ELSEVIER

Pharmacology, Biochemistry and Behavior 67 (2000) 331–337

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

# Olfactory bulbectomy increases met-enkephalin- and neuropeptide-Y-like immunoreactivity in rat limbic structures

Stefany DePrato Primeaux, Philip V. Holmes*

*Biopsychology Program, Psychology Department, The University of Georgia, Athens, GA 30602, USA*

Received 4 February 2000; received in revised form 22 May 2000; accepted 1 June 2000

**Abstract**

Bilateral olfactory bulbectomy (OBX) in rats produces a well-characterized syndrome of behavioral, physiological, and neurochemical changes identical to those seen in depression. Previous experiments using in situ hybridization histochemistry have demonstrated that OBX increases prepro-neuropeptide-Y (NPY) and prepro-enkephalin (ENK) mRNA levels in limbic structures. The present experiments determined whether increases in peptide immunoreactivity occur in conjunction with increases in mRNA levels following OBX. In situ hybridization analyses in olfactory bulbectomized and sham-operated rats revealed increased prepro-ENK mRNA in the piriform cortex (PIR) and olfactory tubercles (OTs) of bulbectomized rats. Prepro-NPY mRNA levels were significantly increased in the PIR of bulbectomized rats as comapred to controls. Radioimmunoassays (RIAs) revealed significant elevations in ENK-like immunoreactivity in the OTs following OBX. NPY-like immunoreactivity was significantly elevated in the PIR following OBX. These data reveal that OBX-induced increases in ENK-like immunoreactivity occur concomitantly with increases in prepro-ENK mRNA, and NPY-like immunoreactivity occur concomitantly with increases in prepro-NPY mRNA. 

*Keywords:* Olfactory bulbectomy; Enkephalin; Neuropeptide-Y; Radioimmunoassay; In situ hybridization; Depression

Bilateral ablation of the rat olfactory bulbs (OBX) produces a well-established behavioral syndrome typically characterized by increased locomotor reactivity in a novel open field [2,18–21,30,36,42,43], deficits in learning [4,29,47,48,53], deficits in aversively motivated behaviors [17,29,33,36,37,40,44,49], and deficits in appetitively motivated behaviors [5,19,24,27,28,40,43]. The role of anosmia in the OBX syndrome has been well studied, and the most widely reported behavioral effects of OBX cannot be attributed to sensory deficits alone [5,41,49,51,52].

OBX leads to changes in various classical neurotransmitters by disrupting neural circuits in the brain. Neurochemical effects of OBX include overall decreases in norepinephrine (NE) [13,14,44,51], serotonin (5-HT) [15,44] and dopamine (DA) [13,14,26]. Increases in the number of $\beta$-adrenoceptors and $\alpha_2$-adrenoceptors [16,50], 5-$HT_2$ receptors [12], and $D_2$ receptors [25] are found in various brain regions following OBX. These neurochemical alterations resemble those seen in depression and are attenuated by antidepressant drug treatment [15,16,26,44,51].

Since neuropeptides are often colocalized with classical neurotransmitters, one would expect changes in neuropeptides to occur in depression and models thereof. The neuropeptides enkephalin (ENK) and neuropeptide-Y (NPY) are of particular interest to this experiment. ENK, an endogenous opioid peptide, is abundant in the striatum of rats and has been found to be inversely related to DA levels in the striatum; DA depletion leads to increased ENK levels [6,7,24,31]. NPY, a 36 amino acid peptide, is one of the most abundant peptides in the central nervous system and is colocalized with NE, 5-HT, and gamma-aminobutyric acid (GABA) [1,3,8,38]. Alterations in these neuropeptide levels have been found in both bulbectomized rats and clinically depressed patients [8,10,11,32]. Reduced ENK and NPY levels have been found in the cerebrospinal fluid of clinically depressed patients [8,32]. Prepro-ENK mRNA levels are increased in the olfactory tubercle (OT) of bulbectomized rats, and

* Corresponding author. Tel.: +1-706-542-2174; fax: +1-706-542-3275.

*E-mail address*: pvholmes@arches.uga.edu (P.V. Holmes).

0091-3057/00/$ – see front matter 
PII: S0091-3057(00)00358-0

prepro-NPY mRNA levels are increased in the piriform cortex (PIR) and dentate gyrus of bulbectomized rats [10,11].

The following experiment examined changes in ENK and NPY mRNA levels and immunoreactivity in the OT and PIR of bulbectomized and sham-operated rats. Quantitative in situ hybridization was used to replicate previous findings that OBX increases prepro-ENK and prepro-NPY mRNA in the OT and PIR, respectively. ENK-like immunoreactivity and NPY-like immunoreactivity in the OT and PIR were measured by radioimmunoassays (RIAs) in order to determine whether peptide levels increase concomitantly with increase in mRNA. A correlational analysis was conducted to determine whether OBX-induced changes in peptide gene expression are associated with deficits in footshock-induced immobility, which serves as a reliable behavioral marker of the OBX syndrome [36].

## 1. Methods

### *1.1. Subjects*

Twenty-one male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 240–300 g at time of surgery were used in this study. Rats were group housed with three to five rats per cage under a 12-h light/dark cycle (lights on at 07:00 hours). All rats had free access to food and water. For behavioral testing, rats were individually transported to a testing room dimly lit with red lights in which a white noise generator provided low-level masking noise. All behavioral tests were conducted between 09:00 and 14:00 hours.

