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CHRONIC FLUOXETINE TREATMENT ATTENUATES STRESSOR-INDUCED CHANGES IN TEMPERATURE, HEART RATE AND NEURONAL ACTIVATION IN THE OLFACTORY BULBECTOMIZED RAT

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Abbreviated title: Stress-induced changes in physiology and cFOS in the OB rat

ABSTRACT AND KEYWORDS

The olfactory bulbectomized (OB) rat is a well characterized animal model that exhibits a number of behavioural and neurochemical changes that have relevance to clinical depression. Hyperactivity in the open field is the most widely used parameter assessed in this model and is reversed following chronic, but not acute, antidepressant treatment. This study investigated OB-induced alterations in heart rate, body temperature and neuronal activation following open field exposure and the impact of chronic treatment with fluoxetine on these parameters. Upon placement in the open field OB rats exhibited a characteristic hyperactivity response. Heart rate and body temperature were increased in sham-operated rats following open field exposure, a predictable response to stress which was significantly reduced in OB rats. Moreover bulbectomy reduced open field-induced cFOS expression in the basal nucleus of the stria terminalis while concurrently increasing expression in the hippocampus, amygdala, paraventricular nucleus of the thalamus and dorsal raphe nucleus. Chronic fluoxetine treatment (10 mg/kg s.c. once daily for 5 weeks) attenuated all of these OB associated changes. In conclusion, OB rats exhibit alterations in behaviour, body temperature, heart rate and neuronal activation in response to open field exposure which are reversed following chronic fluoxetine administration. These results identify stress sensitive regions within the brain which are altered following bulbectomy and which may underlie the abnormal behavioural and physiological changes observed in this rodent model of depression.

Keywords: Olfactory bulbectomy, Hyperactivity, Temperature, Heart Rate, cFOS, Fluoxetine

List of abbreviations:

3V - 3rd ventricle; 5HT – Serotonin; ABC - avidin-biotin-peroxidase complex ; AP - anterior-posterior; BLA - Basolateral nucleus of the amygdala ; BNST - Basal nucleus of the stria terminalis; BPM – beats per minute; BSA – bovine serum albumin; CA1 - CA1 region of the hippocampus; CeAMY - Central nucleus of the amygdala; CPM – counts per minute; DAB - 3,3-diaminobenzidine-4HCl; DG - Dentate gyrus; DRN - Dorsal raphe nucleus; FC - Frontal Cortex; H_2O_2 - hydrogen peroxide; HPA – Hypothalamic-Pituitary-Adrenal axis; LS - Lateral Septum; LV - Lateral Ventricle; LW - lateral wing of the DRN; NaAz – sodium azide; OB – Olfactory bulbectomy; PAG - Periaqueductal grey; PB – Phosphate buffer; PBS – Phosphate buffered saline; PVN - Paraventricular nucleus of the hypothalamus; PVT - Paraventricular nucleus of the thalamus; Tx – Triton X; VR - ventral region of DRN.

INTRODUCTION

As depression is causally linked to stressful life events (Kendler et al., 2001), exposure to stressful events is often employed in attempts to model depression and anxiety disorders in animals. A well documented and validated model of depression is the olfactory bulbectomized (OB) rat, which exhibits a number of behavioural, neurochemical, neuroendocrine and immune alterations correlating with changes observed in depressed patients (Kelly et al., 1997, Harkin et al., 2003; Song and Leonard, 2005). Hyperactivity on exposure to a stressful open field environment is the most commonly assessed behavioural change in the model, a response which is attenuated following chronic, but not acute, antidepressant treatments (Kelly et al., 1997).

Exposure to a novel environment is regarded as a mild psychological stressor eliciting an increase in temperature and heart rate, increased plasma corticosterone levels and behavioural activation in naive animals (Van Den Buuse et al., 2001; 2002; Harkin et al., 2002). Although studies have demonstrated neuroendocrine (Marcilhac et al., 1999) and neurochemical (Connor et al., 1999; Ho et al., 2000; Masini et al., 2004) activation concurrently with behavioural alterations in the OB rat, a paucity of data exists regarding assessment of physiological parameters in the model. This is of particular interest as a number of physiological changes such as reduced heart rate variability, altered autonomic tone and sleep disturbances are known to occur during stress and depression (Van Den Buuse et al., 2001; 2002; Carney et al., 2001; Grippo and Johnson, 2002).

The behavioural changes associated with the OB model have been proposed to be due to neuronal reorganisation and plasticity following removal of the olfactory bulbs (Grecksch et al., 1997). Although many critical brain regions have demonstrated adaptational changes in the model (Nesterova et al., 1997; Wrynn et al., 2000a), the neuroanatomical regions involved in OB-induced hyperactivity have not been defined. The immediate early gene, c-fos is induced in response to stressful stimuli and has been extensively used as a tool to map neuronal functional activation (Kovacs, 1998). For example, exposure of rats to a stressful environment increases c-fos expression in cortical areas (medial prefrontal, cingulate, orbital, parietal), olfactory bulb, lateral septum, amygdala, hippocampus, thalamus, caudate, hypothalamus and periaquaductal grey (Handa et al., 1993; Mulders et al., 1995; Nagahara and Handa, 1997; Wirtschafter et al., 1998; Babai et al., 2001; Klejbor et al., 2003). Furthermore, antidepressants have been shown to modify stress-induced expression of c-fos (Morinobu et al., 1995; Duncan et al., 1996).

The aim of this study was to examine changes in activity, temperature and heart rate following open field exposure in OB rats. A further objective was to identify if specific brain regions are differentially activated in response to open field exposure following OB when compared to sham-operated controls. This experimental approach was employed to determine the regions associated with the characteristic OB-related response to a stressful challenge. The effect of repeated treatment with the prototypical antidepressant fluoxetine on behavioural and physiological parameters and neuronal activation in the OB rat was also assessed.

