THE OLFACTORY BULBECTOMIZED RAT: EVIDENCE FOR ENDOCANNABINOID DYSREGULATION

by

SARAH A. EISENSTEIN

(Under the Direction of Andrea G. Hohmann)

ABSTRACT

Endocannabinoids, which activate cannabinoid CB_1 receptors, modulate neurotransmission. The current studies used the olfactory bulbectomy rodent model, which induces neurobiological changes in the brain and behaviors that are indicative of dopaminergic dysfunction, to test the hypothesis that this behavioral and neurochemical syndrome is attributable to endocannabinoid signaling dysregulation. Locomotor responsivity to a novel open field and repeated administration of amphetamine (1 mg/kg i.p.) was investigated in olfactory bulbectomized and sham-operated rats. CB_1 receptor antagonists/inverse agonists rimonabant or AM251 were administered at 1 mg/kg (i.p.) prior to exposure to a novel open field. To investigate whether enhanced cannabinoid signaling would decrease development of sensitization to amphetamine in a CB_1-dependent manner, the fatty acid amide hydrolase inhibitor URB597 (0.3 mg/kg i.p.) was administered to olfactory bulbectomized and sham-operated rats alone or coadministered with rimonabant (1 mg/kg i.p.) prior to amphetamine administration. Cannabinoid receptor density and endocannabinoid content were measured using radioligand binding with [^{3}H]-CP55,940 and receptor autoradiography and high performance liquid chromatography mass spectrometry, respectively. Olfactory bulbectomy increased locomotor activity upon exposure to novelty and

amphetamine administration relative to sham surgery. Olfactory bulbectomized rats exhibited increased locomotor responsivity to amphetamine on the first day of administration but did not develop the typical sensitization pattern that was observed in sham-operated animals. URB597 attenuated the development of locomotor sensitization to amphetamine in sham-operated animals but not in olfactory-bulbectomized rats. Rimonabant (1 mg/kg) prevented full habituation to a novel open field in olfactory bulbectomized but not sham-operated rats. AM251 (1 mg/kg) also tended to prevent full habituation in olfactory bulbectomized rats. Olfactory bulbectomy decreased endocannabinoid levels in the ventral striatum relative to sham surgery. By contrast, endocannabinoid content in the pirifirom cortex, hippocampus, and cerebellum was not altered by olfactory bulbectomy. Cannabinoid receptor levels in several brain regions and endocannabinoid content in the ventral striatum were differentially correlated with distance traveled at behaviorally relevant time points in olfactory bulbectomized and sham-operated rats. Our data provides evidence that olfactory bulbectomy induces dysregulation of the endocannabinoid signaling system which affects locomotor responses to a novel open field and amphetamine.

INDEX WORDS: Olfactory bulbectomy, locomotor, novelty, sensitization, amphetamine, URB597, rimonabant, AM251, CB_1, [^{3}H]CP55,940, anandamide, 2-arachidonoyl glycerol

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SARAH A. EISENSTEIN

B.S., Tulane University, 2000

M.A., Southeastern Louisiana University, 2004

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2008

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SARAH A. EISENSTEIN

Major Professor:	Andrea Hohmann
Committee:	Philip Holmes
	Jonathon Crystal
	John Wagner

Electronic Version Approved:
Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2008

DEDICATION

This work is dedicated to friends and family, F and M, and Eric.

ACKNOWLEDGEMENTS

I would like to thank my dissertation committee for their very helpful advice and patience. Most of all I would like to thank my advisor, Andrea Hohmann, whose intelligence and work ethic have been nothing short of inspiring.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER ONE

GENERAL INTRODUCTION

Endocannabinoids, the body's own endogenous cannabis-like substances, act at cannabinoid receptors in the central nervous system to modulate neurotransmission. The most well-characterized endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG) are synthesized postsynaptically (Devane et al., 1992) in response to binding of postsynaptic receptors by other neurotransmitters (i.e. glutamate) (Stella et al., 1997; Giuffrida et al., 1999; Stella and Piomelli, 2001) and act at presynaptic CB_1 receptors (Devane et al., 1988; Howlett et al., 1990; Matsuda et al., 1990). CB_1 receptors are G-protein coupled and inhibit adenlyate cyclase activity, resulting in the inhibition of neurotransmitter release (Matsuda et al., 1990). These characteristics of the endocannabinoid system together with the distribution of CB_1 receptors (Herkenham et al., 1991b) strongly suggest that this system is highly involved in modulating emotional and pain responsivity.

One area in which the endocannabinoid system plays a modulatory role is the mesocorticolimbic dopaminergic system. CB_1 receptors are located presynaptically throughout the basal ganglia and ventral striatum on GABAergic and glutamatergic neurons (Herkenham et al., 1991b; Mailleux and Vanderhaeghen, 1992; Hohmann and Herkenham, 2000; Gerdeman and Lovinger, 2001; Kofalvi et al., 2005), which modulate responsivity to stimuli that affect dopaminergic transmission (Blandini et al., 2000). Although it is well known that endocannabinoids affect GABAergic and glutamatergic transmission in the basal ganglia and ventral striatum (van der Stelt and Di Marzo, 2003), it is uncertain how and when the

endocannabinoid system is activated. Stimuli such as stress and drugs of abuse are also known to increase dopaminergic transmission in the nucleus accumbens of the ventral striatum. However, whether endocannabinoids are mobilized to decrease hyperdopaminergic activity or further contribute to the heightened dopaminergic transmission, thereby participating in striatal plasticity in learned responsivity, remains poorly understood.

One way to investigate the role endocannabinoids in modulating dopaminergic transmission is to employ an animal model in which dopaminergic transmission is dysfunctional. The bilateral olfactory bulbectomy rodent model induces behavior and neurobiology resembling hyperdopaminergia. Behaviorally, olfactory bulbectomized animals exhibit heightened sensitivity to the locomotor effects of novelty (Klein and Brown, 1969; van Riezen and Leonard, 1990), the dopamine reuptake blocker cocaine (Chambers et al., 2004), and the indirect dopaminergic agonist amphetamine (Gaddy and Neill, 1976; Holmes et al., 2002). Neurobiologically, olfactory bulbectomy induces dopaminergic hypersensitivity in the ventral striatum (Gilad and Reis, 1979; Lingham and Gottesfeld, 1986; Holmes, 1999; Masini et al., 2004). The role of endocannabinoids in dopaminergic dysfunction induced by this model has not previously been investigated.

The development of pharmacological tools that directly or indirectly act at CB_1 receptors has aided in determining the role of the endocannabinoid system in behavior. The enzyme fatty acid amide hydrolase (FAAH), which degrades anandamide (Cravatt et al., 1996), has been identified as a therapeutic target. The FAAH inhibitor URB597 has been shown by our own laboratory (Moise et al., 2008) and others to have anxiolytic and antidepressant effects (Kathuria et al., 2003; Patel and Hillard, 2006; Bortolato et al., 2007; Marco et al., 2007). The CB_1 receptor competitive antagonist/inverse agonist SR141716A (rimonabant), which antagonizes the

classical hypomotor effects induced by potent CB_1 agonists (Rinaldi-Carmona et al., 1994) , is used to show that a particular phenomena is CB_1-mediated in animal models, but also has been shown to have its own therapeutic benefits through blockade of endocannabinoid neurotransmission (Jagerovic et al., 2008).

The present studies aim to elucidate the contribution of the endocannabinoid system in dopaminergic dysfunction induced by bilateral olfactory bulbectomy. In the first study (Chapter 3), the selective FAAH inhibitor URB597 was administered to olfactory bulbectomized and sham-operated animals to elucidate the role of endocannabinoids in the observed heightened locomotor sensitization to amphetamine that is induced by olfactory bulbectomy. Rimonabant was also employed to determine whether the effects of URB597 are CB_1-mediated. In the second study (Chapter 4), the role of the endocannabinoid system in the heightened locomotor response following exposure to a novel open field (i.e. novelty-induced locomotor activity) induced by olfactory bulbectomy was investigated. Olfactory bulbectomized and sham-operated rats that underwent exposure to novelty were assessed for endocannabinoid content using high-performance liquid chromatography (HPLC). Cannabinoid receptor density and distribution was also assessed in the same animals using [^{3}H]-CP55,940 binding and quantitative autoradiography. The impact of blockade of CB_1 receptors with antagonists/inverse agonists on locomotor responses to novelty in olfactory bulbectomized and sham-operated rats was also investigated.

CHAPTER TWO

LITERATURE REVIEW

Cannabinoid receptors are widely distributed throughout the brain of several mammalian species, including human and the rat (Herkenham et al., 1990; Herkenham et al., 1991b). The best characterized substrates, or endogenous cannabinoids, for these receptors are anandamide (Devane et al., 1992; Fride and Mechoulam, 1993; Mechoulam et al., 1994) and 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995). Endocannabinoids are synthesized and released on demand from postsynaptic neurons (Devane et al., 1992). Release may occur in response to rises in intracellular calcium in the postsynaptic cell (Di Marzo et al., 1994; Cadas et al., 1996; Sugiura et al., 1996; Cadas et al., 1997; Sasaki and Chang, 1997) and/or binding of postsynaptic neurotransmitter receptors (Stella et al., 1997; Giuffrida et al., 1999; Stella and Piomelli, 2001). The actual mechanism of endocannabinoid release may be passive diffusion through and/or eased by the existence of lipid binding proteins embedded in the cell membrane (Akerstrom et al., 2000).

In the central nervous system, the most fully characterized cannabinoid receptor is the CB_1 receptor (Devane et al., 1988; Howlett et al., 1990; Matsuda et al., 1990). CB_1 receptors are localized predominantly to presynaptic sites, and are densely expressed in the hippocampus (Katona et al., 1999; Hajos et al., 2000; Hoffman and Lupica, 2000; Irving et al., 2000; Katona et al., 2000), amygdala (Katona et al., 2001), striatum (Szabo et al., 1998), nucleus accumbens (Hoffman and Lupica, 2001; Manzoni and Bockaert, 2001) and cortical areas (Ferraro et al., 2001b; Ferraro et al., 2001a). Activation of G-protein-coupled CB_1 receptors inhibits adenlyate

cyclase and subsequently inactivates voltage-gated calcium channels and/or activates inward-rectifying potassium channels, thereby exerting an inhibitory effect on neurotransmission (Matsuda et al., 1990). The postsynaptic, on-demand release of endocannabinoids together with the presynaptic location of CB_1 receptors suggest that endocannabinoids act as retrograde messengers. Many lines of evidence have established that endocannabinoids inhibit release of glutamate, GABA, norepinephrine (Schlicker et al., 1997; Kathmann et al., 1999b) and acetylcholine (Gifford and Ashby, 1996; Gifford et al., 1997a; Gifford et al., 1997b; Kathmann et al., 1999a; Gifford et al., 2000; Kathmann et al., 2001a; Kathmann et al., 2001b).

Anandamide and 2-AG are deactivated primarily by the enzymes fatty amino acid hydrolase (FAAH) (Devane et al., 1992; Cravatt et al., 1996) and monoacylglyceride lipase (MGL) (Dinh et al., 2002), respectively. FAAH is located in the somata and dendrites of cells in the hippocampus, in Purkinje cells and their dendrites in the cerebellum, and, similar to CB_1 receptors, in the somata and proximal dendrites of cells in the basolateral nucleus of the amygdala (Gulyas et al., 2004). MGL is located on axon terminals of cells in the hippocampus, axons of the cells in the cerebellum and on somata and proximal dendrites in cells of the basolateral nucleus in the amygdala (Gulyas et al., 2004). Like CB_1 mRNA, FAAH mRNA is heterogeneously distributed in the striatum and throughout the brain (Thomas et al., 1997). The above evidence suggests that endocannabinoids modulate a number of neurotransmitter systems throughout the brain and affect cognitive, affective, and motivational systems.

Endocannabinoids Modulate Dopaminergic Transmission

The role of endocannabinoid involvement in dopaminergic transmission has been the subject of recent intense study. In the striatum, CB_1 receptors are located presynaptically on GABA-ergic medium-spiny neurons (Herkenham et al., 1991b; Mailleux and Vanderhaeghen,

1992; Hohmann and Herkenham, 2000) and glutamatergic neurons (Gerdeman and Lovinger, 2001; Kofalvi et al., 2005). Interestingly, FAAH mRNA expression is distributed homogenously throughout the caudate-putamen, indicating that the hydrolase is localized to medium spiny neurons (Thomas et al., 1997). Using whole-cell patch-clamp electrophysiology techniques and CB_1 knockout mice, Gerdeman et al. found that endocannabinoid signaling is necessary for long-term depression of glutamatergic neurons in the striatum, indicating the importance of endocannabinoids in striatal synaptic plasticity (Gerdeman et al., 2002). The authors hypothesized that anandamide is released as a retrograde messenger from medium spiny neurons and taken up at presynaptic glutamatergic sites in the striatum, thus inducing long term depression. The induction of long-term depression in the striatum by anandamide is blocked by administration of the CB_1 antagonist SR141617A (rimonabant) (Gerdeman et al., 2002).

Endocannabinoids modulate GABAergic and glutamatergic transmission in both the direct and indirect striatal output pathways. The direct striatal output pathway includes the medium spiny GABAergic neurons of the dorsal striatum which project to the output regions of the substantia nigra pars reticulata and the globus pallidus interna (Blandini et al., 2000). The indirect striatal output pathway includes the medium spiny neurons of the dorsal striatum which project to the globus pallidus externa (Blandini et al., 2000). Thus, activation of glutamatergic or GABAergic CB_1 receptors in the indirect pathway may ultimately increase locomotion. By contrast, inhibition by endocannabinoids of the direct pathway decreases locomotion (van der Stelt and Di Marzo, 2003). Activation of CB_1 receptors located on cortical glutamatergic afferents in the nucleus accumbens typically increases ventral tegmental area dopaminergic firing through indirect inhibition of GABAergic medium spiny neurons that normally inhibit this firing (Robbe et al., 2001; Pistis et al., 2002; Robbe et al., 2002). The quality of

endocannabinoid modulation also depends at least partially on the type and location of dopamine receptor that is affected by external input. Systemic administration of D_2-like but not D_1-like agonists increase striatal anandamide levels (Giuffrida et al., 1999; Centonze et al., 2004). While there is currently no evidence of direct modulation of dopaminergic transmission by endocannabinoids, the multiple CB_1 receptor sites in the basal ganglia and ventral striatum allow for indirect modulation of this system through direct modulation of GABA- and glutamatergic transmission (van der Stelt and Di Marzo, 2003). Thus, endocannabinoids play a compensatory role in modulating plastic and locomotor responses to stress, novelty, and rewarding stimuli (Spanagel and Weiss, 1999).

Behavioral evidence supports a role for endocannabinoids in regulating neuronal activity in the striatum. Inhibitors of endocannabinoid transport AM404 and VDM11 attenuate spontaneous hyperlocomotion in CB_1 knockout mice (Tzavara et al., 2006). The typical yawning induced by dopamine agonist apomorphine and later-phase hyperactivity induced by dopamine receptor agonist quinpirole are attenuated by intracerebroventricular and systemic administration of AM404. The reduction in yawning induced by AM404 is mediated by a CB_1-dependent mechanism and mimicked by exogenous anandamide administration (Beltramo et al., 2000). A decrease in locomotor activity is also observed subsequent to d-amphetamine administration in rats treated with the potent CB_1 partial agonist Δ^9-tetrahydrocannabinol (Hattendorf et al., 1977; Pryor et al., 1978; Moss et al., 1984). AM404 also attenuates early hyperactivity observed in a model of Huntington's disease induced by bilateral intrastriatal injections of a toxin, 3-nitroproprionic acid (3-NP), that selectively damages striatal GABAergic efferent neurons. Furthermore, rats that receive 3-NP exhibit significantly fewer CB_1 receptor binding sites and mRNA in the basal ganglia compared to control rats (Lastres-Becker et al., 2002).

Further evidence that the endocannabinoid system modulates the midbrain dopaminergic system is reported in locomotor activity and amphetamine sensitization studies. The CB_1 antagonist/inverse agonist SR141716A (rimonabant) attenuates hypoactivity produced by the direct CB_1 agonist WIN55,212 (Jarbe et al., 2006). Rimonabant potentiates the locomotor response to amphetamine (Masserano et al., 1999; Thiemann et al., 2008b) in otherwise naive animals whereas AM251, a CB_1 antagonist/inverse agonist structurally similar to rimonabant, decreases this response (Thiemann et al., 2008a). Furthermore, CB_1 knockout mice exhibit reduced amphetamine sensitization (Thiemann et al., 2008b). These seemingly contradictory effects of CB_1 antagonists have been attributed to their inverse agonist properties (Pertwee, 2005). However, vanilloid TRPV1, cannabinoid and non-cannabinoid receptors may also be affected by rimonabant and AM251. Clearly, the effects of CB_1 antagonism/inverse agonism on dopaminergic responsivity warrants further investigation.

