DEPRESSION, DRUG ABUSE, AND DOPAMINE: IN VIVO MICRODIALYSIS OF DOPAMINE IN VENTRAL STRIATUM AND SELF-ADMINISTRATION OF D-AMPHETAMINE IN THE OLFACTORY BULBECTOMIZED RAT

by

CHER VIRGINIA MASINI

(Under the direction of PHILIP V. HOLMES)

ABSTRACT

Affective disorders and substance abuse frequently coexist, yet few previous studies have examined drug self-administration using animal models of depression. The present studies employed the olfactory bulbectomy rat model of depression. Bilateral olfactory bulbectomy produces behavioral, physiological and neurochemical changes that closely resemble depression symptomology. Bulbectomized, sham-operated, and anosmic control rats were given a choice between a 0.1 mg/ml *d*-amphetamine solution and distilled water for 3 days. Bulbectomized rats exhibited greater intake of amphetamine solution than sham-operated and anosmic rats. *In vivo* microdialysis of dopamine, DOPAC, and HVA in the ventral and dorsal striatum of bulbectomized and sham-operated rats was performed. Bulbectomized rats had significantly higher basal levels of dopamine than sham-operated controls. Differences between regions were not seen. Bulbectomized and sham-operated rats revealed no significant differences in dopamine, DOPAC, or HVA when dialysis samples were taken while an oral amphetamine solution was available. The results suggest that dysregulation of the mesolimbic dopamine system in bulbectomized rats leads to increased drug seeking behavior.

INDEX WORDS: Olfactory Bulbectomy, Depression, Animal Model, Dopamine, *In Vivo* Microdialysis, Olfactory Tubercle, Self-Administration, Amphetamine

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DEDICATION

This dissertation is dedicated to Domenic Paul Masini, my grandfather. I would not be the person I am today without his watchful eye and loving guidance. My Nono was my hero and my greatest ally. I miss him greatly.

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CHAPTER 1: INTRODUCTION

The present experiments were designed to further our understanding of the relationship between endogenous depression (American Psychiatric Association's *DSM-IV* major depression with melancholia) and drug abuse. Epidemiological studies show a clear relationship between affective disorders and substance abuse. A high comorbidity between depression and drug dependence has been found in both institutional and community populations (Markou, Kosten, & Koob, 1998). The National Institute of Mental Health's Epidemiological Catchment Area Program (ECA), which was a combined community and institutional survey of about 17,500 respondents suggested that comorbidity of major depressive disorder and daily drug use was over 40% (Anthony & Helzer, 1991). In a more recent study, Roeloffs, Fink, Unutzer, Tang, & Wells (2001) reported that 26.3% of women and 29.4% of men who suffered from symptoms of depression also abused drugs.

Epidemiological studies show that there is a relationship between depression and drug abuse but can not prove causality. However, most epidemiological studies focus on primary depression. The distinction between a primary and secondary affective disorder is usually based on the presence of more than one psychiatric condition and the chronology of the disorders (Weissman, Pottenger, Kleber, Ruben, Williams, & Thompson, 1977). Primary depression occurs prior to any other psychiatric disorder and secondary depression occurs after the onset of another psychiatric disorder like substance abuse (Winokur, Turvey, Akiskal, Coryell, Solomon, Leon, et al., 1998). Reliance on

interviews and questionnaires also put into question the results of epidemiological studies. There is the possibility of underreporting of substance abuse disorders and inaccuracy of age of onset self-reports (Christie, Burke, Regier, Rae, Boyd, & Locke, 1988).

Although the phenomenon of the comorbidity of depression and drug abuse is well documented, few previous studies have examined drug self-administration experimentally. One way to test this relationship experimentally is to use an animal model of depression. The temporal relationship between depression and drug abuse can then be experimentally studied. Animal models also make it possible to use more invasive techniques that could not be used with human participants for ethical reasons. It may be possible to use an animal model of depression to examine why clinically depressed humans are more likely to abuse drugs than non-depressed humans.

The present studies employed the olfactory bulbectomized rat model of depression. Bilateral olfactory bulbectomy (OBX) produces a well-described syndrome of behavioral, physiological, and neurochemical changes that resemble clinical depression symptoms. Effects of OBX that are similar to clinical depression include changes in eating patterns (Jesberger & Richardson, 1986; Meguid, Gleason, & Yang, 1993), cognitive impairments (Hall & Macrides, 1983; Thorne & Rowles, 1988; van Rijzingen, Gispen, & Spruijt, 1995; Yamamoto, Jin, & Watanabe, 1997), circadian rhythm and sleep disruptions (Lumia, Teicher, Salchi, Ayers, & Possidente, 1992; Possidente, Lumia, McGinnis, Pratt, & Page, 2000; Sakurada, Shima, Tadano, Sakurada, & Kirara, 1976), hyper-reactivity to novelty or "agitation-like" behavior (Baumbach & Sieck, 1977; Kelly & Leonard, 1995; Primeaux & Holmes, 1999; Tiffany, Mollenauer,

Plotnik, & White, 1979), immunosupression (Song, Earley, & Leonard, 1996; Song & Leonard, 1994), and increases in corticosteroid levels (Cairncross, Cox, Forster, & Wren, 1979; Jesberger, et al.). OBX also produces changes in monoaminergic systems throughout the brain resembling those seen in depression (Cairncross, et al.; Early, Glennon, Lally, Leonard, & Junien, 1994; Grecksch, et al.; Mudunkotuwa & Horton, 1996). These changes are independent of anosmia (Sieck & Baumbach, 1974; Tiffany, et al.) and can be reversed by chronic but not acute antidepressant treatment (Cairncross, et al.; Kelly, Wrynn, & Leonard, 1997; Mudunkotuwa, et al.; Song, et al.; van Riezen & Leonard, 1990; van Riezen, Schnieden, & Wren, 1977). The OBX model is thus well validated and meets the criteria of being a good model of depression.

CHAPTER 2: EXPERIMENT 1

Oral self-administration of *d*-amphetamine in olfactory bulbectomized rats.

The psychostimulant amphetamine is highly abused by humans. The 2000 National Household Survey on Drug Abuse estimated that 8.8 million Americans have tried methamphetamine, the potent analog of amphetamine (SAMHSA, 2000). And the Drug Abuse Warning Network (DAWN) found that methamphetamine-related emergencies increased 30% from 1999 to 2000 (SAMHSA, 2000).

In a study of hospitalized drug abusers, researchers found that 63% of the patients reported using their drug of choice to relieve symptoms of depression (Weiss, Griffin, & Mirin, 1992). Another important finding of this study was that there was no relationship found between feeling mood enhancing effects and having used the drug for depression (Weiss et al.). Epidemiological studies have found a relationship between amphetamine use and depression. Myers, Weissman, Tischler, Holzer, Leaf, Orvaschel, et al. (1984) using data from part of the National Institute of Mental Health's Epidemiological Catchment Area Program (ECA) found that the lifetime rates of major depressive disorder were 32% in stimulant-dependent patients as compared to only 7% in the community population. McCuller, Sussman, Dent, & Teran (2001) also found that depression predicted hard drug use such as stimulants, like cocaine and amphetamine, but not marijuana, alcohol, or smoking in a sample of 1,315 high school students.

Using the olfactory bulbectomized model of depression, Holmes & Masini (1999) examined oral self-administration of *d*-amphetamine (0.1mg/ml) in tap water.

Bulbectomized rats showed a higher intake of *d*-amphetamine on the first day of intake compared to sham-operated rats. In this experiment, the rats were given the *d*-amphetamine solution as their sole source of drinking water and were not given a choice, the latter would be a better model of human drug use. Holmes, Masini, Primeaux, Garrett, Zellner, Stogner, et al. (in press) also examined intravenous self-administration of *d*-amphetamine in the bulbectomized rat. Using a standard operant response procedure, rats lever pressed for *d*-amphetamine infusions. Bulbectomized rats acquired self-administration of a low dose (0.1mg/kg, IV) quicker and had higher stable administration rates than sham-operated rats.

The present study examined the acquisition of self-administration of oral *d*-amphetamine in the bulbectomized rat using a choice paradigm. Rats had *ad libitum* access to both distilled water and 0.1mg/ml *d*-amphetamine in distilled water for 3 days. In two-bottle choice procedures with oral d-amphetamine, previous researchers have found that rats will consume equal amounts of 0.1mg/ml amphetamine and water for the first few days of self-administration testing (Carey, 1973; Janicke & Coper, 1984; Janicke, Janicke, Schulze, & Coper, 1990; Stolerman, Kumar, & Steinberg, 1971). It is well documented that rats develop an aversion to oral amphetamine (Carey; Janicke et al., 1984; Janicke et al., 1990; Ufer, Dadmarz, & Vogel, 1998; Wolf, Jacquet, & Carol, 1978). Most researchers suggest that intake of oral amphetamine declines because of aversive post-ingestional effects (Stolerman et al.). Janicke, Heil, & Coper (1989) suggested that the development of the aversion to oral amphetamine is caused by the formation of *p*-hydroxynorphedrine (*p*-HNE) in the brain. *p*-HNE is a *p*- and β-hydroxylated metabolite of *d*-amphetamine and is a false transmitter in the noradrenergic

system (Kongyingyoes, Janicke, & Coper, 1988). Janicke et al. (1989) found a dose-dependent decrease in *d*-amphetamine intake with the formation of *p*-HNE. They suggested that when a threshold concentration of *p*-HNE is reached, the aversive effects outweigh the reinforcing effects. The present experiment focused on the acquisition phase of oral amphetamine self-administration during three days of self-administration. Janicke & Coper (1984) found that after 3 days, the amount of oral 0.01% or 0.02% *d*-amphetamine solution intake by rats was significantly decreased.

The present experiment also included an additional control group, anosmic rats. Anosmia was produced by zinc sulfate treatment, which produces a reversible olfactory deficit that lasts approximately 5 to 7 days (Alberts, 1974). This controlled for the possibility that differences in *d*-amphetamine self-administration are simply because of lack of olfaction in the bulbectomized rat. It was hypothesized that OBX rats self-administer more amphetamine solution than sham-operated and anosmic rats.

MATERIALS AND METHODS

Subjects & Design.

Thirty-three experimentally naïve male Sprague-Dawley rats (Harlan, Inc., Indianapolis, IN) weighing 250 – 300g at the time of surgery served as subjects. Rats were randomly assigned to 1 of 3 groups consisting of olfactory bulbectomy, sham surgery, or anosmia. Rats were group housed (3 – 4 per cage) prior to surgery in polycarbonate plastic cages (49.5 x 38.1 x 20.3 cm). Post-surgery rats were housed individually in cylindrical polycarbonate plastic metabolic chambers (22.9 x 17.8) on 44.5 cm stainless steel stands (Harvard Apparatus, Holliston, MA) with two 100ml water bottles attached. Chambers were kept in a humidity and temperature-regulated animal

housing facility with lighting maintained on a reverse 12 hour schedule (lights out at 0700h). Food was available *ad libitum* and water available 8 hours a day post-surgery during the dark phase. Rats were weighed daily throughout the experiment. All procedures were approved by the University of Georgia (UGA) Animal Care and Use Committee and followed the guidelines of the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

Olfactory Bulbectomies

Surgeries began after a one-week habituation period which began upon the arrival of the rats at the animal facilities. For surgery, they were anesthetized with intraperitoneal injections of 25mg/kg pentobarbital (Abbott Laboratories, Chicago, IL) and 40mg/kg ketamine hydrochloride (Mallinckrodt, Mundelein, IL). A midline scalp incision was made and 3mm diameter burr holes were drilled bilaterally, 5mm anterior to bregma and 1mm lateral to the midline. The dura mater was pierced and the olfactory bulbs were aspirated with a plastic pipette tip with an approximately 2mm opening. The cavity was filled with GelFoam (Upjohn, Kalamazoo, MI) and the wound closed with silk sutures. Sham-operated rats underwent the same procedure except that the dura mater was left intact and the olfactory bulbs were not aspirated.

Acute Reversible Anosmia

One day before self-administration testing 13 rats were treated with zinc sulfate to render them anosmic. They were anesthetized with intraperitoneal injections of 25mg/kg pentobarbital (Abbott Laboratories, Chicago, IL) and 40mg/kg ketamine hydrochloride (Mallinckrodt, Mundelein, IL). The rats were placed on their backs and a curved 20-gauge syringe was put into the pharynx at the caudal end of the palate and slowly

retracted rostrally to allow the tip to enter the nasal cavity via the posterior choanae. $ZnSO_4$ (5% w/v in 0.9% saline) was slowly perfused until a few drops were seen to drain out of the external nares. The mouth of the rat was aspirated to remove saliva and excess solution during the procedure and during recovery from the anesthesia (Alberts & Galef, 1971).

Anosmia Testing

To ensure that rats were anosmic during testing, a cookie-finding task was employed after self-administration testing. An Oreo cookie with a diameter of 4.5 cm (Nabisco), which rats were previously habituated to, was placed in a polycarbonate plastic cage (49.5 x 27.9 x 20.3 cm) and corncob bedding (Andersons, Maumee, OH) poured on top of the cookie and spread in the cage to a depth of 1 inch. The rat was placed in the middle of the cage and a stopwatch was started. If the rat actively found the cookie in less than 10 minutes, the rat was not considered anosmic and was removed from the study. Previous unpublished observations conducted in this laboratory consistently reveal that intact rats typically find the cookie within 2 minutes.

Open Field Test

An open field test was used to assess the increased locomotor activity typically observed in bulbectomized rats. One day after self-administration each rat was placed in the apparatus for 3 minutes. The apparatus was a 43.2 x 43.2 x 30.5 cm Plexiglas chamber. A 100-watt light (1690 lumens) illuminated the chamber from 1 m above. A white-noise generator was set at 80 dB. The distance traveled (cm) by the rat was recorded by an automated activity monitor (Med Associates, St. Albans, VT). An investigator observed and manually recorded rearing (both front paws of rat leave floor),

immobility (time in seconds) and fecal boli produced. The chamber was cleaned with a 10% chlorine bleach solution between each subject.