MATERIALS AND METHODS

Subjects

Experiments were conducted on male Sprague Dawley rats (weight at start of experiment 220-270g; Harlan, UK), housed singly in a plastic bottomed cage (45 X 25 X 20 cm) containing wood shavings as bedding. The animals were maintained at a constant temperature (20 ± 2°C) and at standard lighting conditions (12:12 h light–dark, lights on from 0800 to 2000 h). Food and water were available *ad libitum*. The experimental protocol was carried out in accordance with the guidelines of the Animal Welfare Committee, National University of Ireland, Galway under licence from the Irish Department of Health and Children and in compliance with the European Communities Council directive 86/609.

Bilateral Olfactory Bulbectomy Surgery

Bilateral olfactory bulbectomy was performed on rats anaesthetised with 7.5% chloral hydrate (375 mg/kg i.p: Merck, Germany) using an injection volume of 5 ml/kg, essentially as previously outlined by Van Riezen and Leonard (1990). In brief, the head was shaven and a midline sagittal incision was made in the skin overlying the skull. Two burr holes of 2mm diameter were drilled into the skull, 5mm rostral to bregma and 2mm lateral to the midline. The olfactory bulbs were removed by gentle aspiration with a water vacuum pump and care was taken not to damage the frontal cortex. The burr holes were then plugged with a haemostatic sponge to control

bleeding. Sham-operated animals were treated in the same manner but the bulbs were left intact. All animals were allowed 7 days to recover following surgery prior to undergoing implantation of bioradiotelemetric transponders (see below). Lesions were verified after completion of the study. Animals were eliminated from the analysis if the bulbs were not completely removed or if damage extended to the frontal cortex. Sham-operated animals were removed if there was any damage to the bulbs or the frontal cortex.

Implantation of Bioradiotelemetric Transponders

Body temperature (°C), heart rate [beat per minute (BPM)] and locomotor activity [counts per minute (CPM)] were recorded by bioradiotelemetry using fully implantable series PDT-4000 E-mitter transponders and Vital View (Mini Mitter Co., Oregon, US) as previously described (Harkin et al., 2002a,b). One week following olfactory bulbectomy, rats were anesthetised with 7.5% chloral hydrate (375 mg/kg i.p) using an injection volume of 5 ml/kg. A small abdominal incision was made and the transponders were implanted into the abdominal cavity along the sagittal plane, dorsal to the digestive organs. Animals were allowed one week to recover prior to drug treatment. Previous studies demonstrate that locomotor activity does not differ between transponder-implanted, sham-operated or naive rats 3 weeks post-surgery (Harkin et al., 2002b).

Drug Treatment

Rats received the selective serotonin re-uptake inhibitor (SSRI) fluoxetine (Clonmel Chemicals, Ireland) (10mg/kg/day) or vehicle (distilled H_2O). Fluoxetine was administered to rats by the subcutaneous (s.c.) route due to the presence of the radiotelemetric transponder in the abdominal cavity. Drug treatment continued for a period of 5 weeks. Performance in the open field was assessed 24 hours following the last drug administration in order to minimise any acute effect of drug administration.

Open Field Test

Each animal was removed from the home cage and placed singly into a brightly lit (lux 200-250) novel open field environment (diameter 75cm) where locomotor activity was assessed using an electronic video tracking system (Noldus EthoVision, Version 3.0) for a 15 minute period. Locomotor activity (distance moved: cm) was monitored in minute intervals for the entire duration of the test period. Following exposure to the open field, rats were returned to their home cages and telemetric data were collected for a further 75 minutes.

cFOS immunohistochemistry

Rats were deeply anesthesised with chloral hydrate (800 mg/kg i.p.) 75 minutes after the end of the open field stress and transcardially perfused with 100ml of heparinized (5 IU/ml) saline solution, followed by 500ml of 4% (w/v) paraformaldehyde in 0.1M phosphate buffer (PB) at pH 7.4 and 4°C. Brains were removed and stored in the same fixative for 90 minutes at 4°C followed by immersion in 20% (w/v) sucrose solution in 0.1M PB containing 1% (w/v) sodium azide (NaAz) for at least 24 hours. Brains

were rapidly frozen on dry ice and coronal sections of 40µm were cut on a cryostat and collected in 0.1M PB.

Prior to immunohistochemical staining sections were washed in 0.1M PB and then placed in 0.75% (v/v) hydrogen peroxide (H_2O_2) for 20 minutes in order to quench the endogenous peroxides in the tissue. cFOS immunolabelling was performed using a polyclonal antibody directed against residues 4-17 of human c-*fos* (Calbiochem, MerckBiosciences, Nottingham, UK). In brief, sections were incubated for 24 hours at room temperature under constant agitation in 0.1M PB-saline (PBS) containing cFOS antisera raised in rabbit (1:20,000), 0.3% (v/v) Triton X (TX), 0.04% (w/v) bovine serum albumin (BSA) and 0.1% (w/v) NaAz. The incubated sections were washed and incubated for 90 minutes in biotinylated donkey-anti-rabbit antisera (1:200; Jackson ImmunoResearch Europe, UK). The secondary antibody had minimal cross-reactivity to non-target species. This was followed by incubation in the avidin-biotin-peroxidase complex (ABC) (1:600; ABC Elite Kit; Vector Laboratories Ltd., Peterborough, UK) for a further 90 minutes followed by sections being immersed in 0.02% (w/v) 3,3-diaminobenzidine-4HCl (DAB) containing 0.01% (v/v) H_2O_2 in PB for 10-15 minutes for a brown reaction product that was terminated by rinses in PB. The sections were mounted on glass-gelatinised slides and air-dried. All sections were lightly counterstained with cresyl violet, dehydrated in graded alcohols, cleared with xylene and coversliped with DePex mounting medium. Photomicrographs were taken with an Olympus microscope BX51 and Olympus C5060 digital camera (Mason Technology, Dublin, Ireland).