Bilateral Olfactory Bulbectomy

The severing of the olfactory bulbs in the rat results in behavioral and neurobiological changes mimicking many of those seen in human affective disorders. Consequently, this procedure is termed the "olfactory bulbectomy rodent model of depression". The subsequent alterations in behavior and neurochemistry are not due simply to the loss of smell or the ability to detect pheromones (Cairncross et al., 1978; Calcagnetti et al., 1996) but rather the loss of input to limbic areas of the brain induced by olfactory bulbectomy. The olfactory bulbs constitute 4% of the rat brain's mass (Cain, 1974). Olfactory bulbectomy induces neuronal degeneration in projections innervating the main and accessory bulbs as well as in primary and secondary projection areas such as the ventral striatum (Scalia and Winans, 1975), hippocampus, piriform cortex (Carlsen et al., 1982), amygdala (Jancsar and Leonard, 1981), dorsal raphe (Nesterova et

al., 1997), and locus coeruleus (Shipley et al., 1985). Importantly, with regards to limbic function, the amygdala projects, via the stria terminalis pathway, to the hypothalamus, caudate putamen, and nucleus accumbens (DeOlmos et al., 1985). The olfactory bulbectomy symptomatology arises approximately two weeks after surgery and persists for several weeks after symptoms appear. The olfactory bulbectomy symptomatology is sensitive to chronic rather than acute treatment with antidepressants (Song and Leonard, 2005). This time course for observing therapeutic efficacy is consistent with the therapeutic window required for demonstrating antidepressant efficacy in human affective disorder (Song and Leonard, 2005). The behavioral and neurobiological changes that occur following olfactory bulbectomy enable identification of neural substrates involved in behavioral abnormalities observed in the model.

Behavioral Changes Following Olfactory Bulbectomy

The olfactory bulbectomy rodent model of depression induces many behavioral changes thought to mimic those of human affective disorders. Perhaps the most striking and most common behavior observed in the olfactory bulbectomized (OBX) rat is hyperlocomotion upon initial exposure to a novel, enclosed arena (Klein and Brown, 1969; van Riezen and Leonard, 1990). This hyperlocomotion effect is thought to resemble increased sensitivity to stress in humans. Increased activity in OBX rats upon exposure to novelty is attenuated with chronic antidepressant treatment (Song and Leonard, 2005). OBX rats also exhibit increased locomotion during the nocturnal phase, which is similarly attenuated by chronic antidepressant treatment (Giardina and Radek, 1991). This symptom may be analogous to sleep disturbances observed in humans with affective disorders. OBX rats show decreased sexual activity (Lumia et al., 1992) and food-motivated behavior (Kelly et al., 1997). These behavioral effects are thought to model anhedonia observed in humans suffering from affective disorders. The OBX rat also exhibits

deficits in learning and/or memory. Defensive freezing behavior after mild foot shock is decreased in OBX rats compared to sham-operated rats (Primeaux and Holmes, 1999). OBX rats exhibit impaired spatial learning in the 8-arm radial maze in which a food reward is placed in one of the arms (Hall and Macrides, 1983). These spatial learning deficits are attenuated with chronic antidepressant treatment and resemble cognitive slowing observed in humans with affective disorders. Finally, learned passive- (Joly and Sanger, 1986; van Riezen and Leonard, 1990) and active (King and Cairncross, 1974) avoidance behaviors are diminished in OBX rats.

Neurochemical Changes Following Olfactory Bulbectomy

The OBX model induces neurobiological alterations in rats that resemble neurobiological pathologies observed in human affective disorders. Noradrenaline function is decreased in brains of OBX rats (Jancsar and Leonard, 1984; Song and Leonard, 1995). Similarly, the levels of serotonin (5-HT) and its metabolite 5-hydroxyindole acetic acid are decreased in the OBX rat (Jancsar and Leonard, 1984; Lumia et al., 1992). Deficits in NE and 5-HT are reversed by chronic antidepressant administration (King and Cairncross, 1974; Song and Leonard, 2005). In addition, changes in glutamatergic, GABAergic, acetylcholinergic, and neuropeptidergic systems that are reminiscent of human pathology have been observed in the OBX rat, some of which are reversed with chronic antidepressant treatment (Song and Leonard, 2005).

Olfactory Bulbectomy Induces Sensitivity to Novelty, Cocaine and Amphetamine

While the olfactory bulbectomy model is usually posited as a model of affective disorders, there is ample evidence that it also functions as a model of hyperdopaminergia. The OBX model induces hyperresponsiveness to novelty (Klein and Brown, 1969; van Riezen and Leonard, 1990) and a "presensitized" state in response to dopaminergic agonists. Hypersensitivity is observed to the dopamine reuptake blocker cocaine (Chambers et al., 2004)

as well as to acute administration of the indirect dopamine agonist amphetamine (Gaddy and Neill, 1976). In addition, OBX rats acquire self-administration of amphetamine at a faster rate than sham-operated controls (Holmes et al., 2002). These behavioral alterations suggest that OBX rats demonstrate neuropathology in a pathway relevant to stress (modeled by increased locomotion in response to a novel environment) and motivation (modeled by increased locomotor sensitization and propensity to self-administer drugs of abuse such as amphetamine). While several neurobiological substrates play a role in either or both of these behaviors, the mesocorticolimbic dopaminergic system is the most likely site of action implicated in these phenomena.

Responsivity to Novelty

Behavior upon exposure to a novel environment relies heavily on dopaminergic tone in the mesocorticolimbic dopaminergic system. Indeed, rats that are high-activity responders to a novel environment, the extent of which is positively correlated with the propensity to self-administer amphetamine, demonstrate increased dopaminergic transmission in the nucleus accumbens (Piazza et al., 1991). In addition, microinjection of the dopamine antagonist fluphenazine into the nucleus accumbens decreases motor activity upon exposure to novelty to levels observed in habituated rats. Microinjection of baclofen, a $GABA_B$ agonist, into the ventral tegmental area (VTA) also attenuates hyperactivity levels in rats exposed to a novel environment. The observed attenuations of hyperactivity specifically occur after microinjections of DA antagonists and $GABA_B$ agonists into structures that comprise the mesolimbic dopamine system; injections of fluphenazine and baclofen into the neighboring motor nuclei and substantia nigra, respectively, do not attenuate novelty-induced hyperactivity to habituation levels (Hooks and Kalivas, 1995). This evidence indicates that the olfactory bulbectomy model, which induces

high locomotor responsivity to novelty, may be useful framework for studying dopaminergic dysfunction.

Responsivity to Cocaine and Amphetamine

The motivational and neural plasticity-inducing effects of cocaine and amphetamine, drugs to which olfactory bulbectomized rats are highly sensitive, appear to converge at the level of the nucleus accumbens in the ventral striatum in the form of dopaminergic transmission. This convergent transmission originates from the ventral tegmental area (Bozarth and Wise, 1986) and the GABA-ergic medium spiny neurons (MSNs) of the striatum that are influenced, in turn, by several cortical inputs (Gerdeman et al., 2003). These inputs include both dopaminergic inputs from the VTA and regulatory glutamatergic input from limbic areas such as the hippocampus, amygdala and other cortical areas. The convergence of cortical input to the striatal MSNs suggests that these neurons play a major role in regulating synaptic plasticity of the mesolimbic dopamine system in response to drugs of abuse and stress (Gerdeman et al., 2003). Pierce and Kalivas term this entire system and its projections to other relevant cortical areas the "motive circuit" and suggest that it is necessary for the expression of sensitization to cocaine (Pierce and Kalivas, 1997). Opiates (Bozarth and Wise, 1986; Di Chiara and Imperato, 1988) and other classes of drugs including alcohol, nicotine and cannabinoids have also been shown to enhance dopaminergic transmission in the nucleus accumbens (Di Chiara and Imperato, 1988; Chen et al., 1990), reinforcing the idea that the mesocorticolimbic dopamine system influences motivational behavior towards reward in general. Berridge and Robinson (Berridge and Robinson, 1998) propose that this system mediates the "incentive salience" value of stimuli with rewarding properties independent of the actual feeling of enjoyment and reward learning (wanting vs. liking). The increased sensitivity to pharmacological compounds that induce

hyperdopaminergic activity in OBX animals further indicates that olfactory bulbectomy is an appropriate model of dopaminergic dysfunction in the "motive circuit".

Olfactory Bulbectomy Induces Heightened Dopaminergic Sensitivity

Olfactory bulbectomy profoundly affects the dopaminergic system. Dopamine D_2 receptor and preproenkephalin mRNA levels are increased in the olfactory tubercle of bulbectomized rats at behaviorally relevant time points (Holmes, 1999). The olfactory tubercle is part of the ventral striatum, which receives dopaminergic input from the VTA, and is implicated in the reinforcing properties of drugs of abuse (Kornetsky et al., 1991). Deafferentation of olfactory bulb output induces sprouting of dopaminergic axons in the olfactory tubercle, as evidenced by increases in both DA uptake as well as the amount and activity of tyrosine hydroxylase (Gilad and Reis, 1979). Postsynaptically, adenylate cyclase activity is increased at 7, 14 and 20 days post deafferentation of the olfactory bulb. D_1 and D_2 receptor levels are also increased at 20 days post-surgery in the olfactory tubercle following the same manipulation (Lingham and Gottesfeld, 1986). Basal dopamine release in the olfactory tubercle and dorsal striatum is also increased in OBX rats compared to sham rats at behaviorally relevant time points (Masini et al., 2004).

Cannabinoid Pharmacological Manipulations in Animal Models

Enzymes that terminate the activity of the endocannabinoids are emerging as promising pharmacotherapeutic targets. Unlike direct agonists of CB_1 receptors, inhibitors of these enzymes are less likely to induce psychoactive side-effects and motor depression. The FAAH inhibitor URB597 has beneficial effects on behavior mediated by the limbic system in several rodent models. URB597 increases the duration of time spent in the open arms of an elevated zero-maze (Kathuria et al., 2003) and plus maze (Patel and Hillard, 2006; Moise et al., 2008) and

decreases isolation-induced ultrasonic vocalizations in rat pups, indicating that URB597 has anxiolytic properties (Kathuria et al., 2003). Importantly, all of these anxiolytic effects are blocked by CB_1 receptor antagonist rimonabant, indicating that they are CB_1-mediated. In addition, URB597 decreases helplessness behavior in the forced swim test, which is used to screen drugs for antidepressant efficacy (Kathuria et al., 2003). Chronic administration of URB597 reverses weight loss and sucrose intake deficits in animals exposed to chronic mild stress, a model of depression (Bortolato et al., 2007). Impulsive behavior is decreased in maternally deprived rats, another model of psychiatric disorders, by a subchronic regimen of URB597 treatment (Marco et al., 2007). Finally, URB597 and other FAAH inhibitors are effective at raising the threshold for intracranial self-stimulation of electrodes implanted in the medial forebrain bundle, indicating that endocannabinoids block reward stimulation (Vlachou et al., 2006). The exact conditions (environment, dose, paradigms) under which URB597 and other FAAH inhibitors are effective at reversing affective symptomatology are still largely unknown. Additional basic research studies are required to better understand how inhibition of FAAH ameliorates behavioral and neuropathological symptomatology associated with psychiatric disorders (Naidu et al., 2007).

The development of drugs that act directly or indirectly on CB_1 receptors have also been elucidated using animal models of diseases that involve dopaminergic dysfunction. SR141617A (rimonabant), a CB_1 antagonist/inverse agonist with high affinity for CB_1 receptors in the brain (CB_1 K_d = 0.23 nM) (Rinaldi-Carmona et al., 1994), antagonizes the classical behavioral effects of CB_1 agonists (Rinaldi-Carmona et al., 1994). AM404, a CB_1 indirect agonist, alleviates hyperkinesias induced by 3-nitroproprionic acid in an animal model of Huntington's disease that

also induces loss of CB_1 binding in the basal ganglia (Lastres-Becker et al., 2002; Lastres-Becker et al., 2003).

Cannabinoid pharmacological intervention may therefore be effective in reducing symptomatology induced by animal models of affective and dopaminergic dysfunction. Olfactory bulbectomy, due to its effects on neurobiology and behavior influenced by dopamine, would seem to be an excellent model to elucidate the role of the endocannabinoid system in the modulation of the dopaminergic responsivity. Pharmacological tools, including compounds that inibit the degradation of endocannabinoids such as anandamide as well as direct and indirect agonists and competitive antagonists that act at CB_1 receptors, provide us the opportunity to do so.

CHAPTER THREE

AMPHETAMINE SENSITIZATION IN THE OLFACTORY BULBECTOMIZED RAT: EVIDENCE FOR ENDOCANNABINOID DYSREGULATION

ABSTRACT

The endocannabinoid system modulates excitatory and inhibitory neurotransmission throughout the central nervous system and regulates activity of the midbrain dopaminergic system. The present studies aimed to investigate the role of the endocannabinoid system in modulating dopaminergic responsivity using an animal model of dopaminergic dysfunction. The olfactory bulbectomy rodent model induces behavioral and neurobiological symptomatology due to increased dopaminergic sensitivity. We hypothesized that increased dopaminergic sensitivity induced by olfactory bulbectomy is mediated, at least in part, by dysregulation of endocannabinoid signaling. Rats underwent olfactory bulbectomy or sham operations and were assessed two weeks later in two tests of hyperdopaminergic responsivity: locomotor response to novelty and locomotor sensitization to amphetamine. Rats were administered amphetamine (1 mg/kg i.p.) for eight consecutive days to induce locomotor sensitization. URB597, an inhibitor of the anandamide hydrolyzing enzyme fatty-acid amide hydrolase (FAAH), was administered daily (0.3 mg/kg i.p.) to sham and olfactory bulbectomized (OBX) rats to investigate the impact of inhibition of FAAH on locomotor sensitization to amphetamine. Pharmacological specificity was evaluated with the CB_1 antagonist/inverse agonist rimonabant (1 mg/kg i.p). OBX rats exhibited heightened locomotor activity in response to novelty and initial amphetamine administration relative to sham-operated rats. URB597 attenuated the development of locomotor sensitization to amphetamine in sham but not OBX rats in a CB_1-mediated manner. The present results provide pharmacological support for the hypothesis that enhanced endocannabinoid transmission attenuates development of sensitization in intact animals but not in animals experiencing dopaminergic dysfunction. Our data collectively suggest that the endocannabinoid system is compromised in olfactory bulbectomized rats.

Introduction

Cannabinoid CB_1 receptors are widely distributed throughout the brain of several mammalian species, including the rat, hamster and human (Herkenham et al., 1990; Herkenham et al., 1991b). The most well-characterized endogenous ligands for this receptor, the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG), are synthesized and released on demand from postsynaptic neurons (Devane et al., 1992; Di Marzo et al., 1994). The CB_1 receptor is localized predominantly to presynaptic sites and acts to inhibit presynaptic neurotransmission in a retrograde manner (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). Thus, endocannabinoids play a modulatory role on neurotransmission throughout the central nervous system.

The endocannabinoid system modulates activity of the mesocorticolimbic dopaminergic system. CB_1 receptors and CB_1 mRNA are located presynaptically on glutamatergic afferents and postsynaptic terminals of GABAergic medium spiny interneurons in the striatum, respectively (Herkenham et al., 1991b; Mailleux and Vanderhaeghen, 1992; Hohmann and Herkenham, 2000). Direct and indirect dopamine agonists increase striatal anandamide levels (Giuffrida et al., 1999; Centonze et al., 2004). Endocannabinoid signaling is necessary for CB_1-mediated long-term depression of glutamatergic neurons in the striatum (Gerdeman et al., 2002). These findings suggest that endocannabinoids indirectly modulate dopaminergic transmission.

We hypothesized that hyperdopaminergic dysfunction observed following olfactory bulbectomy is associated with dysregulation of endocannabinoid signaling. Bilateral olfactory bulbectomy in the rodent induces heightened locomotor responsivity induced by exposure to a novel open field (i.e. novelty) (Klein and Brown, 1969; van Riezen and Leonard, 1990) and a "presensitized" locomotor state in response to indirect dopaminergic agonists such as cocaine

(Chambers et al., 2004) and amphetamine (Gaddy and Neill, 1976). In addition, olfactory bulbectomized rats exhibit faster acquisition of amphetamine self-administration relative to sham rats (Holmes et al., 2002). Olfactory bulbectomy induces sprouting of dopaminergic axons in the olfactory tubercle, a part of the ventral striatum, in which basal dopamine release, receptor levels and adenlyate cyclase activity are increased at behaviorally relevant time points (Gilad and Reis, 1979; Lingham and Gottesfeld, 1986; Holmes, 1999; Masini et al., 2004).

Unlike direct agonists of CB_1 receptors, pharmacological inhibition of endocannabinoid-deactivating enzymes does not induce psychoactive side-effects and motor depression (Piomelli, 2005). Anandamide is deactivated primarily by the enzyme fatty amide acid hydrolase (FAAH) (Devane et al., 1992; Cravatt et al., 1996). The FAAH inhibitor URB597 induces CB_1-mediated anxiolytic effects (Mailleux and Vanderhaeghen, 1992; Kathuria et al., 2003; Patel and Hillard, 2006; Moise et al., 2008) as well as antidepressant (Bortolato et al., 2007) and anti-impulsivity effects (Marco et al., 2007).

We used the olfactory bulbectomy model to investigate the role of endocannabinoids in modulating behaviors influenced by dopaminergic dysfunction - the locomotor response to novelty and sensitization to amphetamine. First, the olfactory bulbectomy model was validated by assessing locomotor activity in response to a novel exposure to an open field (i.e. locomotor response to novelty) in both sham-operated and olfactory bulbectomized (OBX) rats. Next, locomotor sensitization to amphetamine was profiled in OBX and sham rats over eight consecutive days of amphetamine administration. The FAAH inhibitor URB597 was then administered prior to amphetamine administration to evaluate the effect of increasing the bioavailability of anandamide on amphetamine sensitization in OBX and sham rats. The

contribution of CB_1 receptors to URB597-mediated actions was evaluated by blocking cannabinoid CB_1 receptors with the competitive antagonist/inverse agonist rimonabant.