Oral Self-Administration of *d*-Amphetamine

A two-bottle choice test was employed to examine the preference for *d*-amphetamine consumption. Self-administration testing began 14 days after olfactory bulbectomy surgery or 1 day after anosmia induction. Twenty-four hours prior to self-administration testing, the rats were deprived of water. Two 100ml drinking bottles with dripless sipper tubes were attached to the chamber with 100ml of distilled water and 100ml of 0.1mg/ml *d*-amphetamine sulfate in distilled water for 8 hours each day. Volume of each liquid consumed was recorded for 3 days by weighing (g) bottles. Data were also expressed as preference for *d*-amphetamine (amphetamine intake / total intake) x 100.

Histological Analysis

On completion of the experiments, rats were euthanized and their brains removed. Olfactory bulb lesions were verified by weighing tissue recovered from the olfactory bulb cavity. Only data from rats with 85% of the olfactory bulb removed (less than 5mg of tissue) were included.

Data Analysis

Open field data were analyzed using one-way ANOVA. Data from the self-administration experiment were analyzed with a mixed 3 x 3 ANOVA (group x day). Planned comparisons testing the hypotheses of increased amphetamine self-administration in bulbectomized rats compared to sham surgery and anosmic rats were conducted using t-tests. The α was set at $p < 0.05$

RESULTS

Histology.

Olfactory bulb lesions were verified by weighing tissue recovered in the olfactory bulb cavity post-mortem. All the rats in the olfactory bulbectomy group had complete lesions therefore no rats were excluded.

Self-Administration.

Self-administration of *d*-amphetamine and distilled water intake was measured for 3 days. A mixed 3 x 3 ANOVA revealed differences for surgery, $F(2,27) = 7.21$, $p < 0.005$, and days, $F(2,54) = 4.22$, $p < 0.05$. Olfactory bulbectomized rats drank more amphetamine than sham operated controls day 1, $t(18) = 2.85$, $p < 0.05$, day 2, $t(18) = 2.51$, $p < 0.05$, and day 3, $t(18) = 4.20$, $p < 0.005$ (see Figure 1). Olfactory bulbectomized rats also drank more amphetamine than anosmic rats on day 1, $t(18) = 2.70$, $p < 0.05$, and day 3, $t(18) = 4.66$, $p < 0.005$. A significant difference was also revealed for water intake, $F(2,27) = 4.83$, $p < 0.05$ (see Figure 2). When data were expressed as preference for *d*-amphetamine (amphetamine intake / total intake x 100) significant differences were revealed for day 2, $F(2,27) = 4.84$, $p < 0.05$, and day 3, $F(2,27) = 11.47$, $p < 0.0005$ (see Figure 3). OBX rats had a significantly higher preference for amphetamine than sham-operated controls on day 2, $t(18) = -3.43$, $p < 0.005$, and day 3, $t(18) = -2.94$, $p < 0.05$. OBX rats also had a significantly higher amphetamine preference than anosmic rats on day 3, $t(18) = -5.23$, $p < 0.0005$. There were no significant differences in amphetamine preference between sham-operated and anosmic animals.

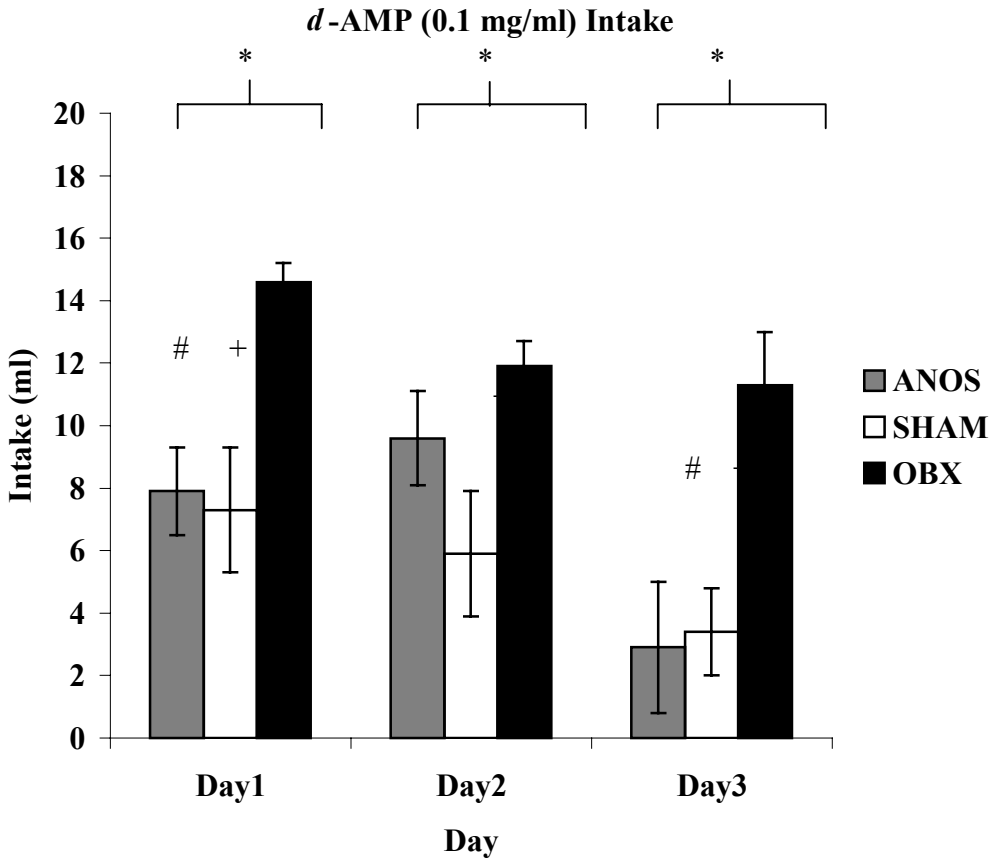


Figure 1. Intake of 0.1mg/ml *d*-amphetamine was measured for 3 days. OBX increased self-administration of amphetamine, ANOS (n = 10) SHAM (n = 10) OBX (n = 10). Mean ± SEM are represented. * indicates a significant difference, $p < 0.05$. + indicates significant t-tests between OBX and SHAM rats, $p < 0.05$. # indicates significant t-tests between OBX and ANOS rats, $p < 0.05$.

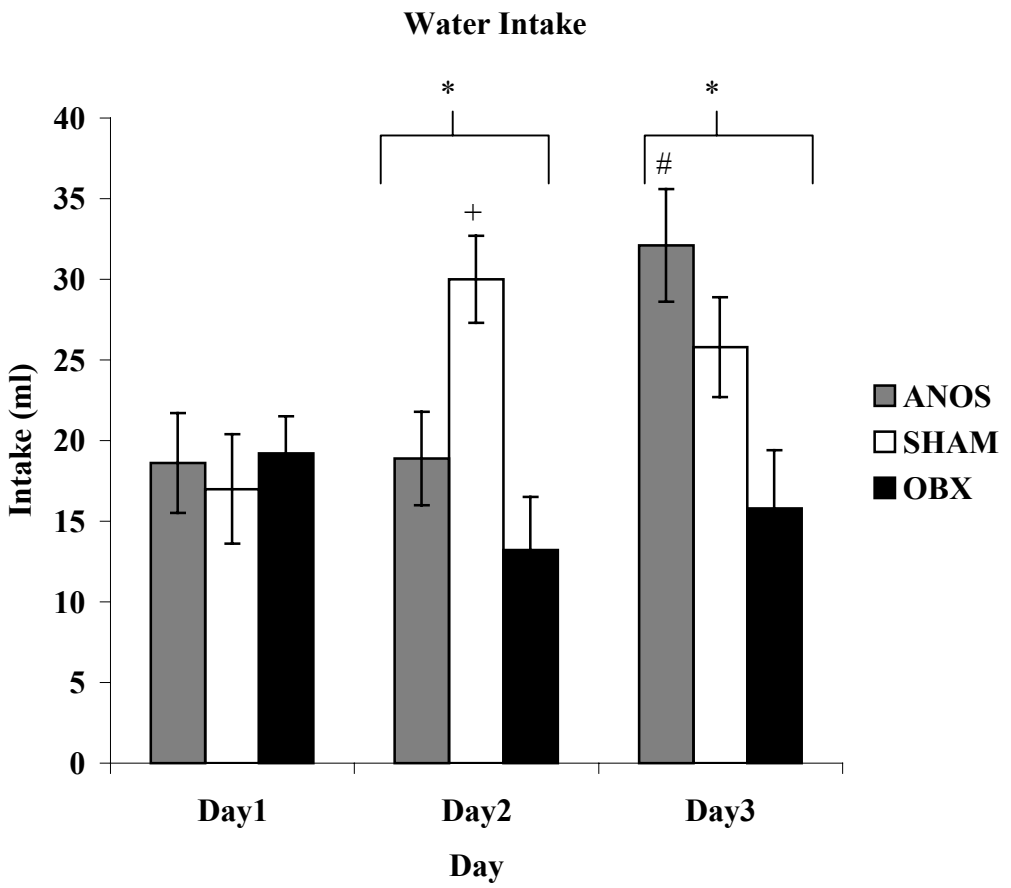


Figure 2. Intake of distilled water was measured for 3 days. OBX increased self-administration of distilled water, ANOS (n = 10) SHAM (n = 10) OBX (n = 10). Mean ± SEM are represented. * indicates a significant difference, $p < 0.05$. + indicates significant t-tests between OBX and SHAM rats, $p < 0.05$. # indicates significant t-tests between OBX and ANOS rats, $p < 0.05$.

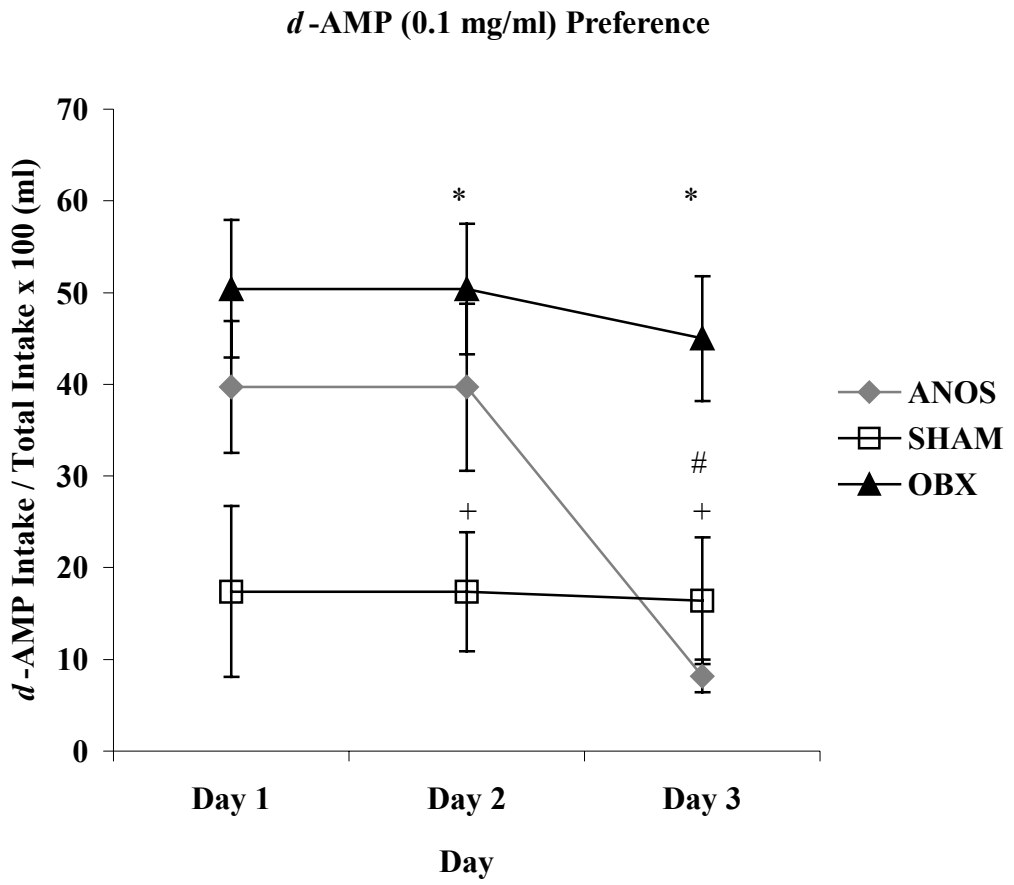


Figure 3. Preference for *d*-amphetamine (amphetamine intake / total intake x 100) was measured for 3 days. OBX increased amphetamine preference, ANOS (n = 10) SHAM (n = 10) OBX (n = 10). Mean ± SEM are represented. * indicates a significant difference, $p < 0.05$. + indicates significant t-tests between OBX and SHAM rats, $p < 0.05$. # indicates significant t-tests between OBX and ANOS rats, $p < 0.05$.

Anosmia Test.

Rats in the anomic group were placed in a chamber with a buried cookie for 10 minutes to determine anosmia. Three rats actively found the cookie in less than 10 minutes and were removed from the study.

Open Field Test.

Rats were placed in an open field chamber for 3 minutes. An automated activity monitor calculated distance traveled and the researcher counted rearing, time immobile and fecal boil. A significant difference was revealed for rearing, $F(2,26) = 10.12$, $p < 0.005$ (See Figure 4). Olfactory bulbectomized rats reared more than sham-operated, $t(18) = -3.04$, $p < 0.005$, and anosmic rats, $t(18) = -3.66$, $p < 0.005$. Distance traveled, time immobile, and fecal boli produced were not significantly affected by surgery.