### *1.2. Surgical procedure*

Olfactory bulbectomies were performed as previously described [36]. Surgeries began 7 days following arrival to the University of Georgia animal facilities. Following anesthetization with sodium pentobarbital (Nembutal, 25 mg/kg, i.p., Abbott Laboratories, N. Chicago, IL) and ketamine hydrochloride (Ketaset, 40 mg/kg, i.p., Fort Dodge, IA), the scalp was shaved and a midline incision was made. Two 3-mm-diameter burr holes were drilled 6 mm rostral of bregma and 1 mm to the right and left of the midline. The olfactory bulb was aspirated in the bulbectomized rats with a 2-mm-diameter plastic pipette tip, and the cavity was filled with gel–foam (Upjohn) to control bleeding. Special care was taken to avoid damaging the frontal cortex. Sham rats were treated identically, however the olfactory bulbs were not removed. All animals were treated in accordance with the University of Georgia Animal Use and Care Committee and followed guidelines described in the National Research Council Guide for the Care and Use of Laboratory Animals.

### *1.3. Foot-shock induced immobility*

Fourteen days following surgery, OBX rats ($n=14$) and sham-operated rats ($n=7$) were tested in a footshock induced immobility paradigm. The footshock apparatus is a Lafayette Shuttlebox apparatus (Lafayette, IN; model 85102), which consists of a chamber measuring $60\times21\times19$ cm. The chamber is illluminated by two 6-W bulbs encased in the lid of the chamber. A one-way observation window allowed constant viewing of the subject during testing. The experimenter recording behavior was unaware of the rat's surgical condition. Rats were placed into the apparatus for a 3-min habituation period prior to footshock, and the number of crossings of the midline of the chamber and the total duration of immobility were measured. Following this period, rats were given three 2-s, 0.3-mA scrambled foot shocks at 20-s intervals. The current was delivered by a Lafayette Instruments shock generator (model 82404/5-SS). Following the three footshocks, rats were observed for 10 min and the number of crossings and total duration of immobility were measured. The chamber was cleaned with a 10% chlorine bleach solution between each subject to remove olfactory cues.

Three days following behavioral testing, rats were killed by rapid decapitation and lesions were confirmed. The extent of damage to the frontal lobes was also assessed. The criteria for a complete lesion to the olfactory bulb was a tissue weight of less than 30% of the tissue weight of an intact olfactory bulb. The data from one bulbectomized rat was removed from the study due to an incomplete lesion. All behavioral data were analyzed by a two-tail between-subjects $t$ test. Levene's test for equality of variances was conducted for each $t$ test. If the variances were heterogeneous ($p<10$), then the Welch correction for heterogeneity of variance was used to determine the exact $p$ value of the test. The significance level for all behavioral measures was set at $p<0.05$.

### *1.4. Quantitative in situ hybridization histochemistry*

Following rapid decapitation, rat brains from each experiment were removed, frozen on dry ice, and stored at $-80°C$. Twelve-micrometer sections of the OT and PIR were sliced using a Microm cryostat and sections were mounted on gelatin/chromium potassium sulfate-coated microscope slides. Approximately every fifth section was stained with 0.1% thionin in order to visualize anatomical landmarks. The brain regions analyzed in this study, the OT and PIR, correspond to plates 15 (bregma +0.70) and 31 (bregma −3.30), respectively, in the rat brain atlas of Paxinos and Watson [34]. For in situ hybridization, sections were fixed in 4% formaldehyde in 0.12 M sodium phosphate-buffered saline (PBS, pH 7.4) for 5 min, rinsed three times with 1× PBS, and placed in 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% NaCl, pH 8.0,

for 10 min. Sections were then dehydrated in 70%, 80%, 90%, and 100% ethanol washes, dilapidated in chloroform for 5 min, rinsed in 100% and 90% ethanol, and air dried. Oligodeoxynucleotide probes were labeled at the 3′ end using terminal deoxynucleotidyl transferase (15 units/μl; GIBCO-BRL), $^{35}$S-dATP (1000–1500 mCi/mmol; New England Nuclear, Boston, MA), and tailing buffer. The ENK probe is complementary to bases 388–435 of rat prepro-ENK mRNA (5′-ATCTGCATCCTTCTTCATGAAACCGCCATACCTCTTGGCAAGGATCTC-3′) [54]. The NPY probe is complementary to bases 3146–3194 of rat prepro-NPY mRNA (5′-ATGAGATGTGGGGGGAAACTAGGAAAAGTCAGGAGAGCAAGTTTCATT-3′) [23]. Unincorporated nucleotides were separated from DNA probes using Stratagene Nuctrap chromatographic columns (La Jolla, CA). Sections were hybridized with $0.25-0.50\times10^6$ cpm of radiolabeled probes in buffer containing 50% formamide, 600 mM NaCl, 80 mM Tris–HCl, 4 mM EDTA, 0.1% sodium pyrophosphate, 0.2% SDS, 0.2 mg/ml heparin sulfate, and 10% dextran sulfate and incubated for 20–24 h in a humid chamber (VWR 18101R, Shel-Lab, Sheldon Mfg, Cornelius, OR) at 37°C. Sections then received several washes of $1\times$SSC and $2\times$SSC/50% formamide. All slides were then exposed to autoradiographic films (BioMax MR, Eastman Kodak) for 2 to 4 weeks.