Quantification of cFOS profiles

cFOS positive nuclei were quantified from a fixed area size using a Kodak ID (Version 3.5) image analysis software. The system was calibrated to ignore background staining. The group identity of the rat was obscured during the counting procedure. Regions of interest were defined based on the extent of cellular groups comprising specific landmarks in accordance with Paxinos and Watson (1986) rat brain atlas. The planes of the sections were standardized as far as possible. The anterior-posterior (AP) level from bregma of the regions was analyzed as follows: Frontal cortex (AP: 0.20 mm), lateral septum (AP: 0.20 mm), basal nucleus of the stria terminalis (BNST: AP: -0.26 mm), paraventricular nucleus of the hypothalamus (PVN; AP: -2.12 mm), central nucleus of the amygdala (CeAMY; AP: -2.8 mm) , basolateral amygdala (BLA; AP: -2.8 mm), paraventricular nucleus of the thalamus (PVT; AP: -2.8 mm), Dentate Gyrus (DG; AP: -2.8 mm), CA1 region of the hippocampus (CA1; AP: -2.8 mm), habenula (AP: -3.14 mm), dorsal raphe nucleus (DRN; AP: -8.0 mm), locus coeruleus (AP: -9.68 mm). cFOS immunoreactive profiles were counted from a fixed area under 100-200X in at least two sections per region, bilaterally and averaged to give the mean number of cFOS positive profiles for that region per animal.

Statistical analysis

Data were analysed by analysis of variance (ANOVA) using a GB-STAT (Version 8) statistical package. Distance travelled in the open field was analysed in 1-minute time bins over the entire course of the 15-minute trial by repeated measures ANOVA with lesion (Sham and OB), drug treatment (vehicle and fluoxetine) and time as factors. Additional analyses were performed on data totalled over the first 5 minutes of the

trial using two-way ANOVA with lesion and drug treatment as factors. Body temperature and heart rate were continuously assessed, except during the open field test, on account of a technical limitation of the telemetric receiving devices, and an average computed at 5-minute intervals for 30 minutes prior to and 75 minutes following open field exposure. For determination of the change in temperature and heart rate, a baseline was calculated by averaging samples collected before open field exposure, which was subtracted from all values to determine change from baseline average. Locomotor activity data were assessed as the sum of the activity counts per 5 minute interval. Data post-stressor exposure were compared to the baseline average. Analyses were performed using repeated measures ANOVA with lesion, drug treatment and time as factors. Neuronal activation data were analysed by two-way ANOVA with lesion and drug treatment as factors. All inter-group comparisons were assessed using a Student Newman Keuls (SNK) *post-hoc* comparison test where appropriate. Data are expressed as mean ± standard error of the mean (SEM) and were deemed significant when $P<0.05$.

RESULTS

Chronic fluoxetine treatment attenuates OB-induced hyperactivity

ANOVA of locomotor activity over the 15-minute open field test showed an effect of lesion ($F_{1,16}$ = 6.17 P = 0.024), lesion x drug treatment interaction ($F_{1,16}$ = 5.83 P = 0.028), time ($F_{14,224}$ = 15.08 P <0.0001), lesion x time interaction ($F_{14,224}$ = 5.37 P <0.0001) and drug treatment x time interaction ($F_{14,224}$ = 2.00 P = 0.019). Post-hoc comparisons revealed that vehicle-treated OB rats display an increase in distance travelled in the 1st and 3rd minute of open field exposure when compared to sham-operated controls (Figure 1A). OB-induced hyperactivity was attenuated by chronic fluoxetine treatment. Fluoxetine treatment did not alter locomotor activity of sham-operated animals when compared to vehicle treated controls. Analysis of behaviour over the first 5 minutes of the test trial demonstrated a lesion ($F_{1,19}$ = 14.77, P = 0.001) and lesion x drug interaction effect ($F_{1,19}$ = 7.06, P = 0.017). OB induced an increase in distance travelled in the open field when compared to sham-operated controls (Figure 1B). Chronic fluoxetine treatment did not affect behavioural responses of sham-operated rats but attenuated OB-induced hyperactivity in the test arena when compared to their vehicle-treated OB counterparts (Figure 1B).

Open field-induced hyperthermia is attenuated in OB rats; normalisation by chronic fluoxetine treatment

No lesion, drug treatment or interaction effects were found on baseline temperature prior to open field exposure (Figure 2A). ANOVA of core body temperature

following the open field test showed an effect of time ($F_{15,270}$ = 44.65, P < 0.001), lesion x time interaction ($F_{15,270}$ = 7.36, P < 0.001) and drug treatment x time interaction ($F_{15,270}$ = 4.08, P < 0.001). Post-hoc analysis revealed that body temperature of sham-operated controls was increased from the 20th to 60th minute interval when compared to their pre-stress baseline levels (Figure 2A). This robust hyperthermic response to open field exposure in sham-operated animals was not altered by chronic fluoxetine treatment. In contrast, open field exposure did not increase core body temperature of OB rats from baseline level. However, fluoxetine-treated OB rats exhibited a hyperthermic response to open field exposure for the 20th to 70th minute interval. In addition, OB rats demonstrated a reduced hyperthermic response following open field exposure for the 20th to 45th minute when compared to sham-operated controls. Chronic fluoxetine treatment normalised the temperature response of OB rats when compared to their vehicle-treated counterparts (Figure 2A).

Open field-induced tachycardia is attenuated in OB rats; reversal following chronic fluoxetine treatment.

No lesion, drug treatment or interaction effects were found on baseline heart rate prior to open field exposure (Figure 2B). ANOVA of heart rate following the open field test showed an effect of lesion ($F_{1,19}$ = 15.33, P < 0.001), time ($F_{15,270}$ = 20.39, P < 0.001), lesion x time interaction ($F_{15,270}$ = 1.73, P = 0.044) and drug treatment x time interaction ($F_{15,270}$ = 1.87, P < 0.026). Post-hoc analysis revealed that sham-operated animals exhibited an increase in heart rate in response to open field exposure over the 20th to 90th minute interval when compared to their pre-stress baseline (Figure 2B). This tachycardic response was not altered by chronic fluoxetine treatment. Heart rate

of OB rats was increased for the 20th to 40th minute interval following the open field test when compared to baseline levels. In comparison, a robust tachycardic response was obtained in OB rats following fluoxetine treatment over the 20th-70th minute interval following the open field test when compared to pre-stress baseline. OB rats demonstrated an attenuated tachycardic response following open field exposure for the 25th to 30th, 40th and 65th to 70th minute when compared to sham-operated controls. Chronic fluoxetine treatment blocked the reduced tachardiac response of OB rats in the 65th minute when compared to their vehicle-treated counterparts (Figure 2B).