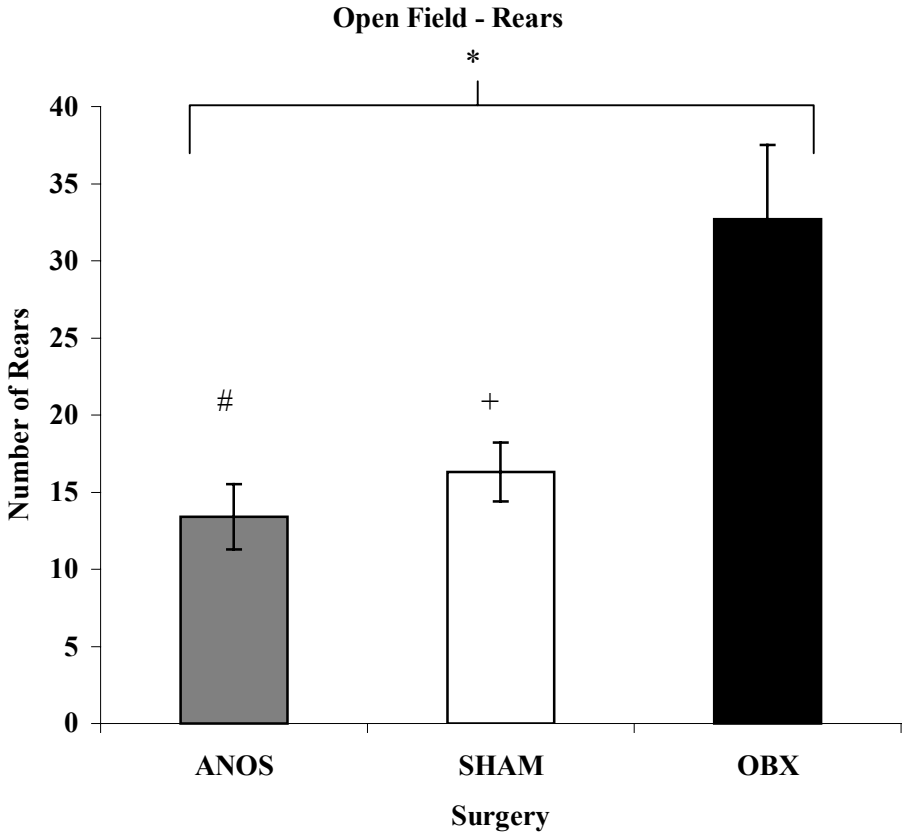


Figure 4. Rats were placed in an open field for 3 minutes. A researcher counted number of rears. OBX increased number of rears, ANOS (n = 10) SHAM (n = 10) OBX (n = 10). Mean ± SEM are represented. * indicates a significant difference, $p < 0.05$. + indicates significant t-tests between OBX and SHAM rats, $p < 0.05$. # indicates significant t-tests between OBX and ANOS rats, $p < 0.05$.

CONCLUSIONS

In the present experiment, OBX rats drank more 0.1 mg/ml *d*-amphetamine solution than sham-operated and anosmic rats for 3 days. Previous studies have found differences in oral amphetamine intake only on day 1 when amphetamine solution was the sole source of drinking water (Holmes & Masini, 1999). The present study examined the acquisition of amphetamine intake but in the future it would be useful to examine how long the differences persist.

Differences in distilled water intake were observed on days 2 and 3. On day 2, sham-operated rats drank more water than OBX and anosmic rats. On day 3, anosmic rats drank more water than OBX and sham-operated rats.

Data in the present experiment were also presented as preference for amphetamine. Representing intake this way makes it clear that OBX rats do not necessarily prefer amphetamine since preference is around 50%. OBX rats just have less of an aversion to amphetamine than sham-operated and anosmic rats. The differences seen in intake of amphetamine by OBX rats are not due to the lack of sense of smell. Rats rendered anosmic by zinc sulfate treatment did not differ significantly from sham-operated rats.

OBX rats reared more in the open field test though a difference between groups was not seen for exploratory activity (distance traveled). Previous researchers typically found that OBX increases activity in novel open fields (Earley, Glennon, Lally, Leonard, & Junien, 1994; Ho, Chang, Liu, Tai, Wong, & Tsai, 2000; Mar, Spreekmeester, & Rochford, 2000; O'Connor & Leonard, 1988; Primeaux & Holmes, 1999; Stock, Hand, Ford, & Wilson, 2000; Tiffany, Mollenauer, Plotnik, & White, 1979). To efficiently

measure liquid intake for the self-administration test, rats were housed in cylindrical 22.9 x 17.8 cm metabolic chambers. The size of these cages limited the exploration and movements of the rats. It is feasible that if housed in the more typical rectangular 49.5 x 27.9 x 20.3 cm cages, the increased exploratory behavior of the OBX rats would have occurred.

In general, OBX rats self-administer more amphetamine solution than control animals. These results support the self-medication hypothesis. Based on evidence that clinically depressed humans are more likely to abuse drugs than non-depressed humans (Christie, Burke, Regier, Rae, Boyd, & Locke, 1988; Gilman & Abraham, 2001; Glassman, Helzer, Covey, Cottler, Stetner, Tipp, et al., 1990; Grant & Pickering, 1998; McCuller, Sussman, Dent, & Teran, 2000; Winokur, Turvey, Akiskal, Coryell, Solomon, Leon, et al., 1998). This experiment utilized an animal model of depression and revealed a similar phenomenon. The next two experiments were designed to investigate the neurochemical basis of this phenomenon.

CHAPTER 3: EXPERIMENT 2

Baseline levels of dopamine, DOPAC, and HVA in the striatum of olfactory bulbectomized rats.

The monoamine neurotransmitter dopamine (DA) is involved in both endogenous depression and drug abuse. According to the incentive salience hypothesis proposed by Berridge & Robinson (1998) the mesolimbic dopamine system may mediate the "wanting" of a reward. Such motivation is inferred from goal-directed behavior or apparent attraction to an incentive stimulus and consumption of the goal object (Berridge & Robinson, 1998). Drugs abused by humans all have effects on the mesolimbic DA system (Giorgetti, Hotsenpiller, Froestl, & Wolf, 2002; Pulvirenti & Diana, 2001; Spanagel & Weiss, 1999). DiChiara, Acquas, & Carboni (1992) suggested that the motivation to abuse drugs by humans is related to a change in the mesolimbic DA system. Differences in dopamine have also been examined in rodent models of drug abuse. Mice genetically bred to exhibit a high preference for ethanol show low DA concentrations in the terminal regions of mesolimbic DA pathways (George, Fan, Ng, Jung, O'Dowd, & Naranjo, 1995).

Several hypotheses about the etiology of depression suggest that deficits in catecholamines, including norepinephrine, serotonin, and DA, lead to clinical depression (Bunney & Davis, 1965; Schildkraut, 1965; Willner, 1983). Though DA does not account for all aspects of depression, several categories of evidence implicate an important role of DA in depression. Lower levels of the DA metabolite, homovanillic

acid (HVA) were found in the cerebral spinal fluid (CSF) of depressed suicide attempters (Brown & Gershon, 1993; Roy, Agren, Pickar, Linnoila, Doran, Cutler, et al., 1986). Several researchers have also found lower values of CSF HVA after probencid administration in clinically depressed compared to normal humans (Goodwin, Post, Dunner, & Gordon, 1973; van Praag, Korf, & Schut, 1973). Probencid is a drug that blocks the exit of HVA from the CSF to the blood and thus amplifies the change in HVA levels caused by altered turnover of DA (Korf & van Praag, & Sebens, 1971). Roy, Pickar, Linnoila, Doran, Ninan, & Paul (1985) found significantly lower levels of the DA metabolites HVA, dihydroxyphenylacetic acid (DOPAC), and conjugated DOPAC, in melancholic compared to nonmelancholic depressives. DOPAC concentrations reflect the amount of monoamines recaptured by nerve terminals (Roth, Murrin, & Walters, 1976).

Dopamine is also implicated in the mechanism of action of electroconvulsive shock therapy (ECS) and is affected by antidepressant treatment. Concentrations of DA in microdialysates from the rat striatum increased to 127% of baseline levels after acute ECS (Nomiko, Zis, Damsma, & Fibiger, 1991). Acute ECS also increased interstitial concentrations of DOPAC and HVA in nucleus accumbens though chronic ECS decreased the metabolites (Nomiko et al.). *In vivo* experiments have found that mesolimbic DA function is enhanced by both tricyclic and atypical antidepressants (De Montis, Devoto, Gessa, Porcella, Serra, & Tagliamonte, 1990; Maj, Rogez, Skuza, & Sowinska, 1984; Nomiko et al., 1991; Spyraki & Fibiger, 1981; Willner & Montgomery, 1981). Many DA agonists have also been found to have antidepressant effects (Bouras & Bridges, 1982; Post, Gerner, Carman, Gillin, Jimerson, Goodwin, & Bunney, 1978;

Willner, 1983). The stimulant drug, amphetamine, has been found to improve mood in some depressed patients (Ayd & Zohar, 1988; Silberman, Reus, Jimerson, Lynott, & Post, 1981). Little (1988) found that a single dose of amphetamine or methylphenidate causes a mood elevation in about 50% of depressed patients and that the response to many antidepressants can be predicted by an amphetamine challenge test.

It is well known that Parkinson's disease is caused by a deficiency of DA produced by the degeneration dopaminergic neurons (Hornykiewicz, 1966). A high incidence of depression is seen in Parkinson's disease (Asnis, 1977; Guze & Barrio, 1991; Jacobs & Silverstone, 1988; Randrup, Munkvad, Fog, Gerlach, Molander, Kjellberg, et al. 1975). And the onset of depression often precedes physical disabilities that accompany Parkinson's (Guze et al.).

Researchers using the olfactory bulbectomized (OBX) rat model of depression have found evidence for decreased DA transmission in the brain (Nakamura & Nakamura, 1975). Of particular importance to the present study is evidence of decreased DA found in the striatum of OBX rats (Gottesfeld, Garcia, Lingham, & Chronister, 1989; Lumia, Teicher, Salchli, Ayers, & Possidente, 1992). Dopamine has been shown to suppress prepro-enkephalin (ENK) gene expression in striatal neurons and therefore is commonly used as a marker of DA depletion or dysfunction (Kowalski & Giraud, 1993). Dopamine deafferentation produced by 6-OHDA or electrolytic lesions lead to elevated prepro-ENK mRNA in the striatum (Gerfen, McGinty, & Young, 1991; Nisenbaum, Kitai, & Gerfen, 1994; Normand, Popovivi, Onteniente, Fellman, Piatier-Tonneau, Auffray, et al., 1988). Researchers have found that OBX increases prepro-ENK gene

expression in the striatum (Holmes, 1999; Primeaux & Holmes, 2000), a finding consistent with decreased dopamine function.

The present study examined levels of dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the ventral and dorsal striatum of OBX rats. Holmes (1999) using *in situ* hybridization found 41% higher prepro-ENK mRNA levels in the olfactory tubercle (OT) component of the ventral striatum of OBX rats compared to sham-operated controls days 14 and 28 post-surgery. These time points were similar to other behavioral and neurochemical changes seen after OBX (Baumbach & Sieck, 1977; Broekkamp, O'Connor, Tannaer, Rijk, & Van Delft, 1986; Holmes, Davis, Masini, & Primeaux, 1998). Though ENK changes were seen in the OT, they were not found in the nucleus accumbens of OBX rats (Holmes, 1999; Holmes & Masini, 1999). This may be because the OT but not the nucleus accumbens receives extensive olfactory bulb input (Shipley, McLean, & Ennis, 1995; Heimer, Zahm, & Alheid, 1995). The OT is also densely innervated by dopaminergic neurons of the ventral tegmental area, which is implicated in drug abuse (Ungerstedt, 1971) and also supports ICSS (Prado-Alcala & Wise, 1984).

The present study utilized *in vivo* microdialysis combined with high-performance liquid chromatography with electrochemical detection (HPLC-EC) to examine dopamine and metabolite levels in OBX and sham-operated rats. It was hypothesized that OBX rats will have lower basal concentrations of extracellular DA and metabolites in microdialysates from the OT component of the ventral striatum compared to sham-operated controls.

MATERIALS AND METHODS

Subjects & Design.

Sixty experimentally naïve male Sprague-Dawley rats (Harlan, Inc., Indianapolis, IN) weighing 250 – 300g at the time of surgery served as subjects. Rats were group housed (3 – 4 per cage) prior to surgery in polycarbonate plastic cages (49.5 x 38.1 x 20.3 cm). Post-surgery rats were housed individually in polycarbonate plastic cages (49.5 x 27.9 x 20.3 cm). Rats were randomly assigned to 1 of 4 groups consisting of OBX-OT (n = 15), OBX-DS (n = 15), SHAM-OT (n = 15), and SHAM-DS (N = 15). Chambers were kept in a humidity and temperature-regulated animal housing facility with lighting maintained on a reverse 12 hour schedule (lights out at 0700h). Food and water were available *ad libitum*. Rats were weighed daily throughout the experiment. All procedures were approved by the University of Georgia Animal Care and Use Committee and followed the guidelines of the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

Open Field Testing.

Open field test procedures were conducted as described in Experiment 1.

In Vivo Microdialysis.

Sterile stereotaxic surgery was performed following the guidelines of the University of Georgia Animal Care and Use Committee. The rats were anesthetized with intraperitoneal injections of 25mg/kg pentobarbital (Abbott Laboratories, Chicago, IL) and 40mg/kg ketamine hydrochloride (Mallinckrodt, Mundelein, IL) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). A midline incision was made and olfactory bulbectomy or sham surgery was performed as previously described. Then

a burr hole was drilled dorsal to the right olfactory tubercle following the coordinates found in Paxinos & Watson's *The Rat Brain in Stereotaxic Coordinates* (1997): anterior-posterior +1.0 mm from bregma, lateral -2.5 mm and dorsal-ventral –7.0 mm from the skull surface. Control animals had the hole drilled dorsal to the right dorsal striatum following the coordinates anterior-posterior +1.0 from bregma, lateral –2.5 and dorsal-ventral –3.5 from the skull surface. A 20-gauge stainless steel guide cannula with a removable stylette was lowered to the specified coordinates and secured to the skull with jeweler's screws and dental acrylic.