Methods

Subjects and Surgical Procedures

Male Sprague-Dawley rats (N = 57), Harlan, Indianapolis, IN) that weighed approximately 250 - 300 g at surgery were used. All behavioral and surgical procedures were approved by the University of Georgia Animal Care and Use Committee. Rats were housed in groups of two to five in a humidity- and temperature-controlled animal housing facility. The lighting schedule was reversed so that lights were on at 0600 and off at 1800. All behavioral testing was initiated during the light phase. Rats were randomly assigned to either sham or olfactory bulbectomy (OBX) surgery. For OBX surgery, rats (n = 18) were anesthetized with a combination of pentobarbital (65 mg/kg intraperitoneally (i.p.); Sigma, St. Louis, MO) and ketamine hydrochloride (100 mg/kg i.p.; Fort Dodge Laboratories, Fort Dodge, IA) or isoflurane (Abbott Laboratories, North Chicago, IL). Burr holes measuring 3 mm in diameter were bilaterally drilled approximately 5 mm anterior to bregma and 1 mm lateral to the midline. The dura mater was pierced and a curved plastic pipette tip was used to aspirate the olfactory bulbs. The resulting cavity was filled with Gelfoam (Upjohn, Kalamazoo, MI). Rats receiving the sham surgery (n = 39) underwent the same procedure except that the olfactory bulbs were not aspirated. Confirmation of olfactory bulb lesion was determined by brain dissection at the end of the experiment. Lesions were considered complete if the bulbs were completely severed from the forebrain, the weight of the tissue dissected from the olfactory bulb cavity did not exceed 5 mg, and frontal lobes were not bilaterally damaged. Histological verifications were performed by an experimenter blinded to the surgical condition.

Pharmacological Manipulations

URB597 was purchased from Cayman Chemical (Ann Arbor, MI). Rimonabant was a gift from NIDA. D-amphetamine sulfate was purchased from Sigma (St. Louis, MO). URB597 (0.3 mg/kg) and rimonabant (1 mg/kg) were dissolved in a 1:1:8 ratio of 100% ethanol:emulphor:saline. D-amphetamine sulfate (1 mg/kg) was dissolved in 0.9% saline. Drugs were administered intraperitoneally (i.p.) in a volume of 1 ml/kg body weight. Animals were randomly assigned to drug conditions that included ethanol:emulphor: saline vehicle (sham n = 4, OBX n =4), saline (sham n = 10, OBX n = 5), URB597 (0.3 mg/kg) (sham n = 13, OBX n = 9), URB597 (0.3 mg/kg) coadministered with rimonabant (1 mg/kg) (sham n = 9), or rimonabant (1 mg/kg) alone (sham n = 4). All animals (n = 57) received amphetamine fifteen minutes following these pharmacological manipulations.

Locomotor Sensitization to Amphetamine

Locomotor sensitization to amphetamine was assessed using a model that was validated previously using cocaine (Chambers et al., 2004) (see Figure 3.1 for diagrammed procedure). At least two weeks following surgery, rats were placed individually in the center of a polycarbonate activity monitor chamber (Med Associates, St. Albans, VT) measuring 44.5 x 44 x 34 cm housed in a darkened, quiet room. A 25- watt bulb shone over the chamber. Activity was automatically measured by computerized analysis of photobeam interrupts (Med Associates). Total distance traveled in the arena was obtained from the computer program and used for data analysis. Rats remained undisturbed in this chamber for 30 min. At the end of this period, according to previously randomly assigned drug conditions, rats were injected i.p. with vehicle, saline, URB597 (0.3 mg/kg), URB597 (0.3 mg/kg) coadministered with CB_1 antagonist rimonabant (1 mg/kg) or rimonabant (1 mg/kg) alone. Rats were then placed back in the center of the chamber

and remained undisturbed for 15 min. Activity was again automatically recorded by the computer software. At the end of this period, rats were injected i.p. with d-amphetamine sulfate and placed back into the chamber, undisturbed, for 45 min. Activity was automatically recorded by Med Associates computer software during the entire interval. The exact same procedure was employed for the next seven days (see Figure 1).

Data Analysis

Distance traveled was recorded in three consecutive phases on each day of behavioral testing: pre-injection open field activity (for 30 min); pre-amphetamine open field activity after injection of vehicle/saline, URB597, or URB597 plus rimonabant (for 15 min); and post-amphetamine open field activity after injection of amphetamine (for 45 min). The first 30 min open field session on the first day was analyzed with a between subjects (sham vs. OBX) Student's t-test. Differences between surgical and drug groups were analyzed with repeated measures analysis of variance (ANOVA) with Fisher's Least Significant Difference post hoc tests. In the case of significant interactions, Student's t-tests were performed as appropriate. Friedman's test for nonparametric repeated measures ANOVA was used to analyze sensitization trends. "Distance traveled" counts obtained from activity meter software at various time points served as the dependent variable. In a small minority of cases (0.7% of data points or 28 out of 4104 data points), because of technical difficulties with computer software or the open field arena, data points were incomplete. Missing data points never occurred on more than one day out of eight consecutive days of testing for any animal. Missing data values were therefore replaced with group means for that specific time point on that day. $P \leq 0.05$ was considered significant.

Results

Control Conditions

In both sham and OBX groups, distance traveled post-amphetamine did not differ between saline- and ethanol: emulphor: saline vehicle-treated animals [$P > 0.05$, both comparisons]. Therefore, saline-treated animals were combined with the ethanol:emulphor:saline-treated animals to form what will be referred to hereafter as the "vehicle"-treated control group.

Exposure to Novelty

OBX animals traveled greater distances than sham rats during the 30 min exposure to the novel open field arena ($t_{55} = 1.95$, $P < 0.05$, one-tailed) (see Figure 3.2).

Habituation (Pre-injection Sessions)

Distance traveled was assessed during the 30 min pre-injection sessions on days 2-7 (i.e. when the open field arena is no longer novel). Olfactory bulbectomy did not affect locomotor activity during this period. Both OBX and sham groups that received vehicle exhibited decreased activity over consecutive days of testing ($F_{6, 126} = 13.87$, $P = 0.001$). Cannabinoid pharmacological manipulations did not affect distance traveled in sham or OBX animals during the 30 min habituation session on days 2-7 ($P>0.05$ for both analyses) (data not shown).

Cannabinoid Pharmacological Manipulations (Pre-amphetamine Sessions)

Distance traveled during the 15 min pre-amphetamine interval following administration of vehicle, URB597 (0.3 mg/kg), URB597 (0.3 mg/kg) in the presence or absence of rimonabant (1 mg/kg), or rimonabant (1 mg/kg) alone was assessed over eight days of testing. Olfactory bulbectomy did not affect distance traveled during this pre-amphetamine interval in animals that received vehicle ($P > 0.05$) (data not shown). To ensure that any possible locomotor effects of

URB597 during the preamphetamine session did not coincide with effects of URB597 on locomotor sensitization to amphetamine in sham animals (see post-amphetamine session results below), the first and last four days of preamphetamine locomotor activity were analyzed separately. In sham animals, during the first four days of testing, URB597 produced a modest but reliable decrease in distance traveled during the preamphetamine session ($F_{2, 32} = 6.11$, $P < 0.01$) and this effect was blocked by coadministration of rimonabant ($P < 0.05$ for both post hoc comparisons) (see Figure 3.3a). However, URB597 did not affect locomotor activity during the preamphetamine interval during the last four days of testing ($P > 0.05$) (see Figure 3.3b). This latter interval corresponds to the period in which effects of URB597 on locomotor sensitization to amphetamine were observed in sham animals (see post-amphetamine session results below). Rimonabant, administered alone to sham animals at a dose that was inactive (1 mg/kg i.p.) when administered by itself, did not affect distance traveled during the pre-amphetamine sessions relative to vehicle ($P > 0.05$) (see Figure 3.3c). Finally, URB597 did not affect distance traveled during the preamphetamine sessions in OBX animals relative to vehicle ($P > 0.05$), although both OBX groups exhibited decreases in activity over eight days of testing ($F_{7, 112} = 2.27$, $P < 0.05$) (see Figure 3.3d).

Amphetamine-induced Locomotor Activity (Post-amphetamine Sessions)

Amphetamine-induced locomotor activity was greater in OBX than sham-operated animals that similarly received vehicle on the first day of amphetamine administration ($F_{1, 21} = 6.88$, $P < 0.05$) (see Figure 3.4a). Amphetamine-induced distance traveled increased but then leveled out over the 45 min observation interval in both groups ($F_{4, 84} = 27.36$, $P < 0.001$) (see Figure 3.4a). Olfactory bulbectomy differentially affected distance traveled over time on the first day of amphetamine administration ($F_{4, 84}=3.72$, $P < 0.01$) : OBX animals traveled more than

shams from 27-45 min post-amphetamine administration ($P < 0.05$ for all comparisons, t-tests) (see Figure 3.4a).

Amphetamine-induced distance traveled was compared in sham and OBX rats that received vehicle over all eight days of testing. A time-dependent sensitization to amphetamine developed in sham-operated groups ($Fr_{8, 14}$=32.64, $P< 0.001$); distance traveled on days 5-8 was greater than that observed on day 1 ($P < 0.05$ for all comparions, Dunn's multiple comparisons), By contrast, OBX animals did not further sensitize to locomotor effects of amphetamine ($P>0.05$) (see Figure 3.4b).

Drug Effects on the Development of Amphetamine-induced Sensitization

Sham-operated and OBX groups were analyzed separately because OBX animals responded very differently to both amphetamine and URB597 relative to sham-operated rats with regards to distance traveled.

In sham animals that received vehicle and URB597 in the presence or absence of rimonabant, amphetamine-induced distance traveled was greater during the last four days of testing than that observed during the first four ($Fr_{8,35} = 47.48$, $P < 0.0001$, Friedman's test); distance traveled on day 8 was greater than that observed on days 1, 2 or 3. Amphetamine-induced locomotor activity was also greater on days 5-7 than on day 1 ($P < 0.05$ for all comparisons, Dunn's multiple comparisons) (see Figure 3.5a, b). Furthermore, in sham animals that received URB597, development of sensitization did not occur ($P > 0.05$, Friedman's). Therefore, the effect of cannabinoid pharmacological manipulations was analyzed during the first four and last four days of testing separately to best describe the effect of URB597 on the development of sensitization in sham animals. During the first four days of testing, amphetamine-induced distance traveled was not affected by pharmacological manipulations ($P >$

0.05) (see Figure 3.5a). Pharmacological manipulations affected distance traveled during post-amphetamine sessions over the last four days of amphetamine administration ($F_{2, 32} = 3.72$, $P < 0.05$). Shams that received URB597 prior to amphetamine administration traveled less distance than those that received vehicle and this effect was blocked by rimonabant ($P < 0.05$ for both post hoc comparisons) (see Figure 3.5b). Development of locomotor sensitization to repeated amphetamine injection was observed in sham animals during the last four days of testing ($F_{3, 96} = 5.73$, $P = 0.001$) (see Figure 3.5b). Rimonabant alone did not affect development of sensitization relative to vehicle ($P > 0.05$) but both vehicle-treated and rimonabant alone-treated animals exhibited increased activity over repeated amphetamine injections ($F_{7, 112} = 40.01$, $P = 0.001$) (see Figure 3.5c).

In OBX animals, amphetamine-induced distance traveled was similar across all 8 days of amphetamine administration ($P > 0.05$), reflecting the lack of further sensitization to amphetamine in OBX animals (see Figure 3.5d). Furthermore, URB597 did not alter the development of sensitization in OBX rats ($P>0.05$) (see Figure 3.5d).

Discussion

The olfactory bulbectomy model is a model in which dopaminergic transmission is profoundly altered (Gilad and Reis, 1979; Lingham and Gottesfeld, 1986; Mailleux and Vanderhaeghen, 1992; Holmes, 1999; Masini et al., 2004). This model was employed to examine the role of the endocannabinoid signaling system in modulating behaviors known to rely at least partially on dopaminergic transmission: locomotion in response to novelty and development of locomotor sensitization to amphetamine. In line with other studies (Klein and Brown, 1969; van Riezen and Leonard, 1990), OBX animals traveled greater distances than sham animals in response to novelty. Sham and OBX animals also responded quite differently to

the FAAH inhibitor URB597. Sham animals that received the FAAH inhibitor URB597 traveled less than those that received vehicle or URB597 coadministered with rimonabant during the preamphetamine interval. By contrast, the locomotor activity of OBX animals was not affected by inhibition of FAAH over the same intervals. Finally, sham animals exhibited a time-dependent sensitization to amphetamine that was apparent across successive days and was decreased by URB597 in a CB_1-dependent manner. In agreement with previous research (Gaddy and Neill, 1976), OBX animals displayed a heightened locomotor response to acute treatment with amphetamine. Our results verify and extend this observation by documenting that, unlike shams, further sensitization to amphetamine was absent in OBX animals following repeated amphetamine. Our data suggest that OBX animals are "presensitized" to indirect dopaminergic agonists. Thus, sensitization could not be further enhanced in OBX animals with repeated amphetamine treatment (Chambers et al., 2004). The inability to detect enhanced amphetamine-induced locomotor sensitization in OBX animals likely reflects the fact that OBX animals were already presensitized to amphetamine. By contrast, sham rats were able to sensitize to the effects of amphetamine. Furthermore, OBX animals were insensitive to the FAAH inhibitor URB597, with regards to development of sensitization to amphetamine. Rimonabant alone did not affect sensitization to amphetamine relative to vehicle, indicating that the dose used in the current study (1 mg/kg i.p.) did not alter locomotor activity induced by novelty or amphetamine.

A recent study employing an amphetamine sensitization paradigm (7 consecutive days of amphetamine administration) similar to the one used here, demonstrated that the CB_1 antagonist/inverse agonist AM251 decreased development as well as expression of sensitization in otherwise naive animals (Thiemann et al., 2008a). By contrast, another study conducted in the

same laboratory showed that rimonabant (3 mg/kg i.p.) increased amphetamine-induced hyperactivity on the first day of amphetamine administration as well as the expression of sensitization on a challenge day (Thiemann et al., 2008b). Masserano et al. (1999) also found that rimonabant potentiated the locomotor response to amphetamine. In our study, a lower, behaviorally inactive dose (1 mg/kg i.p.) of rimonabant was administered than that used in the Thiemann studies. Thus, rimonabant alone did not affect locomotor activity in our studies. The differences in findings regarding the effects of AM251 and rimonabant may be explained by the fact that these drugs are known to have inverse agonist properties and may differentially block CB_1 receptors (Pertwee, 2005).

Inhibition of FAAH with URB597 decreased the development of amphetamine sensitization in sham animals in our study. This observation contrasts with the decrease in amphetamine sensitization observed with AM251 (Thiemann et al., 2008a). Administration of URB597, which ultimately results in increased extracellular availability of anandamide (Piomelli, 2005), would be expected to exert an effect opposite to that of CB_1 blockade. However, URB597 did not affect development of sensitization until day 5 of our paradigm. Perhaps subchronic administration of URB597 produces a more prolonged activation of CB_1 receptors by increasing extracellular anandamide concentration in an environment in which the endocannabinoid system is better able to modulate dopaminergic transmission in response to chronic amphetamine treatment or other stimuli that affect the midbrain dopaminergic system. This enhanced modulation may not be apparent until development of sensitization is well under way (i.e. approximately day 5 of amphetamine administration in the current study) and there is sufficient alteration of dopaminergic tone to mobilize endocannabinoids in a manner sensitive to

these behavioral measurements. Notably, FAAH inhibition had no effect on the development of amphetamine sensitization in OBX animals.

OBX animals exhibited a presensitized locomotor state that is likely to result from an inability of the endocannabinoid system to modulate dopaminergic activity. The removal of the olfactory bulbs may induce gross structural changes in the striatum and/or basal ganglia that include losses of glutamatergic and GABAergic neurons, thereby removing CB_1 receptors that may reside presynaptically on these afferent inputs. Alternatively, olfactory bulbectomy may disrupt the synthesis of endogenous cannabinoids in postsynaptic neurons in the striatum and/or basal ganglia that are affected by deafferentation due to olfactory bulbectomy. These alterations would be expected to induce lower than optimal levels of 2-AG and anandamide. Finally, olfactory bulbectomy-induced changes in structures that are directly innervated by the olfactory bulb and involved in limbic responses (e.g. amygdala, piriform and entorhinal cortices, and olfactory tubercle) (Kelly et al., 1997) may disrupt regulatory input downstream from these structures into the dopaminergic system, including the striatum and other areas in the basal ganglia. Our data suggest that this disruption may render OBX animals more vulnerable to stimuli, such as novelty and amphetamine, that heighten dopaminergic transmission.

It is noteworthy that acute administration of URB597 decreased activity in sham but not OBX animals during the pre-amphetamine interval. This suppression, however, dissipated over time; by days 4-8 URB597 no longer suppressed pre-amphetamine locomotor activity. This interval occurred immediately after injection and may reflect a URB597-mediated anxiolytic effect in respose to the stress of exposure to a novel environment or injection. Thus FAAH inhibition may exert an immediate anxiolytic effect rather than a locomotor effect per se. This finding is in agreement with those of other studies in which FAAH inhibition has been found to

exert anxiolytic effects without altering locomotor activity (Kathuria et al., 2003; Patel and Hillard, 2006; Moise et al., 2008). As with sensitization, OBX animals were insensitive to this putative anxiolytic effect. Interestingly, FAAH inhibition did not influence the development of amphetamine sensitization until later days in the sensitization protocol relative to vehicle in sham-operated rats. Overall, these findings suggest that endocannabinoids may be more involved in modulating anxiety responses than responses to indirect dopaminergic agonists such as amphetamine. Nevertheless, subchronic (8 days) administration of URB597 does eventually aid in lessening development of sensitization to amphetamine. Our data suggests that endocannabinoid transmission plays a role in regulating plasticity in response to stimuli that enhance dopaminergic transmission.