Quantification of autoradiographic films was performed using a computerized image analysis system (Image 1.38 software, W. Rasband, NIMH; Power Macintosh 8100 computer; light box and camera, Imaging Research, St. Catharines, Ontario; video interface, Data Translation, Marlboro, MA). Brain regions were analyzed by tracing the area to be measured with the aid of a rat brain atlas [34] and measuring transmittance values (optical gray scale) within that area of the PIR. A density slice threshold procedure was used to isolate the area of the OT to be analyzed. Levels of prepro-ENK were measured in both the PIR and the OT of experimental and control rats, and levels of prepro-NPY were measured in the PIR of experimental and control rats. Data were recorded and analyzed with a one-tail between-subjects *t* test. Levene's test for equality of variances was conducted for each *t* test. If the variances were heterogeneous ($p<0.10$), then the Welch correction for heterogeneity of variance was used to determine the exact *p* value of the test. Correlational analyses were conducted to test the association between OBX-induced prepro-ENK and prepro-NPY plasticity, and the association between neurochemical measures and behavior. A significance level of $p<0.05$ was set for all tests.

### *1.5. RIA*

After approximately thirty 12-μm sections for each region were mounted on slides, the remaining PIR and OT were dissected from each brain and stored at −80°C. Unilateral sections of dissected tissue were weighed and placed in $12\times75$ glass test tubes. The tissues were then homogenized (PowerGen 125, Fisher Scientific) for 15 s following the addition of 250 μl of 0.5 M acetic acid with 2.5% aprotinin. The glass test tubes were placed in boiling water for 10 min and then centrifuged at 3000 rpm ($1500\times g$) at 4°C (Beckman Model TJ-6 Centrifuge and refrigeration unit) for 30 min. Following centrifugation, the supernatant was aspirated off and placed in $12\times75$ plastic test tubes. The remaining pellet was discarded. The plastic test tubes containing the supernatant were placed under vacuum (20,000 mm Hg) in a Labconco Centrivap concentrator at 60°C until all liquid was evaporated (approximately 3 h) and then stored at −20°C. RIA kits (Peninsula Laboratories, Belmont, CA) were used to asses the levels of and Met-ENK and NPY in bulbectomized and sham-operated rats. The kits contained all the reagents needed for the RIA. Each test tube containing extracted peptide from the experimental/control tissue was reconstituted with 250 μl of RIA buffer. Tubes for determining a standard curve, non-specific binding, total binding and total counts were included in each assay. On day 1 of the RIA procedure, 100 μl of the primary antibody, rabbit anti-peptide serum, was added to 100 μl of the reconstituted peptide from the experimental/control tissue. On day 2, 100 μl of $^{125}$I-peptide ($1.0-1.5\times10^4$ cpm per tube) was added to each test tube. On day 3, 100 μl of goat anti-rabbit IgG serum and 100 μl of normal rabbit serum were added to every test tube. Following a 90-min incubation period, all the tubes were centrifuged (Beckman Model TJ-6 Centrifuge and refrigeration unit) at 3000 rpm (approximately $1500\times g$) for 20 min at 4°C. The supernatant was then carefully aspirated off. The remaining pellets were placed in scintillation vials with 3 ml of Scintiverse, and levels of $^{125}$I were counted in a scintillation counter (Beckman Scintillation System LS6500).

A standard curve was generated for each peptide according to protocols described by the manufacturer. Picograms of peptide were determined from the standard curve and divided by the wet tissue weight of each experimental sample. Data were recorded and analyzed with a two-tail between-subjects *t* test. Levene's test for equality of variances was conducted for each *t* test. If the variances were heterogeneous ($p<0.10$), then the Welch correction for heterogeneity of variance was used to determine the exact p value of the test.

## 2. Results

### *2.1. Footshock-induced immobility test*

There was no difference between OBX and sham-operated rats in the number of crossings made during habituation, $t(18)=-1.963$, $p<0.05$. Following footshock, OBX rats spent significantly less time immobile than sham-

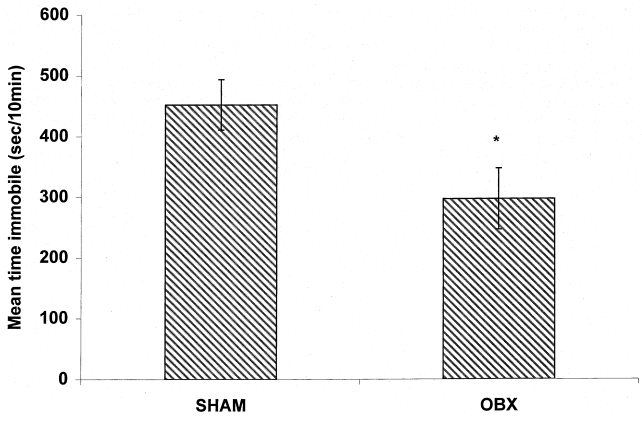


Fig. 1. The footshock-induced immobility test measured time spent immobile (s) for 10 min following three 0.3-mA scrambled footshocks, OBX ($n=13$) and SHAM ($n=7$). Means±SEM are represented.

operated rats, $t(18)=2.380$, $p<0.05$ (see Fig. 1), and made more crossings than sham-operated rats, $t(18)=-2.545$, $p<0.05$. The variances for each of these measures were found to be heterogeneous by Levene's test for equality of variances ($p<0.10$), and therefore were given a corrected $t$ statistic and $p$ value by the Welch correction for heterogeneity of variance.

