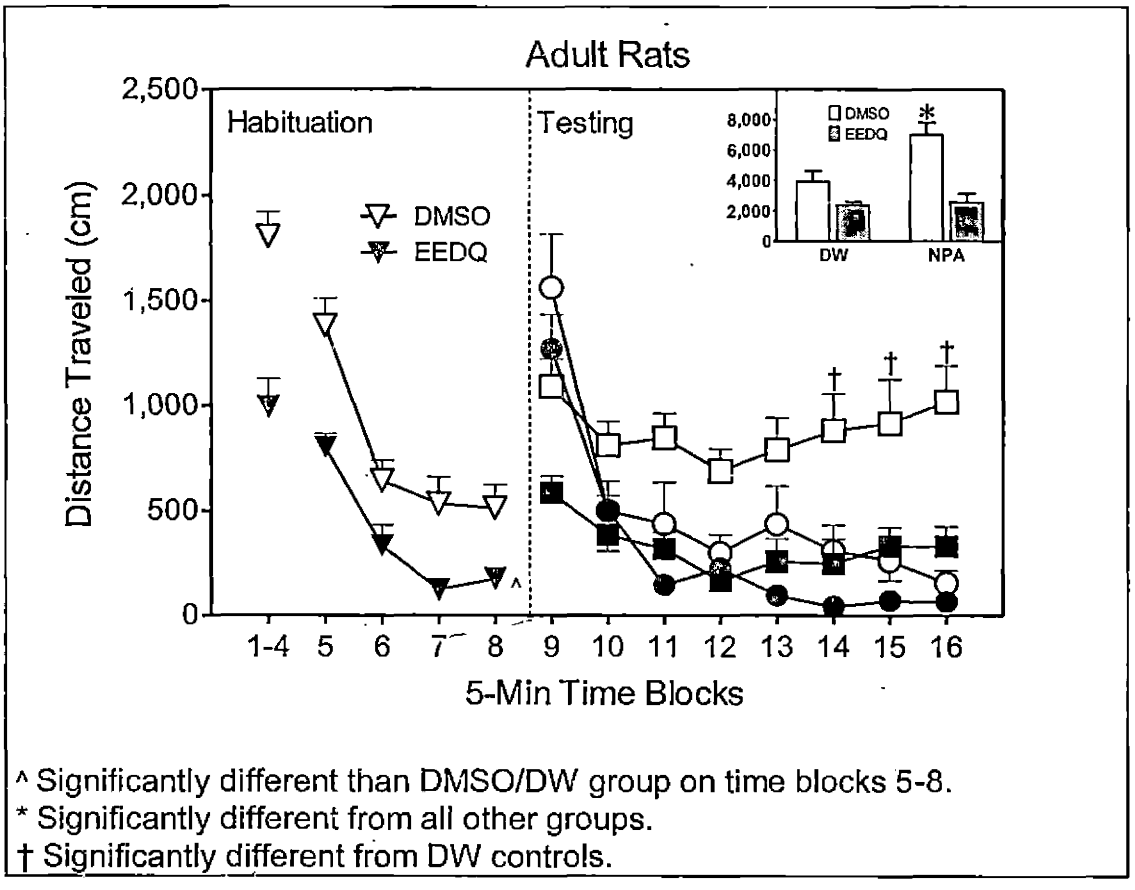


Figure 4. Mean Distance Traveled (±SEM) During the 80-min Behavioral Testing Session on PD 85 (*n* = 8-9 per Group) of Experiment 1. At the Conclusion of Time Block 8 (Indicated by the Dashed Line), Rats Previously given Bilateral Infusions of DMSO or EEDQ (100 µg) into the Dorsal Striatum Received Bilateral Infusions of Distilled Water or NPA (20 µg). Inset Represents Mean Distance Traveled Scores Collapsed Across Time Blocks 9-16. (o) DMSO/DW; (•) EEDQ/DW; (□) DMSO/NPA; (■) EEDQ/NPA.

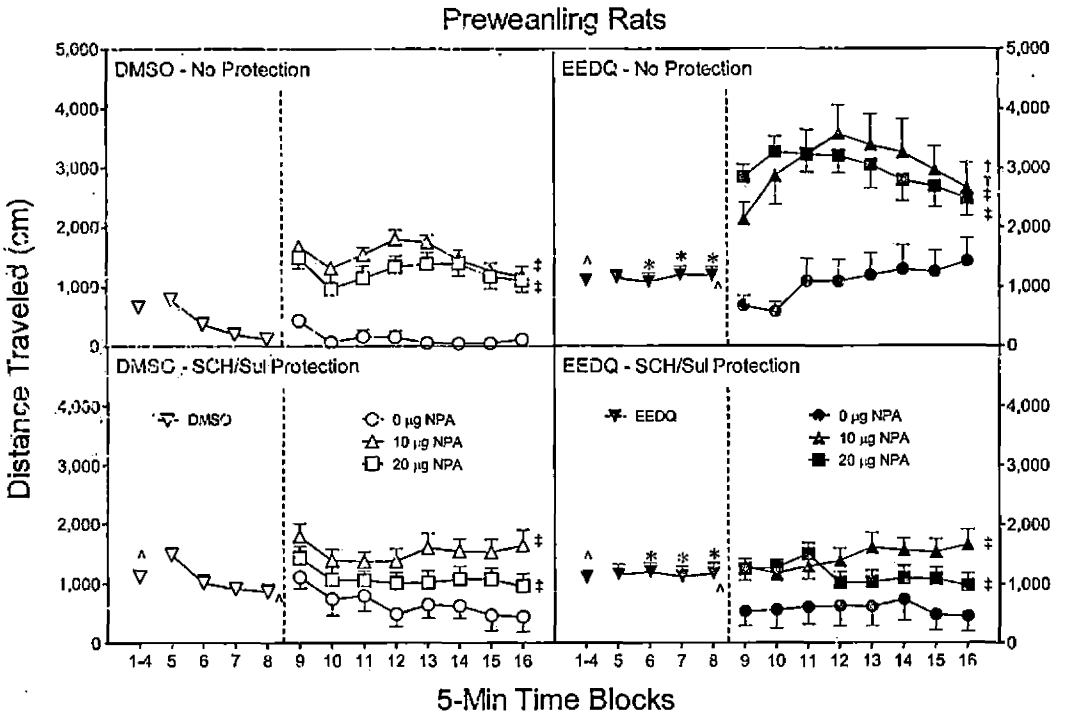
remained at a low, stable rate that was similar to controls. Individual time block analysis showed that microinjecting NPA (20 µg) into the dorsal striatum caused an increase in locomotor activity on time blocks 14-16 [[a]Post-Drug × Time Block interaction $F(5,152)=10.91$, $P<0.001$, and Tukey tests]. These results indicate that EEDQ fully attenuates NPA's locomotor enhancing effects in adult rats.

Experiment 2

Role of D1- and D2-Like Striatal Receptors for the EEDQ-Induced Behaviors of Preweanling Rats

Habituation Phase. During the chamber habituation phases (i.e., time blocks 1-4 and 5-8), distance traveled scores were significantly affected by the pretreatment and condition variables (i.e., SCH23390/sulpiride vs. saline; EEDQ vs. DMSO)(Figure 5, panels to the left of dashed vertical lines)[Pretreatment × Condition interactions, $F(1,92)=5.51$, $P<0.05$; $F(1,92)=8.95$, $P<0.01$, respectively]. Specifically, rats treated with either EEDQ or SCH23390/sulpiride had significantly greater distance traveled scores than the control group (i.e., the DMSO/No Protection group)[Tukey tests]. Therefore, blocking D1 and D2 receptors, either reversibly or irreversibly, increased basal distance traveled scores. Although the Pretreatment × Condition × Time Block interaction did not reach significance, EEDQ-treated rats did have significantly greater distance traveled scores than DMSO controls on time blocks 6-8 [[a]Condition × Time Block interaction, $F(2,178)=20.98$, $P<0.001$, and Tukey tests].