Home cage locomotor activity

No lesion, drug treatment or interaction effects were found on baseline home cage activity prior to open field exposure (Figure 2C). On returning to the home cage following the open field test, home cage activity was increased in both sham and OB animals for the first five minutes when compared to baseline scores ($F_{15,270} = 33.57$, $P < 0.001$). A slower acclimatization was observed in the fluoxetine treated groups where increased activity was observed over the 20th to 35th minute interval when compared to baseline scores ($F_{15,270} = 2.04$, $P = 0.013$) (Figure 2C).

Differential cFOS expression in the OB rat following open field exposure

ANOVA showed an effect of lesion on cFOS expression following the open field test in several discrete brain regions when compared to sham-operated controls. An effect of bulbectomy lesion was observed in the basal nucleus of the stria terminalis (BNST; $F_{1,19} = 6.20$, $P = 0.024$), central (CeAMY; $F_{1,20} = 4.69$, $P = 0.046$) and basolateral

nucleus (BLA; $F_{1,20} = 8.60$, $P = 0.009$) of the amygdala, paraventricular nucleus of the thalamus (PVT; $F_{1,19} = 6.20$, $P = 0.024$), dentate gyrus (DG; $F_{1,22} = 7.11$, $P = 0.015$) and CA1 region ($F_{1,21} = 6.77$, $P = 0.018$) of the hippocampus and dorsal raphe nucleus (DRN; $F_{1,20} = 9.18$, $P = 0.008$). Post-hoc comparisons revealed that cFOS expression was increased in the CeAMY, BLA, PVT, DG, CA1 and DRN of OB rats when compared to sham-operated controls (Figure 3 and 4). Concurrently a decrease in cFOS activation was observed in the BNST in the OB rat when compared to sham-operated controls. Fluoxetine treatment did not alter cFOS expression in any brain region when compared to vehicle-treated sham-operated rats. However, altered cFOS expression in the OB brain following open field exposure was attenuated by chronic fluoxetine treatment [BNST ($F_{1,21} = 8.07$, $P = 0.011$), CeAMY ($F_{1,20} = 6.25$, $P = 0.023$), BLA ($F_{1,20} = 8.60$, $P = 0.009$), DG ($F_{1,22} = 5.27$, $P = 0.033$), PVT ($F_{1,19} = 7.20$, $P = 0.016$); CA1 ($F_{1,21} = 4.70$, $P = 0.044$), DRN ($F_{1,20} = 4.60$, $P = 0.047$)] when compared to their vehicle-treated counterparts (Figure 3). cFOS expression in the frontal cortex [Sham Vehicle (367 +/- 56), Sham Fluoxetine (437 +/- 44), OB Vehicle (395 +/- 41), OB Fluoxetine (459 +/- 78)], lateral septum [Sham Vehicle (263 +/- 16), Sham Fluoxetine (244 +/- 26), OB Vehicle (249 +/- 18), OB Fluoxetine (221 +/- 41)], paraventricular nucleus of the hypothalamus [Sham Vehicle (82 +/- 6), Sham Fluoxetine (93 +/- 12), OB Vehicle (62 +/- 11), OB Fluoxetine (88 +/- 13)], habenula [(Sham Vehicle (21 +/- 2), Sham Fluoxetine (22 +/- 3), OB Vehicle (24 +/- 2), OB Fluoxetine (20 +/- 3)] or locus coeruleus [(Sham Vehicle (49 +/- 5), Sham Fluoxetine (35 +/- 5), OB Vehicle (45 +/- 5), OB Fluoxetine (35 +/- 10)] was not effected by bulbectomy lesion or drug treatment.

DISCUSSION

This study demonstrates that OB-induced hyperactivity in the open field is accompanied by impaired temperature and heart rate responses. This provides behavioural and physiological evidence that OB rats are unable to mount appropriate responses to a stressful stimulus. Additionally, this is the first study to show that neuronal activation is increased in the amygdala (CeAMY and BLA), hippocampus (CA1 and DG), thalamus (PVT) and dorsal raphe nucleus (DRN) of the OB rat upon exposure to a stressful environment. This is accompanied by a decrease in open field-induced cFOS expression in the basal nucleus of the stria terminalis (BNST). Chronic fluoxetine treatment attenuated alterations in behaviour, temperature and heart rate and central cFOS expression in the OB rat following open field exposure.

Stressor exposure elicits a predictable physiological stress response characterised by increased temperature, heart rate and blood pressure (Van Den Buuse et al., 2001; 2002; Harkin et al., 2002). The present findings provide evidence that OB rats are unable to increase core body temperature following open field exposure. Novelty-induced hyperthermia is a fever response due to an elevated thermoregulatory set point (Oka et al., 2001). Although the mechanisms underlying the temperature response of OB rats to stressor exposure remain unresolved, OB rats may have a lower internal thermoregulatory set point, resulting in these animals being unable to mount an appropriate hyperthermic response to open field exposure. In addition, a reduced heart rate response to the open field was also observed in these animals. Although physiological responses were not assessed during the open field test

previous studies have demonstrated reduced heart rate activation in the first 15 minutes of novelty exposure 5 and 10 days following bulbectomy (Kawaski et al., 1980). Cardiovascular responses to novelty are due to sympathetic activation and/or vagal withdrawal (Van Den Buuse et al., 2001, 2002), alterations in which may account for the reduced heart rate response to the open field in the OB rat. A reduced cardiosympathetic response was reported on exposure of OB rats to other stressors, namely hypotension, air jet stress and smoke exposure (Moffit et al., 2002). Thus, removal of the olfactory bulbs may result in autonomic dysfunction correlating with alterations also observed in the clinical setting (Carney et al., 2001; Grippo and Johnson, 2002). The reduced temperature and heart rate responses were concurrently observed with increased activity in the test arena suggesting that the physiological responses to open field exposure are independent of activity levels. Thus, separate anatomical pathways may modulate behavioural and physiological responses to stress in the model.