### 2.2. Quantitative in situ hybridization

Bilateral OBX produced a 47.5% increase of prepro-ENK mRNA in the PIR (mean optical gray scale±SEM; OBX: 44.4304±4.3228; SHAM: 30.1193±2.0895; $t(18)=-2.981$, $p<0.01$) and a 2.6% increase in the OT (mean integrated density scale±SEM; OBX: 145.0475±1.1419; SHAM: 141.0475±1.5438; $t(18)=-1.908$, $p<0.05$). Bilateral OBX produced a 27.9% increase of prepro-NPY mRNA in the PIR (mean optical gray scale±SEM; OBX: 39.0069±2.7223; SHAM: 30.5107±0.5138; $t(18)=-3.067$, $p<0.01$). Representative autoradiographs of prepro-ENK and prepro-NPY hybridization are depicted in Figs. 2–4.

### 2.3. RIA

Bilateral OBX significantly increased Met-ENK-like immunoreactivity in the OT, $t(17)=-2.069$, $p<0.05$ (see Fig. 5). Bilateral OBX significantly increased the levels of NPY-like immunoreactivity in the PIR, $t(18)=-2.096$, $p<0.05$ (see Fig. 6). The variances for data from the PIR were found to be heterogeneous by Levene's test for equality of variances ($p<0.10$), and therefore were given a corrected $t$ statistic and $p$ value by the Welch correction for heterogeneity of variance.

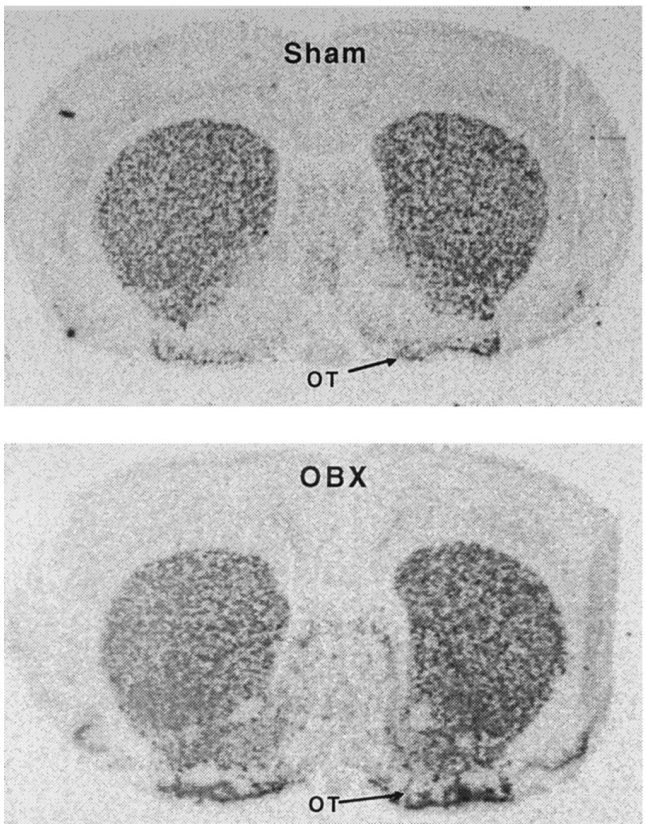


Fig. 2. Computer-generated representative autoradiographs depicting prepro-ENK mRNA hybridization in the OT of OBX and SHAM rats.

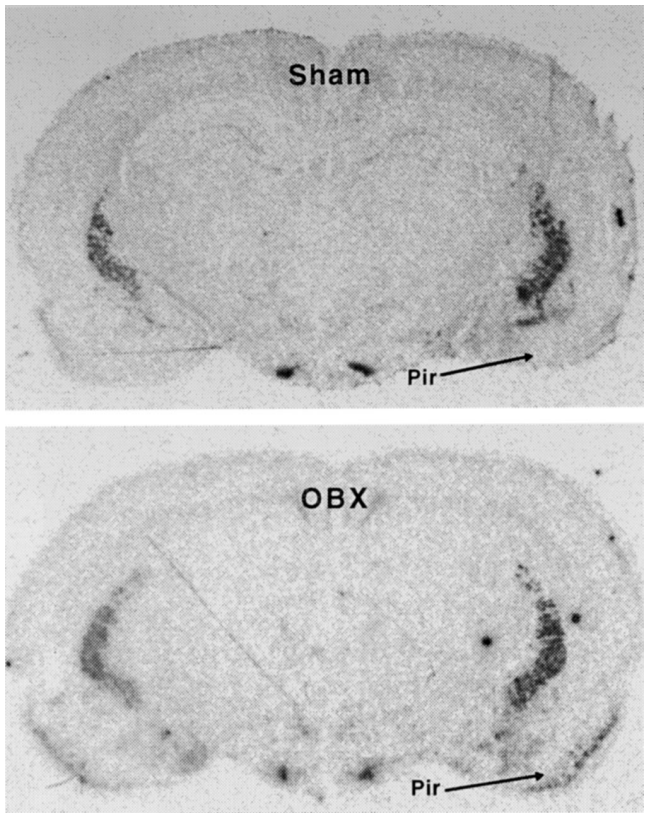


Fig. 3. Computer-generated representative autoradiographs depicting prepro-ENK mRNA hybridization in the PIR of OBX and SHAM rats.