Testing Phase. During the testing phase (i.e., time blocks 9-16), EEDQ-treated rats had significantly greater distance traveled scores than DMSO-treated rats (Figure 5,



* Significantly different from DMSO-treated rats.
^ Significantly different from the DMSO/No Protection condition (collapsed across time blocks 1-4 and 5-8).
† Significantly different from rats in the other treatment conditions infused with 10 or 20 µg NPA (collapsed across time blocks 9–16).
‡ Significantly different from rats in the same treatment condition infused with 0 µg NPA (collapsed across time blocks 9-16).

Figure 5. Mean Distance Traveled (±SEM) During the 80-min Behavioral Testing Session on PD 18 (*n* = 8 per group) of Experiment 2. Rats were Previously (i.e., PD 17) Injected with Saline or SCH23390/sulpiride and given Bilateral Infusions of either DMSO or EEDQ (100 µg) into the Dorsal Striatum. At the Conclusion of Time Block 8 (PD 18; indicated by the dashed line), Rats Received Bilateral Infusions of Distilled Water or NPA (10 or 20 µg).

panels to the right of dashed vertical lines) [Condition main effect, $F(1,84)=28.68$, $P<0.001$]. However, the locomotor enhancing properties of EEDQ were only evident in the EEDQ-No Protection group [Pretreatment × Condition interaction, $F(1,84)=25.39$, $P<0.001$, and Tukey tests]. Specifically, the EEDQ-treated group that received no receptor protection (i.e., D1- and D2-like receptors were inactivated by EEDQ), exhibited greater distance traveled scores than both DMSO-treated groups (Figure 5, upper and lower left graphs) as well as the EEDQ-Protected group (Figure 5, lower right graph).

Infusing NPA (10 or 20 µg) into the dorsal striatum caused a significant increase in locomotor activity [Post-Drug main effect, $F(2,84)=32.09$, $P<0.001$, and Tukey tests]. The effects of the agonist varied according to protection condition [Pretreatment × Post-Drug interaction, $F(2,84)=5.07$, $P<0.01$]. Specifically, NPA-treated rats in the receptor protection groups (i.e., the SCH23390/sulpiride groups) had smaller distance traveled scores than NPA-treated rats in the No Protection groups.

Even though the Pretreatment × Condition × Post-Drug × Time Block four-way interaction was not significant, two

three-way interactions involving time block were statistically significant [[a]Pretreatment × Post-Drug × Time Block interaction $F(14,288)=2.05$, $P<0.05$; [a]Condition × Post-Drug × Time Block interaction, $F(14,288)=1.88$, $P<0.05$]. Most importantly, EEDQ treatment caused an increase in locomotor activity scores across time blocks [[a]Condition × Time Block interaction, $F(3,288)=8.51$, $P<0.001$]. This potentiated locomotor effect was completely eliminated when EEDQ-treated rats received receptor protection (i.e., SCH23390/sulpiride), because distance traveled scores of the EEDQ-Protection group were similar to rats pretreated with distilled water and infused with DMSO (Figure 5, top left and bottom right panel). Thus, NPA potentiated the locomotion of preweanling rats only if D1- and D2-like receptors were simultaneously inactivated by EEDQ.

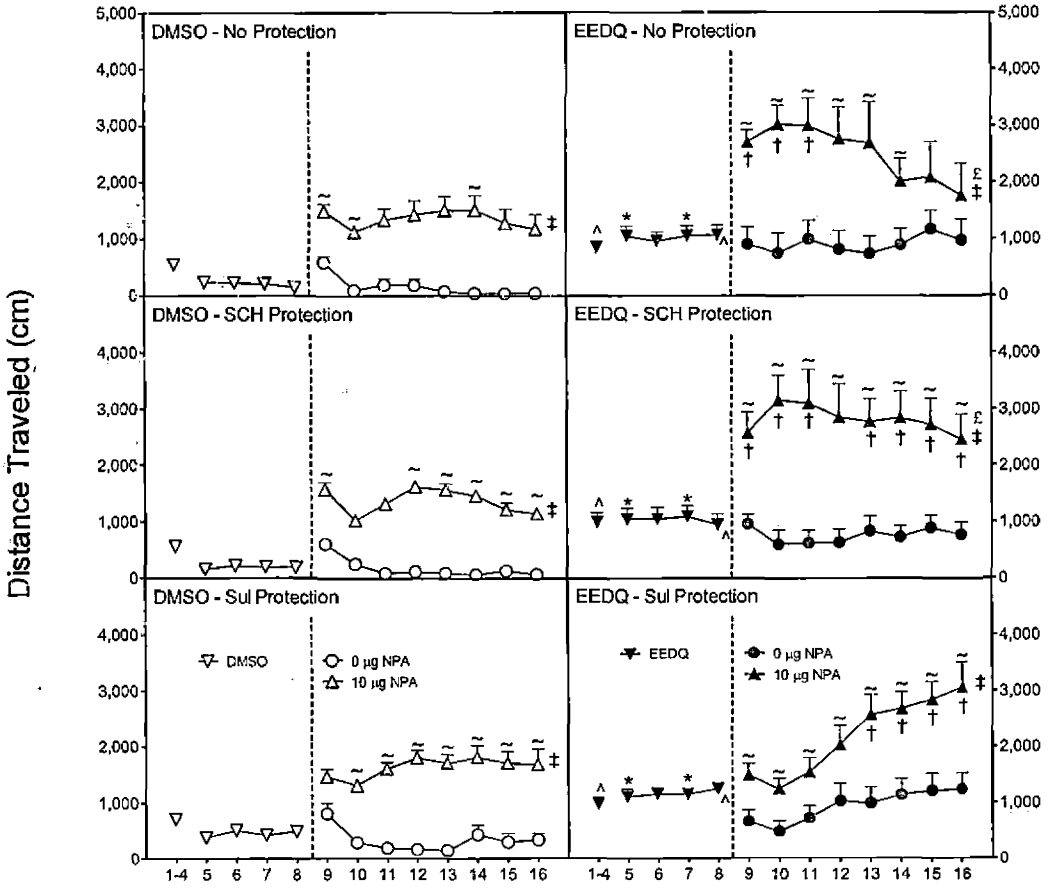
Experiment 3

Effects of D1- or D2-Like Striatal Receptor Inactivation on the EEDQ-Induced Behaviors of Preweanling Rats

Habituation. During the chamber habituation phases (i.e., time blocks 1-4 and 5-8), distance traveled scores were significantly affected by condition (EEDQ or DMSO)(Figure 6, panels to the left of dashed vertical lines)[Condition main effects, $F(1,90)=14.88$, $P<0.001$; $F(1,90)=68.78$, $P<0.001$, respectively]. Specifically, EEDQ-treated rats had greater distance traveled scores when compared to DMSO-treated rats. Individual time block analysis showed that EEDQ-treated rats had significantly greater distance traveled scores than all DMSO-treated rats on time blocks 5 and 7 [[a]Condition × Time Block interaction, $F(5,406)=34.29$, $P<0.001$, and Tukey tests].

Testing Phase. During the testing phase (i.e., time blocks 9-16), EEDQ-treated rats had significantly greater distance traveled scores than rats receiving DMSO (Figure 6, compare panels to the right of the dashed vertical line) [Condition main effect $F(1,84)=35.65$, $P<0.001$]. Regardless of pretreatment (i.e., saline, SCH23390, or sulpiride) and receptor inactivation conditions (i.e., EEDQ or DMSO), microinjecting NPA (10 µg NPA) into the dorsal striatum caused a significant increase in distance

Preweanling Rats



* Significantly different from the DMSO condition.

^ Significantly different from the DMSO condition (collapsed across time blocks 1-4 and 5-8).