Two hours before testing, a concentric-style microdialysis probe was inserted through the guide cannula to a depth of 9.0 mm (olfactory tubercle) or 5.5mm (dorsal striatum) from skull surface. The rat was then placed in the metabolic chamber and perfused with artificial CSF (0.752g NaCl, 0.031g $KHCO_3$, 0.020g $MgCl_2 \bullet 6H_20$, 0.180g $NaHCO_3$, 0.050g dextrose, 0.013g $CaCl_2$ with pH 7.4).

The probe was connected to a dual channel swivel (Instech, Plymouth Meeting, PA) and perfusion started with an infusion syringe pump (Harvard Apparatus, Holliston, MA) at a flow rate of 2 µl / min. Dialysis was performed during the dark phase (0700 – 1900h) and rats were allowed to habituate to the chamber for 2 hours prior to collection. Dialysate samples were collected for 20 minutes yielding 40 µl samples. Samples were collected in sterile polypropylene microcentrifuge vials (Fisher Scientific, Pittsburgh, PA) and frozen immediately and stored at - 80° until analyzed.

HPLC-EC.

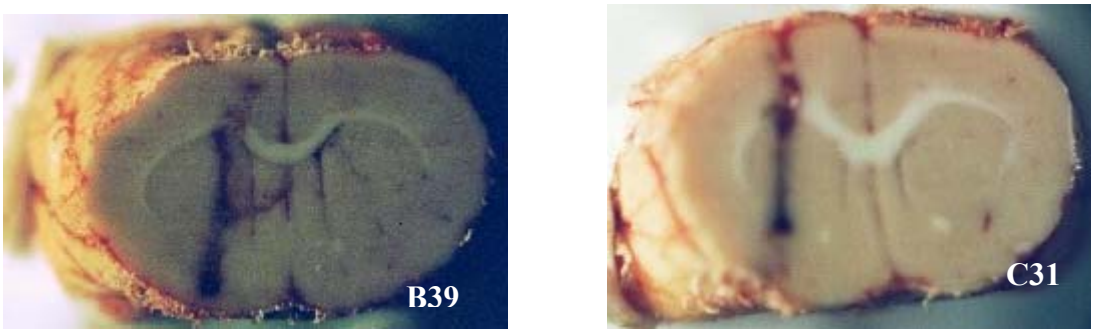
Untreated samples were thawed and injected into the HPLC system. The system consisted of a Waters 717 autoinjector (Waters, Milford, MA) and a C12 bonded silica

gel column (Phenomenex Inc., Torrance, CA). The mobile phase (55.196g $NaPO_4 \bullet H_20$, 0.148g [0.1 mM] EDTA, 0.173g [0.2 mM] octane sulfonic acid, 5% [200 mL] acetonitrile, pH 3.1) was delivered at a constant flow of 0.5 ml/min. An EC detector functioning with a glassy carbon electrode (Waters, Milford, MA) with a potential maintained at 0.1 nAMP was used. The position and height of peaks of DA, DOPAC, and HVA were compared with reference standard solutions (Sigma Chemical Co., St. Louis, MO). Peak areas were quantified by Millennium 32 software (Waters, Milford, MA). The average of 3 samples was used to determine baseline DA, DOPAC, and HVA levels for each animal.

Histological Analysis.

On completion of the experiments, rats were euthanized and their brains removed. Olfactory bulb lesions were verified by weighing tissue recovered from the olfactory bulb cavity. Only data from rats with 85% of the olfactory bulb removed (less than 5 g of tissue) were included. Cannula placement was verified for each subject after completion of the experiment by injecting 1.0 μl fast-green dye, euthanizing the rat with CO_2, and then removing the brain. Each brain was sectioned into 12 μm coronal slices with a Microm HM505E cryostat (Walldorf, Germany). The sections were photographed with a camera with a magnifying lens and visually examined by a researcher who was blind to the experimental conditions. Only data from rats with at least 75% of the probe membrane located in the ventral or dorsal striatum were included. See Figure 5 for representative photographs.

PANAL A:



PANAL B:

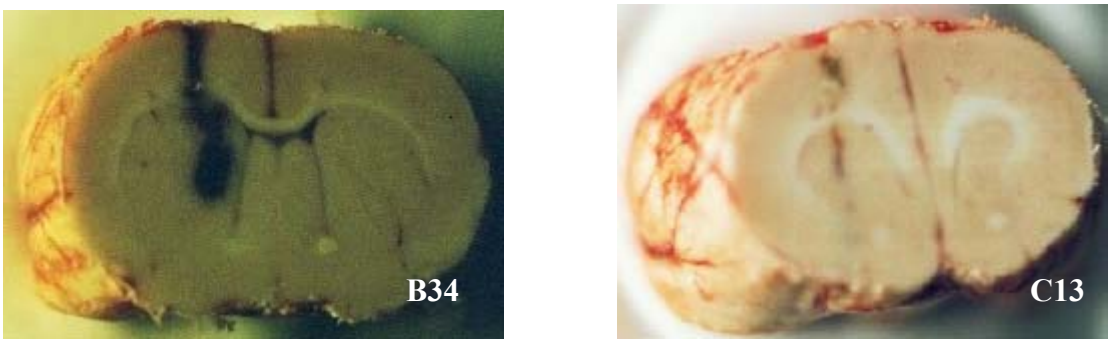


Figure 5. Representative photomicrographs of cannula placement in olfactory tubercle (Panal A) and dorsal striatum (Panal B).

Data Analysis.

Open field data was analyzed using one-way analyses of variance (ANOVA). Data from microdialysis sampling was analyzed with a one-way ANOVA. Baseline data was expressed as the average of 3 sample (MEAN ± SE) levels of DA, DOPAC, and HVA (fmol).

RESULTS

Histology.

Olfactory bulb lesions were verified by weighing tissue recovered in the olfactory bulb cavity post-mortem. Six rats were excluded from the study due to incomplete olfactory bulb lesions. Cannula placement was verified for each subject. Seven rats were excluded because the cannula track was not in the intended region. Three rats also died due to post-surgery complications.

In Vivo Microdialysis.

Samples of DA, DOPAC, and HVA were taken for 6 hours. Means ± SE were determined by averaging 3 samples taken at 2 hour intervals. A main effect for surgery was found, $F(1,29) = 4.57$, $p < 0.05$ (See Figures 6 & 7). OBX rats with samples taken from dorsal striatum had significantly higher DA levels than sham-operated rats with samples taken from dorsal striatum, $t(14) = -1.74$, $p < 0.05$. No main effects of region from which samples were taken, dorsal striatum or olfactory tubercle, were found. Levels of DOPAC and HVA did not differ by groups.

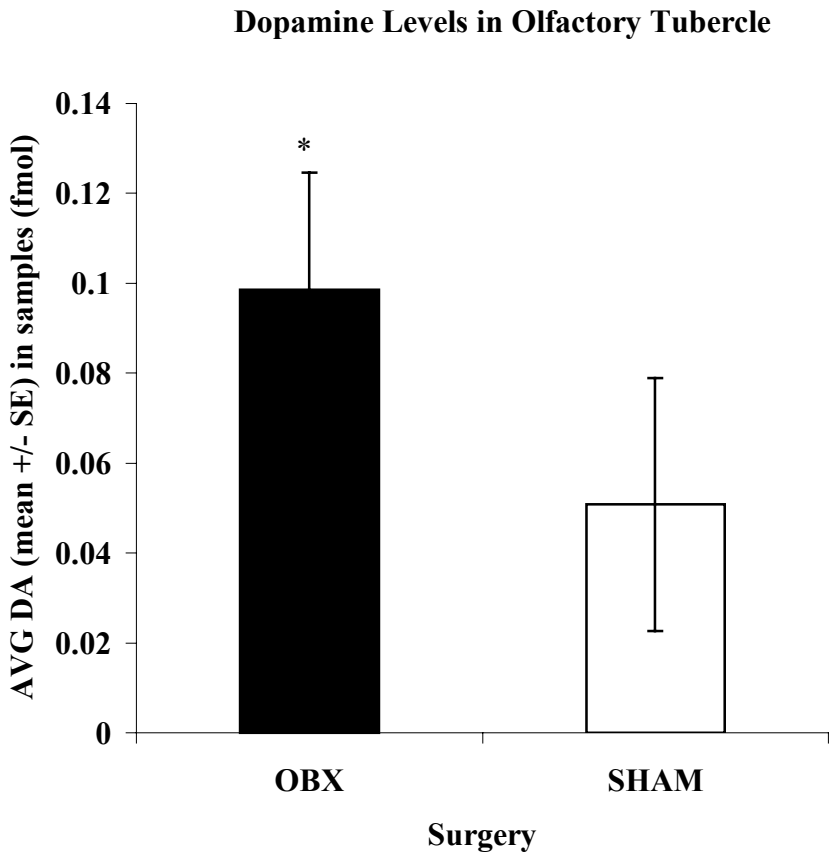


Figure 6. *In vivo* microdialysis samples of DA from OT were taken for 6 hours. OBX rats had higher DA levels than sham-operated rats, OBX (n = 8) SHAM (n = 10). Average of 3 sample means ± SEM are represented. * indicates a significant difference, $p < 0.05$.

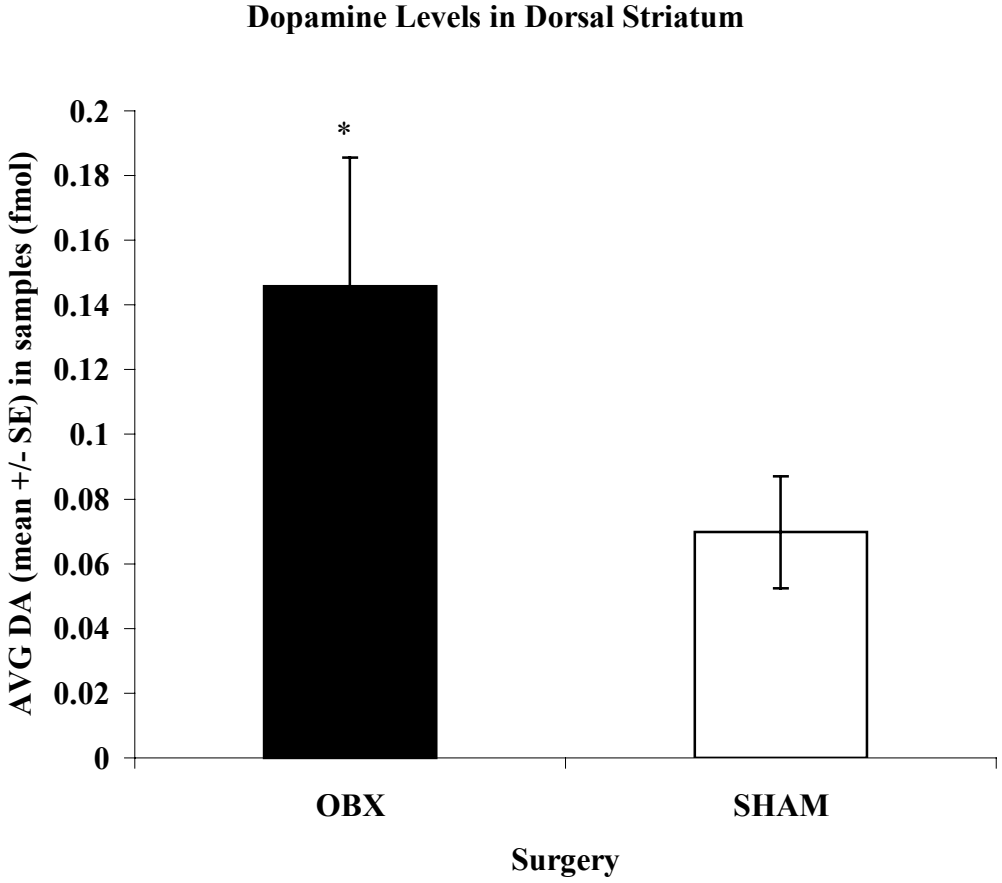


Figure 7. *In vivo* microdialysis samples of DA from DS were taken for 6 hours. OBX rats had higher DA levels than sham-operated rats, OBX (n = 8) SHAM (n = 10). Average of 3 sample means ± SEM are represented. * indicates a significant difference, $p < 0.05$. + indicates significant t-tests between, $p < 0.05$.

Open Field Test.

Open field activity was measured for 3 minutes. A significant difference was found for rearing, $F(1,33) = 19.11$, $p < 0.05$, (see Figure 8) and distance traveled, $F(1,33) = 9.38 = p < 0.05$ (See Figure 9). OBX rats reared significantly more than sham-operated rats, $t(33) = -4.45$, $p < 0.0005$ and exhibited greater locomotor activity, $t(33) = -3.17$, $p < 0.005$. No main effects were found for fecal boli produced and immobility.