Figure 3.1. Experimental design. All animals underwent the diagrammed procedure daily for 8 days beginning 14 days following OBX or sham surgery. First, animals were placed in the open field arena (30 min). Animals were then injected with either URB597 (0.3 mg/kg i.p.), URB597 (0.3 mg/kg i.p.) plus rimonabant (1 mg/kg i.p.), rimonabant alone (1 mg/kg i.p.) or vehicle (i.p.) and placed back into the arena (15 min). Animals were then treated with amphetamine (1 mg/kg i.p.) and placed back into the arena (45 min). Activity levels were monitored for each of the three phases described.

Figure 3.1

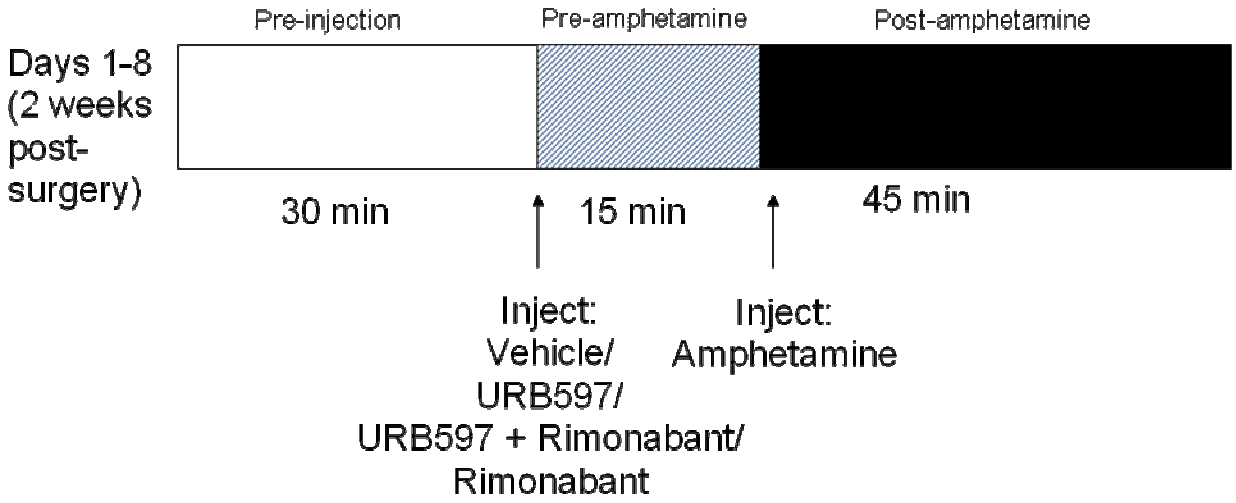

Pre-injection
Pre-amphetamine
Post-amphetamine
Days 1-8 (2 weeks post-surgery)
30 min
15 min
45 min
Inject: Vehicle/ URB597/ URB597 + Rimonabant/ Rimonabant
Inject: Amphetamine

Figure 3.2. Exposure to novelty in olfactory bulbectomized and sham-operated rats. Olfactory bulbectomy induced a heightened locomotor response to novelty relative to sham surgery during the initial 30 min open field session on day 1. Mean + SEM shown; *, $P < 0.05$ compared to sham.

Figure 3.2

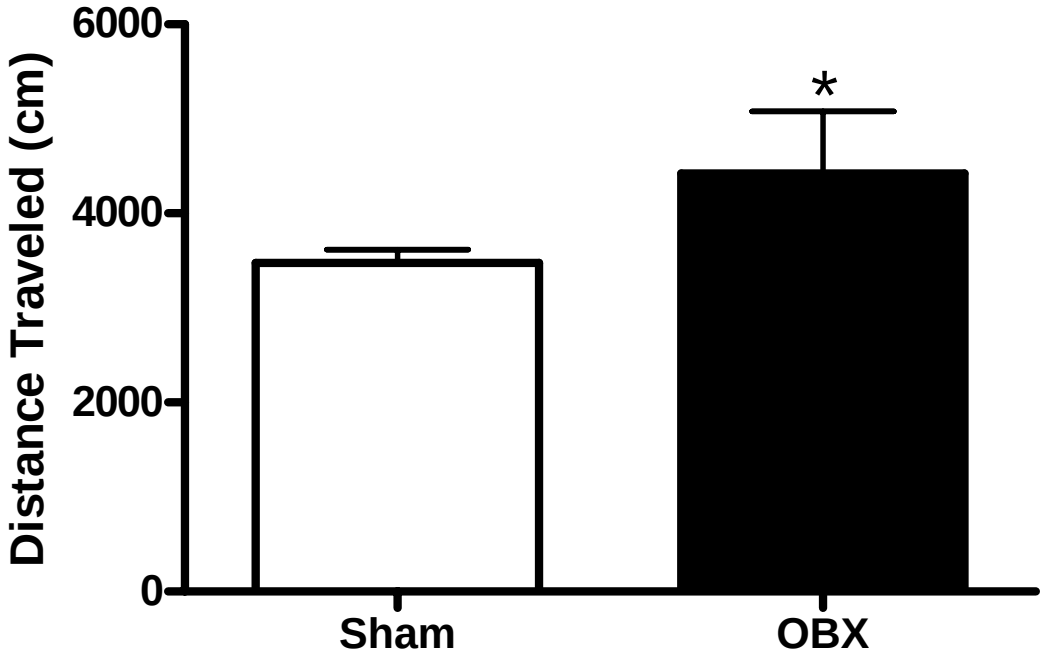


Figure 3.3 Preamphetamine activity in sham-operated and olfactory bulbectomized rats. Pre-amphetamine locomotor activity is (a) decreased by URB597 (0.3 mg/kg i.p.) in a CB_1-mediated manner in sham animals during days 1-4 of testing. This effect was blocked by rimonabant (1 mg/kg i.p).. (b) Neither URB597 (0.3 mg/kg i.p.) nor URB597 (0.3 mg/kg i.p.) coadministered with rimonabant (1 mg/kg i.p.) affects locomotor activity relative to vehicle during days 5-8 of testing in sham animals. (c) Rimonabant (1 mg/kg i.p.) alone does not affect pre-amphetamine locomotor activity relative to vehicle in sham rats. (d) URB597 (0.3 mg/kg i.p.) does not affect pre-amphetamine locomotor activity in OBX animals relative to vehicle. Mean + SEM shown.

Figure 3.3

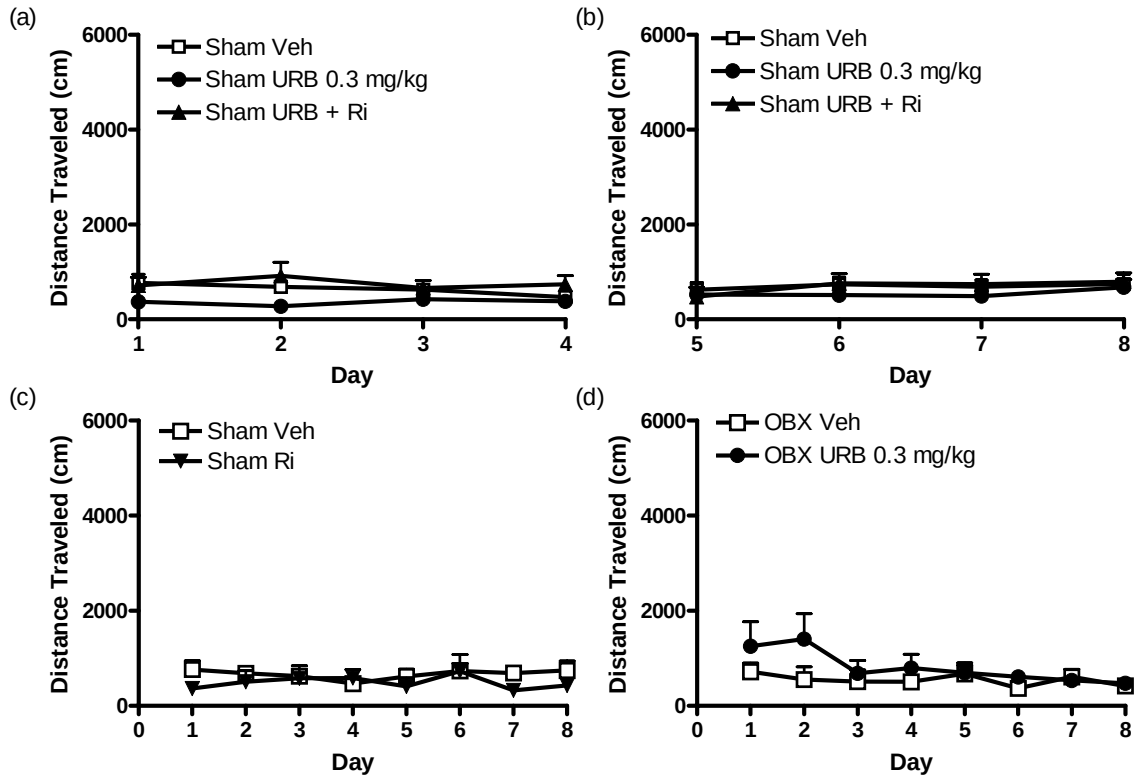

(a)
Sham Veh
Sham URB 0.3 mg/kg
Sham URB + Ri
Distance Traveled (cm)
Day
(b)
Sham Veh
Sham URB 0.3 mg/kg
Sham URB + Ri
Distance Traveled (cm)
Day
(c)
Sham Veh
Sham Ri
Distance Traveled (cm)
Day
(d)
OBX Veh
OBX URB 0.3 mg/kg
Distance Traveled (cm)
Day

Figure 3.4. Olfactory bulbectomy and development of amphetamine sensitization. (a) Olfactory bulbectomy induces a heightened locomotor state in response to initial (day 1) amphetamine (1 mg/kg i.p.) administration. (b) Sham animals develop a typical sensitization to repeated injections of amphetamine, as defined by increased locomotor activity. By contrast, OBX animals are presensitized to amphetamine. Mean + SEM shown; *, $P < 0.05$ relative to sham; ** $P < 0.01$ relative to sham.

Figure 3.4

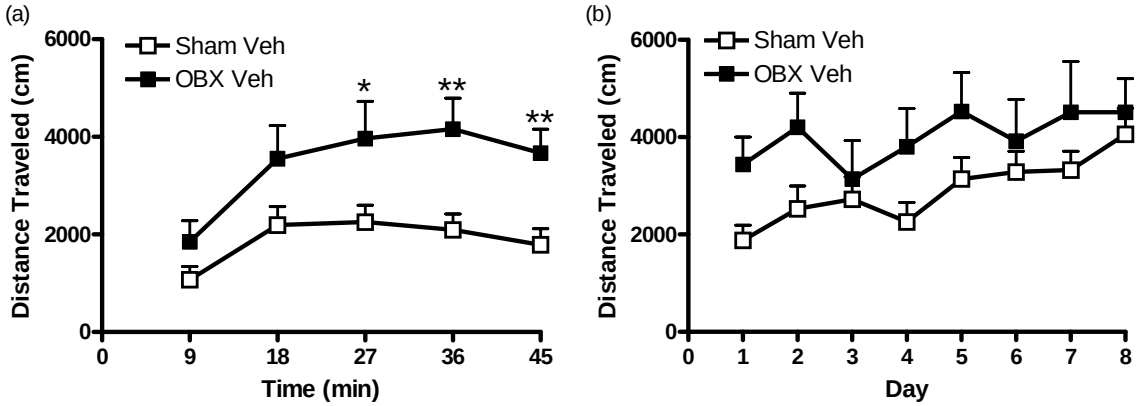
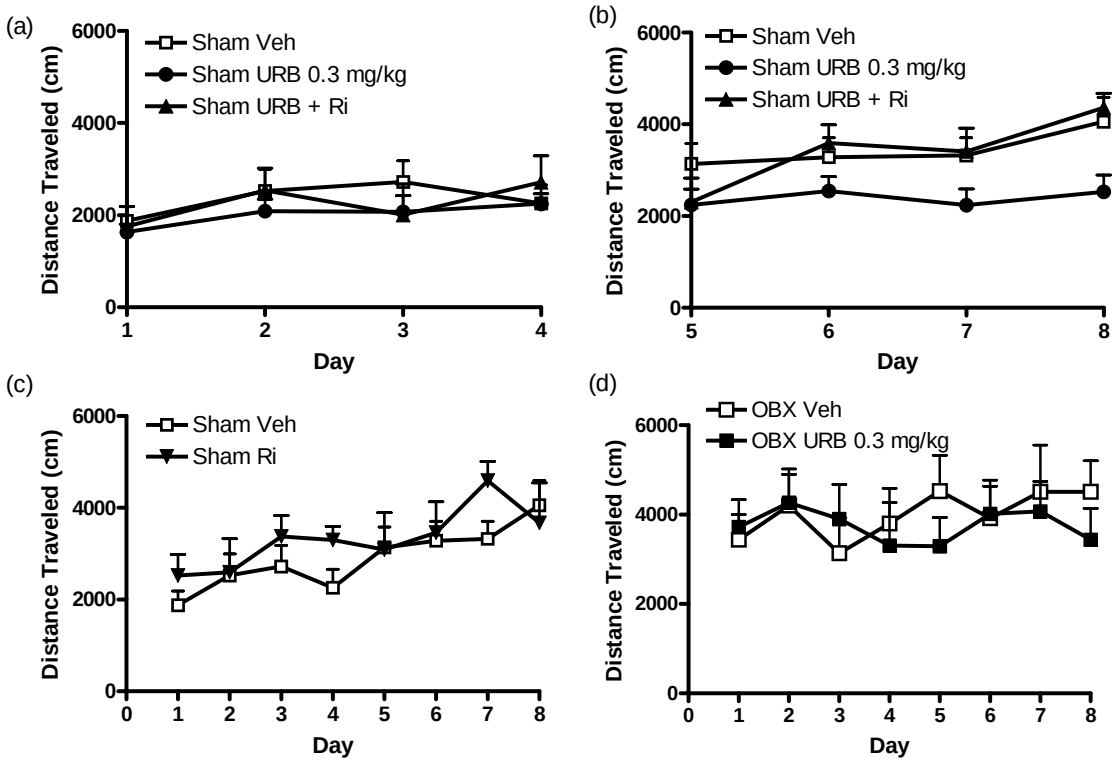

(a)
Sham Veh
OBX Veh
Distance Traveled (cm)
6000
4000
2000
0
0
9
18
27
36
45
Time (min)
*
**
**
(b)
Sham Veh
OBX Veh
Distance Traveled (cm)
6000
4000
2000
0
0
1
2
3
4
5
6
7
8
Day

Figure 3.5. Cannabinoid pharmacological manipulations and amphetamine sensitization in sham-operated and olfactory bulbectomized rats. (a) Sham animals that received vehicle (i.p.), URB597 (0.3 mg/kg i.p.), or URB597 (0.3 mg/kg i.p.) coadministered with rimonabant (1 mg/kg i.p.) exhibit similar locomotor activity in response to amphetamine (1 mg/kg i.p.) during the first four days of amphetamine administration. (b) URB597 (0.3 mg/kg i.p.) attenuated amphetamine sensitization in sham animals relevant to vehicle (i.p.) and URB597 (0.3 mg/kg i.p.) coadministered with rimonabant (1 mg/kg i.p.). (c) Rimonabant (1 mg/kg i.p.) did not affect the development of locomotor sensitization to amphetamine relative to vehicle (i.p.) in sham animals. (d) Olfactory bulbectomy blocked the effect of URB597 on the development of amphetamine sensitization. Mean + SEM shown.

Figure 3.5


(a)
Sham Veh
Sham URB 0.3 mg/kg
Sham URB + Ri
Distance Traveled (cm)
Day
(b)
Sham Veh
Sham URB 0.3 mg/kg
Sham URB + Ri
Distance Traveled (cm)
Day
(c)
Sham Veh
Sham Ri
Distance Traveled (cm)
Day
(d)
OBX Veh
OBX URB 0.3 mg/kg
Distance Traveled (cm)
Day

CHAPTER FOUR

THE ROLE OF ENDOCANNABINOID DYSREGULATION IN THE HYPERLOCOMOTOR RESPONSE TO NOVELTY INDUCED BY OLFACTORY BULBECTOMY IN THE RAT

ABSTRACT

The endocannabinoid system modulates excitatory and inhibitory neurotransmission throughout the central nervous system and regulates activity of the midbrain dopaminergic system. Bilateral olfactory bulbectomy induces a hyperlocomotor response to a novel environment, a behavior attributed to dopaminergic dysfunction. The present studies aimed to investigate the role of the endocannabinoid system in the regulation of this behavior in olfactory-bulbectomized and sham-operated rats. Drug-naive olfactory bulbectomized and sham-operated rats were exposed to a novel open field for 30 min. High performance liquid chromatography and [^{3}H]-CP55,940 and binding autoradiography were performed to quantify endocannabinoid content and cannabinoid receptor densities, respectively, in brains of drug-naïve olfactory bulbectomized and sham-operated rats exposed to novelty. CB_1 receptor antagonists/inverse agonists rimonabant and AM251 were administered (1 mg/kg i.p.) prior to exposure to the novel arena in both olfactory bulbectomized and sham-operated rats. Olfactory bulbectomy increased the locomotor response elicited by exposure to a novel open field. Cannabinoid receptor levels in several brain regions were differentially correlated with distance traveled at behaviorally relevant time points during exposure to the novel open field in olfactory bulbectomized and sham-operated rats. 2-arachidonoylglycerol (2-AG) levels were negatively correlated with distance traveled in sham-operated rats and positively correlated with total distance traveled in olfactory bulbectomized rats. Rimonabant (1 mg/kg i.p.) increased distance traveled during the habituation phase of the exposure to the novel environment in olfactory bulbectomized but not in sham-operated rats. AM251 (1 mg/kg i.p.) also tended to increase distance traveled in olfactory bulbectomized rats during the habituation phase. The present findings suggest that the endocannabinoid system is dysregulated in olfactory bulbectomized rats. These studies may provide insight into how the

endocannabinoid signaling system may interact with the midbrain dopaminergic system to modulate dopamine-dependent behaviors.