£ Significantly different from the DMSO condition with the same pretreatment (i.e., No Protection, SCH23390, or sulpiride) receiving 10 µg NPA group (collapsed across time blocks 9–16).

~ Significantly different from rats in the same treatment condition infused with 0 µg NPA at the same time block.

‡ Significantly different from rats in the same treatment condition infused with 0 µg NPA (collapsed across time blocks 9-16).

† Significantly different from DMSO group with the same pretreatment (i.e., No Protection, SCH23390, or sulpiride) receiving 10 µg NPA group at the same time block.

Figure 6. Mean Distance Traveled (±SEM) During the 80-min Behavioral Testing Session on PD 18 (n = 8 per group) of Experiment 3. Rats were Previously (i.e., PD 17) Injected with Saline, SCH23390, or Sulpiride and given Bilateral Infusions of either DMSO or EEDQ (100 µg) into the Dorsal Striatum. At the Conclusion of Time Block 8 (PD 18; indicated by the dashed line), Rats Received Bilateral Infusions of Distilled Water or NPA (10 µg).

traveled scores when compared to rats receiving 0 µg NPA [Post-Drug main effect, $F(1,84)=101.87$, $P<0.001$].

More importantly, there was a significant four-way interaction (Figure 6) [[a]Pretreatment × Condition × Post-Drug × Time Block interaction, $F(7,307)=3.61$, $P<0.001$]. Due to this higher-order interaction, three two-way ANOVAs were conducted. Each Condition × Time Block ANOVA compared NPA-treated rats given either DMSO or EEDQ. A separate ANOVA was conducted for each protection condition (i.e., No Protection, SCH23390 Protection, and sulpiride Protection). An individual time block analysis of the No Protection groups (upper graphs, Figure 6) showed that NPA caused a potentiated locomotor response in EEDQ-treated rats on time blocks 9-11 (i.e., when compared to DMSO-treated rats receiving NPA) [[a]Condition × Time Block interaction, $F(3,45)=3.80$, $P<0.05$, and Tukey tests]. Likewise, when comparing the SCH23390 Protection groups (middle graphs, Figure 6), NPA caused a potentiated response in EEDQ-treated rats on time blocks 9-11 and 13-16) [[a]Condition × Time Block interaction, $F(4,50)=3.80$, $P<0.05$, and Tukey tests]. And, when comparing the sulpiride Protection groups (lower graphs, Figure 6), NPA caused a potentiated response in EEDQ-treated rats on time

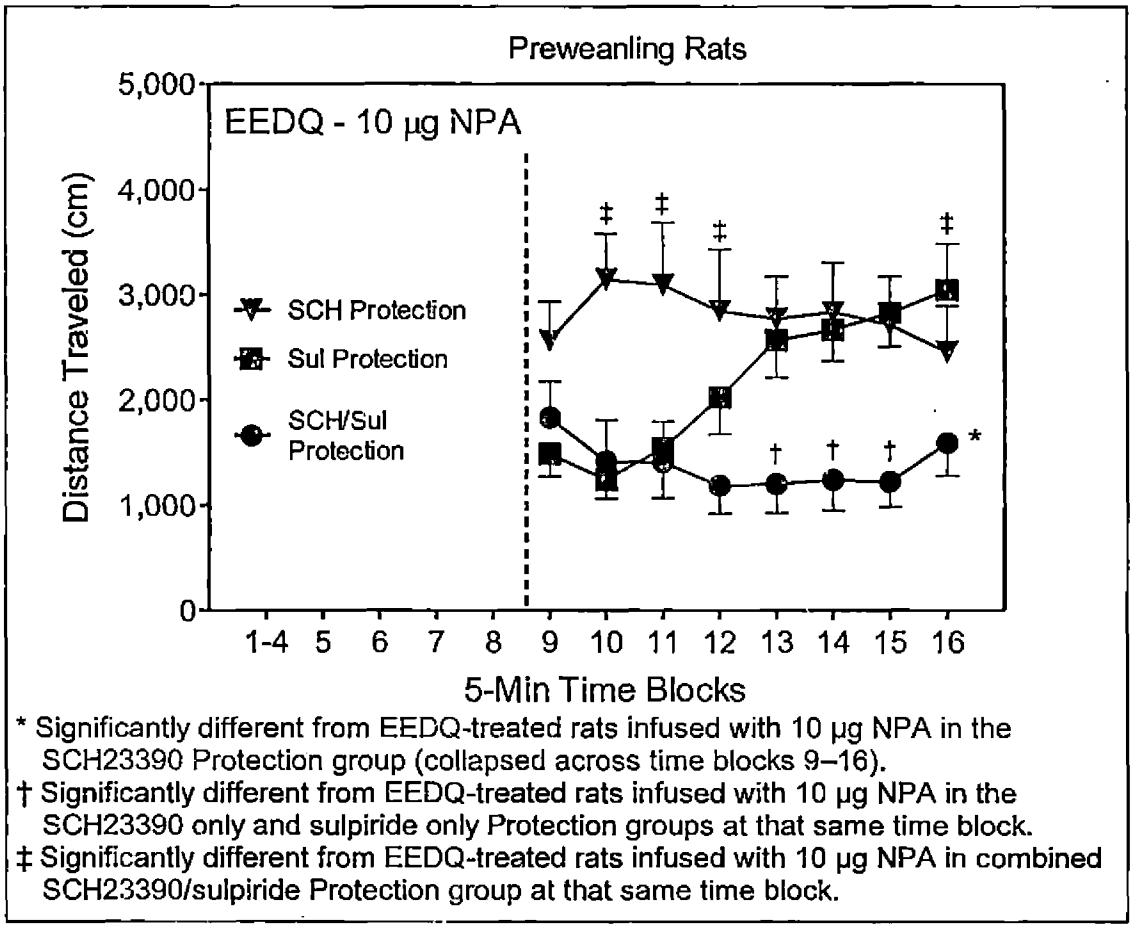


Figure 7. Mean Distance Traveled (±SEM) During the 80-min Behavioral Testing Session on PD 18 (n = 8 per group) of Experiment 3. Rats were Previously (i.e., PD 17) Injected with Combined SCH23390/sulpiride, SCH23390 alone, or Sulpiride Alone and given Bilateral Infusions of EEDQ (100 µg) into the Dorsal Striatum (the SCH23390 alone and sulpiride alone rats are from Figure 6). At the Conclusion of Time Block 8 (PD 18; indicated by the dashed line), Rats Received Bilateral Infusions of NPA (10 µg).

blocks 13-16) [[a]Condition × Time Block interaction, $F(3,37)=6.05$, $P<0.01$, and Tukey tests].

In this experiment an additional group of 8 rats were infused with NPA 24 hr after receiving combined SCH23390/sulpiride pretreatment and EEDQ (i.e., these rats were treated as in Experiment 2). A 3 × 8 (Pretreatment × Time Block) ANOVA was conducted to compare EEDQ-treated rats infused with NPA within the three protection groups (i.e., SCH23390, sulpiride, or combined SCH23390/sulpiride). EEDQ-treated rats in the combined SCH23390/sulpiride protection group had smaller distance traveled scores than EEDQ-treated rats in the SCH23390 protection group infused with NPA (Figure 7) [Pretreatment main effect, $F(2,21)=4.64$, $P<0.05$, and Tukey tests]. Importantly, EEDQ-treated rats given SCH23390 Protection did not differ from rats given sulpiride Protection. Additionally, the effects of NPA on distance traveled scores varied across time blocks [[a]Pretreatment × Time Block interaction, $F(6,68)=7.01$, $P<0.001$, and Tukey tests]. Specifically, the combined SCH23390/sulpiride protection group differed from both the SCH23390 alone and sulpiride alone protection groups on time blocks 13-15, from the SCH23390 alone protection group on time blocks 10-12, and from the sulpiride alone protection group on time block 16.