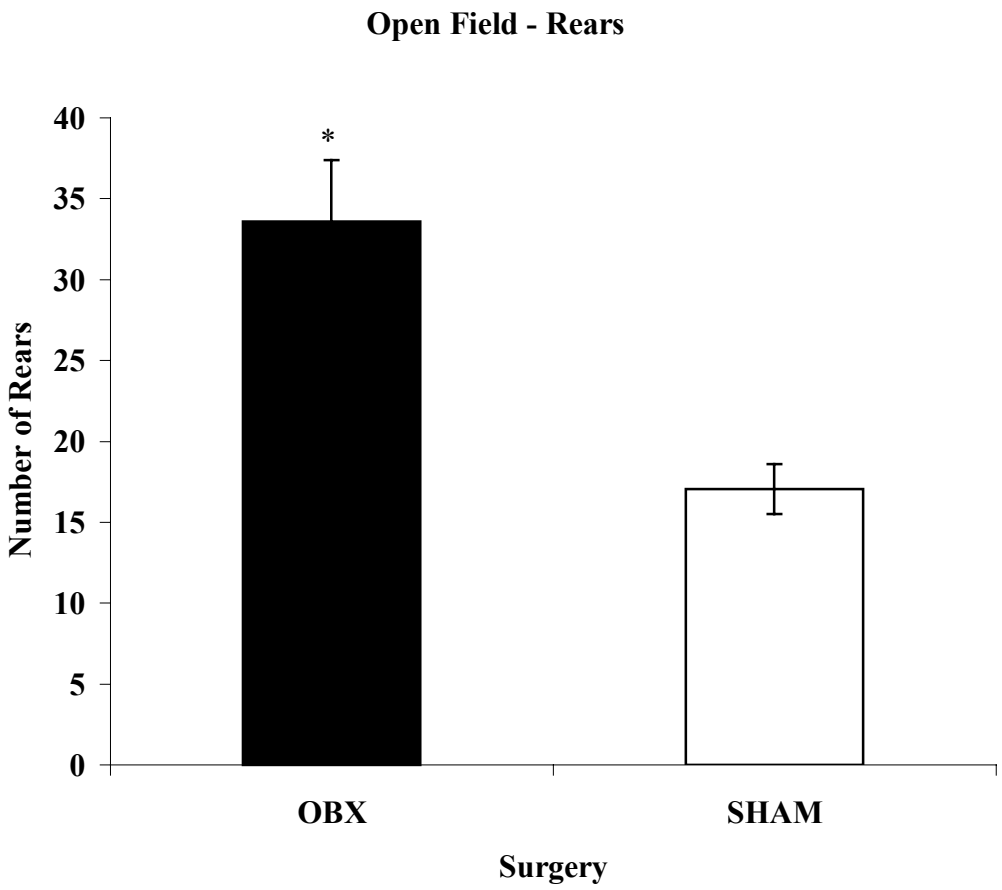


Figure 8. Rats were placed in an open field for 3 minutes. A researcher counted number of rears. OBX increased number of rears, OBX (n = 15) SHAM (n = 20). Mean ± SEM are represented. * indicates a significant difference, $p < 0.05$.

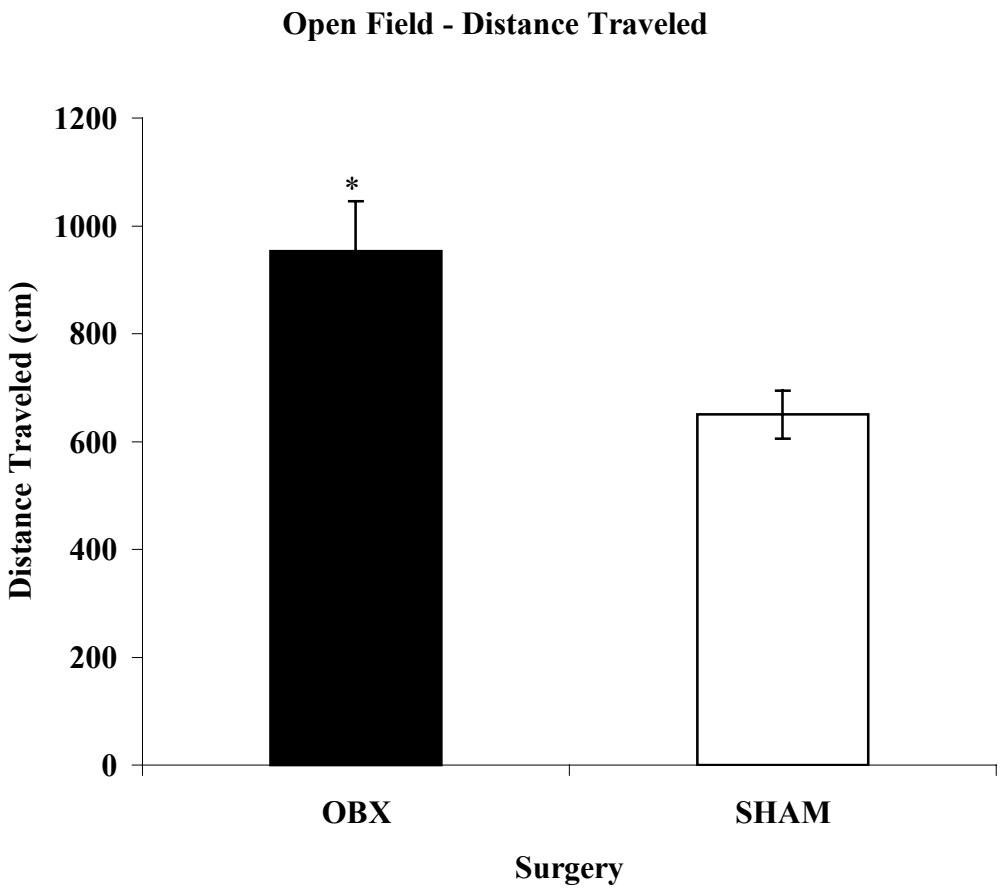


Figure 9. Rats were placed in an open field for 3 minutes. An automated activity monitor calculated distance traveled, OBX (n = 18) SHAM (n = 20). Mean ± SEM are represented. * indicates a main effect of surgery, $p < 0.05$.

CONCLUSIONS

The present study was intended to identify differences in basal levels of endogenous dopamine and metabolites between olfactory bulbectomized and sham-operated rats using *in vivo* microdialysis with electrochemical detection. Using post-mortem analysis of tissue levels of DA previous researchers have found evidence that olfactory bulbectomy decreases dopamine levels in striatum (Gottesfeld, Garcia, Lingham, & Chronister, 1989; Lumia, Teicher, Salchli, Ayers, & Possidente, 1992). In contrast, the present study revealed that olfactory bulbectomy increased basal dopamine release in striatum. Axonal degeneration produced by lesions can indirectly affect neurons that become deprived of their affect input and lead to degeneration (Gilad & Reis, 1979). But changes eventually occur and remaining intact afferents may undergo collateral sprouting. Lingham & Gottesfeld (1986) found that 14 to 20 days after olfactory bulbectomy, sprouting of dopaminergic axon terminals in the olfactory tubercle occurred. These authors did not suggest a functional consequence of the dopaminergic hyper-innervation but it is feasible that increased dopamine release could occur.

The differences in dopamine levels seen between olfactory bulbectomized and sham-operated rats were similar in the ventral and dorsal striatum. It was hypothesized that the olfactory tubercle component of ventral striatum would reveal different levels of dopamine and metabolites than the dorsal striatum because of its connection to the olfactory bulb. The olfactory tubercle receives direct projections from both the olfactory bulb and ventral tegmental area (VTA)(Shipley, McLean, & Ennis, 1995; Heimer, Zahm, & Alheid, 1995). Though the main projections to dorsal striatum are from substantia nigra, there are some from the VTA (Lindvall & Bjorklund, 1978). The similar levels of

DA and metabolites between dorsal and ventral striatum may reflect this common pathway.

In the present experiment, no differences between basal endogenous levels of dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were found. Other researchers have also found it to be difficult to detect group differences in endogeneous DA and metabolites without electrical stimulation or pharmacological treatment (Ewing et al., 1983; Gonon & Buda, 1985; Justice, 1993; Sharp, Zetterstrom, & Ungerstedt, 1986).

Previous researchers typically found that OBX increased activity in novel open fields (Earley, Glennon, Lally, Leonard, & Junien, 1994; Ho, Chang, Liu, Tai, Wong, & Tsai, 2000; Mar, Spreekmeester, & Rochford, 2000; O'Connor & Leonard, 1988; Primeaux & Holmes, 1999; Stock, Hand, Ford, & Wilson, 2000; Tiffany, Mollenauer, Plotnik, & White, 1979). This experiment also found that OBX increased activity in a novel open field. Both number of rears and exploratory behavior were found to be significantly greater in the open field test.

In conclusion, contrary to expectations, endogenous basal dopamine levels in olfactory bulbectomized rats were higher than sham-operated rats in both dorsal and ventral striatum, and no differences were seen for the dopamine metabolites DOPAC and HVA between bulbectomized and sham-operated controls.

CHAPTER 4: EXPERIMENT 3

Levels of dopamine, DOPAC, and HVA in the ventral striatum during self-administration of *d*-amphetamine in olfactory bulbectomized rats.

This experiment examined the levels of dopamine and its metabolites during self-administration of *d*-amphetamine in olfactory bulbectomized and sham-operated rats. The technique of *in vivo* microdialysis enables the researcher to examine simultaneously the behavior of the rat and neurotransmitter levels. Previous studies have found that *d*-amphetamine injections produced an immediate significant increase in dopamine levels that peaked between 20 and 40 minutes post-drug administration (Anderzhanova, Afanas'ev, Kudrin, & Rayevsky, 2000; Ciano, Coury, Depoortere, Egilmez, Lane, Emmett-Oglesby, et al., 1995; Di Chiara & Imperato, 1988; Hurd, Lindefors, Brodin, Brene, Persson, Ungerstedt, et al., 1992). This experiment attempted to determine if the olfactory bulbectomized rat has the same response to oral amphetamines and if the amount of *d*-amphetamine administered is correlated with extracellular dopamine levels in the ventral striatum. The hypothesis was that olfactory bulbectomized rats have lower basal levels of dopamine and will therefore self-administer more amphetamine than normal rats to increase levels of dopamine to normal levels. This hypothesis is similar to self-medication hypotheses that suggest that humans who are clinically depressed are more inclined to use drugs than non-depressed people (Khantzian, 1985; Markou, Kosten, & Koob, 1998; Weiss, Griffin, & Mirin, 1992).

MATERIALS AND METHODS

Subjects & Design.

Forty-four experimentally naïve male Sprague-Dawley rats (Harlan, Inc., Indianapolis, IN) weighing 250 – 300g at the time of surgery served as subjects. Rats were group housed (3 – 4 per cage) prior to surgery in polycarbonate plastic cages (49.5 x 38.1 x 20.3 cm). Post-surgery rats were housed individually in polycarbonate plastic cages (49.5 x 27.9 x 20.3 cm). Rats were randomly assigned to 1 of 4 groups consisting of OBX-OT (n = 9), OBX-DS (n = 9), SHAM-OT (n = 11), and SHAM-DS (N = 15). Chambers were kept in a humidity and temperature-regulated animal housing facility with lighting maintained on a reverse 12 hour schedule (lights out at 0700h). Food was available *ad libitum* and water available 8 hours a day post-surgery during the dark phase. Rats were weighed daily throughout the experiment. All procedures were approved by the University of Georgia Animal Care and Use Committee and followed the guidelines of the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

Open Field Testing.

Open field test procedures were conducted as described in Experiment 1

In Vivo Microdialysis During *d*-Amphetamine Intake.

In vivo microdialysis was performed as previously described in Experiment 2. The metabolic chamber also had 100ml water bottle attached to it and contained 0.1mg/ml *d*-amphetamine in distilled water. A researcher measured intake by weighing the bottle before and after microdialysis testing.

HPLC-EC.

High performance liquid chromatography was performed as previously described in Experiment 2. The average of 3 samples was used to determine DA, DOPAC, and HVA levels during amphetamine drinking for each animal.

Histological Analysis.

On completion of the experiments, rats were euthanized and their brains removed. Olfactory bulb lesions were verified and cannula placement examined as previously described in Exp 2.

Data Analysis.

Open field data was analyzed using one-way ANOVA. Data from microdialysis sampling and self-administration were also analyzed with one-way ANOVAs. The relationship between DA, DOPAC, and HVA levels and self-administration of *d*-amphetamine will be examined using a Pearson correlation coefficient.

RESULTS

Histology.

Olfactory bulb lesions were verified by weighing tissue recovered in the olfactory bulb cavity post-mortem. All the rats in the olfactory bulbectomy group had complete lesions therefore no rats were excluded. Cannula placement was verified for each subject. Seven rats were excluded because the cannula track was not in the intended region.

Microdialysis.

Samples of DA, DOPAC, and HVA were taken for 6 hours. Means ± SE were determined by averaging 3 samples taken a 2 hour intervals. No main effects for surgery

or region from which samples were taken, dorsal striatum or olfactory tubercle, were found (see Figure 10).

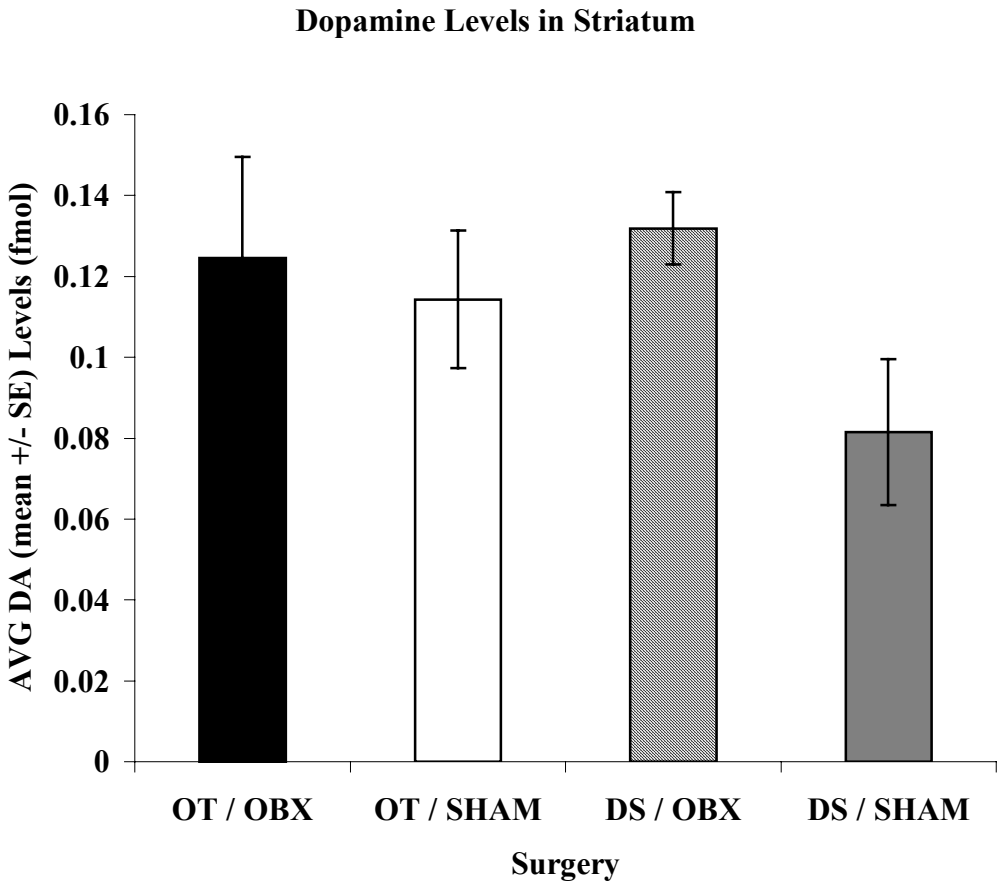


Figure 10. *In vivo* microdialysis samples of DA from were taken for 6 hours. OT/OBX (n = 9) OT/SHAM (n = 8) DS/OBX (n = 9) DS/SHAM (n = 11). Average of 3 sample means ± SEM are represented.