Chronic fluoxetine treatment attenuated the OB-related hyperactivity in the open field and the impaired physiological responses to open field exposure. However, due to the long half-lives of fluoxetine and its active metabolite norfluoxetine (Caccia et al., 1990; Gardier et al., 1994; Lefebvre et al., 1999) the acute effects of fluoxetine administration on cannot be completely ruled out. It should be noted that although the impaired temperature response of OB rats to the open field test was completely normalised by fluoxetine treatment, the effect of this treatment regime on the decreased heart rate response of OB rats was not as pronounced. A longer treatment period with this SSRI may be necessary to fully attenuate the lesion-induced reduction in heart rate response.

Mapping the pattern of neuronal activation in response to open field exposure identifies critical brain regions in the OB rat possibly involved in the hyperactivity and reduced temperature and heart rate responses observed in the model. Removal of the olfactory bulbs is associated with ventricular enlargement, decreased cortical, hippocampal, caudate and amygdaloid volumes, and disruption of the blood brain barrier (Wrynn et al., 2000a), results which correlate with clinical studies in the depressed patient (Videbech, 1997; Sheline, 2000; Drevets, 2000). The olfactory bulbs send direct connections to the cortex (pyriform, entorhinal and parahippocampal), amygdala and BNST (Haberly and Price, 1977; Leonard and Tuite, 1981). Many of the behavioural alterations observed in the OB rat have been attributed to severed connections between the olfactory organs and various brain centres, with much emphasis being placed on the resultant disinhibition of the amygdala following removal of the bulbs (Leonard and Tuite, 1981; Van Riezen and Leonard, 1990; McNish and Davis, 1997; Mucignat-Caretta et al., 2004). Increased neuronal activation in the CeAMY and BLA of the OB rat following open field exposure confirms that the amygdaloid nuclei are integral in mounting a stress response in these animals. Altered gene expression (Wrynn et al., 2000b) and cell proliferation (Keilhoff et al., 2006) has been previously reported in the BLA of OB rats and this region has been proposed as a potential site of antidepressant activity. The decrease in cFOS expression in the BNST of OB rat would suggest that this area is not activated in response to open field exposure in the model. However, it is possible that cells of this region are activated by processes that do not promote the synthesis of the cFOS protein.

The BNST has been implicated in mediating responses relating more to anxiety and apprehension than fear, while the CeAMY is involved in the initiation and maintenance of fear, and perhaps to a lesser extent anxiety (Davis and Shi, 1999; Lang et al., 2000; Walker et al., 2003). Differential activation of neuronal networks in these limbic regions may indicate that exposure of OB rats to a novel environment induced an emotional stress response mediated, in part, by fear via neuronal activation of the CeAMY. In addition, reduced neuronal activation in the BNST may indicate that OB rats exhibit a reduced state of defensive preparedness and anxiety to stressor exposure. This correlates with previous reports of reduced defensive behaviour in OB rats (Primeaux and Holmes, 1999; Stock et al., 2001).

The amygdala and BNST receive inputs from the hippocampus and PVT (Cullinan et al., 1993, Bubser and Deutch, 1999), both of which also exhibit increased cFOS expression in the OB rat brain following open field exposure. In turn, the amygdaloid complex projects to the paraventricular nucleus of the hypothalamus and brain stem nuclei such as the central grey and dorsal motor nucleus of the vagus nerve (Gray and Magnuson, 1987; Gray et al., 1989). Due to these extensive anatomical connections, neural processing involving the BNST and CeAMY controls much of the behavioural, physiological, neuroendocrine and autonomic responses to stress (Davis and Shi, 1999; Van de Kar and Blair, 1999; Carrasco and Van de Kar, 2003). Thus, the pattern of open field-induced changes in neuronal activation following OB suggest that the neural circuit mediating stress responses are altered in this model, accounting for the behavioural and physiological responses observed. Chronic fluoxetine treatment acts on neuronal pathways in these discrete brain regions to attenuate the maladaptive

response of OB rats to stress as measured by changes in cFOS expression, behavioural activity and reduced temperature and heart rate responses.

Further studies are required in order to determine the neurotransmitter or transmitter systems associated with these alterations. In this regard, the OB rat has been proposed as a model of hyposerotonergic depression with many of the behavioural and biochemical aspects of the model also observed following destruction of serotonergic innervation of the bulbs (Lumia et al., 1992). Bulbectomy induces a reterograde loss of cells in the DRN (Nesterova et al., 1997), decreased basal 5HT levels and rate of 5HT synthesis in the amygdala and hippocampus (Marcilhac et al., 1999; van der Stelt et al. 2005), increased $5HT2_A$ receptor density and sensitivity in the frontal cortex (Gurevich et al., 1993; Nakagawasai et al., 2003) and increased density of serotonin transporter sites in the cortex and hippocampus (Slotkin et al., 1999). In response to stress, OB rats demonstrate an increase in serotonin neurotransmission in the hypothalamus (Marcilhac et al., 1999) and nucleus accumbens (Connor et al., 1999). Combining these earlier studies with our evidence for increased neuronal activation in the DRN and other forebrain regions highly innervated by the raphe nuclei, the central serotonergic system may be implicated in mediating the stress responses to open field exposure observed in the model. Moreover, the attenuation of altered neuronal activation in the OB rat by chronic treatment with the SSRI fluoxetine lends further support for the involvement of the DRN-5HT system in stress-induced changes in activity, temperature and heart rate in the OB rat model of depression. However, uncertainty remains as to whether the effects observed with fluoxetine extend to antidepressants with different acute modes of action.