Introduction

Endocannabinoids, the most well characterized of which are anandamide and 2-arachidonoylglycerol (2-AG), are released on demand from postsynaptic neurons upon receptor-stimulated or activity-dependent cleavage of membrane phospholipid precursors (Di Marzo et al., 1994). Endocannabinoids activate presynaptic CB_1 cannabinoid receptors to inhibit presynaptic transmission throught a retrograde mechanism (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). One system in which endocannabinoids modulate neurotransmission is the midbrain dopaminergic system. CB_1 receptor protein and mRNA are densely expressed in the main components of the basal ganglia, including dorsal and ventral striatum, globus pallidus, and substantia nigra (Herkenham et al., 1990; Hohmann and Herkenham, 2000). Pharmacological manipulation of dopaminergic transmission in the striatum increases anandamide levels (Giuffrida et al., 1999; Centonze et al., 2004). Endocannabinoid signaling is necessary for CB_1-mediated long-term depression of glutamatergic neurons in the striatum (Gerdeman et al., 2002). The multiple CB_1 receptor sites in the basal ganglia and ventral striatum allow for indirect modulation of dopaminergic transmission through direct modulation of GABAergic and glutamatergic transmission (Herkenham et al., 1991b; Mailleux and Vanderhaeghen, 1992; Spanagel and Weiss, 1999; Hohmann and Herkenham, 2000), indicating that endocannabinoids play a compensatory role in modulating plastic, locomotor responses to stress, novelty, and rewarding stimuli (Spanagel and Weiss, 1999).

The olfactory bulbectomy rodent model induces dopaminergic dysfunction (Gilad and Reis, 1979). Glutamatergic innervation to limbic areas of the brain including the amygdala, olfactory tubercle, entorhinal and piriform cortices is disrupted, thus inducing an array of symptomalogy reminiscent of mental illness in humans (Song and Leonard, 2005) (see Figure

4.8). Deafferentation of olfactory bulb output induces sprouting of dopaminergic axons and increases in adenlyate cyclase activity, basal dopamine release, and D_1 and D_2 levels (Gilad and Reis, 1979; Lingham and Gottesfeld, 1986; Holmes, 1999; Masini et al., 2004) in the olfactory tubercle, a region of the ventral striatum.

Most pertinent to the currently described studies, the OBX model induces hyperlocomotion in response to exposure to a novel open field (Klein and Brown, 1969; van Riezen and Leonard, 1990). This hyperresponsivity to novelty is believed to result from hyperdopaminergic transmission (Gilad and Reis, 1979). The current study investigates the role of endocannabinoids in the increased locomotor response to novelty in the olfactory bulbectomized rat. First, motor activity in response to a novel environment was assessed in olfactory bulbectomized (OBX) and sham-operated rats. Second, the effect of CB_1 receptor blockade on motor responses to novelty in OBX and sham rats was investigated by administering CB_1 antagonists/inverse agonists rimonabant and AM251. We hypothesized that the ability of endocannabinoids to suppress a hyperdopaminergic state is impaired in the olfactory bulbectomized rat, whereas this modulation is effective in sham-operated rats. Thus, olfactory bulbectomized rats would be more sensitive to the antagonist/inverse agonist effects of rimonabant and AM251 than sham-operated animals. Endocannabinoid levels in sensorimotor and limbic brain regions derived from OBX and sham brains were quantified with high performance liquid chromatography-mass spectrometry (LC/MS). Cannabinoid receptor densities in sensorimotor and limbic areas of OBX and sham rats were measured using binding and quantitative autoradiography with the radioligand and potent cannabinoid agonist [^{3}H]-CP55,940.

Methods

Subjects and Surgical Procedures

Male Sprague-Dawley rats (N = 66, Harlan, Indianapolis, IN) that weighed approximately 250 - 300 g at surgery were used. The University of Georgia Animal Care and Use Committee approved all behavioral and surgical procedures. Rats were housed in groups of two to five per cage in a humidity- and temperature-controlled animal housing facility. The lighting schedule was reversed so that lights were on at 0600 and off at 1800. Behavioral testing took place during the light phase. Rats were randomly assigned to either sham or olfactory bulbectomy (OBX) surgery. For OBX surgery, rats (n = 31) were anesthetized with a combination of pentobarbital (65 mg/kg intraperitoneally (i.p.); Sigma, St. Louis, MO) and ketamine hydrochloride (100 mg/kg i.p.; Fort Dodge Laboratories, Fort Dodge, IA) or isoflurane (Abbott Laboratories, North Chicago, IL). Burr holes measuring 3 mm in diameter were bilaterally drilled approximately 5 mm anterior to bregma and 1 mm lateral to the midline. The dura mater was pierced and a curved plastic pipette tip was used to aspirate the olfactory bulbs. The resulting cavity was filled with Gelfoam (Upjohn, Kalamazoo, MI). Rats receiving the sham surgery (n = 35) underwent the same procedure except that the olfactory bulbs were not aspirated. Confirmation of olfactory bulb lesion was determined by brain dissection at the end of the experiment. Lesions were considered complete if the bulbs were completely severed from the forebrain, the weight of the tissue dissected from the olfactory bulb cavity did not exceed 5 mg, and frontal lobes were not bilaterally damaged. Histological verifications were performed by an experimenter blind to the surgical condition.

Chemicals

[$^2H_4$]-Anandamide was prepared by the reaction of fatty acyl chlorides (Nu-Chek Prep, Elysian, MN) with a 10-fold molar excess of [$^2H_4$]-ethanolamine (Cambridge Isotope Laboratories, Andover, MA) in dichloromethane at 0-4˚C for 15 min, with stirring. The products were washed with water, dehydrated over sodium sulphate, filtered, and dried under N_2 and characterized by liquid chromatography/mass spectrometry (LC/MS) and ^1H nuclear magnetic resonance spectroscopy. Purity was >98% by LC/MS. Rimonabant and ^3H-CP55,940 were gifts from NIDA. AM251 was purchased from Tocris Bioscience (Ellisville, MO) and Cayman Chemical Company (Ann Arbor, MI). All other chemicals were purchased from commercial sources. Rimonabant and AM251 were dissolved in 1:1:8 (100% ethanol: emulphor: saline) and 1:1:1:17 (Dimethyl sulfoxide: cremophor: 100% ethanol: saline) vehicle, respectively. All drug injections were administered intraperitoneally (i.p.) in a volume of 1 ml/kg body weight.

Exposure to Novelty

Approximately two weeks following surgery, rats were placed individually in the center of a polycarbonate activity monitor chamber (Med Associates, St. Albans, VT) measuring 44.5 x 44 x 34 cm housed in a darkened, quiet room. A 25- watt bulb shone over the chamber. Activity was automatically measured by computerized analysis of photobeam interrupts (Med Associates). Total distance traveled in the arena was used for data analysis. Rats remained undisturbed in this chamber for 30 min. Locomotor responses upon exposure to the novel open field were compared in OBX (n = 10) and sham (n = 10) rats in the absence of pharmacological manipulations. The effect of CB_1 receptor blockade on locomotor responses to novelty was evaluated in OBX rats receiving either 1:1:8 vehicle (n = 5), 1:1:1:17 vehicle (n = 3), rimonabant, 1 mg/kg (n = 7), or AM251 1 mg/kg (n = 6). Sham rats similarly received either

1:1:8 vehicle (n = 4), 1:1:1:17 vehicle (n = 5), rimonabant 1 mg/kg (n = 8) or AM251 1 mg/kg (n = 8). Drugs were administered 30 min prior to exposure to the novel open field. Animals remained in their home cages prior to assessment of open field activity.

Tissue Extraction

Drug-naive OBX and sham rats (n = 20) were decapitated immediately following the exposure to the novel open field. Brains were rapidly dissected, divided into two hemispheres along the longitudinal axis and snap frozen in isopentane precooled to -30°C on dry ice. Frozen brains were stored at low temperature (-80°C or -30°C) until use. One hemisphere was used to obtain tissue punches for use in LC/MS studies and the other hemisphere was used to measure cannabinoid receptor density and distribution using [^{3}H]-CP55,940 binding and autoradiography.

Receptor Binding and Autoradiography

Single hemisphere coronal brain (n = 20) tissue sections (14 μM thickness) were cryostat cut and mounted four sections per slide. Cannabinoid receptor binding was performed using [^{3}H]CP55,940 (specific activity 77.5 Ci/mmol; Research Triangle Institute, Research Triangle Park, NC) as described previously (Herkenham et al., 1991a; Hohmann and Herkenham, 1998; Hohmann et al., 1999). Nonspecific binding was determined in the presence of 10 μM CP55,940. Briefly, binding was performed in cytomailers (3 h at 37° C) in 50 mM Tris-HCl (pH 7.4) containing 5% bovine serum albumin (BSA) and 5 nM [^{3}H]CP55,940. Slides were washed (4 h at 0° C) in the same buffer containing 1% BSA, fixed in 0.5% formalin in 50 mM Tris-HCl (pH 7.4 at 25° C) and blown dry. Sections were apposed to [^{3}H]-sensitive film (Amersham Hyperfilm, GE Healthcare LifeSciences, Piscataway, NJ) together with [^{3}H] standards (^{3}H microscales, Amersham, Arlington Heights, IL) for 14 weeks. Images were captured using a scanner.

Densitometry

Densitometry was performed using the public domain NIH Image software (U.S. National Institutes of Health, http://rsb.info.nih.gov/nih-image/) on a Macintosh computer (Macintosh, Cupertino, CA). The mean densities for relevant brain regions of the scanned tissue images were calculated and converted to nCi/mg tissue wet weight based on a best-fit polynomial equation calibration formula that takes into account tissue equivalent values provided by Amersham. Brain areas were outlined using the rat brain atlas (Paxinos & Watson, 1986) as a guide. The nCi/mg values for each tissue section of each animal were averaged. These averages were pooled into separate averages for sham and OBX animals. Non-specific binding values were subtracted from total binding values to obtain specific binding values used in data analysis. Specific binding values were converted to pmol/mg using an equation that takes into account the specific activity of the radioligand.

Lipid Extractions

Punches derived from single-hemisphere frozen brains of drug-naive rats (n = 16) were weighed and homogenized in methanol (1 ml per 100 mg tissue) containing [$^2H_4$]-anandamide and [$^2H_8$]-2-arachidonoyl-*sn*-glycerol (2-AG) (Cayman Chemicals, Ann Arbor, MI) as internal standards. Lipids were extracted with chloroform (2 vol) and washed with water (1 vol). Endocannabinoids and related lipids were fractionated by open-bed silica gel column chromatography, as described previously (Giuffrida et al., 2000). Briefly, the lipids were reconstituted in chloroform and loaded onto small glass columns packed with Silica Gel G (60-Å 230-400 Mesh ASTM; Whatman, Clifton, NJ). Fatty acid ethanolamides and 2-AG were eluted with 9:1 chloroform/methanol (vol/vol). Eluates were dried under N_2 and reconstituted in 0.1 ml of chloroform/methanol (1:4, vol/vol) for LC/MS analyses.

LC/MS Analyses.

An 1100-LC system coupled to a 1946D-MS detector (Agilent Technologies, Inc., Palo Alto, CA) equipped with an electrospray ionization (ESI) interface was used to measure anandamide, OEA, PEA and 2-AG levels in select brain regions punched from a single hemisphere of each frozen sample. Lipids were separated using a XDB Eclipse C18 column (50 x 4.6 mm i.d., 1.8 μm, Zorbax), eluted with a gradient of methanol in water (from 75% to 85% in 2.5 min and then to 90% in 7.5 min) at a flow rate of 10.0 ml/min. Column temperature was kept at 40˚C. MS detection was in the positive ionization mode, capillary voltage was set at 3 kV and fragmentor voltage was varied from 120V. N_2 was used as drying gas at a flow rate of 13 liters/min and a temperature of 350˚C. Nebulizer pressure was set at 60 PSI. Quantifications were conducted using an isotope-dilution method, monitoring Na^+ adducts of the molecular ions ($[M+Na]^+$).

Statistical Analysis

Data were analyzed by repeated measures analysis of variance (ANOVA), univariate ANOVA and Student's *t*-tests. In animals that received pharmacological manipulations, distance traveled was analyzed in 3 min blocks. Significant interactions were further analyzed with Student's t-tests or ANOVA, as appropriate. Planned comparisons were performed using one-tailed t-tests, as appropriate. Relationships between variables were analyzed using Pearson's *r* correlation. $P \leq 0.05$ was considered significant.

Results

Exposure to Novelty in Drug-naïve Rats

Animals placed in the open field arena showed exploratory behavior that declined progressively over the 30 min observation interval ($F_{9, 162} = 61.84$, $P < 0.0001$) (see Figure 4.1a). OBX animals traveled greater cumulative distance than sham animals during the first three

minutes of exposure to the novel open field (t_{18}=-2.28, $P < .05$) (see Figure 4.1b). By contrast, no difference in levels of exploratory behavior was observed between sham and OBX animals for the remainder of the observation interval (3-30 min following introduction into the open field) ($P > 0.05$) (see Figure 4.1c). We therefore divided the open field session into two phases for subsequent analyses: "novelty" (0-3 min post exposure to the novel open field) and "habituation" (3-30 min post exposure to the novel open field). Brains derived from these drug-naïve animals were used to determine the impact of olfactory bulbectomy on endocannabinoid levels and cannabinoid receptor densities in discrete brain regions.

To assess whether the observed hyperactivity in drug-naive olfactory bulbectomized rats during the novelty phase was due to anxiety or increased exploratory behavior induced by the novel open field, the open field arena was divided into a center zone (23.4 x 23.4 cm) and residual zone (all of the area outside the center area) for analysis. During the first three minutes of exposure to the novel open field, OBX animals spent a larger percent of the session time in the center area ($t_{18} = 1.72$, $P = 0.05$, one-tailed *t*-test) (see Figure 4.2a) and a lesser percent of the session time in the residual area ($t_{18} = 1.72$, $P = 0.05$, one-tailed *t*-test) (see Figure 4.2b). OBX animals did not differ from sham animals in time spent per entry into the center area during the novelty phase ($P > 0.05$) (see Figure 4.2c) although they spent less time per entry into the residual area ($t_{18} = 2.46$, $P < 0.05$) (see Figure 4.2d). During the entire 30 min session, OBX animals did not differ from sham-operated animals on any of these parameters (see Figure 4.2e-h).

Control conditions

Distance traveled during the 30 min exposure to the novel open field did not differ in sham animals that received rimonabant vehicle (1:1:8) or AM251 vehicle (1:1:1:17) from that

observed in drug-naïve animals. Therefore, data from these animals were pooled into a single "sham control" group for subsequent analysis of the effects of rimonabant and AM251 on locomotor activity. Likewise, distance traveled during the 30 min exposure to a novel open field was similar in OBX animals that received rimonabant vehicle (1:1:8) or AM251 vehicle (1:1:1:17) to that observed in drug-naive animals. Therefore, data from these animals were similarly pooled into a single "OBX control" group for subsequent analysis of the effects of rimonabant and AM251 on locomotor activity.

CB_1 Blockade with Rimonabant and AM251

Distance traveled during the "novelty" phase (0-3 min) of exposure to the open field was increased in OBX controls (1110.08 ± 75.39 cm) relative to sham controls (828.3 ± 34.77 cm) (t_{35} = -3.46, $P < 0.01$). To further evaluate this difference between OBX and sham rats in the presence of rimonabant and AM251, drug effects were analyzed seperately in each surgical group. In sham rats, rimonabant decreased distance traveled relative to control while AM251 increased distance traveled relative to control and rimonabant ($F_{2, 34}$ = 9.98, $P < 0.0001$, $P < .05$ for all comparisons) (see Figure 4.3a). In OBX rats, drug treatment did not affect distance traveled relative to control during the "novelty" phase ($P > 0.05$) (see Figure 4.3b).