Self-Administration.

A solution of 0.1mg/ml of *d*-amphetamine in distilled water was available during microdialysis testing. A researcher measured intake by weighing the bottle before and after microdialysis testing. A significant difference was revealed for amphetamine intake, $F(35, 1) = 18.88$, $p < 0.005$ (see Figure 11). OBX rats drank more amphetamine solution than SHAM rats, $t(35) = -4.35$, $p < 0.0005$. A Pearson correlation coefficient was calculated to test the association between mean amphetamine intake and mean DA levels, $r = 0.026$, $p = 0.88$.

Open Field Test.

Open field activity was measured for 3 minutes. A significant difference was found for rearing, $F(1,33) = 34.43$, $p < 0.005$, (See Figure 12) and distance traveled, $F(1,33) = 22.52$, $p < 0.005$ (see Figure 13). OBX rats reared more, $t(32) = 5.82$, $p < 0.005$, and explored more, $t(32) = -4.49$, $p < 0.005$. No significant differences were found for fecal boli produced and immobility.

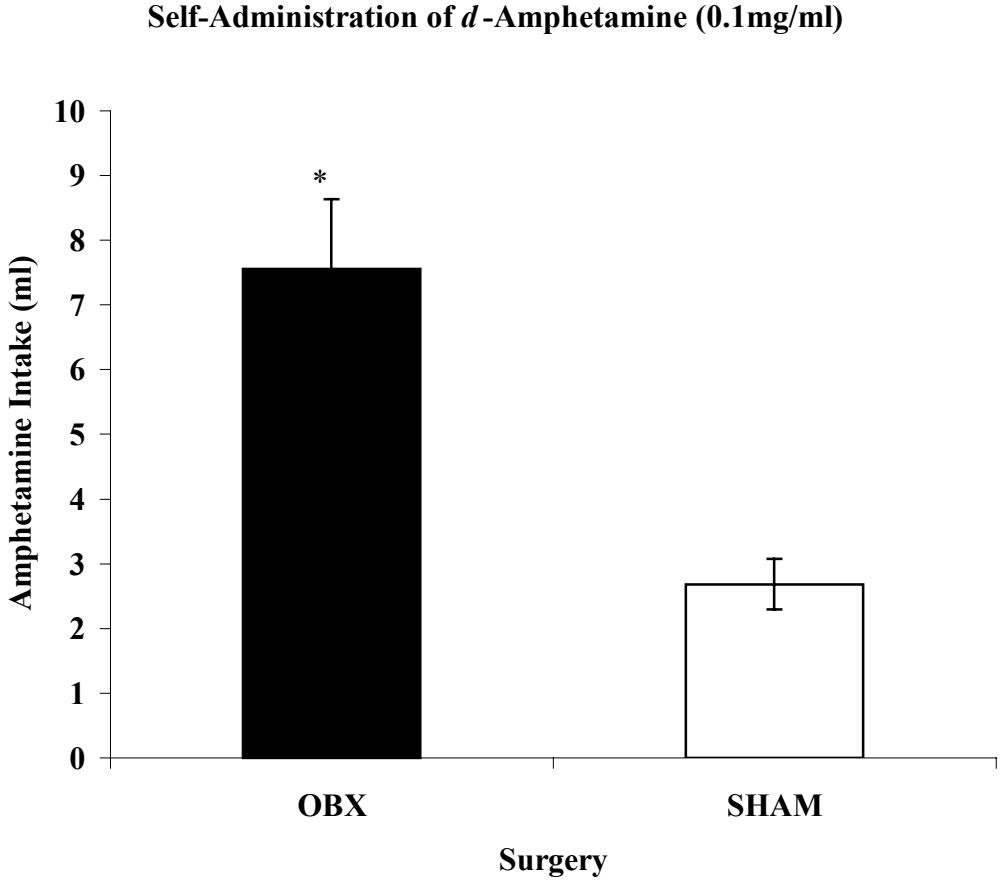


Figure 11. Intake of 0.1mg/ml *d*-amphetamine was measured during microdialysis. OBX increased self-administration of amphetamine, OBX (n = 18) SHAM (n = 19). Mean ± SEM are represented. * indicates a significant difference, $p < 0.05$.

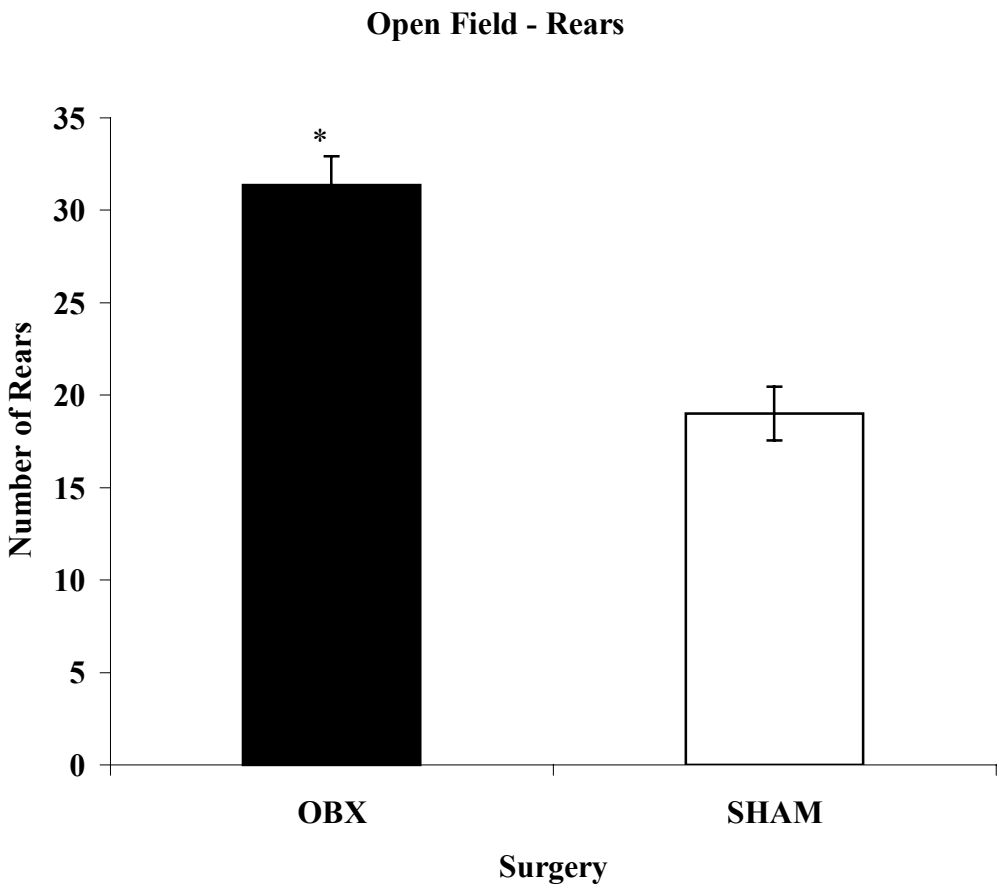


Figure 12. Rats were placed in an open field for 3 minutes. A researcher counted number of rears. OBX increased number of rears, OBX (n = 16) SHAM (n = 18). Mean ± SEM are represented. * indicates a significant difference, $p < 0.05$.

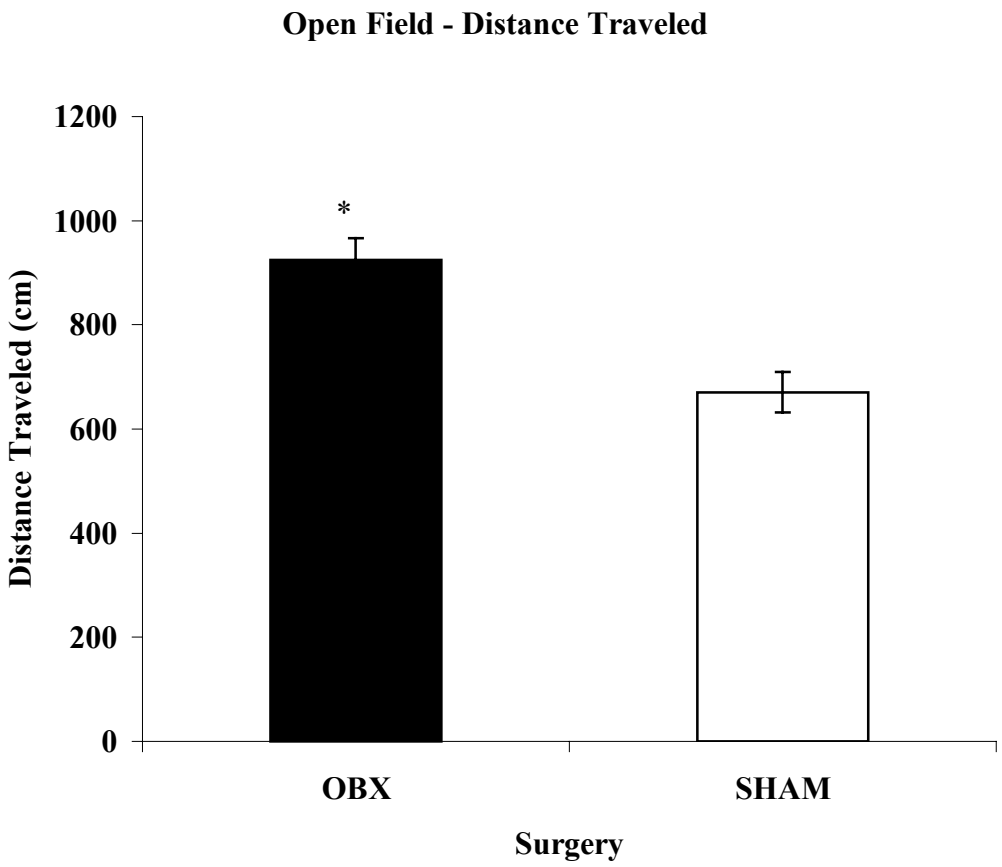


Figure 13. Rats were placed in an open field for 3 minutes. An automated activity monitor calculated distance traveled, OBX (n = 16) SHAM (n = 18). Mean ± SEM are represented. * indicates a significant difference, $p < .05$.

CONCLUSIONS

The present study was intended to identify differences between levels of dopamine, DOPAC, and HVA during amphetamine intake between olfactory bulbectomized and sham-operated rats using *in vivo* microdialysis with electrochemical detection. No differences between groups were found for dopamine, DOPAC, or HVA. Previous studies examining amphetamine-stimulated release of dopamine have found large increases in dopamine levels in the striatum (Anderzhanova, Afanas'ev, Kudrin, & Rayevsky, 2000; Di Chara & Imperato, 1988; Di Ciano, Coury, Depoortere, Egilmez, Lane, Emmett-Oglesby, et al., 1995; Fuentese & Del Rio, 1972). A major difference between the present study and these previous microdialysis studies is that amphetamine was administered here orally. Previous studies have examined intravenous, intraperitoneal, and subcutaneous injections of *d*-amphetamine but not oral self-administration. The small amount of amphetamine drank by the rats in the present study (0 – 14ml during a 6 hour period) may not have been sufficient to influence dopamine release. Alternatively, the small amount of amphetamine consumed by sham-operated rats may have increased dopamine to the same levels observed in bulbectomized rats. This interpretation is based on the assumption that that bulbectomized rats did not respond to the oral amphetamine self-administration.

Olfactory bulbectomized rats drank significantly more *d*-amphetamine solution during microdialysis. It is interesting that though there were differences in amphetamine intake as previously seen in Experiment 1, there were no differences in DA levels between bulbectomized and sham-operated rats as seen in Experiment 2. A Pearson correlation coefficient was calculated to test the association between mean amphetamine

intake and mean dopamine levels. No significant association was found but visual inspection of the scatterplot suggested a trend for higher amphetamine intake and higher dopamine levels for bulbectomized rats.

In conclusion, olfactory bulbectomized rats drank more 0.1mg/ml *d*-amphetamine solution during *in vivo* microdialysis than sham-operated rats. Bulbectomized and sham-operated rats, however, did not differ in levels of dopamine, DOPAC, or HVA.

CHAPTER 5: DISCUSSION

The present studies examined the phenomenon of comorbidity of depression and drug abuse. Epidemiological studies have clearly identified the existence of a relationship between endogenous depression and drug abuse. People with major depressive disorder tend to use nicotine, alcohol, and hard drugs more frequently than non-depressed people (see Appendix A). This observation led to the formation of the self-medication hypothesis that suggests that people are treating their symptoms with drugs of abuse (Khantzian, 1985; Markou, Kosten, & Koob, 1998; Weiss, Griffin, & Mirin, 1992).