In conclusion, this study identifies stress-sensitive neuroanatomical regions which are altered in the OB rat. Altered neuronal expression in these regions reflects changes in the central stress circuitry in the model and may underlie the behavioural and physiological changes observed on exposure to the open field. Moreover these studies indicate neuroanatomical regions that may underlie antidepressant activity in the model.

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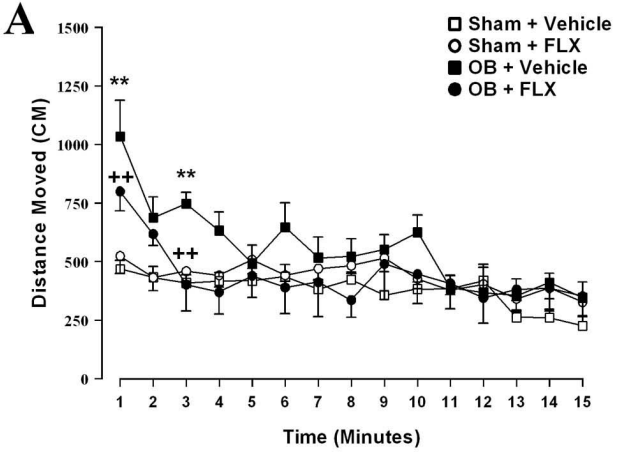
TITLES AND LEGENDS TO FIGURES

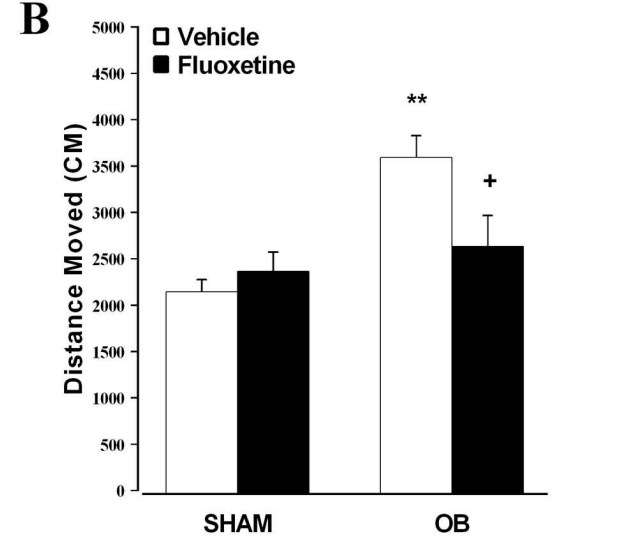
Figure 1: The effect of bulbectomy and chronic fluoxetine treatment on distance travelled (cm) over (A) the entire 15-minute open field exposure and (B) the first 5 minutes in the open field arena. Data expressed as means ± SEM (n = 5-6). ** $P < 0.01$ compared to vehicle-treated sham-operated controls. $^{+}$ $P < 0.05$, $^{++}$ $P < 0.01$ when compared to vehicle-treated OB group.

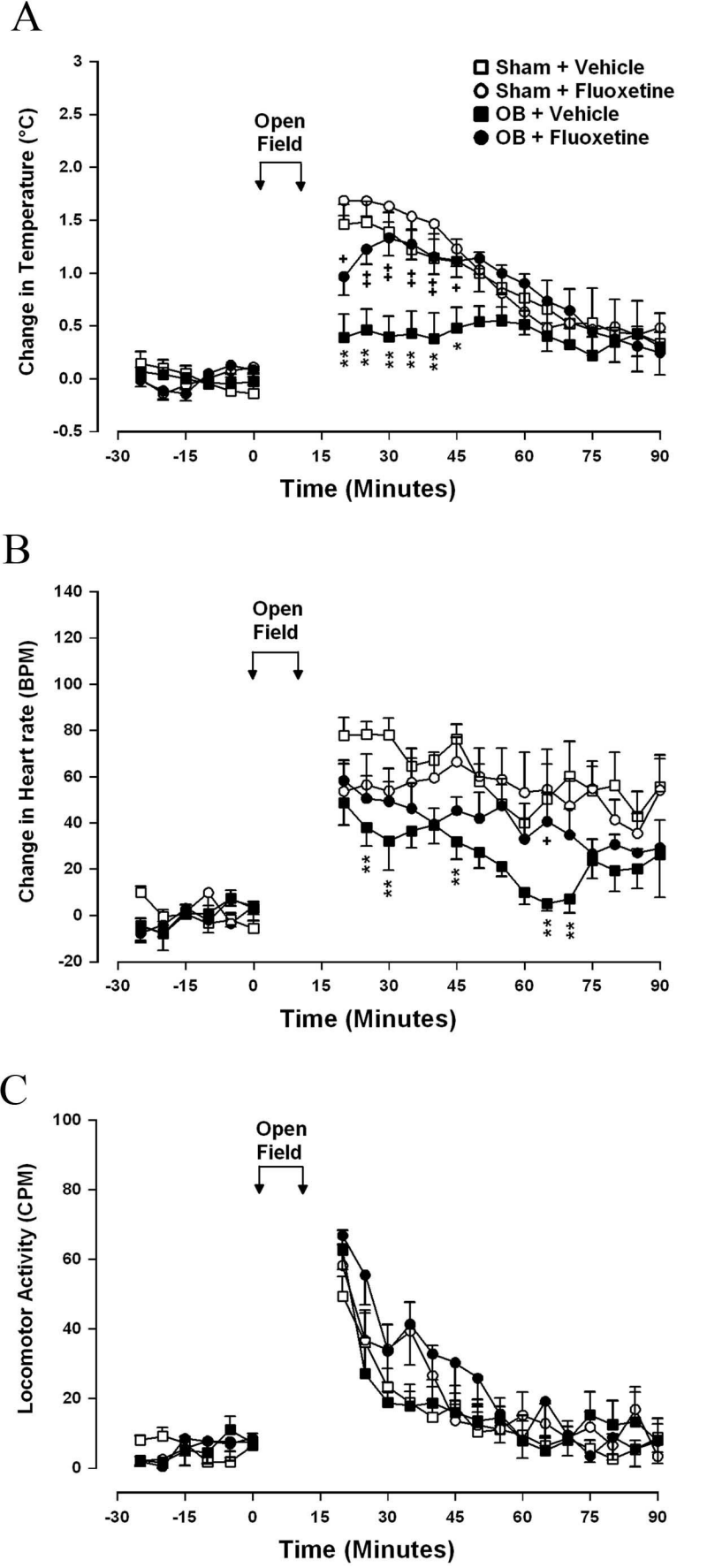
Figure 2: The effect of olfactory bulbectomy and chronic fluoxetine treatment on open field-induced changes in (A) core body temperature, (B) heart rate and (C) locomotor activity. N = 5-6 per group. Data expressed as means ± SEM of mean change from baseline for temperature and heart rate recorded at 5-minute intervals. Data expressed as mean ± SEM of sum of counts per 5-minute interval for locomotor activity. * $P < 0.05$, ** $P < 0.01$ compared to vehicle-treated sham-operated controls. $^{+}$ $P < 0.05$, $^{++}$ $P < 0.01$ when compared to vehicle-treated OB group. Baseline averages for body temperature (Sham + Vehicle: 36.78 ± 0.23°C; Sham + Fluoxetine: 37.11 ± 0.30°C; OB + Vehicle: 36.55 ± 0.10°C; OB + Fluoxetine: 36.50 ± 0.21°C), heart rate (Sham + Vehicle: 334 ± 7 BPM; Sham + Fluoxetine: 319 ± 25 BPM; OB + Vehicle: 337 ± 19 BPM; OB + Fluoxetine: 334 ± 16 BPM) and locomotor activity (Sham + Vehicle: 6 ± 1 CPM; Sham + Fluoxetine: 3 ± 2 CPM; OB + Vehicle: 5 ± 2 CPM; OB + Fluoxetine: 6 ± 2 CPM). □ Sham + Vehicle ○ Sham + Fluoxetine ■ OB + Vehicle ● OB + Fluoxetine.