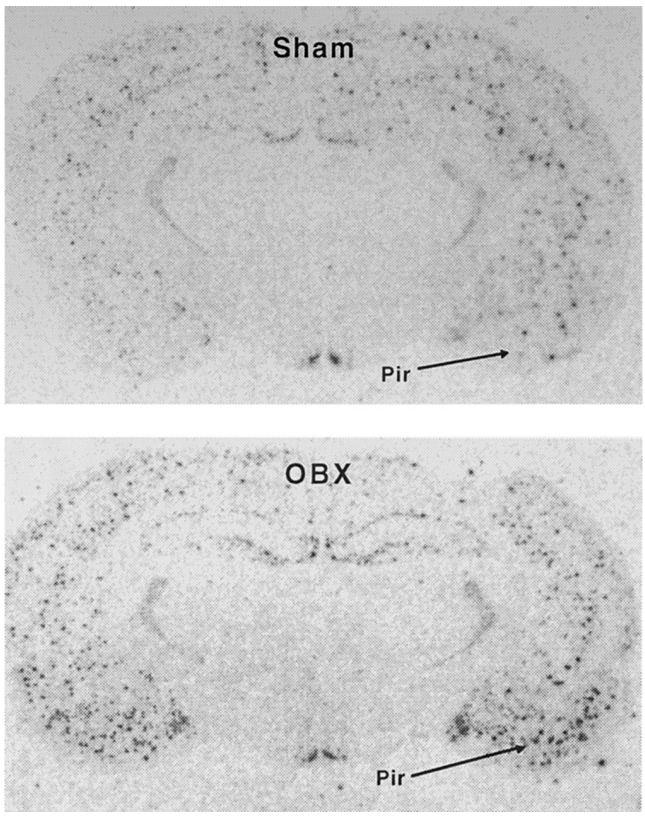


Fig. 4. Computer-generated representative autoradiographs depicting prepro-NPY mRNA hybridization in the PIR of OBX and SHAM rats.

### 2.4. Tests of association between behavioral and neurochemical changes

Correlational analyses were performed between time spent immobile in the footshock-induced immobility test and prepro-ENK and prepro-NPY mRNA levels. Similar correlations between peptide-like immunoreactivity and behaviors were also performed. Several marginal effects were observed in correlations between in situ hybridization data and behavioral data, suggesting an association between neurochemical and behavioral changes. A marginal negative correlation was found between time spent immobile and prepro-NPY mRNA levels in the PIR of bulbectomized rats, $r^2 = -0.513$, $p = 0.07$. A marginal negative correlation was also found between time spent immobile and prepro-ENK mRNA levels in the PIR of bulbectomized rats, $r^2 = -0.475$, $p < 0.10$. Correlational analyses were also conducted between ENK and NPY gene expression and peptide levels in the regions of interest. An analysis of prepro-ENK and prepro-NPY gene expression revealed a significant association. Prepro-NPY mRNA levels in the PIR were positively correlated with prepro-ENK mRNA levels in the PIR of bulbectomized rats, $r^2 = 0.604$, $p < 0.05$. In addition, a marginal positive correlation was found between prepro-NPY mRNA levels in the PIR and prepro-ENK mRNA levels in the OT of bulbectomized rats, $r^2 = -0.476$, $p < 0.10$. A marginal positive correlation was also found between prepro-ENK mRNA levels in the PIR and OT of bulbectomized rats, $r^2 = -0.487$, $p < 0.10$. No other significant or marginal correlations between peptide-like immunoreactivity and behavior were found. Surprisingly, no significant correlations between prepro-peptide gene expression or peptide levels were detected. Although OBX increases both prepro-peptide mRNA levels and peptide-like immunoreactivity, the lack of a statistically significant correlation reveals that there is not a simple linear relationship between these two variables.

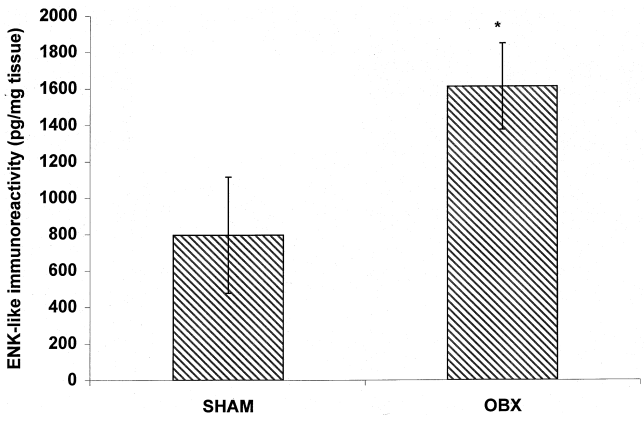


Fig. 5. Radioimmunoassays measured the levels of Met-ENK-like immunoreactivity (pg/mg tissue) in the OT of OBX ($n = 12$) and SHAM ($n = 7$) rats. Means ± SEM are represented.