Although the phenomenon of the comorbidity of depression and drug abuse is well documented, few previous studies have examined drug self-administration experimentally. To experimentally examine this phenomenon, an animal model of depression was used. Few previous studies have utilized this method. Although not models of depression per se, previous paradigms employing various stressors have revealed that chronic or early neonatal stress may increase self-administration. For example, Lemaire, Deminiere, & Mormede (1994) found that chronic stress increased self-administration of intravenous amphetamine in rats. Researchers have also found enhanced acquisition of cocaine self-administration for rats that were separated from their mothers during postnatal days 2 to 9 (Kosten, Miserendino, & Kehoe, 2000) and Huot, Thrivikraman, Meaney, & Plotsky (2001) found higher intake of oral ethanol for rats that were separated during postnatal days 2 to 14 than rats that were not separated.

Researchers using the Fawn-Hooded rat, a potential animal model of depression, found high rates of ethanol intake and preferences for ethanol over 50% (Overstreet, Rezvani, & Janowky, 1992). There are no previous studies examining self-administration of amphetamine using a validated model of depression such as the olfactory bulbectomized rat model. The present studies found that olfactory bulbectomy increases self-administration of oral amphetamine solution. This observation supports the self-medication hypothesis.

The present studies intended to examine the neurochemical changes that might lead to the increased amphetamine intake of bulbectomized rats. Using *in vivo* microdialysis with electrochemical detection, levels of dopamine and its metabolites, DOPAC and HVA, were examined. Olfactory bulbectomized rats had significantly higher basal levels of dopamine than sham-operated rats. Researchers examining individual differences in locomotor activity have found that "high responding" or hyperactive rats both self-administer more amphetamine (Klebaur, Bevins, Segar, & Bardo, 2001; Piazza, Deminiere, Le Moal, & Simon, 1989; Piazza, Maccari, Deminiere, Le Moal, Mormede, & Simon, 1991) and have greater dopamine release in the striatum (Bradberry, Gruen, Berridge, & Roth, 1991; Hooks, Colvin, Juncos, & Justice, 1992; Piazza et al., 1991; Piazza, Rouge-Pont, Deminiere, Kharoubi, Le Moal, & Simon, 1991; Rouge-Pont, Piazza, Kharoubi, Le Moal, & Simon, 1993). These studies and Experiment 2 suggest that increased dopamine transmission may lead to increased drug taking behavior. Berridge & Robinson (1998) suggested that heightened dopamine transmission enhances the subjective "wanting" or incentive salience for drugs like cocaine and amphetamine. The present results thus provide further support for this theory using a

model that involves hyperdopaminergic activity. *In vivo* microdialysis of monoamines has not been examined in the olfactory bulbectomized rat previously. A replication of this study might strengthen the belief that this is a consistent effect.

In conclusion, the olfactory bulbectomized model of depression can be successfully used to experimentally examine the comorbidity of depression and drug abuse. Future studies will include replicating the microdialysis studies and examining the effects of antidepressant treatment on *d*-amphetamine intake.

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APPENDIX A: COMORBIDITY STUDIES

Reference:	Participants:	Results:
Ahmad, Mufti, & Farooq, 2001	50 patients admitted for drug treatment at Lady Reading Hospital, Peshawar in 1997	30% diagnosed with both opioid dependence and major depression.
Cargill, Emmons, Kahler, & Brown, 2001	350 hospitalized smokers at Miriam Hospital, RI	Depressed smokers had less confidence that they could quit smoking.
Corvin, O'Mahony, O'Regan, Comerford, O'Connell, Craddock, et al., 2001	92 patients with bipolar affective disorder in Republic of Ireland	57% of the patients were smokers, 21.7% abused alcohol, & 20.7% abused drugs.
Degenhardt, Hall, & Lynskey, 2001	10,641 adult Australians from Australian National Survey of Mental Health and Well-Being	Among those with cannabis dependence, 14% met criteria for an affective disorder, compared to 6% of non-users.
Gilman & Abraham, 2001	14,480 people from the U.S. Epidemiologic Catchment Area community survey	Increased odds of depression after 1-year follow-up associated with baseline alcohol abuse.
Roeloffs, Fink, Unutzer, Tang, & Wells, 2001	1,187 adults who reported symptoms of depression in managed primary care clinics in MD, MO, CA, TX, & CO	8.3% of women and 19% of men reported alcohol abuse and 26.3% of women and 29.4% of men reported drug abuse.
Roy, 2001	214 cocaine dependent patients admitted to a substance abuse treatment program at Department of Veterans Affairs, NJ	39.3% cocaine abusers had attempted suicide and reported significantly more major depression.
Dodge & Potocky, 2000	64 women in a large public alcohol treatment center in a metropolitan area of Florida	Sample had a mean depression score 65.5 on Costello-Comrey Depression Scale, indicating that they were clinically depressed.

McCuller, Sussman, Dent, & Teran, 2000	1,315 high school students	Depression predicted only hard drug use (stimulants, cocaine, hallucinogen, inhalant, PCP, depressants, heroin, & steroids) but not marijuana, alcohol, or smoking.
Merikangas & Avenevoli, 2000	1,626 first degree relatives of probands from Yale Family Study	Both social phobia and bipolar affective disorder preceded that of substance use disorder.
Schmitz, Stotts, Averill, Rothfleisch, Bailley, Sayre, & Grabowski, 2000	151 consecutive admissions to the Treatment Research Clinic (TRC) in Houston, TX	Depressed cocaine abusers had higher prevalence of antisocial personality disorder, higher craving for cocaine, lower self-efficacy to refrain from drug use, & lower perceived social support.
Abraham & Fava, 1999	375 outpatients with major depressive disorder in Depression Research Program at Massachusetts General Hospital	31% of patients with major depressive disorder had a comorbid substance use disorder. 14% also abused multiple substances.
Hanna & Grant, 1999	42,862 adult respondents from the 1992 National Longitudinal Alcohol Epidemiologic Survey (NLAES)	Early onset smokers had significantly more depressive episodes. Only late onset smokers had more treatment for depressive disorders than did lifetime nonsmokers (54.2% vs. 47.3%). Relative to lifetime nonsmokers, the odds of ever having been diagnosed with major depression were almost twice as great for respondents who began smoking before age 13.
Breslau, Peterson, Schulz, Chilcoat, & Andreski, 1998	1,200 people selected from all 21 – 30 year old members of a large health maintenance organization in southeastern Michigan	Incidence of first-onset major depression in daily smokers was 12.1% vs. 6.5% in persons who had ever smoked a cigarette but never smoked daily for 1 month or more up to the

		time of the baseline interview. History of major depression at baseline was associated with a 3-fold increase in the risk for progression to daily smoking.
Charney, Paraherakis, Negrete, & Gill, 1998	75 patients who sought treatment at the Montreal General Hospital (MGH) Addictions Unit	Lifetime comorbidity rates were 60.2% for mood disorders. Found that dually diagnosed patients had a decreased rate of remission, increased vulnerability to relapse, higher readmission rates, and a need for more inpatient and outpatient services. Women were more likely to be diagnosed with both primary & substance-induced depression than men & stayed in treatment longer.
Grant & Pickering, 1998	42,862 adult respondents from the 1992 National Longitudinal Alcohol Epidemiologic Survey (NLAES)	14% of cannabis abusers and 29% of cannabis dependents met criteria for major depression
Winokur, Turvey, Akiskal, Coryell, Solomon, Leon, et al., 1998	746 adults from the NIMH Collaborative Depression Study	Of the 277 bipolar patients, 66%were primary depressives. For the bipolar drug users median time to relapse after recovery was 47 days longer than the bipolar non-abusers. The use of amphetamines, cocaine and alcohol was more frequent for bipolars.

Darke & Ross, 1997	222 heroin injectors in Australia	67% of addicts met the criteria for a lifetime anxiety &/or depressive disorder. In the depressed addicts, the onset of depression preceded heroin dependence in 61% of the cases.
Schuckit, Tipp, Bucholz, Nurnberger, Hesselbrock, Crowe, et al., 1997	2713 alcoholics entering inpatient or aftercare treatment at 6 sites	43.9% lifetime diagnosis of an affective disorder.
Hasin, Tsai, Endicott, Mueller, Coryell, & Keller, 1996	127 psychiatric patients in the clinical studies portion of the NIMH Collaborative Depression Study (between 1978 and 1981) with both major depression and alcoholism	Improvement in depression status significantly improved remission in alcoholism.
Kessler, Nelson, McGonagle, Liu, Swartz, & Blazer, 1996	5,877 respondents of U.S. National Comorbidity Survey	28% of drug abusers and 39% of drug dependents met criteria for an affective disorder.
Kranzler, Del Boca, & Rounsaville, 1996	225 alcoholics (74% male)	38.3% lifetime diagnosis of depression.
Mason, Kocsis, Ritvo, & Cutler, 1996	71 alcoholics	39.4% met criteria for depression.
Miller, Klamen, Hoffmann, & Flaherty, 1996	6,355 patients at addiction treatment programs at 41 sites	43.7% met criteria for lifetime major depression.
Patton, Hibbert, Rosier, Carlin, Caust, & Bowes, 1996	2,525 adolescents in Victoria, Australia	38% of male & 59% of female regular smokers fell into the high psychiatric morbidity group. Teens with psychiatric symptoms had a two time greater risk for regular smoking.
Sellman & Joyce, 1996	93 alcohol dependent men	25.8% lifetime diagnosis of depression.

Grant, 1995	42,862 respondents of the National Institute on Alcohol Abuse and Alcoholism's National Longitudinal Alcohol Epidemiologic Survey	Respondents with major depression had 6.4 times the odds of abusing cannabis as those without depression.
Grant, & Harford, 1995	42,862 respondents of the National Institute on Alcohol Abuse and Alcoholism's National Longitudinal Alcohol Epidemiologic Survey	21.36% respondents with an episode of major depression in the past year were also classified with an alcohol dependence and / or abuse diagnosis. The increased risk of having alcohol dependence among those with major depression was 4 times greater than those without major depression.
Penick, Powell, Nickel, Bingham, Riesenmy, Read, & Campbell, 1994	928 male patients being treated for alcoholism in 6 veterans administration medical centers	36.4% lifetime diagnosis of major depression and of the 159 alcoholic patients that also abused drugs, 44% had a comorbid diagnosis of depression.
Kendler, Neale, MacLean, Heath, Eaves, & Kessler, 1993	727 complete pairs & 112 unpaired white female same-sex twin pairs from Virginia Twin Registry	Among the 263 individuals with recorded ages of onset for both smoking and major depression, the onset of smoking preceded that of major depression in 61.6%, major depression preceded smoking in 29.3%, and they both began in the same year in 9.1%.
Ingraham & Wender, 1992	48 female and 23 male adoptees born in Denmark between 1924 & 1947 and who had been placed in the homes of non-relatives	Significantly greater incidence of affective disorders and substance abuse seen in the biological relatives of the index cases. An increased frequency of substance abuse in the biological relatives of adoptees with a diagnosis of an affective disorder.

Weiss, Griffin, & Mirrin, 1992	494 hospitalized drug abusers	10% had major depression and reported using drugs in response to feeling depressed.
Rounsaville, Anton, Carroll, Budde, Prusoff, & Gawin, 1991	149 patients seeking treatment for cocaine abuse at an outpatient cocaine abuse clinic and 149 in an inpatient drug abuse unit within a psychiatric hospital setting in southern Connecticut	The rate of current major depression was 4.7% and 60.7% for lifetime affective disorders in cocaine abusers.
Roy, DeJong, Lamparski, George, & Linnoila, 1991	339 alcoholics	33% had history of major depression. Alcoholics with histories of major depression had earlier onsets of problem drinking and higher daily alcohol intake than non-depressed alcoholics.
Ziedonis & Kosten, 1991	94 patients being treated in the APT Methadone Maintenance Program of Yale University	Depressed patients had poorer retention, stronger depressive symptoms, and far fewer instances of 2 weeks of cocaine-free urine (0% vs. 32%).
Anda, Williamson, Escobedo, Mast, Giovino, & Remington, 1990	2963 respondents (39% current smokers)	The % of current smokers increased significantly as the Depression Scale scores increased. Depressed respondents had the lowest incidence of quitting of any subgroup.
Glassman, Helzer, Covey, Cottler, Stetner, Tipp, et al., 1990	3213 respondents of the Epidemiologic Catchment Area (ECA) Program in St. Louis (1980 & 1983)	The lifetime prevalence of major depressive disorder was 5.1% of sample. Depressed subjects were more likely to have smoked, and were less likely to stop smoking.

Hirschfeld, Hasin, Keller, Endicott, & Wunder, 1990	298 patients with primary major depression recruited when they sought treatment for alcoholism through the inpatient and outpatient psychiatric units of NIMH Collaborative Program on the Psychobiology of Depression	Recovery and relapse rates for major depression were unaffected by concurrent alcoholism.
Regier, Farmer, Rae, Locke, Keith, Judd, et al., 1990	20,291 people from the Epidemiologic Catchment Area (ECA) study of the NIMH	Some form of substance dependence or abuse was found in 32.0% of individuals with at least one affective disorder diagnosis.
Grant, Hasin, & Harford, 1989	120 hospitalized alcoholics (70% male)	67% had lifetime diagnosis of major depression.
Christie, Burke, Regier, Rae, Boyd, & Locke, 1988	4,778 young adult respondents of the NIMH Epidemiologic Catchment Area Program	6.2% had both substance use disorder & depressive or anxiety disorders. Overall association between having had a major depressive episode & having had a substance use disorder (odds ratio of 2.7).
Penick, Powell, Liskow, Jackson, & Nickel,1988	273 male inpatients at alcohol rehabilitation center	25.7% diagnosed with major depression.
Ross, Glaser, & Stiasny, 1988	501 males and females seeking assistance for alcohol – drug problems	34.3% had lifetime diagnosis for an affective disorder.
Deykin, Levy, & Wells, 1987	424 college students aged 16 to 19 attending 2 Boston area colleges	Subjects who reported a history of alcohol abuse were 4 times more likely to have a history of major depressive disorder. Drug abusers were 3.3 times more likely than non-abusers to have depression.
Willenbring, 1986	52 male alcoholic inpatients	34% had lifetime history of major depression.
McLellan, Woody, & O'Brien, 1979	51 men who were admitted to the Coatesville Veterns Administration Hospital for inpatient drug-abuse treatment	Men who abused psychodepressants had more symptoms of depression, including 5 of 14 patients who had attempted suicide

Weissman, Slobetz, Prusoff, Mezritz, & Howard,1976	106 consecutive 1st admissions of a Methadone Maintenance Clinic & 44 patients who had been in maintenance treatment for an average of 1 year	64% reported a history of mental illness in their parents or siblings, usually depression in the mother (14%) or alcoholism in the father (12%). 54% themselves had psychiatric treatment in the past & 24% reported that they had received treatment before their addiction problem. According to the Raskin Depression Scale criteria, 32% of the patients were clinically depressed.