Experiment 4

Quantitative Autoradiography

Representative D1- and D2-like receptor autoradiograms of EEDQ- and DMSO-treated rats are shown in Appendix B (preweanling rats) and Appendix C (adult rats). Autoradiograms were analyzed using *t*-tests (condition: EEDQ and DMSO) at each age to assess D1- and D2-like receptor densities. Additionally, separate 2 × 2 (condition × age) ANOVAs were conducted to analyze receptor densities across ontogeny.

[^{3}H]SCH23390 Autoradiography

Intrastriatal EEDQ injections significantly reduced D1-like receptor densities in both preweanling and adult rats (see Table 1) [$t(8)=7.70$, $P<0.001$; $t(8)=8.41$, $P<0.001$, respectively].

[^{3}H]Spiperone Autoradiography

Microinjecting EEDQ into the dorsal striatum caused a significant reduction in D2-like receptor binding sites in both preweanling and adult rats (see Table 1) [$t(7) = 3.92$, $P < 0.01$; $t(8) = 4.53$, $P < 0.01$, respectively].

Ontogenetic Differences

A 2 × 2 (Age × Condition) ANOVA was used to assess D1-like receptor densities. Microinjecting EEDQ into the dorsal striatum of both preweanling (30%) and adults (34%)

Table 1. Quantitative *in Vitro* Autoradiography Used to Determine [^{3}H]SCH23390 and [^{3}H]spiperone Labeling Intensities in Coronal Sections of the Dorsal Striatum after Intrastriatal Microinjections of DMSO and EEDQ in Preweanling and Adult Rats

Treatment	B_{max} (fmol/mg protein) D1-like receptors	B_{max} (fmol/mg protein) D2-like receptors
PD 18		
DMSO	19.17 (±1.63)	8.12 (±0.29)[b]
EEDQ	5.71 (±0.95)[a]	4.85 (±0.56)[ab]
%DMSO	30%	60%
PD 85		
DMSO	22.10 (±1.41)	15.28 (±2.58)
EEDQ	7.56 (±1.06)[a]	5.50 (±0.57)[a]
%DMSO	34%	36%

Preweanling and adult rats received bilateral infusions of DMSO or EEDQ (100 mg/kg) on PD 17 and PD 84, respectively. Tissue was harvested 24 hr after EEDQ or DMSO infusions. [a] Significantly different from DMSO-treated rats of the same age; [b] Significantly different from EEDQ-treated PD 85 rats.

rats significantly reduced [^{3}H]SCH23390 binding, and this effect did not vary according to age (Figure 8)[Condition main effect, $F(1,16) = 129.88$ $P < 0.001$]. In contrast, adult rats had markedly more dorsal striatal D2-like receptors than preweanling rats (see Table 1)[Age main effect, $F(1,5) = 10.02$ $P < 0.01$]. EEDQ infusions significantly reduced [^{3}H]spiperone binding in the dorsal striatum [Condition main effect, $F(1,15) = 28.05$ $P < 0.001$], and this effect varied between age groups [Age × Condition interaction, $F(1,15) = 6.99$ $P < 0.05$]. More specifically, D2-like receptor densities were reduced to 60% in preweanling rats; while, in adult rats, EEDQ reduced D2-like receptor densities to 36% of control values.

Taken together, these data show that adult and preweanling rats have similar levels of D1-like receptors in the dorsal striatum, while adults have approximately twice the number of D2-like receptors. EEDQ caused similar reductions of D1-like receptor densities in preweanling and adult rats. Interestingly, EEDQ depleted the D2-like receptors of preweanling rats to a lesser extent than adults.

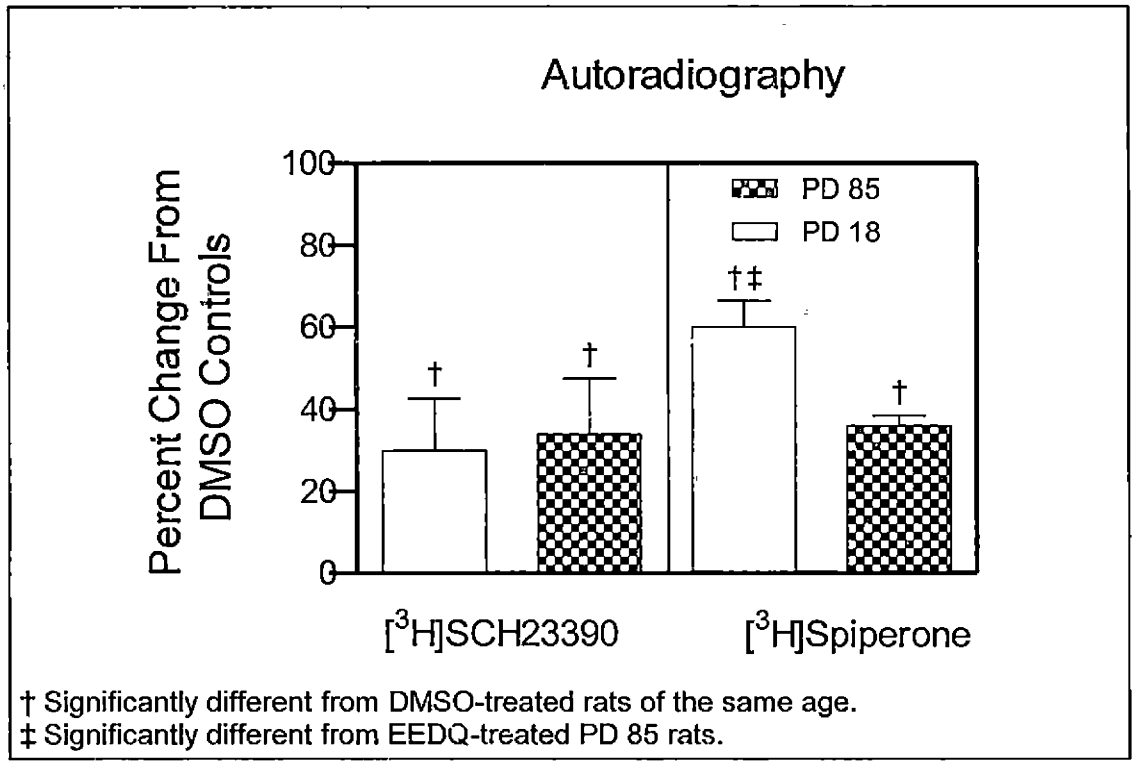


Figure 8. Effects of Intrastriatal Microinjections of EEDQ on [^{3}H]SCH23390 and [^{3}H]Spiperone (i.e., D1- and D2-like Receptors, Respectively) Densities in Dorsal Striatal Coronal Sections of Preweanling and Adult Rats. Values Represent Percent Change from DMSO-treated Control Rats (100%).

Experiment 5

D1-Like Receptor Binding

When assessed on PD 18, D1-like receptor binding site densities (B_{max}) were significantly reduced 24 hr after EEDQ infusions (Table 2) [$t(10) = 5.70$, $P < 0.001$]. D1-like receptor affinity (K_D) was not altered by EEDQ, with the overall K_D value being 0.840 (±0.138) nM.

Similarly, infusing EEDQ on PD 84 caused a significant decrease in D1-like receptor binding site densities (Table 2) [$t(9) = 6.11$, $P < 0.001$]. In contrast to the preweanling age group, EEDQ infusions significantly enhanced K_D values in the dorsal striatum of adult rats [$t(9) = 2.40$, $P < 0.05$].

D2-Like Receptor Binding

Infusing EEDQ into the dorsal striatum of PD 17 rats caused a significant decrease in D2-like receptor binding site densities when assessed 24 hr later (Table 3) [$t(10) = 3.16$, $P < 0.05$]. D2-like receptor affinity was not altered when EEDQ was infused into the dorsal stratum.