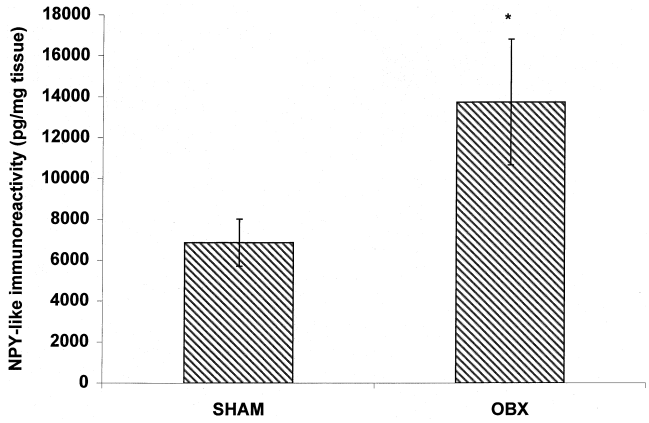


Fig. 6. Radioimmunoassays measured the levels of NPY-like immunoreactivity in the piriform cortex of OBX ($n = 13$) and SHAM ($n = 7$) rats. Means ± SEM represented.

## 3. Discussion

The aim of the present experiments was to determine whether increases in peptide levels accompany the increased prepro-peptide mRNA observed in prior studies. This experiment as well as previous research has demonstrated that OBX leads to increased prepro-NPY mRNA in the PIR

[10] and increased prepro-ENK mRNA in the OT [10] as measured by quantitative in situ hybridization histochemistry. The present results revealed that bilateral OBX also increases NPY-like immunoreactivity in the PIR, and ENK-like immunoreactivity in the OT.

Another aim of this experiment was to determine the nature of the relationship between increased prepro-ENK and prepro-NPY gene expression in bulbectomized rats. Prepro-NPY mRNA levels in the PIR were found to be positively associated with both prepro-ENK mRNA levels in the PIR and OT, though only marginally in the OT. Prepro-ENK mRNA levels in the PIR were positively, though marginally, associated with prepro-ENK mRNA levels in the OT of bulbectomized rats. The positive correlations between ENK gene expression and NPY gene expression suggests that these peptide systems are similarly involved in the neurochemical adaptations that occur in limbic structures following OBX.

The final goal of this experiment was to evaluate the relationship between time spent immobile following footshock and peptide gene expression in bulbectomized rats. The marginally significant correlation between prepro-NPY mRNA and time spent immobile in bulbectomized rats suggests a relationship between increased NPY gene expression and the severity of the OBX syndrome. A marginally significant correlation was also found between ENK gene expression in the PIR and time spent immobile in bulbectomized rats, once again suggesting a relationship between increased ENK in the PIR and the severity of the OBX syndrome. The present findings provide further support for the hypothesis that altered neuropeptide gene expression may underlie certain aspects of depressive disorders.

ENK, an endogenous opioid peptide, is abundant in the striatum of rats and is inversely related to DA levels in the striatum [6,7,24,31]. The functional significance of OBX-induced increases in ENK levels in the OT is unclear. The OT is functionally and anatomically a component of the ventral striatum along with the nucleus accumbens and portions of the ventral caudate–putamen [9]. Behaviorally, the OT supports intracranial self-stimulation, suggesting a role in reward [22,35]. Decreased dopaminergic function in the OT may explain the deficits in appetitively motivated behaviors observed in the OBX syndrome. Increased ENK activity in the OT may underlie the decreased footshock-induced immobility exhibited by bulbectomized rats. Previous studies demonstrating that microinjections of opioid receptor agonists into the ventral striatum produce behavioral activation support this hypothesis [39,55].

The functional significance of increased NPY levels in the PIR following OBX is less clear. Beyond its role in olfactory discrimination and seizure activity, the behavioral function of the PIR is not well known [45,46]. The connections of the PIR with other limbic structures suggests a role for this structure in deficits in aversively motivated behaviors following OBX. The present finding of a negative correlation between NPY gene expression in the PIR and stress-induced immobility suggests that NPY may mediate OBX-induced decreases in footshock-induced immobility. However, further research involving direct manipulations of NPY in this structure is needed to test this hypothesis.

## References

[1] Aoki C, Pickel VM. Neuropeptide Y in the cortex and striatum: ultrastructural distribution and coexistence with classical neurotransmitters and neuropeptides. Ann NY Acad Sci 1990;611:186–205.

[2] Baumbach HD, Sieck MH. Temporal effects of discrete lesions in the olfactory and limbic systems on open field behavior and dyadic encounters in male hooded rats. Physiol Behav 1977;18:617–37.

[3] Bouna S, Gysling K, Calas A, Araneda S. Some noradrenergic neurons of locus coeruleus–olfactory pathways contain neuropeptide Y. Brain Res Bull 1994;34(4):413–7.