Distance traveled during the "habituation" phase (3-30 min) of exposure to the novel open field was increased by olfactory bulbectomy relative to sham surgery ($F_{1, 60}$ = 9.72. $P <$ 0.01) and increased by AM251 relative to control treatment ($F_{2, 60}$ = 3.5, $P < 0.05$, $P < 0.05$ for comparison) (see Figure 4.4a). To further evaluate the difference between OBX and sham rats in the presence of rimonabant and AM251, drug effects were analyzed in each surgical group separately. In sham rats, drug treatment impacted distance traveled in a time-dependent manner ($F_{16, 256}$ = 2.17, $P < 0.01$) but further analysis failed to reveal any time points at which

rimonabant-, AM251- and control-treated sham rats differed from each other (see Figure 4.4b). In OBX rats, rimonabant increased distance traveled relative to control treatment during the "habituation phase" of exposure to the novel open field ($F_{2, 28} = 3.25$, $P = 0.054$, $P < 0.05$ for comparison). A trend towards increased activity in OBX animals that received AM251 was observed ($P = 0.075$). Effects of rimonaband did not differ from AM251 on locomotor behavior during the habituation phase (see Figure 4.4c).

Receptor Binding and Autoradiography

Olfactory bulbectomy did not alter cannabinoid receptor density in any brain region analyzed (see Table 4.1). However, cannabinoid receptor density in several regions, including the periaqueductal gray, CA3 region of the ventral hippocampus, substantia nigra, ventromedial hypothalamus, caudate putamen, entopeduncular nucleus, and the amygdaloid nucleus, was differentially correlated with distance traveled in the novel open field at behaviorally relevant time points (0-3 min and 0-30 min) in drug-naïve OBX and sham rats (see Table 4.2). Notably, the area of cannabinoid receptor binding sites in the substantia nigra was markedly increased in OBX relative to sham rats ($t_{13} = -2.13$, $P = 0.05$) (see Figure 4.5).

LC/MS Analyses

Olfactory bulbectomy decreased 2-AG ($t_{14} = 2.16$, $P < 0.05$) and anandamide ($t_{14}=1.81$, $P<0.05$, one-tailed) levels in the ventral striatum (see Figure 4.6a,b). A nonsignificant trend towards decreased 2-AG and anandamide was also observed in the amygdala of OBX rats ($P =$ 0.07, 0.09, respectively) (see 4.6c, d). Endocannabinoid levels in the piriform cortex, hippocampus, or the cerebellum were not altered by olfactory bulbectomy ($P > 0.05$ for all comparisons) (see Figure 4.6e, f). In sham rats, distance traveled during the novelty phase (0-3 min) of exposure to the open field was negatively correlated with 2-AG levels in the ventral

striatum (r_9= -.667, $P < 0.05$) (see Figure 4.7a). This correlation was absent in OBX rats. However, in OBX rats, 2-AG levels were positively correlated with total distance traveled (30 min after exposure to novelty) (r_7 = .789, $P < 0.05$) (see Figure 4.7b).

Discussion

Olfactory bulbectomized rats exhibit increased locomotor activity in response to a novel open field, a behavior attributed to hyperdopaminergic activity (Gilad and Reis, 1979; Saigusa et al., 1999; Verheij et al., 2008). The modulatory role of endocannabinoids in the midbrain dopaminergic system was explored by investigating behavior, receptor binding, and endocannabinoid levels in the olfactory bulbectomized rat, a model of dopaminergic dysfunction (Gilad and Reis, 1979; Lingham and Gottesfeld, 1986; Holmes, 1999; Masini et al., 2004). Olfactory bulbectomy reliably increased locomotor activity relative to sham surgery during the "novelty" phase (0-3 min) of the exposure to a novel open field session. Rimonabant (1 mg/kg) increased distance traveled relative to control treatment during the "habituation" phase (3-30 min) of the exposure to a novel open field session in olfactory bulbectomized but not sham-operated rats. Surprisingly, rimonabant (1 mg/kg) decreased locomotor activity during the "novelty" phase in sham-operated but not olfactory bulbectomized rats. AM251 (1 mg/kg) tended to increase distance traveled in both sham-operated and olfactory bulbectomized rats during the "habituation" phase but only in sham-operated rats during the "novelty" phase. Cannabinoid receptor density in several brain regions and ventral striatal cannabinoid content were differentially correlated with distance traveled at behaviorally relevant time points in sham and OBX rats. Endocannabinoid levels were diminished in the ventral striatum of OBX relative to sham rats.

The observation of elevated locomotor activity in response to a novel open field in drug-naive OBX relative to sham rats is in agreement with the results of other studies (Klein and Brown, 1969; van Riezen and Leonard, 1990). However, the present studies are the first to document a role for endocannabinoids in the most characteristic behavioral phenotype of the olfactory bulbectomized rat. We show evidence that exposure to a novel open field for 30 min can be divided into a "novelty" phase (0-3 min), during which olfactory bulbectomized exhibit hyperlocomotor activity relative to sham animals, and a "habituation" phase (3-30 min) during which olfactory bulbectomized animals habituate to sham levels. The hyperlocomotor activity does not seem to be due to anxiety upon exposure to the open field because olfactory bulbectomized rats actually spent less time in the residual area than the sham-operated rats. During the novelty phase, rimonabant decreases distance traveled in sham-operated rats. However, locomotor activity during this phase is not affected by CB_1 blockade in olfactory bulbectomized animals. During the habituation phase, the ability of olfactory bulbectomized but not sham animals to habituate to the environment is reliably decreased by rimonabant. Effects of rimonabant did not differ from AM251 during this phase. Our study also demonstrated that ventral striatal endocannabinoid content is decreased by olfactory bulbectomy. Thus, a possible explanation for the prevention of full habituation to novelty in OBX rats by CB_1 blockade is that rimonabant more effectively competes with already low levels of endocannabinoids in the striatum for CB_1 receptors in OBX rats (see Figure 4.8). Rimonabant is known to antagonize the hypolocomotor effects of CB_1 agonists and typically does not affect motor behavior when administered by itself (Rinaldi-Carmona et al., 1994; Navarro et al., 1997; Jarbe et al., 2002). A 1 mg/kg dose may not be high enough to completely block endocannabinoid actions in the ventral striatum of sham animals, in whom cannabinoid levels are presumably normal, in a

behaviorally relevant way (i.e. habituation remains intact in sham animals). The decrease in activity induced by rimonabant during the novelty phase of exposure to the novel open field in sham animals warrants further investigation. It is possible that rimonabant causes stereotypic behavior that blocks horizontal locomotor behavior. Rimonabant may also activate cannabinoid receptors differently depending on the level of endocannabinoid tone induced by external stimuli (i.e. novel environment). Overall, the results of our study with rimonabant suggest that hyperdopaminergic function, as in OBX rats exposed to novelty, elicits a situation in which a low dose of rimonabant increases locomotor activity. This observation may be attributed to more effective competition for CB_1 receptors in the diminished endocannabinoid environment in the ventral striatum of the olfactory bulbectomized rat.

The CB_1 antagonist/inverse agonist AM251 generally increased locomotor activity in response to the novel open field in both sham-operated and olfactory bulbectomized rats. However, when surgical groups were examined separately, AM251 did not reliably increase distance traveled relative to control except in sham animals during the novelty phase. This effect may be absent in olfactory bulbectomized rats because they are the most active during the novelty phase; therefore effects of AM251 may be masked by already high levels of activity relative to sham animals. Furthermore, levels of endocannabinoid tone sufficient to modulate locomotor activity may not emerge until later periods in the olfactory bulbectomized rat (i.e. habituation). Unlike rimonabant, the effects of AM251 on locomotor activity during the habituation phase did not differ between olfactory bulbectomized and sham-operated rats. It is likely that increased locomotor activity in olfactory bulbectomized animals during the habituation phase would be observed with large sample sizes because effects of AM251 did not differ from rimonabant during the habituation phase. However, differences in the effects of

rimonabant and AM251 on locomotor activity clearly warrant further investigation (for review, see Pertwee, 2005).

Olfactory bulbectomy was associated with an increased area of termination of cannabinoid receptor binding sites in the substantia nigra pars reticulata (SNR) relative to sham-operated rats. This increase in cannabinoid receptor area was not, however, accompanied by increases in cannabinoid receptor levels. However, olfactory bulbectomy was associated with cannabinoid receptor binding in the SNR relative to sham surgery (see Figure 4.4). Furthermore, cannabinoid receptor binding was positively correlated with distance traveled by OBX rats over the first 3 min during the 30 min exposure to the novel open field. This interval reflects the same interval during which OBX rats traveled more than sham rats and sham activity was negatively correlated with 2-AG levels in the ventral striatum. Cannabinoid receptor levels in the caudate putamen, entopeduncular nucleus, and amygdaloid nucleus were associated with total distance traveled during the open field session, suggesting that the endocannabinoid signaling system may play a role in habituation to a novel environment in OBX animals. In sham animals, cannabinoid receptor levels in the ventromedial hypothalamus were associated with distance traveled during initial exposure to the novel open field (3 min). It is not possible to determine exactly how the endocannabinoid signaling system modulates locomotor activity in response to novelty based on correlational data. However, it is notable that cannabinoid receptors in several areas of the basal ganglia are associated with distance traveled by OBX animals at behaviorally relevant time points, suggesting that the endocannabinoid system does interact with the dopaminergic system. This interaction would seem to be more evident in animals or environments in which the dopaminergic system is especially sensitive since these associations were not observed in sham animals at behaviorally relevant time points.

Furthermore, these observations highlight brain regions of interest for future studies focused on uncovering interactions of the endocannabinoid and dopaminergic systems.

Olfactory bulbectomy decreased endocannabinoid content in the ventral striatum but not in other brain regions (piriform cortex, hippocampus) known to be deafferented by olfactory bulbectomy. Moreover, brain regions not innervated by the olfactory bulb (e.g. cerebellum) showed not change in endocannabinoid content following olfactory bulbectomy. 2-AG content in the ventral striatum was differentially associated with locomotor activity in the open field at behaviorally relevant time points in OBX and sham rats. Specifically, in sham animals, 2-AG content was negatively correlated with distance traveled at 3 min following exposure to the novel open field, the time point at which distance traveled was found to be increased by olfactory bulbectomy in both drug-naive and vehicle-treated rats. In OBX rats this correlation was notably absent, suggesting that the endocannabinoid system is dysfunctional. In fact, 2-AG levels were positively correlated with total distance traveled during the entire open field session. Taken together, these findings are consistent with the hypothesis that in sham animals 2-AG is mobilized early upon exposure to the novel open field and effectively modulates behavioral responsivity (see Figure 4.8). In OBX animals, 2-AG content is low and/or is released too late to effectively modulate behavioral responsivity to novelty. The loss of olfactory bulb input to important limbic regions of the brain, including primary projection areas such as the piriform and entorhinal cortices, amygdala and olfactory tubercle would be expected to affect secondary regions important to regulation of striatal signaling such as the hippocampus and cingulate cortex (see Figure 4.8). Our findings suggest that endocannabinoid signaling is altered in olfactory bulbectomized rats. Further investigation is necessary to confirm that endocannabinoid modulation of dopaminergic transmission is dysfunctional.

Table 4.1 Cannabinoid receptor densities did not differ between sham-operated and olfactory bulbectomized rats in any brain region analyzed. Mean nCi/mg tissue wet weight ± S.E.M. shown. *n* = 5 -10 animals per surgical group.

	Surgical Group	
Brain region	**Sham**	**OBX**
Basal ganglia/striatum		
Dorsolateral quadrant	9.68 ± 0.66	9.05 ± 1.58
Dorsomedial quadrant	6.41 ± 0.36	5.48 ± 0.84
Ventrolateral quadrant	8.38 ± 0.52	6.17 ± 1.12
Ventromedial quadrant	6.53 ± 0.41	4.89 ± 0.83
Nucleus accumbens	5.95 ± 0.38	4.92 ± 0.77
Olfactory tubercle	4.15 ± 0.33	3.96 ± 0.59
Caudate putamen	3.84 ± 0.19	3.54 ± 0.23
Lateral globus pallidus	4.37 ± 0.41	3.87 ± 0.24
Entopeduncular nucleus	3.98 ± 0.58	3.58 ± 0.65
Substantia nigra	9.23 ± 1.04	11.75 ± 1.19
Ventral tegmental area	1.78 ± 0.78	2.0 ± 0.35
Cerebral cortex		
Rostral cingulate cortex	5.48 ± 0.33	5.98 ± 0.47
Anterior cingulate cortex	2.66 ± 0.25	2.83 ± 0.1
Posterior cingulate cortex	2.8 ± 0.45	2.85 ± 0.23
Piriform cortex	2 ± 0.17	2.26 ± 0.3
Entorhinal cortex	4.65 ± 0.85	5.29 ± 0.53
Amygdala		
General amygdala	2.13 ± 0.25	2.1 ± 0.22
Amygdaloid nucleus	6.06 ± 1.38	4.62 ± 0.57
Hippocampus		
Dorsal formation	3.51 ± 0.3	3.59 ± 0.47
Dorsal CA1	5.21 ± 1.0	5.13 ± 1.02
Dorsal CA2	6.71 ± 1.85	7.51 ± 2.83
Dorsal CA3	6.08 ± 1.25	8.25 ± 3.09
Dorsal dentate gyrus	5.04 ± 0.88	4.83 ± 0.89
Ventral formation	6.21 ± 1.25	6.08 ± 0.53
Ventral CA1	13.56 ± 2.57	12.3 ± 2.24
Ventral CA2	10.9 ± 2.29	10.5 ± 1.01
Ventral CA3	10.69 ± 3.63	8.25 ± 0.5
Ventral dentate gyrus	8.26 ± 1.59	7.86 ± 0.67
Ventral subiculum	7.64 ± 1.15	7.63 ± 0.69
Diencephalon		
Ventromedial hypothalamus	2.32 ± 0.2	2.61 ± 0.58
Arcuate nucleus	1.48 ± 0.16	2.14 ± 0.34
Brain stem		
Periaqueductal gray	4.2 ± 0.82	3.91 ± 0.55
Superior colliculus	3.65 ± 0.62	3.52 ± 0.35
Dorsal raphe nucleus	2.03 ± 0.39	1.55 ± 0.25
Cerebellum	2.94 ± 0.19	2.66 ± 0.13

Table 4.2 Distance traveled during both the first 3 min of exposure to a novel open field as well the entire 30 min open field session correlated with cannabinoid receptor density in a regionally-specific manner in sham and OBX rats. Data are Pearson's product-moment correlations. Significant correlations are in bold. *n* = 6-9 per surgical group.

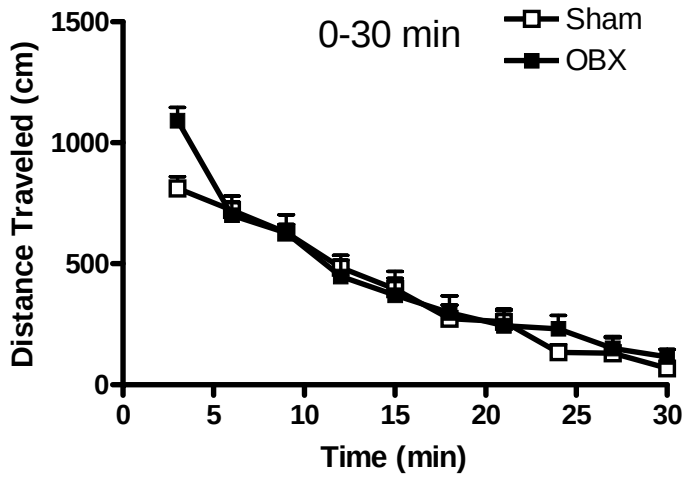
	Time (min post exposure to novel arena)			
	0-3		**30**	
Brain region	**Sham**	**OBX**	**Sham**	**OBX**
Substantia nigra	0.243	**0.739***	0.287	0.36
Ventromedial hypothalamus	**-0.78***	-0.519	-0.332	-0.16
Caudate putamen	0.42	0.09	-0.034	**-0.814***
Entopeduncular nucleus	-0.014	0.552	0.689	**0.752***
Amygdaloid nucleus	0.097	0.785	-0.068	**0.955****

*P < 0.05; **P < 0.01.

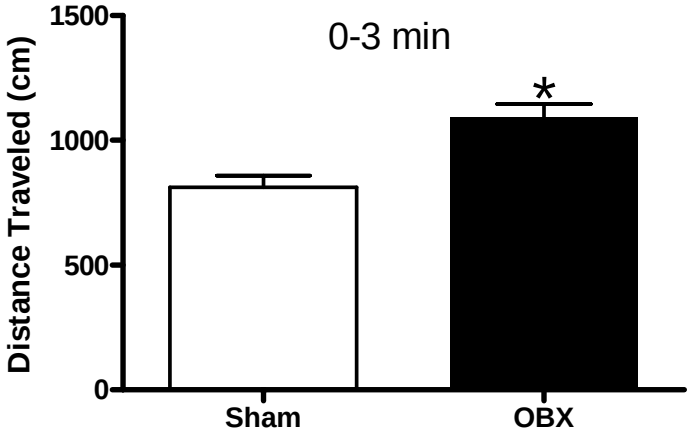
Figure 4.1. Locomotor activity elicited by response to a novel open field exposure consists of novelty and habituation phases in drug-naive rats. (a) Sham-operated and OBX animals exhibited decreased distance traveled over the 30 min exposure to a novel open field session. (b) OBX animals traveled greater distance than sham rats during the novelty phase (0-3 min) of the 30 min exposure to a novel open field session. (b) Olfactory bulbectomy and sham-operated rats did not differ from each other in distance traveled over the habituation phase (3-30 min) of exposure to the novel open field. (Mean + SEM shown, $n = 10$ per group). *, $P < 0.05$ compared to shams.