Although EEDQ infusions on PD 79 caused a substantial decrease in D2-like receptor binding like densities (i.e., 35% reduction), this effect did not reach statistical significance (Table 3) [$P = 0.12$]. However, the mean K_D

Table 2. Effect of DMSO and EEDQ on D1-like Receptor Density (fmol/mg Protein, ±SEM) and Affinity (nM, ±SEM) for [^{3}H]SCH23390 Binding in the Dorsal Striatum of Preweanling and Adult Rats

Treatment	B_{max} (fmol/mg protein)	K_d (nM)
PD 18 (n=12)		
DMSO	3631.44[b] (±483.63)	0.699 (±0.098)
EEDQ (100 mg/kg)	802.28[a] (±110.98)	0.981 (±0.258)
%DMSO	22%	
PD 85 (n=11)		
DMSO	4771.8 (±459.01)	0.684 (±0.055)
EEDQ (100 mg/kg)	1413.4[a] (±234.78)	1.069[a] (±0.166)
%DMSO	30%	

Preweanling and adult rats received bilateral infusions of DMSO or EEDQ (100 mg/kg) on PD 17 and PD 84, respectively. Tissue was harvested 24 hr after EEDQ or DMSO infusions. [a] Significantly different from DMSO-treated rats of the same age; [b] Significantly different from PD 85 rats.

Table 3. Effect of DMSO and EEDQ on D2-like Receptor Density (fmol/mg Protein, ±SEM) and Affinity (nM, ±SEM) for [^{3}H]Spiperone Binding in the Dorsal Striatum of Preweanling and Adult Rats

Treatment	B_{max} (fmol/mg protein)	K_d (nM)
PD 18 (n=12)		
DMSO	606.1[b] (±83.79)	0.140 (±0.013)
EEDQ (100 mg/kg)	276.0[a] (±63.49)	0.124 (±0.035)
%DMSO	46%	
PD 85 (n=12)		
DMSO	800.8 (±147.67)	0.114 (±0.019)
EEDQ (100 mg/kg)	520.4 (±73.32)	0.289[a] (±0.067)
%DMSO	65%	

Preweanling and adult rats received bilateral infusions of DMSO or EEDQ (100 mg/kg) on PD 17 and PD 84, respectively. Tissue was harvested 24 hr after EEDQ and DMSO infusions. [a] Significantly different from DMSO-treated rats of the same age; [b] Significantly different from PD 85 rats.

value of D2-like receptors was significantly increased after EEDQ treatment (Table 3) [$t(10) = 2.51$, $P < 0.05$].

Ontogenetic Differences

When comparing adult and preweanling rats, adult rats had significantly more D1- and D2-like receptor binding sites than preweanling rats [Age main effects, $F(1,19) = 5.82$ $P < 0.05$; $F(1,20) = 5.06$, $P < 0.05$, respectively]. At the two ages, EEDQ infusions caused approximately similar reductions in B_{max} values for both D1- (PD 18, 78%; PD 85, 70%) and D2-like (PD 18, 54%; PD 85, 35%) receptors. In both adult and preweanling rats, EEDQ seemed to produce a greater decline in D1- than D2-like receptors, even though this trend did not reach statistical significance.

Collectively, these data suggest that B_{max} values for both D1- and D2-like receptors were significantly reduced after EEDQ infusions in preweanling rats, while K_D values were unaltered. In adult rats, however, EEDQ caused a significant reduction in the B_{max} values of D1-like, and not D2-like, receptors. Interestingly, K_D values for both D1- and D2-like receptors were significantly elevated in EEDQ-treated adult rats.

CHAPTER TWELVE

DISCUSSION

The purpose of this thesis was three-fold: a) to investigate the effects of intrastriatally administered EEDQ on NPA-mediated locomotor activity in preweanling and adults rats; b) to determine which dopamine receptor subtype (D1- or D2-like) is responsible for modulating EEDQ's paradoxical behavioral effects in preweanling rats; and c) to examine the magnitude of EEDQ-induced D1- and D2-like receptor inactivation in both adult and preweanling rats. It was predicted that: a) microinjecting EEDQ into the dorsal striatum of adult and preweanling rats would block NPA-induced locomotor activity; b) both D1- and D2-like receptors in the dorsal striatum are responsible for mediating NPA-induced locomotor potentiation in preweanling rats; and c) intrastriatal infusions of EEDQ were predicted to cause a substantial reduction of D1- and D2-like receptor binding sites in both preweanling and adult rats.

Effects of EEDQ on the NPA-Mediated Locomotor Activity of Adult Rats

Previous research has shown that systemic co-administration of the selective D1- and D2-like agonists SKF38393 and quinpirole elicits a dose-dependent

increase in locomotor activity as well as stereotyped grooming and sniffing (Braun & Chase, 1986; Clark & White, 1987; Kashihara et al., 1996). Systemic injections of the non-selective agonists apomorphine or (+)3-PPP increases the locomotor activity of adult rats (Bradbury et al., 1984; Costall et al., 1981). Similarly, low-dose infusions of apomorphine or NPA into the dorsal striatum causes both locomotion and rearing (Bordi et al., 1989; Carrera et al., 1998; Dias et al., 2006; Dickson et al., 1994).

In the current experiment, adult rats were microinjected with NPA (0 or 20 µg) 24 hr after EEDQ or DMSO infusions. As predicted, NPA caused a significant increase in the locomotor activity of DMSO-treated adult rats; however, EEDQ was able to fully attenuate NPA-induced increases in locomotor activity. These results are not surprising considering that systemic EEDQ treatment blocks NPA-induced locomotion in adults (Bordi et al., 1989; Hamblin & Creese, 1983; Meller et al., 1989). Therefore, consistent with previous research, it appears that reductions in both D1- and D2-like receptors due to irreversible inactivation by EEDQ was sufficient to block the locomotor activating properties of NPA in adult rats.

Effects of Dorsal Striatal NPA Infusions on the Locomotor Activity of Preweanling Rats

In Experiments 2 and 3, locomotor activity was assessed after intrastriatal infusions of the non-selective dopamine agonist NPA. Treatment with either 10 or 20 µg NPA increased locomotor activity in control rats. This result was not surprising because previous studies have reported that infusing NPA into the dorsal striatum stimulates locomotion (Charntikov et al., 2008, 2011). Additionally, systemically administering NPA, as well as other non-selective dopamine agonists (i.e., cocaine and amphetamine), increases locomotor activity in preweanling rats (McDougall et al., 1995, 1993, 1999; Nazarian et al., 1999).

Effects of EEDQ on the NPA-Induced Locomotor Activity of Preweanling Rats

Contrary to our predictions, EEDQ did not attenuate the NPA-induced locomotor activity of preweanling rats. Instead, EEDQ potentiated the locomotor activity of PD 18 rats, while it attenuated the NPA-induced locomotor activity of adult rats. Due to these striking results, we investigated which dopamine receptor subtype, if any, was responsible for this effect. Using receptor protection techniques (i.e., combined SCH23390/sulpiride treatment), Experiment 2 showed that dopamine receptors are

responsible for EEDQ's paradoxical behavioral effects in preweanling animals. Specifically, there is a blunting of NPA-induced locomotion in EEDQ-treated rats pretreated with SCH23390/sulpiride, when compared to EEDQ-treated rats given saline protection (see Figure 5). In other words, NPA-induced locomotor potentiation was only evident if EEDQ was allowed to alkylate D1- and D2-like receptors.

The purpose of Experiment 3 was to determine which dopamine receptor subtype (i.e., D1- or D2-like) is responsible for the potentiated locomotor response to NPA that is evident after irreversible antagonism with EEDQ. NPA (10 µg) caused a significant increase in the locomotor activity of EEDQ-treated rats regardless of individual receptor protection conditions (i.e., SCH23390 or sulpiride pretreatment)(see Figure 6). Therefore, these results show that inactivating either D1- or D2-like receptors was sufficient to potentiate NPA-induced locomotor activity. Only when both D1- and D2-like receptors were protected from EEDQ (i.e., after combined SCH23390/sulpiride treatment) was NPA-induced locomotor potentiation blocked. Taken together, these data strongly suggest that the NPA-induced locomotor potentiation exhibited by EEDQ-treated preweanling rats is due to the alkylation of D1- and D2-like receptors.