[4] Broekkamp CL, O'Connor WT, Tonnaer JADM, Rijk HW, Van Delft AML. Corticosterone, choline acetyltransferase and noradrenaline levels in olfactory bulbectomized rats in relation to changes in passive avoidance acquisition and open field activity. Physiol Behav 1986;37:429–34.

[5] Calcagnetti DJ, Quatrella LA, Schechter MD. Olfactory bulbectomy disrupts the expression of cocaine-induced conditioned place preference. Physiol Behav 1996;59:597–604.

[6] Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Sibley DR. D1 and D2 dopamine receptor-regulated gene expression of striatornigral and striatopallidal neurons. Science 1990;250:1429–32.

[7] Gerfen CR, Keefe KA, Gauda EB. D1 and D2 dopamine receptor function in the striatum: coactivation of D1 and D2 dopamine receptors on separate populations of neurons results in potentiated immediate early gene response in D1-containing neurons. J Neurosci 1995;15(12):8167–76.

[8] Heilig M, Widerlov E. Neuropeptide Y: an overview of central distribution, functional aspects, and possible involvement in neuropsychiatric illnesses. Acta Psychiatr Scand 1990;82:95–114.

[9] Heimer L, Zaborsky L, Zham DS, Alheid GF. The ventral striatopallidothalamic projection: I. The straitopallidal link originating in the striatal parts of the olfactory tubercle. J Comp Neurol 1987;255(4):571–91.

[10] Holmes PV. Olfactory bulbectomy increases prepro-enkephalin mRNA levels in the ventral striatum in rats. Neuropeptides 1999;33(3):206–11.

[11] Holmes PV, Davis RC, Masini CV, Primeaux SD. Effects of olfactory bulbectomy on neuropeptide gene expression in the rat olfactory/limbic system. Neuroscience 1998;86(2):587–96.

[12] Hong KW, Lee WS, Rhim BY. Role of central α2-adrenoceptors on the development of muricidal behaviors in olfactory bulbectomized rats: effect of α2-adrenoceptors antagonists. Physiol Behav 1987;39:535–9.

[13] Iwasaki K, Fujiwara M, Shibata S, Ueki S. Changes in brain catecholamine levels following olfactory bulbectomy and the effect of acute and chronic administration of desipramine in rats. Pharmacol Biochem Behav 1986;24(6):1715–6.

[14] Iwasaki K, Fujiwara M, Ueki S. In vivo changes in brain catecholamine release from rat hypothalamus following olfactory bulbectomy. Pharmacol Biochem Behav 1989;34:879–85.

[15] Janscar S, Leonard BE. The effect of antidepressant drugs on conditioned taste aversion learning in the olfactory bulbectomised rat. Neuropharmacology 1981;20:1341–5.

[16] Jesberger JA, Richardson JS. Effects of antidepressant drugs on the behavior of olfactory bulbectomized and sham-operated rats. Behav Neurosci 1986;100(2):256–74.