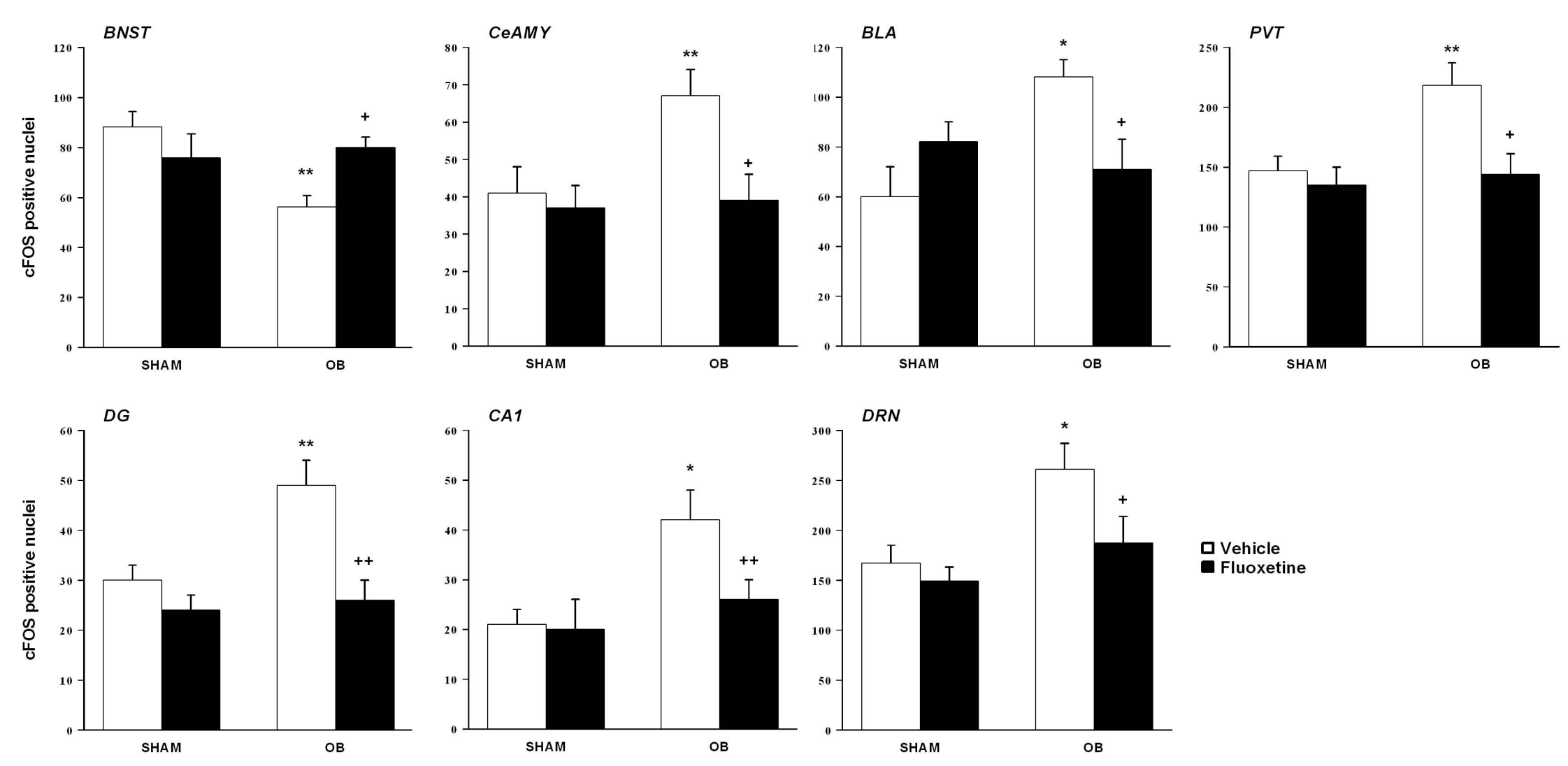
Figure 3: The mean number (+ SEM) of cFos immunoreactive profiles in the brain of sham or OB rats chronically treated with vehicle or fluoxetine. N = 5-6 per group. BNST - Basal nucleus of the stria terminalis; CeAMY - central nucleus of the amygdala; BLA - basolateral nucleus of the amygdala; PVT - paraventricular nucleus of the thalamus; DG - dentate gyrus; CA1 - CA1 regions of the hippocampus; DRN - dorsal raphe nucleus. * $P < 0.05$, ** $P < 0.01$ compared to vehicle-treated sham-operated controls. $^{+}$ $P < 0.05$, $^{++}$ $P < 0.01$ when compared to vehicle-treated OB group.