Figure 4.1

(a)



(b)



(c)

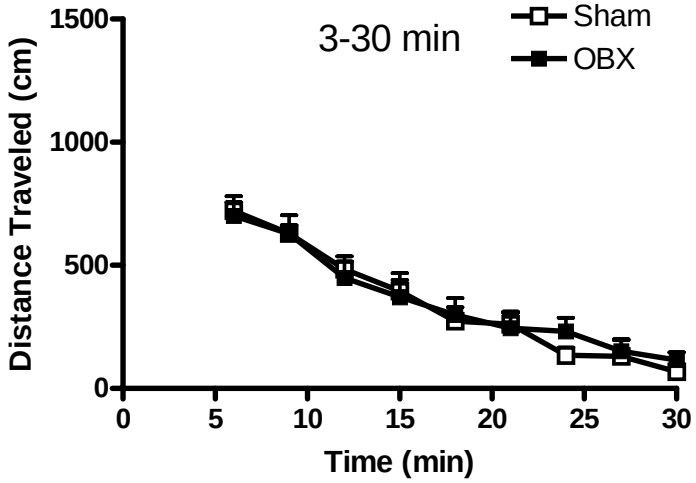


Figure 4.2. Sham-operated and olfactory bulbectomized rats differ in exploratory patterns during the novelty but not the habituation phase during exposure to a novel open field. Olfactory bulbectomy (a) increases the percentage of time spent in the center area of the open field and (b) decreases the percentage of time spent in the residual area of the open field during the novelty phase relative to sham. Olfactory bulbectomy (c) does not affect time spent per entry into the center area but (d) decreases time spent per entry into the residual area relative to sham surgery. (e-h) Olfactory bulbectomy does not affect exploratory patterns during the entire 30 min exposure to the novel open field relative to sham surgery. Mean + S.E.M. shown. *, $P < 0.05$ relative to sham.

Figure 4.2

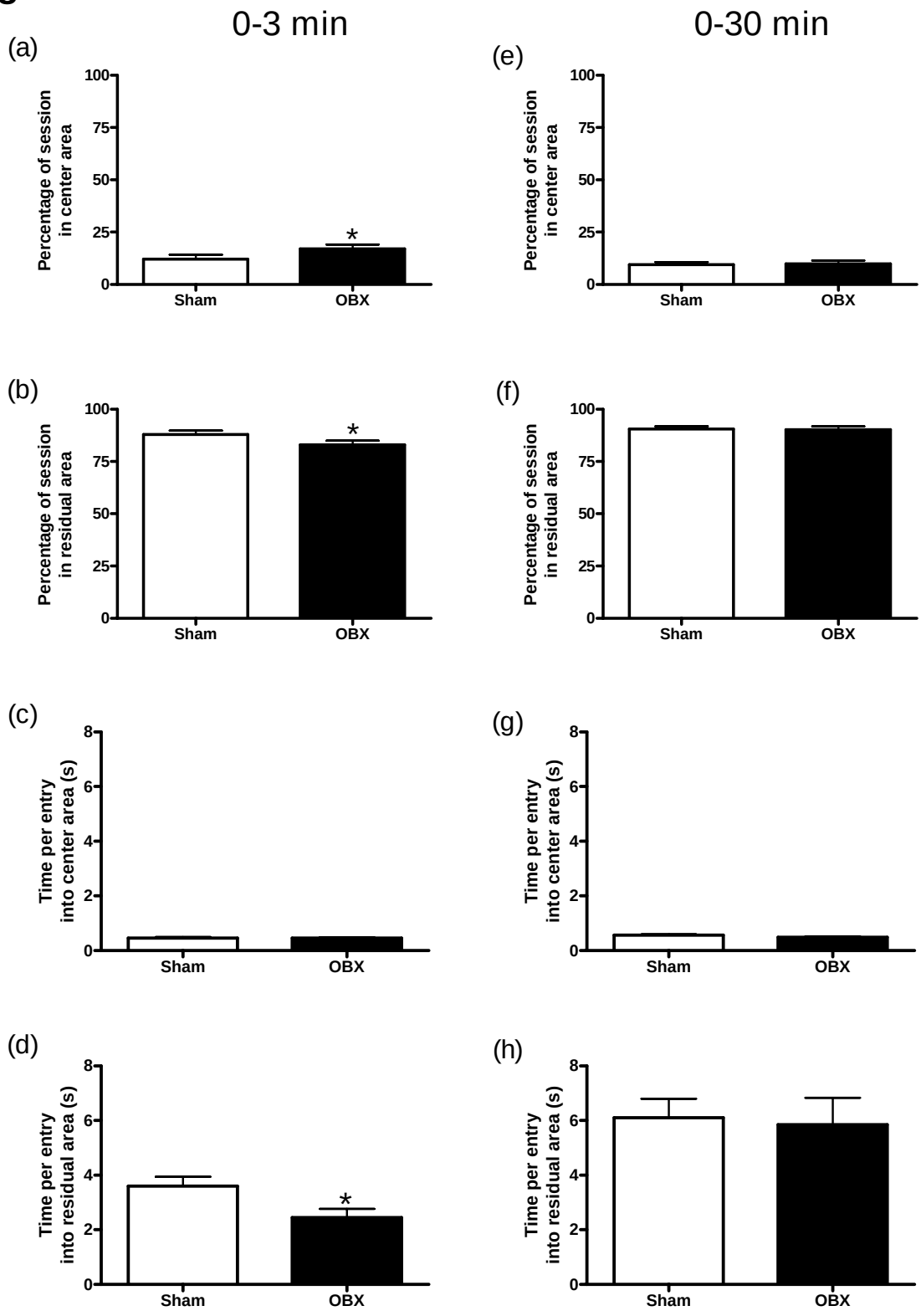

0-3 min
0-30 min
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Percentage of session in center area
Percentage of session in residual area
Time per entry into center area (s)
Time per entry into residual area (s)
Sham
OBX
*

Figure 4.3. CB_1 blockade affects sham-operated but not olfactory bulbectomized rats during the novelty phase of exposure to a novel open field. (a) In sham animals rimonabant (1 mg/kg i.p.) decreased and AM251 (1 mg/kg i.p.) increased distance traveled during the "novelty" phase (0-3 min) of the 30 min exposure to a novel open field session. (b) CB_1 blockade did not reliably alter distance traveled during the novelty phase in olfactory bulbectomized animals. Mean + S.E.M. shown. *, $P < 0.05$, **, $P < 0.01$ relative to sham control; $^{###}$, $P < .001$ relative to sham rimonabant.

Figure 4.3

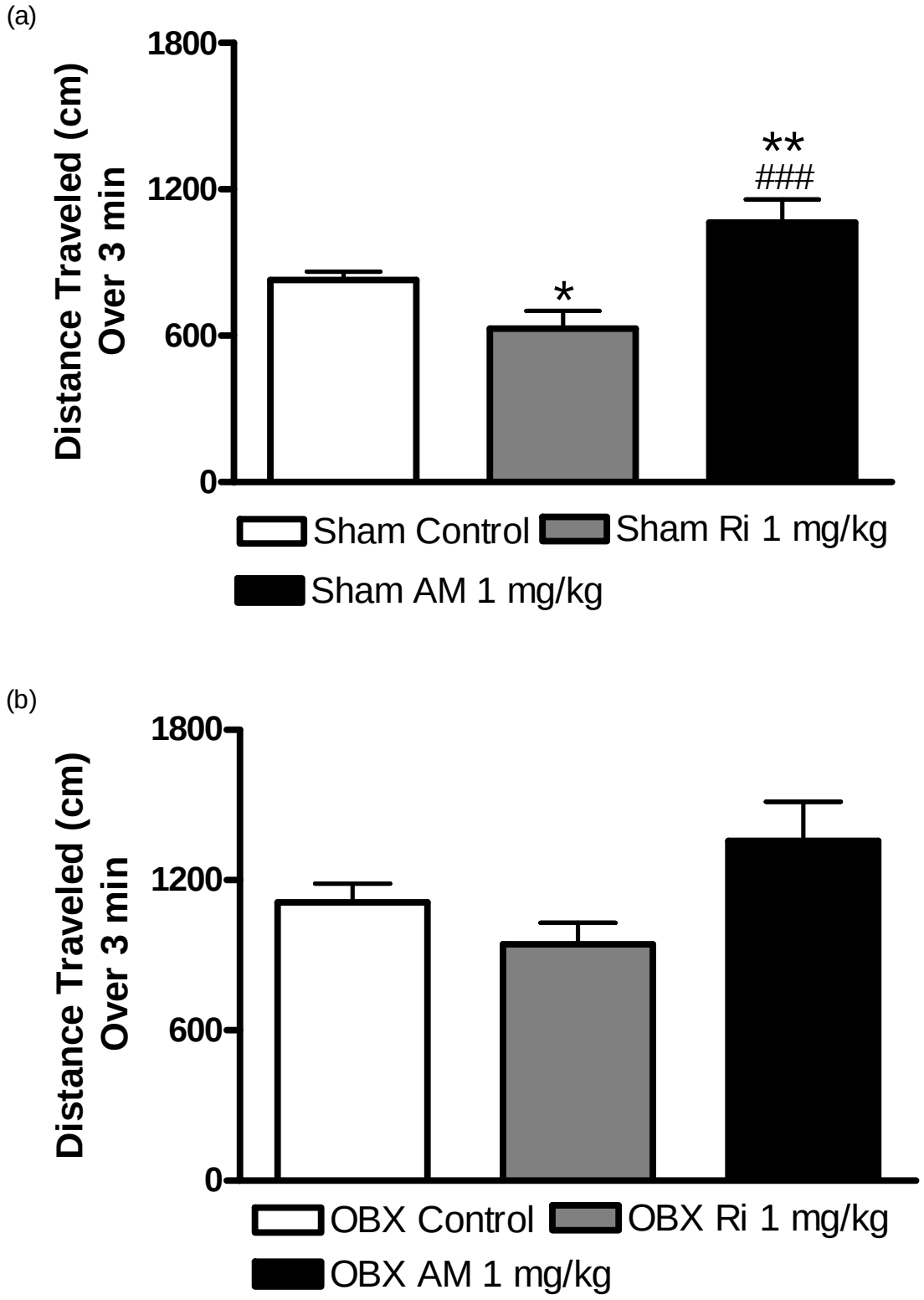

(a)
1800
1200
600
0
Distance Traveled (cm)
Over 3 min
**
###
*
Sham Control
Sham Ri 1 mg/kg
Sham AM 1 mg/kg
(b)
1800
1200
600
0
Distance Traveled (cm)
Over 3 min
OBX Control
OBX Ri 1 mg/kg
OBX AM 1 mg/kg

Figure 4.4. Blockade of CB_1 receptors prevents full habituation to a novel environment in olfactory bulbectomized but not in sham-operated animals. (a) Blockade of CB_1 receptors with rimonabant (1 mg/kg i.p.) or AM251 (1 mg/kg i.p.) elevates locomotor activity in olfactory bulbectomized rats relative to sham surgery during the "habituation" phase (3-30 min) of exposure to a novel open field. (b) CB_1 blockade does not affect habituation to novelty in sham animals. (c) Rimonabant (1 mg/kg) prevents full habituation to novelty relative to control conditions in olfactory bulbectomized rats. Mean + SEM shown.

Figure 4.4

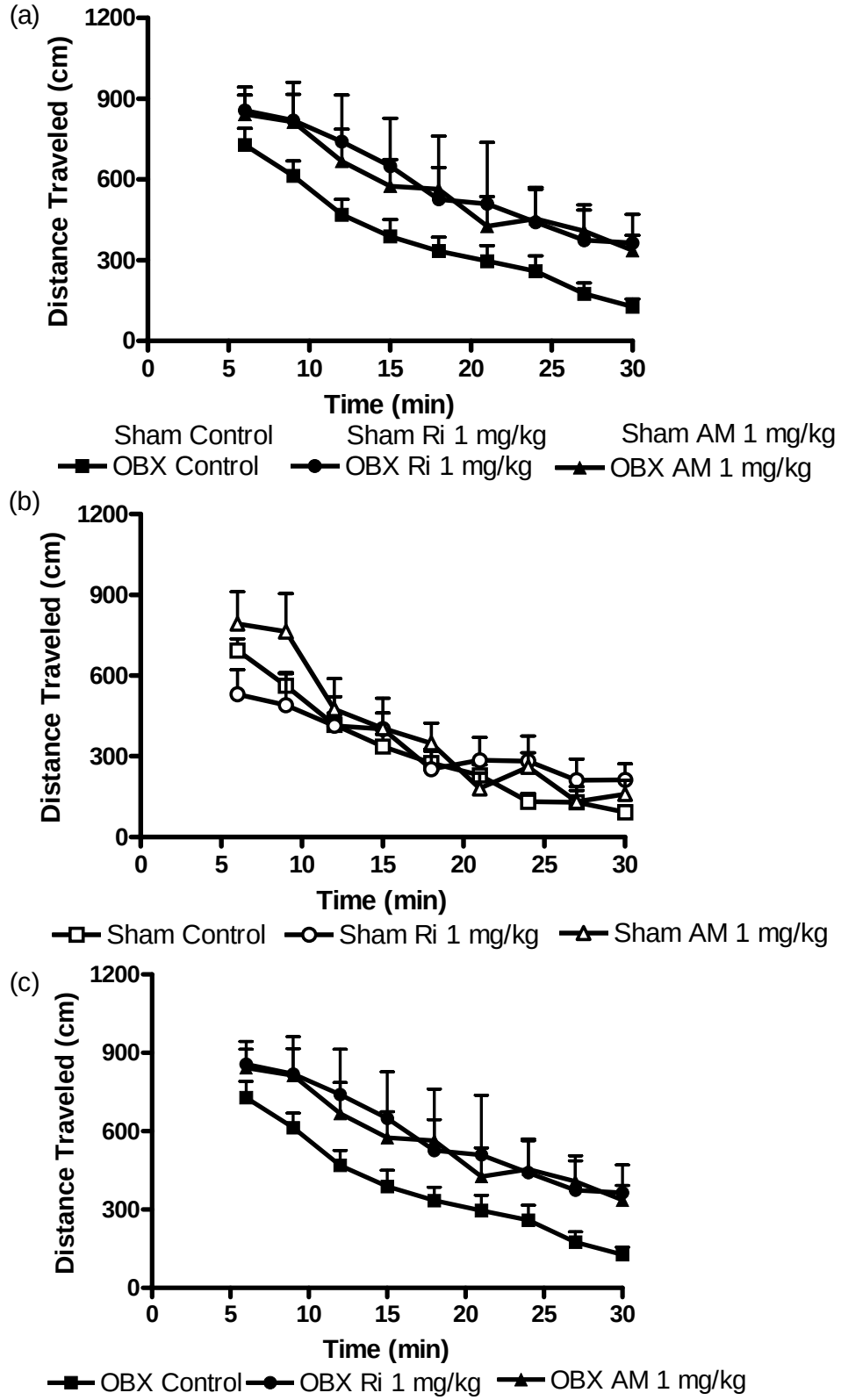
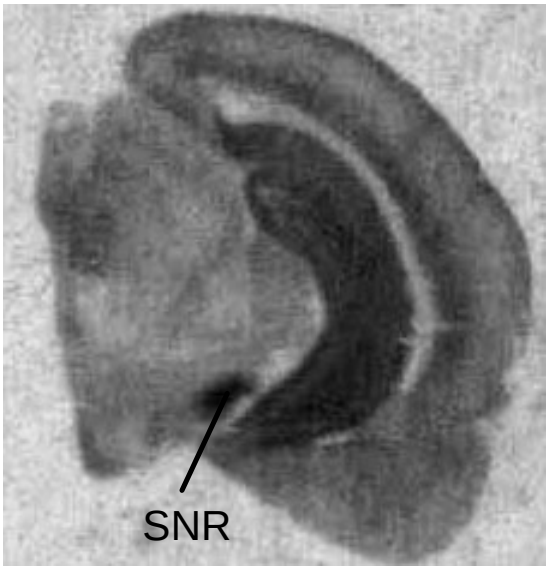

(a)
Distance Traveled (cm)
1200
900
600
300
0
0
5
10
15
20
25
30
Time (min)
Sham Control
Sham Ri 1 mg/kg
Sham AM 1 mg/kg
OBX Control
OBX Ri 1 mg/kg
OBX AM 1 mg/kg
(b)
Distance Traveled (cm)
Time (min)
Sham Control
Sham Ri 1 mg/kg
Sham AM 1 mg/kg
(c)
Distance Traveled (cm)
Time (min)
OBX Control
OBX Ri 1 mg/kg
OBX AM 1 mg/kg

Figure 4.5. Olfactory bulbectomy increases the area of cannabinoid receptor binding sites in the substantia nigra pars reticulata. (a) Olfactory bulbectomy increased the area of cannabinoid receptor binding in the substantia nigra pars reticulata relative to sham surgery. Autoradiographic images depict cannabinoid receptor binding area in the substantia nigra pars reticulata in (b) sham-operated and (c) olfactory bulbectomized rats. (Mean + S.E.M. shown, n = 8 per group). *, $P < 0.05$ relative to sham.