[^{3}H]SCH23390 and [^{3}H]Spiperone Homogenate Ligand Receptor Binding and Autoradiography

I hypothesized that intrastriatal infusions of EEDQ would: (a) cause a substantial reduction of D1- and D2-like receptor binding sites in preweanling and adult rats, and (b) the decline of D1- and D2-like receptors would be greater in adult rats than preweanling rats. It has already been established that systemic injections of EEDQ cause robust declines in both D1- and D2-like receptors in preweanling and adult rats (Crawford et al., 1992; Giorgi et al., 1991, 1992; Meller et al., 1985). Data from the present homogenate receptor binding study showed that EEDQ infusions caused reductions in the B_{max} values of both D1- (PD 18, 78%; PD 85, 70%) and D2-like (PD 18, 54%; PD 85, 35%) receptors, with the decline being greater for D1- than D2-like receptors. In preweanling rats, EEDQ caused a significant reduction in the B_{max} values of D1- and D2-like receptors; whereas, in adult rats, EEDQ caused a significant reduction in the B_{max} values of D1-like, but not D2-like receptors. These data were contrary to my prediction because EEDQ caused a greater reduction of D1- and D2-like receptors sites in preweanling rats than adults.

The D1-like receptor autoradiography data complements the receptor binding data. EEDQ reduced D1-like receptors by 70% in preweanling rats and by 66% in adult rats. Analysis of the autoradiography data showed that the D2-like receptor binding sites of preweanling rats were reduced by 40%, which was similar to the results obtained using homogenate ligand binding techniques (a reduction of 54%). In striking contrast, however, the autoradiography data showed that there was a far greater reduction of D2-like receptor binding sites in EEDQ-treated adult rats (64%) than what was observed in the homogenate binding assay (35%).

When considering D2-like receptors, the homogenate ligand binding and autoradiography results are contradictory to one another. In the homogenate ligand binding experiment, the proportion of D2-like receptor inactivation in the dorsal striatum is somewhat greater in preweanling rats than adults (PD 18, 54%; PD 85; 35%). In terms of the autoradiography data, EEDQ caused significantly greater receptor alkylation in adult rats than preweanling rats (PD 18, 40%; PD 85, 64%). Therefore, the binding data show that adult rats have approximately 25% more D2-like receptors than preweanling rats; whereas, the autoradiography data show that adult rats have

approximately 50% more D2-like receptors. Previous studies employing homogenate binding techniques support the notion of age-dependent differences in the number of binding sites. Specifically, adult rats have significantly more D1- and D2-like receptor binding sites than preweanling rats (Crawford et al., 1992). In contrast, two studies using quantitative autoradiography showed that D1- and D2-like receptor binding reached adult levels by approximately PD 14-21 (Rao et al., 1991; Schambra et al., 1994). Therefore, it is not atypical for homogenate ligand binding and quantitative autoradiography to provide different patterns of results.

In the current homogenate ligand and autoradiography experiments, the same volume of EEDQ (0.75 µl per side) was injected into the dorsal striatum of both preweanling and adult rats. Across ontogeny, the size of the dorsal striatum varies, thus it is possible that more D1- and D2-like receptors were inactivated in preweanling rats than adults due to the relative size of the structure. Moreover, it is uncertain whether intrastriatal dispersion (solubility) is similar in the two age groups. Therefore, it is very likely that more of the dorsal striatum was left intact in adult rats.

Autoradiography is a technique in which the whole brain is imaged, but individual structures are of particular interest. In this case, a radiograph of the tissue is made on a photographic plate from the radiation emitted by tritium and the distribution of the radiation is visualized and quantified. A homogenate receptor binding assay involves gross dissection of a particular brain structure. These methodological differences can account for the discrepancies observed across the two techniques. It is likely that during gross dissection, unaffected areas of the striatum were included in the final tissue sample. Instead, autoradiography is target-area specific such that a discrete area of the structure is analyzed.

Paradoxical Actions of EEDQ in Preweanling Rats

The data from these experiments showed that intrastriatal EEDQ affects preweanling and adult rats in a qualitatively different manner. EEDQ treatment attenuated NPA-induced locomotor activity in adult rats. In contrast, EEDQ treatment potentiated, rather than attenuated, NPA-induced locomotor activity in preweanling rats. This paradoxical behavioral pattern gives rise to some questions: a) Why did EEDQ affect the behavior of adult

and preweanling rats differently; and b) why was the locomotor activity of preweanling rats potentiated? Several possible explanations can be used to elucidate these findings. First, it is possible that some other type of receptor was inactivated and accounted for the potentiated locomotor response in preweanling rats. Second, the proportion of D1- and D2-like receptors inactivated was not sufficient to diminish the locomotor activating properties of NPA in preweanling rats. Third, a disproportionate inactivation of D1- and D2-like receptors may change the relative excitatory/inhibitory effects of dopamine agonists on the direct and indirect pathways. Fourth, a receptor reserve, or the lack thereof, may account for the qualitatively different locomotor patterns observed across ontogeny. Lastly, the receptors that were regenerated or those that remained after alkylation may be more sensitive in preweanling rats.

Even though EEDQ binds to and inactivates D1- and D2-like receptors, this non-competitive antagonist binds to various other receptor types, including α-adrenergic, muscarinic, serotonergic, and GABAergic (Adler et al., 1985; Arnt & Hyttel, 1988; Hamblin & Creese, 1983; Meller et al., 1985; Miller et al., 1991; Nowak et al., 1988; Saller et al., 1989; Vinod, Subhash, & Srinivas, 2001).

Regardless, the protection experiments clearly show that dopamine receptors were responsible for the potentiated locomotor activity exhibited by EEDQ-treated preweanling rats receiving NPA. This is evidenced by the fact that the potentiated locomotor response was attenuated when both D1- and D2-like receptors were selectively protected (i.e., SCH23390/sulpiride pretreatment) from the inactivating effects of EEDQ. Therefore, it is unlikely that our results can be explained by the alkylation of some other receptor subtype.

Secondly, it is doubtful whether the inability of EEDQ to attenuate NPA-induced locomotion can be attributed to insufficient receptor alkylation. First, insufficient receptor alkylation should not result in a *potentiated* locomotor response. Moreover, both the homogenate binding and autoradiography experiments indicate that there was a significant reduction in the D1- and D2-like receptors of EEDQ-treated preweanling rats, and this reduction is comparable to what was observed in adults. Therefore, a significant proportion of D1- and D2-like receptors were inactivated in the dorsal striatum of preweanling rats, thus insufficient receptor alkylation cannot account for the potentiated behavior observed in young animals.

A third possible explanation for the paradoxical behavioral effects observed in preweanling rats stems from the neural organization of D1- and D2-like receptors. Stimulating D1-like receptors activates the direct (striatonigral) pathway; whereas, stimulation of D2-like receptors disinhibits the indirect (striatopallidal) pathway (Gerfen, 1992). The downstream components of these pathways are glutamatergic (excitatory) and GABAergic (inhibitory) projections to major output structures of the basal ganglia (i.e., the substantia nigra pars reticulata and entopeduncular nucleus). If EEDQ caused a disproportional inactivation of D1- and D2-like receptors, it is possible that an imbalance developed that affected the relative activity of the direct and indirect pathways. A change in the activation (or inhibition) of either of these pathways may cause the potentiated locomotor response observed in preweanling rats.