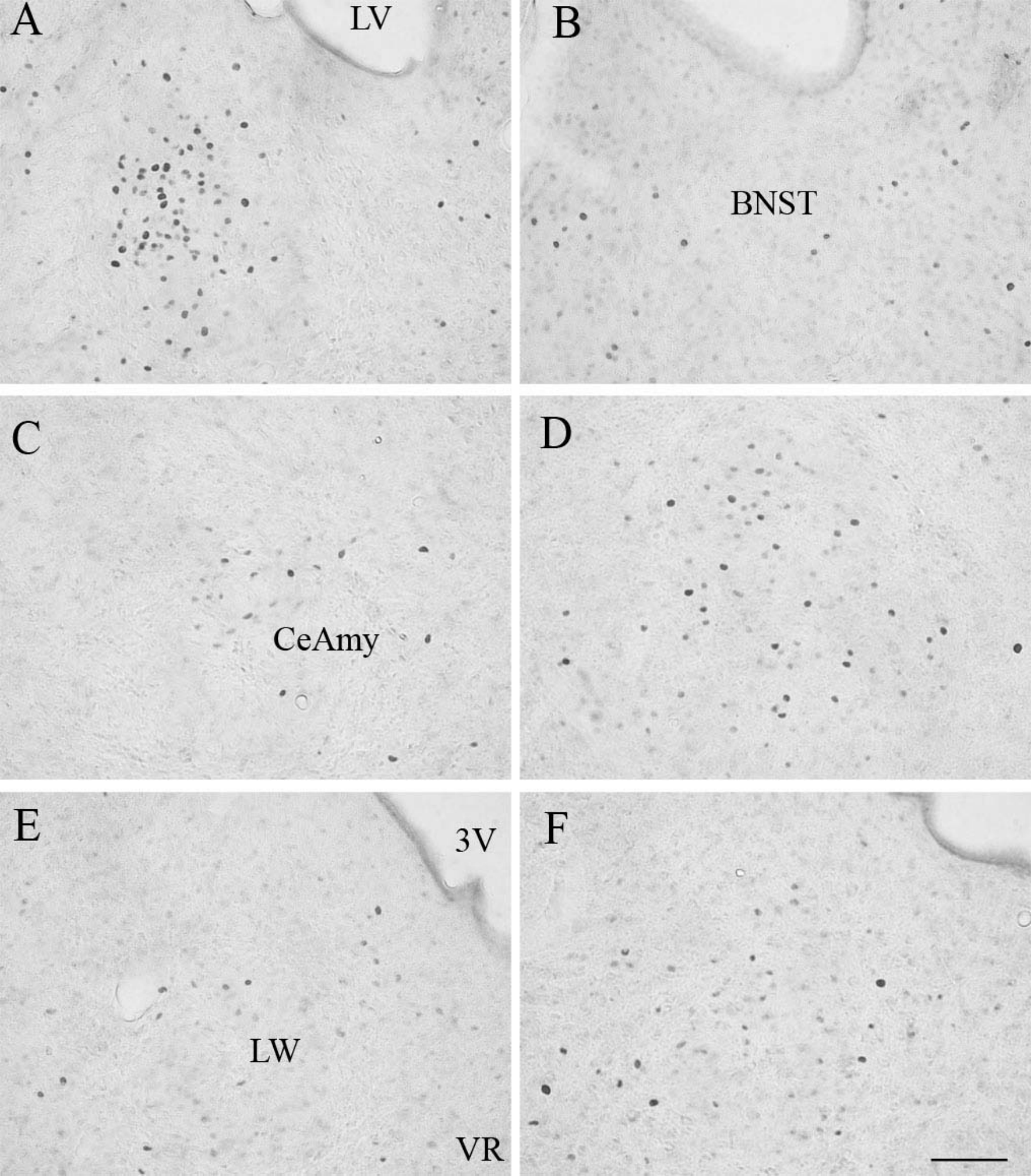
Figure 4: Photomicrographs of cFos expression in three brain regions in vehicle-treated sham-operated control and OB rats. BNST (A) sham (B) OB; CeAMY (C) sham (D) OB and lateral wing of the DRN (D) sham (E) OB. Abbreviations: 3V - 3rd ventricle, BNST - basolateral nucleus of the stria terminalis, CeAMY - central nucleus of the amygdala, DRN - dorsal raphe nucleus, LW - lateral wing of the DRN, LV - lateral ventricle, VR - ventral regions of DRN. Bar represents 0.1mm.


A
Sham + Vehicle
Sham + FLX
OB + Vehicle
OB + FLX
Distance Moved (CM)
1500
1250
1000
750
500
250
0
**
++
**
++
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Time (Minutes)


B
Vehicle
Fluoxetine
Distance Moved (CM)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
**
+
SHAM
OB


A
Sham + Vehicle
Sham + Fluoxetine
OB + Vehicle
OB + Fluoxetine
Open Field
Change in Temperature (°C)
Time (Minutes)
B
Open Field
Change in Heart rate (BPM)
Time (Minutes)
C
Open Field
Locomotor Activity (CPM)
Time (Minutes)


BNST
CeAMY
BLA
PVT
DG
CA1
DRN
cFOS positive nuclei
SHAM
OB
Vehicle
Fluoxetine


A
LV
B
BNST
C
CeAmy
D
E
3V
LW
VR
F