[17] Kawasaki H, Watanabe S, Ueki S. Potentiation of pressor and behavioral responses to brain stimulation following bilateral olfactory bulbectomy in freely moving rats. Brain Res Bull 1980;5:711–8.
[18] Kelly JP, Leonard BE. An investigation of the hyperactive response in the olfactory bulbectomised rat model of depression. Med Sci Res 1995;23:837–8.
[19] Kelly JP, Leonard BE. Effects of chronic desipramine on waiting behaviour for a food reward in olfactory bulbectomized rats. J Psychopharmacol 1996;10(2):153–6.
[20] Kelly JP, Norman TR, O'Halloran A, Leonard BE. Home cage and open-field locomotor activity responses in singly housed olfactory bulbectomised rats. Med Sci Res 1996;24:335–7.
[21] Kelly JP, Wrynn AS, Leonard BE. The olfactory bulbectomized rat as a model of depression: an update. Pharmacol Ther 1997;74(3):299–316.
[22] Konetsky C, Huston-Lyons D, Porrino LJ. The role of the olfactory tubercle in the effects of cocaine, morphine and brain stimulation reward. Brain Res 1991;541:75–81.
[23] Larhammar D, Ericsson A, Persson H. Structure and expression of the rat neuropeptide Y gene. Proc Natl Acad Sci USA 1987;84:2068–72.
[24] Li SJ, Jiang HK, Stachowiak MS, Hudson PM, Owyang V, Nanry K, Tilson HA, Hong JS. Influence of nigrostriatal dopaminergic tone on the biosynthesis of dynorphin and enkephalin in rat striatum. Mol Brain Res 1990;8:219–25.
[25] Lingham RB, Gottesfeld Z. Deafferentation elicits increased dopamine-sensitive adenlyate cyclase and receptor binding in the olfactory tubercle. J Neurosci 1986;6:2208–14.
[26] Lumia AR, Teicher MH, Salchli F, Ayers E, Possidente B. Olfactory bulbectomy as model for agitated hyposerotonergic depression. Brain Res 1992;587:181–5.
[27] Mequid MM, Gleason JR, Yang ZJ. Olfactory bulbectomy in rats modulates feeding pattern but not total food intake. Physiol Behav 1993;54:471–5.
[28] Mequid MM, Koseki M, Yang Z, Gleason JR, Laviano A. Acute adaptive changes in food intake pattern following ablation in rats. NeuroReport 1997;8:1439–44.
[29] Mollenauer S, Plotnik R, Snyder E. Effects of olfactory bulb removal on fear responses and passive avoidance in the rat. Physiol Behav 1973;12:141–4.
[30] Mudunkotuwa NT, Horton RW. Desipramine administration in the olfactory bulbectomized rat: changes in brain β-adrenoceptor and 5-HT2A binding sites and their relationship to behavior. Br J Pharmacol 1996;117:1481–6.
[31] Normand E, Popovici T, Onteniente B, Fellman D, Piatier-Tonneau C, Auffray C, Bloch B. Dopaminergic neurons of the substantia nigra modulate preproenkephalin A gene expression in rat striatal neurons. Brain Res 1988;439:39–46.
[32] Nyberg F, Terenius L. Neurochemical methods in the study of the role of endorphins in psychiatric disorders. In: Rodgers RJ, Cooper SJ, editors. Endorphins, opiates, and behavioral processes. New York: Wiley, 1988.
[33] O'Connor WT, Leonard BE. Behavioural and neuropharmacological properties of the dibenzazepines, desipramine and lofepramine: studies on the olfactory bulbectomized rat model of depression. Prog Neuro-Psychopharmacol Biol Psychiatry 1988;12:41–51.
[34] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego: Academic Press.
[35] Prado-Alcala R, Wise RA. Brain stimulation reward and dopamine terminal fields: I. Caudate–putamen, nucleus accumbens and amygdala. Brain Res 1984;297:265–73.
[36] Primeaux SD, Holmes PV. Role of aversively motivated behavior in the olfactory bulbectomy syndrome. Physiol Behav 1999;67(1):41–7.
[37] Richman CL, Gulkin R, Knoblock K. Effects of bulbectomization, strain, and gentling on emotionality and exploratory behavior in rats. Physiol Behav 1971;8:447–52.
[38] Sawchenko PE, Swanson LW, Grzanna R, Howe PRC, Bloom SR, Polak JM. Colocalization of neuropeptide Y immunoreactivity in brainstem catecholaminergic neurons that project to the paraventricular nucleus of the hypothalamus. J Comp Neurol 1985;241:138–53.
[39] Schildein S, Agmo A, Huston JP, Schwarting RKW. Intraaccumbens injections of substance P, morphine and amphetamine: effects on conditioned place preference and behavioral activity. Brain Res 1998;790(1–2):185–94.
[40] Sieck MH. Selective olfactory system lesion in rats and changes in appetitive and aversive behavior. Physiol Behav 1972;10:731–9.
[41] Sieck MH, Baumbach HD. Differential effects of peripheral and central anosmia producing techniques on spontaneous behavior patterns. Physiol Behav 1974;13:407–25.
[42] Sieck MH, Baumbach HD, Gordon BL, Turner JF. Changes in spontaneous, odor modulated and shock induced behavior patterns following discrete olfactory system lesions. Physiol Behav 1974;13:427–39.
[43] Sieck MH, Gordon BL. Selective olfactory bulb lesions: reactivity changes and avoidance learning in rats. Physiol Behav 1972;9:545–52.
[44] Song C, Leonard BE. Serotonin reuptake inhibitors reverse the impairments in behavior, neurotransmitter and immune functions in the olfactory bulbectomized rat. Hum Psychopharmacol 1994;9:135–46.
[45] Staubli U, Schottler F, Nejat-Bina D. Role of dorsomedial thalamic nucleus and piriform cortex in processing olfactory information. Behav Brain Res 1987;25:117–29.
[46] Stripling JS, Patneau DK. Seizure mechanisms in the piriform cortex. Kindling 1990;4:45–59.
[47] Thorne BM, McDougal Y, Topping JS. Olfactory bulb removal and response suppression in rats. Physiol Behav 1976;17:259–65.
[48] Thorne BM, Rowles JS. Memory deficit in passive avoidance learning in bulbectomized Long–evans hooded rats. Physiol Behav 1988;44:339–45.
[49] Tiffany PB, Mollenauer S, Plotnik R, White M. Olfactory bulbectomy: emotional behavior and defense responses in the rat. Physiol Behav 1979;22:311–7.
[50] Tiong AHK, Richardson JS. Differential effects of olfactory bulbectomy on β-adrenoceptors in rat amygdala, hippocampus, and cerebral cortex. Brain Res 1990;531:269–75.
[51] van Riezen H, Leonard BE. Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rats. Pharmacol Ther 1990;47:21–34.
[52] van Riezen H, Schnieden H, Wren AF. Olfactory bulb ablation in the rat: behavioural changes and their reversal by antidepressant drugs. Br J Pharmacol 1977;60:521–8.
[53] Yamamoto T, Jin J, Watanabe S. Characteristics of memory dysfunction in olfactory bulbectomized rats and the effects of cholinergic drugs. Behav Brain Res 1997;83:57–62.
[54] Yoshikawa K, Williams C, Sabol SL. Rat brain preproenkephalin mRNA: cDNA cloning, primary structure and distribution in the central nervous system. J Biol Chem 1984;259:14301–8.
[55] Zhang M, Kelley AE. Opiate agonists microinjected into the nucleus accumbens enhance sucrose drinking in rats. Psychopharmacology 1997;132:350–60.