The proposed imbalance of outputs might also affect feedback loops within the basal ganglia. The substantia nigra pars compacta contains dopamine cell bodies, which send projections to the dorsal striatum (nigrostriatal pathway), subthalamic nucleus (nigrosubthalamic pathway), and globus pallidus (nigropallidal pathway)(Haber, 1994). In terms of the nigrostriatal pathway, dopamine neurons

synapse on intrinsic GABAergic neurons of the dorsal striatum (Clarke et al., 1988). A hypothetical imbalance of direct and indirect pathway activation would be expected to differentially affect the firing rate of compacta dopamine neurons, thereby indirectly altering the functioning of the direct and indirect pathways (i.e., cause differential firing of the feedback loop).

Fourth, the relative size of a "receptor reserve" may explain why EEDQ differentially affects the behaviors of preweanling and adult rats. Receptor reserve theory refers to the relationship between receptor occupation and response. According to this model, a receptor reserve exists when there are more receptors on the surface of the cell membrane than are needed for the maximal effect (E_{max}) of a drug. Theoretically, if spare receptors are present, the drug concentration that produces 50% occupancy should be greater than the concentration that produces 50% of maximum response.

Evidence suggests the presence of a large receptor reserve at striatal D2-like autoreceptors in adult rats (Furchgott & Burstyn, 1967; Meller, Bohmaker, Namba, Friedhoff, & Goldstein, 1987; Meller, Helmer-Matyjek, Bohmaker, Adler, Friedhoff, & Goldstein, 1986; Roth, 1979), and a much smaller reserve at D2-like postsynaptic

receptors (Meller et al., 1987; Ruffolo, 1982). A recent article by Gubernator and colleagues (2009) utilized fluorescent false neurotransmitters and multiphoton imaging to show a preferential synaptic vesicle reserve for presynaptic D2-like receptors. It has also been postulated that a receptor reserve exists for postsynaptic D1-like receptors, because (a) D1-like receptor agonist-induced behaviors persisted in EEDQ-treated rats (Arnt et al., 1988; Rosengarten et al., 1989) and (b) a reduction in D1-like receptor biding sites in EEDQ-treated rats did not directly correlate with a decrease in adelylate cyclase activity (Hess et al., 1987).

In contrast, because EEDQ-treated preweanling rats continue to exhibit NPA-induced locomotor activity, it is possible that young rats have a larger reserve of D1- and D2-like postsynaptic receptors. Consistent with this idea, our data showed that D1- and D2-like receptor-mediated locomotor activity was preserved in EEDQ-treated preweanling rats even after significant receptor alkylation. That being said, there is no corroborating evidence showing that a large D2-like receptor reserve exists in preweanling rats. Moreover, a receptor reserve explanation cannot account for the *potentiated* locomotor response exhibited by preweanling rats.

Fifth, it is possible that EEDQ leaves the remaining receptors in a supersensitized state or, alternatively, that the newly generated receptors are supersensitive. Dopamine receptor supersentivity refers to a phenomenon where there is a greater than normal physiological, behavioral, or biochemical response to a dopamine agonist (for review, see Kostrzewa, 1995). Evidence suggests that dopamine depletion (e.g., after reserpine or 6-OHDA treatment) causes a decrease in D1-like receptor expression and, conversely, an increase in D2-like receptor expression (Gerfen et al., 1990; Gerfen, McGinty, Young 1991; Young et al., 1986; see also Gerfen 2003). Even though there is either a decrease or no change in D1-like receptor expression (Gerfen et al., 1990; Marshall, Navarrete, & Joyce, 1989), a supersensitive response is evident after D1-like receptor stimulation (Berke, Paletzki, Aronson, Hyman, & Gerfen, 1998; Gerfen et al., 1995; Robertson, Vincent, & Fibiger, 1990; Steiner & Gerfen, 1996). Dopamine depletion can also cause receptor supersensitivity in preweanling rats as evidenced by a potentiated behavioral response to dopaminergic drugs (Farley et al., 2006).

Not surprisingly, EEDQ significantly reduces dopamine content in the dorsal striatum at PD 17 and PD 90

(Crawford et al., 1992, 1994a). Reduced dopamine levels can lead to receptor inactivation and, consequently, receptor supersensitivity (Berke et al., 1998; Gerfen et al., 1995; Robertson et al., 1990; Steiner & Gerfen, 1996). Receptor turnover rates vary across ontogeny, with receptor repopulation occurring at a more rapid pace in preweanling rats than adults (Leff et al., 1984; Kula et al., 1992). It is likely that newly synthesized receptors are supersensitive (see Seeman, Weinshenker, Quirion, Srivastava, Bhardwaj, Grandy, Premont, Sotnikova, Boksa, El-Ghundi, O'Dowd, George, Perreault, Männistö, Robinson, Palmiter, & Tallerico, 2005); thus, with quicker receptor repopulation it is possible that EEDQ-treated preweanling rats have a larger complement of supersensitive receptors than adult rats. If so, preweanling rats may show a potentiated locomotor response because of an abundance of supersensitive D1- and D2-like receptors.

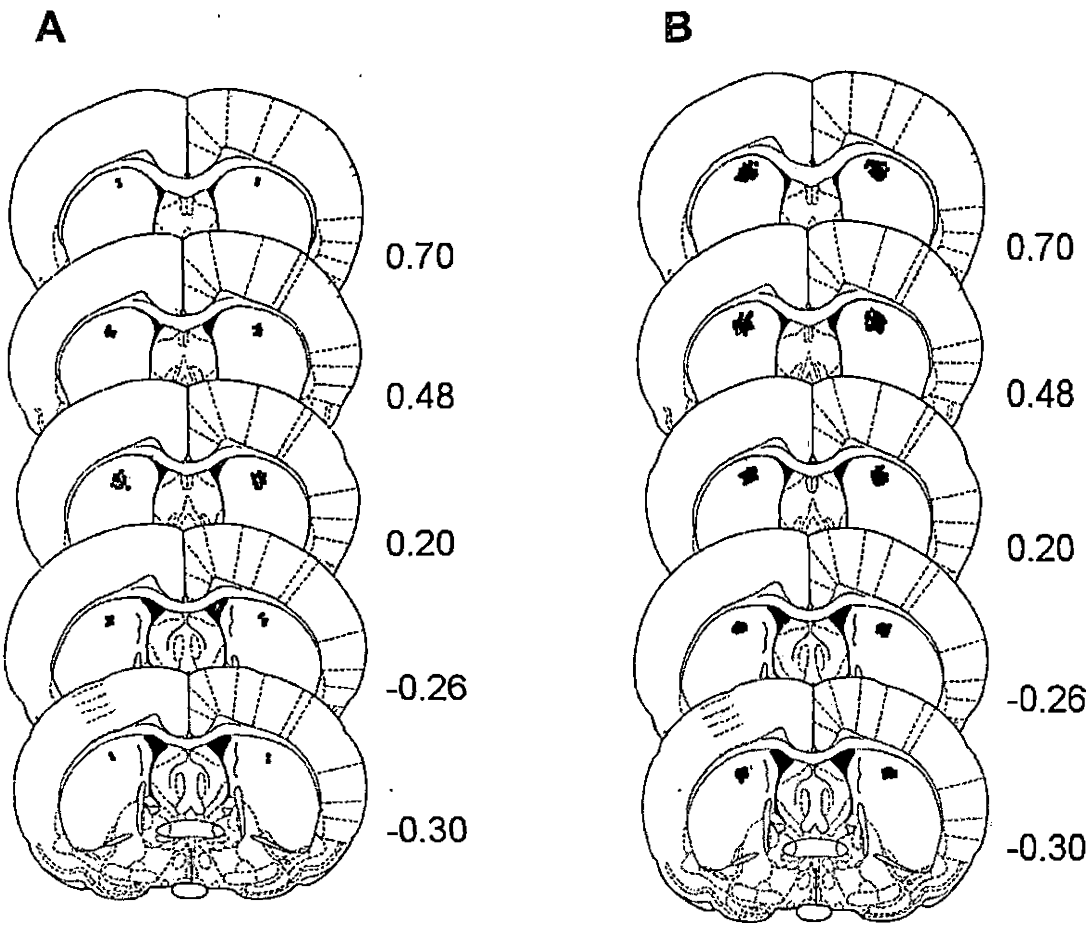
Conclusion

In conclusion, data from this thesis adds to previous research showing that dopaminergic compounds can affect the behaviors of preweanling and adult rats differently. The present results inducate that NPA does not increase the locomotor activity of EEDQ-treated adults; whereas, EEDQ-treated preweanling rats show a potentiated locomotor