Figure 4.5

(a)

(b)



(c)

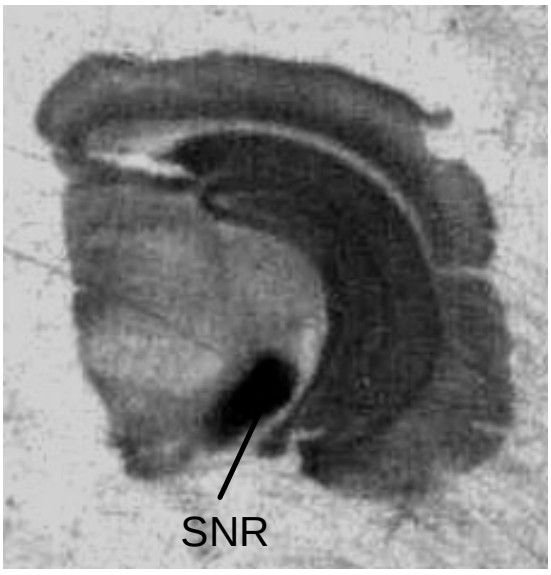


Figure 4.6. Endocannabinoid content in sham-operated and olfactory bulbectomized rats. Olfactory bulbectomy decreased (a) anandamide and (b) 2-AG levels in the ventral striatum. Olfactory bulbectomy also produced a trend toward decreased (c) anandamide and (d) 2-AG levels in the amygdala of olfactory bulbectomized rats relative to sham surgery. Olfactory bulbectomy had no effect on (e) anandamide or (f) 2-AG levels in the cerebellum. (Mean + S.E.M. shown, n = 7-9 per group). *, $P < 0.05$ relative to sham.

Figure 4.6

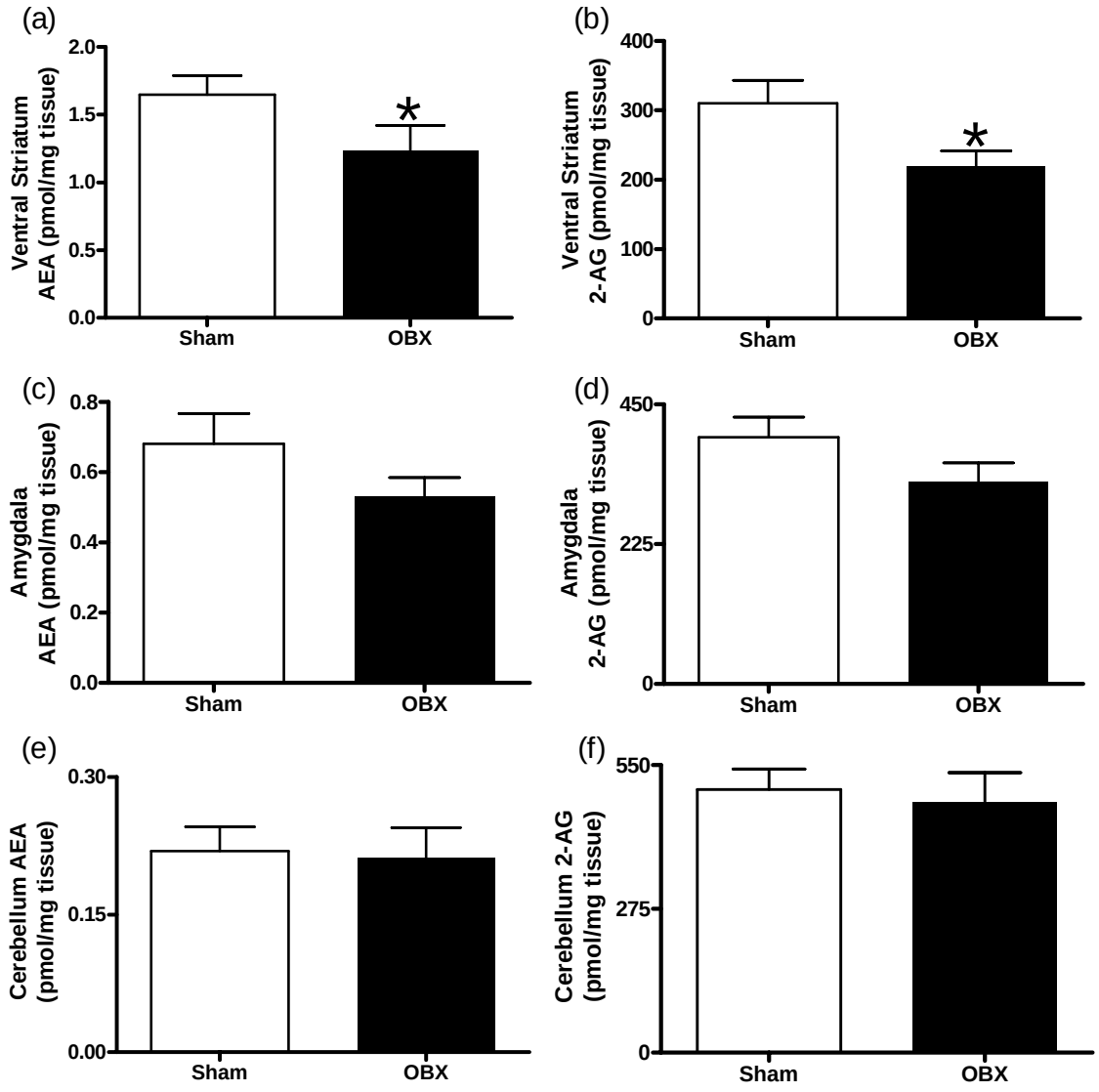

(a)
Ventral Striatum AEA (pmol/mg tissue)
2.0
1.5
1.0
0.5
0.0
*
Sham
OBX
(b)
Ventral Striatum 2-AG (pmol/mg tissue)
400
300
200
100
0
*
Sham
OBX
(c)
Amygdala AEA (pmol/mg tissue)
0.8
0.6
0.4
0.2
0.0
Sham
OBX
(d)
Amygdala 2-AG (pmol/mg tissue)
450
225
0
Sham
OBX
(e)
Cerebellum AEA (pmol/mg tissue)
0.30
0.15
0.00
Sham
OBX
(f)
Cerebellum 2-AG (pmol/mg tissue)
550
275
0
Sham
OBX

Figure 4.7. Endocannabinoid content is differentially related to distance traveled in sham-operated and olfactory bulbectomized rats. 2-AG levels in the ventral striatum were (a) negatively correlated with distance traveled at 3 min post-exposure to a novel open field in sham-operated animals and (b) positively correlated with total distance traveled during the 30 min exposure to the open field session in OBX rats. (n = 7-8 per group). Both correlations were statistically significant ($P < 0.05$).

Figure 4.7

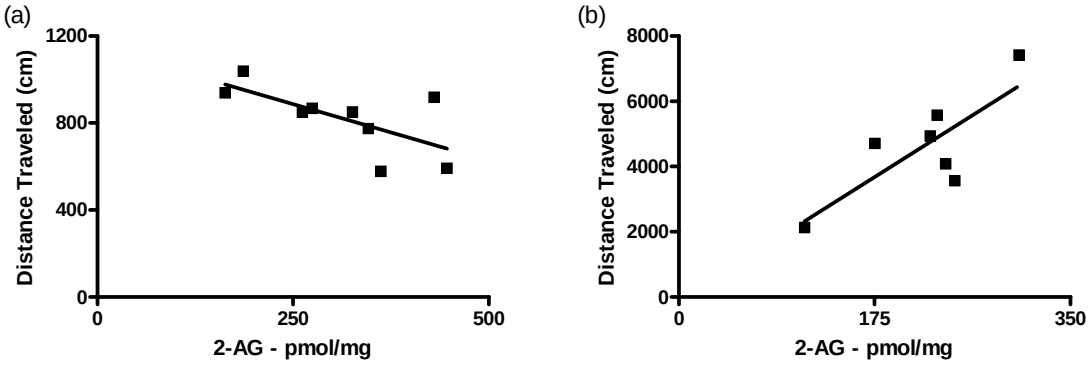
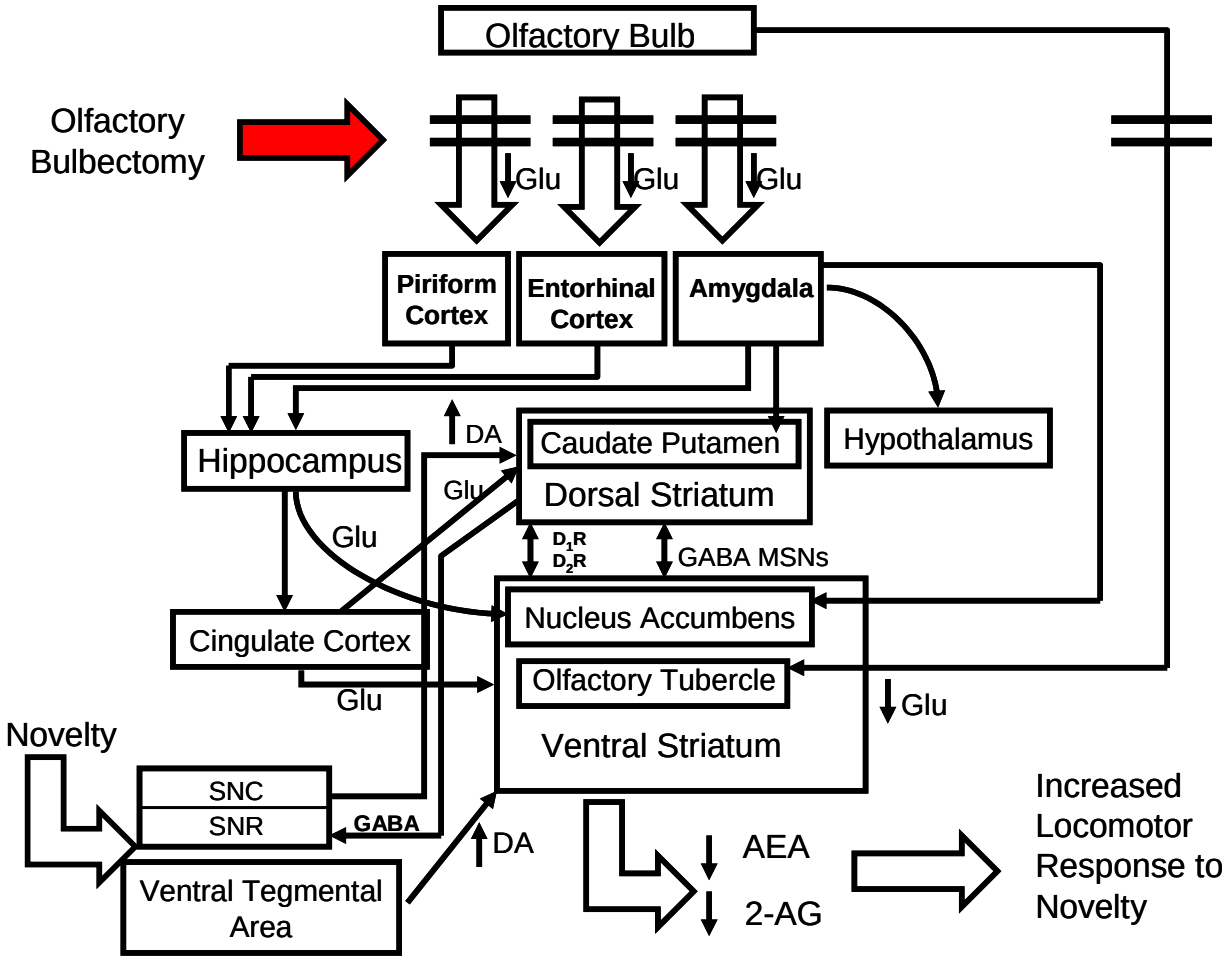

(a)
Distance Traveled (cm)
1200
800
400
0
0
250
500
2-AG - pmol/mg
(b)
Distance Traveled (cm)
8000
6000
4000
2000
0
0
175
350
2-AG - pmol/mg

Figure 4.8. Olfactory bulbectomy disrupts glutamatergic projections to limbic areas including piriform and entorhinal cortices, amygdala and olfactory tubercle. These areas in turn project to the hippocampus and piriform cortex, regions that help regulate dopaminergic transmission in the striatum. We hypothesize that a novel environment increases dopaminergic input from the ventral tegmental area and substantia nigra pars compacta (SNC) into the striatum. In sham animals, the endocannabinoid system is intact and endocannabinoids are mobilized in the striatum to act at CB_1 receptors localized to glutamatergic terminals (derived from the hippocampus and cingulate cortex) and GABAergic terminals (localized to striatal medium spiny neurons) to modulate behavioral responsivity to this increase in dopaminergic transmission. However, this system is dysfunctional (i.e. low endocannabinoid content in the ventral striatum) in olfactory bulbectomized rats due to disrupted glutamatergic inputs and possibly GABAergic interneurons. The endocannabinoid system is unable to effectively modulate dopaminergic responsivity, resulting in increased locomotor response to the novel environment in olfactory bulbectomized animals.

Figure 4.8


Olfactory Bulb
Olfactory Bulbectomy
Glu
Glu
Glu
Piriform Cortex
Entorhinal Cortex
Amygdala
Hippocampus
DA
Caudate Putamen
Dorsal Striatum
Hypothalamus
Glu
Glu
D1R
D2R
GABA MSNs
Nucleus Accumbens
Cingulate Cortex
Olfactory Tubercle
Glu
Glu
Ventral Striatum
Novelty
SNC
SNR
GABA
DA
Ventral Tegmental Area
AEA
2-AG
Increased Locomotor Response to Novelty

CHAPTER FIVE

GENERAL DISCUSSION

The present studies investigated the role of the endocannabinoid system in neurobiological and behavioral symptomatology in the olfactory bulbectomized rat that has been attributed to dopaminergic dysfunction. Olfactory bulbectomy induced hyperlocomotor responsivity to novelty and amphetamine relative to sham surgery. Olfactory bulbectomy blocked the ability of the FAAH inhibitor URB597, a pharmacological inhibitor of anandamide hydrolysis, to attenuate development of sensitization to amphetamine relative to sham surgery. The CB_1 antagonist/inverse agonist rimonabant, at a dose of 1 mg/kg that is usually behaviorally inactive (Rinaldi-Carmona et al., 1994; Navarro et al., 1997; Jarbe et al., 2002), prevented full habituation to the open field in olfactory bulbectomized rats relative to sham surgery. AM251 generally increased locomotor activity in the open field in both surgical groups. Olfactory bulbectomy also decreased anandamide and 2-AG content in the ventral striatum relative to sham surgery but not in other areas of the brain including piriform cortex, hippocampus and cerebellum. 2-AG content in the ventral striatum, along with cannabinoid receptor levels in several discrete brain regions, were differentially related to distance traveled upon exposure to novelty at behaviorally relevant time points in olfactory bulbectomized and sham-operated rats.

The main hypothesis tested in the studies described was that endocannabinoids play a regulatory role in modulating dopaminergic responsivity to stress and psychostimulants. In an animal model such as the olfactory bulbectomy model, dopaminergic functioning is disrupted, providing an environment in which to test the role of endocannabinoid sigaling. The intact

olfactory bulb primarily projects to the piriform and entorhinal cortices, amygdala, and olfactory tubercle (for review, see Kelly et al., 1997). Secondary projections include the hippocampus, cingulate cortex, dorsal and ventral striatum among other brain areas (Niewenhuys et al., 1988; DeOlmos et al., 1995). Given the many connections between these brain regions, it is not surprising that olfactory bulbectomy induces neurochemical and behavioral abnormalities beyond those caused by a loss of sense of smell. In the case of increased locomotor responsivity to novelty and repeated amphetamine administration, it seems that olfactory bulbectomy induces dopaminergic dysregulation in the basal ganglia, particularly in the ventral striatum. Our data provide evidence that part of this dopaminergic dysfunction is due to loss of intact cannabinoid signaling. First, 2-AG levels in the ventral striatum were decreased by olfactory bulbectomy. These levels were negatively correlated with cumulative distance traveled at 3 min post-exposure to novelty in sham rats, indicating that endocannabinoid mobilization in the ventral striatum is associated with normal activity levels in response to novelty. In OBX animals, however, this correlation is absent. In fact, 2-AG levels in the ventral striatum were positively correlated with cumulative activity at 30 min post-exposure to novelty. It seems that mobilization of endocannibinoids in response to the stress of a novel environment in olfactory bulbectomized animals is insufficient to modulate the initial locomotor response in the novelty phase. It is also possible that endocannabinoids are unable to bind to CB_1 receptors on glutamatergic or GABAergic presynaptic neurons in the striatum to modulate the behavioral response to novelty.

The ability of URB597 to attenuate sensitization levels in sham-operated but not olfactory bulbectomized animals may be due to the profound locomotor "presensitization" to amphetamine observed in the latter group. Enhancing anandamide mobilization in these animals with a FAAH inhibitor is likely insufficient to increase endocannabinoid tone and presumably

overcome this level of dopaminergic activation. In other words, olfactory bulbectomized rats seem to be sensitized to psychostimulants before the initial administration (Chambers et al., 2004). This explanation is supported by our findings that rimonabant (1 mg/kg) prevents full habituation to the novel open field relative to sham-operated animals. The antagonist/inverse agonist may be able to better compete for CB_1 receptor binding in the presence of diminished anandamide and 2-AG levels, thus allowing it to exert inverse agonist effects.

Finally, cannabinoid receptor levels in several brain regions, including the basal ganglia and striatum, were differentially correlated with distance traveled at behaviorally relevant time points in olfactory bulbectomized and sham-operated rats. 2-AG levels in the ventral striatum were also differentially correlated with distance traveled at behaviorally relevant time points in olfactory bulbectomized and sham rats. Overall, our findings indicate that olfactory bulbectomy induces changes in the cannabinoid signaling system that are relevant to behaviors, including increased locomotor response to novelty and presensitization to amphetamine, that are attributed to dopaminergic dysfunction.

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