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APPENDIX B: LITERATURE REVIEW

The following is a brief review of the syndrome produced by bilateral removal of the olfactory bulbs of the rodent. According to McKinney & Bunney (1969) the minimum requirements of a good animal model of depression are that the model be based on observable behavioral changes that can be objectively evaluated, the symptoms of syndrome be reasonably analogous to those seen in human depression, that treatments used for humans work in the same manner to reverse the syndrome seen in the animal, and that the model be reproducible by other investigators. The olfactory bulbectomized rat (OBX) model of depression meets these criteria. The syndrome consists of a constellation of behavioral, physiological, and neurochemical changes. Many of these changes are similar to those seen in clinically depressed humans. The syndrome produced is independent of anosmia (Alberts, 1974; Sieck & Baumbach, 1974). And, many of the changes seen can be reversed by chronic antidepressant treatment in a similar manner as depressed humans (Cairncross, Cox, Forster, & Wren, 1979; Kelly, Wrynn, & Leonard, 1997; Mudunkotuwa & Horton, 1996; Song, Earley, & Leonard, 1996; van Riezen & Leonard, 1990; van Riezen, Schnieden, & Wren, 1977). According to Hanin & Usdin (1977) animal models of psychiatric disease should simulate the human syndrome, show symptoms of the disease, and respond to treatment in the same manner as humans. This is a great advantage the OBX model has over other animal models of depression.

Behavioral Changes

Humans are diagnosed with a major depressive disorder when they show behavioral changes such as depressed mood, anhedonia (loss of interest or pleasure), weight changes, sleep pattern changes, psychomotor agitation, suicidal thoughts, inappropriate guilt, and cognitive deficits (American Psychiatric Association, DSM-IV). Some of these symptoms are uniquely human and therefore are not appropriate or possible to study using an animal model. But, changes in eating and sleeping patterns, psychomotor agitation, cognitive deficits, and anhedonic behavior have been examined using the OBX model.

Olfactory bulbectomy produces changes in feeding patterns. Several researchers have found that OBX reduced the amount of food consumed but increased the frequency of eating (Larue, 1975; Larue & Le Magnen, 1970). Meguid, Gleason, & Yang, 1993 though found no differences in the amount of food consumed by OBX rats compared to sham-operated rats but did find that OBX rats ate smaller meals more frequently. Some researchers have also found differences in weight gained post-surgery between OBX and sham-operated rats (Jesberger & Richardson, 1986; Thorne & Rowles, 1988).

Researchers have also found differences in sleep patterns between OBX and sham-operated rats. Giardina & Radek (1991) found OBX rats to be more active during the early and late hours of their dark phase than sham-operated rats. This abnormal behavior was then reversed by 7 daily morning injections of the antidepressant, imipramine (Giardina et al., 1991). Lengthened active periods after OBX have also been found in mice and golden hamsters (Pieper & Lobocki, 1991; Possidente, Lumia,

McGinnis, Teicher, de Lemos, Sterner, et al., 1990). A decrease in REM sleep has also been seen in the OBX rat (Sakurada, Shima, Tadano, Sakurada, & Kisara, 1976).

One of the most frequently seen behavior changes in OBX rats is hyperlocomotion or hyperreactivity in a novel open field. OBX rats consistently show higher ambulation rates in an open field compared to sham-operated rats (Earley, Glennon, Lally, Leonard, & Junien, 1994; Ho, Chang, Liu, Tai, Wong, & Tsai, 2000; Mar, Spreekmeester, & Rochford, 2000; Mudunkotuwa et al., 1996; O'Connor & Leonard, 1988; Primeaux & Holmes, 1999; Song et al., 1997; Stock, Hand, Ford, & Wilson, 2001; Thorne et al., 1988; Tiffany, Mollenauer, Plotnik, & White, 1979; van Riezen et al., 1977). This behavior change in OBX rats has also been found to be consistently reversed by chronic antidepressant treatment (Earley et al.; Mar et al.; Mudunkotuwa et al.; O'Connor et al.; Song et al.; van Riezen et al.). Some researchers believe that this increase in locomotion is because OBX rats are more anxious than normal rats. One way to access anxiety in rats is to use an elevated plus maze (Hogg, 1996; Kulkarni & Sharma, 1991; Wall & Messier, 2001). Researchers have found that OBX rats spend more time in the open arms than closed arms compared to sham-operated rats (Song et al., 1996; Song & Leonard, 1994; Stock et al., 2001). Other researchers suggest that OBX rats are actually more susceptible to stress. McNish & Davis (1997) found that OBX rats showed sensitization to shock intensities that did not produce sensitization in sham-operated and unoperated control rats. Other researchers have also found that OBX rats have an exaggerated startle response (Jesberger et al., 1986; van Riezen et al.). Though, tailflick tests reveal that there are no differences in pain sensitivity between OBX and control rats (Holmes, Koprovica, & Crawley, 1994).

Another behavioral similarity between OBX rats and depressed humans is a deficit in learning. Several researchers have found that OBX rats require significantly more trials to reach criterion in passive avoidance tests than sham-operated controls (Cairncross et al., 1979; Jesberger et al., 1986; Noreika, Pastor, & Liebman, 1981; Primeaux et al., 1999; Thorne et al., 1988; van Riezen et al., 1977). Yamamoto, Jin, & Watanabe (1997) found that OBX rats made more reference and working memory errors in a runway maze and showed impairments in reversal learning compared to sham-operated rats. Hall & Macrides (1983) found that OBX rats made more errors in a radial arm maze than controls 9 days after surgery. The cognitive deficits found after OBX can also be reversed by chronic antidepressant treatment (Cairncross et al.; Greckesh, Zhou, Franke, Schroder, Sabel, Becker et al.,1997; Jesberger et al.; Noreika et al.; van Riezen et al.).

Clinically depressed humans typically show anhedonia or lack of interest in activities that they previously found pleasurable (*DSM-IV*). OBX rats also show some anhedonic-like behaviors like decreases in sex and sucrose. Bulbectomy has been found to produce impairments in male sexual behavior in rats and mice (Cain & Paxinos, 1974; Edwards, 1974; Heimer & Larsson, 1967; Larsson, 1975). Stock, Ford, & Wilson (2000) found that OBX rats have significantly lower sucrose preference levels than sham-operated animals.

Anosmia

The changes seen following bilateral OBX are independent of anosmia. Alberts (1974) found that olfactory bulb removal produced behavioral and physiological effects that were not related to the sensory deficit produced. These changes were not seen after

producing anosmia by lateral olfactory tract lesions, destruction of the olfactory nerve, removal of the neuroepithelium, intranasal zinc sulfate treatment, anethesia with tracheostomy (Alberts). Sieck et al. (1974) found that activity, rearing, and sniffing frequency in rats was increased after bilateral OBX independent of olfactory cue changes. Using a mechanical robot as a novel stimulus, researchers found that OBX caused a reduction of defensive reactions that was not seen in anosmic rats (Tiffany et al., 1979).

Anatomy of the Olfactory Bulb

The olfactory bulbs represent approximately 4% of the entire brain volume of the rat (Cain, 1974). The main olfactory bulbs (MOB) receives primary sensory input from olfactory sensory neurons (Lancet, 1986) and noradrenergic input from the locus coeruleus (Macrides & Davis, 1983). The MOB projects through the lateral olfactory tracts to the piriform cortex, amygdala, olfactory tubercles, and ventrolateral entorhinal area (Baumbach & Sieck, 1977; Heimer, 1968; Kosel, van Hoesen, & West, 1981). The MOB also has polysynaptic connections with the dorsomedial thalamic nucleus, ventromedial thalamic nucleus, lateral habenular nucleus, lateral hypothalamus, and lateral preoptic areas (Price & Powell, 1970).

Morphological Changes

The behavioral changes that make up the OBX syndrome are not immediate. It takes approximately 8 to 14 days for the syndrome to fully emerge and then it only persists for about 30 days (Baumbach et al., 1977; Broekkamp, O'Connor, Tonnaer, Rijk, & van Delft, 1986). This may be because of the degeneration that occurs when the olfactory bulbs are removed. Lesions always result in more than simple destruction of a discrete area in the brain (Brunjes, 1992). Several morphological changes are observed

following OBX. White & Westrum (1964) found that olfactory bulb lesions in the rat resulted in reductions in dendritic branching 4 to 8 days post-surgery in the piriform cortex. Carlsen, De Olmos, & Heimer (1982) also found degeneration in regions outside the olfactory projection area 3 to 4 days following lesions of the olfactory bulbs. Cell shrinkage in the nucleus of the horizontal limb of the diagonal band has been observed in OBX rats (Price, 1969; Price et al., 1970). Also after OBX, sprouting has been observed following the loss of neural input in the olfactory tubercle (Moore, Bjorklund, & Stenevi, 1974) and olfactory cortex, (Devor, 1975).

Physiological Changes

There are physiological changes seen after OBX that are similar to changes seen in depressed humans. Severely depressed patients show increased adrenocortical activity and disturbances in the feedback of ACTH (Carroll & Davies, 1970; Carroll, Martin, & Davies, 1968). Pfohl, Sherman, Schlechte, & Winokur (1985) found that depressed patients had increased ACTH concentrations when they sampled their blood every 20 minutes over a 24 hour period. Researchers have found that even in stress-free conditions, OBX rat plasma concentrations of corticosterone are high (Carincross, Wren, Cox, & Schnieden, 1977; Loyber, Perassi, & Lecnona, 1976; Marcilhac, Faudon, Anglade, Hery, & Siaud, 1999). Similar immunological responses are seen in depressed humans and OBX rats. O'Neill & Leonard (1990) found that neutrophil phagocytosis is suppressed in depressed patients. Likewise, phagocytic response of neutrophils was found to be significantly reduced following OBX (Song et al., 1994; Song & Leonard, 1997). The antidepressant drug desipramine was able to reverse this phagocytic response in the OBX rat (Song et al., 1997).

Neurotransmitter Changes

Bilateral OBX produces changes in neurotransmitters and many of these differences have also been observed in humans with major depressive disorder. Several hypotheses on the etiology of depression suggest that deficits in catecholamines lead to clinical depression (Bunney & Davis, 1965; Schildkraut, 1965; Willner, 1983). Concentrations of norepinephrine have been found to be significantly reduced in the frontal cortex (Redmond, Kelly, & Leonard, 1997), hypothalamus and amygdala (Song et al., 1997) in OBX rats. Following OBX, serotonin levels are reduced in prefrontal cortex (Gurevich, Aleksandrova, Otmakhova, Katkov, Nesterova, & Bobkova, 1993) and amygdala (Jancsar & Leonard, 1981). Decreased DA transmission has been found in the striatum of OBX rats (Gottesfeld, Garcia, Lingham, & Chronister, 1989; Lingham & Gottesfeld, 1986; Lumia, Teicher, Salchli, Ayers, & Possidente, 1992). Dopamine has been shown to suppress prepro-enkephalin (ENK) gene expression in striatal neurons and therefore is commonly used as a marker of DA depletion or dysfunction (Kowalski & Giraud, 1993. Dopamine deafferentation produced by 6-OHDA or electrolytic lesions lead to elevated prepro-ENK mRNA in the striatum (Gerfen, McGinty, & Young, 1991; Nisenbaum, Kitai, & Gerfen, 1994; Normand, Popovivi, Onteniente, Fellman, Piatier-Tonneau, Auffray, et al., 1988). Researchers have found that OBX increases prepro-ENK gene expression in the striatum (Holmes, 1999; Primeaux & Holmes, 2000). Changes in other neurotransmitters are also found following OBX. Using quantitative receptor autoradiography, Webster, Flores, Marcotte, Cecyre, Quirion, & Srivastava (2000) observed significantly more NMDA receptors in the medial prefrontal cortex 1 to 5 weeks after OBX surgery. Differences in NMDA in the frontal cortex of suicide

victims have also been observed (Nowak, Ordway, & Paul, 1995). Higher GABA levels in the amygdala are found in OBX compared to sham-operated animals (Janscar et al.). Lower levels of acetylcholine are found after OBX in the amygdala (Yoshimura, 1981) and cerebral cortex (Yoshimura, Gomita, & Ueki, 1974). Using *in situ* hybridization, Holmes et al. (1998) found significantly higher levels of prepro-NPY mRNA in the piriform cortex and dentate gyrus 14 and 28 days after OBX compared to sham-operated and surgically naïve rats. Holmes & Crawley (1996) found increased prepro-galanin gene expression levels in locus coeruleus 3 and 14 days after OBX.

Conclusion

The olfactory bulbectomy model is a well-validated animal model of depression. It shows physiological, neurochemical and behavioral changes that are similar to those seen in humans with major depressive disorder. It also reacts to antidepressant treatment in a similar way as humans do. Most of the behavioral and neurochemical changes are seen after chronic but not acute antidepressant treatment. This response makes the OBX model a very good tool for researchers to use to test new therapeutics.

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