response after NPA treatment. Importantly, this potentiated locomotion is due to the alkylation of D1- and D2-like receptors, and not some other receptor type. Homogenate ligand binding and autoradiography experiments showed that EEDQ caused a significant reduction in the dorsal striatal D1- and D2-like receptors of preweanling rats. I think that the most plausible explanation for the potentiated locomotor response observed in EEDQ-treated preweanling rats stems from age-dependent differences in D1- and D2-like receptor supersensitivity. Receptor turnover is greater in preweanling rats and it possible that NPA is stimulating an abundance of supersensitized receptors, thereby resulting in a potentiated locomotor response.

APPENDIX A

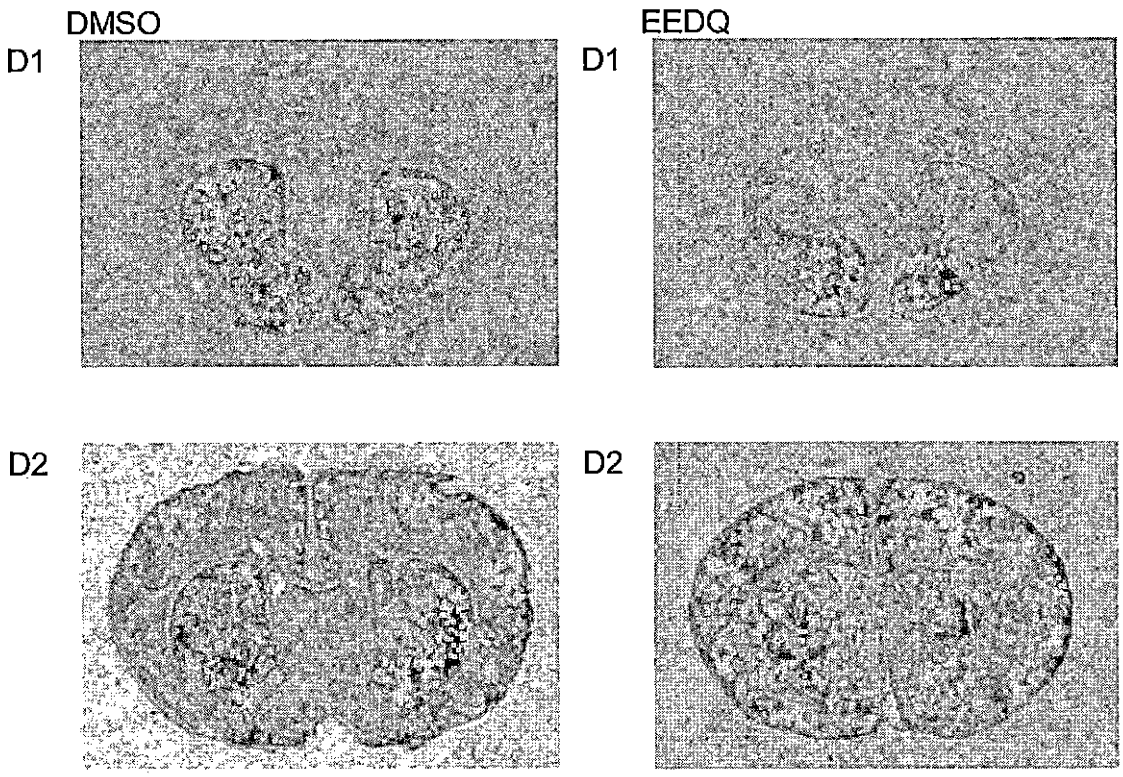
CANNULA PLACEMENT SCHEMATICS



Schematic representations of cannula placements in the dorsal striatum of (A) adult (Experiment 1) and (B) preweanling rats (Experiment 2 and 3). In all cases, numbers on the right indicate distance (mm) from Bregma using coordinates from the rat brain atlas of Paxinos and Watson (1998).

APPENDIX B

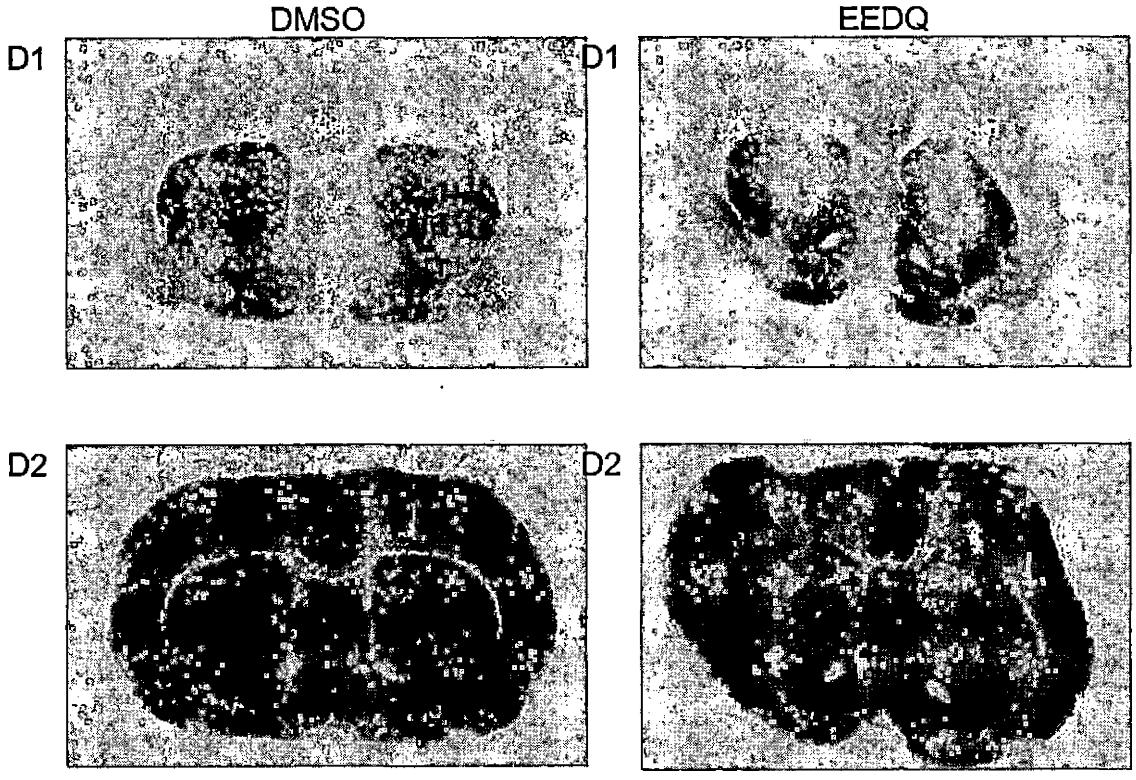
AUTORADIOGRAMS: PREWEANLING RATS



Representative autoradiograms of [^{3}H]SCH23390 and [^{3}H]spiperone binding after bilateral infusions of EEDQ and DMSO into the dorsal striatum on PD 17.

APPENDIX C

AUTORADIOGRAMS: ADULT RATS



Representative autoradiograms of [^{3}H]SCH23390 and [^{3}H]spiperone binding after bilateral infusions of EEDQ and DMSO into the dorsal striatum on PD 84.

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