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Short communication

Trans-species assessment of antidepressant activity in a rodent model of depression

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Abstract:
Olfactory bulbectomy (OB), a preclinical model of depression, has most often been performed and validated in rats, but not as comprehensively in other rodent species. This study demonstrated that bulbectomy induced a hyperactive response in the open field test in three rodent species, namely the rat, mouse and hamster. OB, in all species, produced an increase in the distance travelled in the perimeter of the arena. The OB mouse was the only species to demonstrate increased distance travelled in the central part of the arena. These behavioral disturbances were attenuated in all species following chronic treatment with the antidepressant imipramine.

Key words:
olfactory bulbectomy, open field, rat, mouse, hamster, imipramine

Abbreviations: IMI – imipramine, OB – olfactory bulbectomy, S – sham-operated control, V – vehicle

Introduction

Animal models of depression provide valuable preclinical information on the underlying pathophysiology of depression and in screening potential therapeutic agents for antidepressant activity. One well-characterized model is the olfactory bulbectomized (OB) rat, which exhibits a number of behavioral, neurochemical, neuroendocrine and immune alterations correlating with changes observed in depressed patients [2, 3, 16]. Of the behavioral changes which occur following bulbectomy, hyperactivity in the open field, a novel exploratory environment, responds selectively to chronic antidepressant treatment thus mimicking the clinical time-course of antidepressant action [3, 18]. The necessity for repeated antidepressant administration to correct the behavioral aberration observed in the open field test distinguishes the OB model from many other simulations of depression and tests of antidepressant action in rodents.

In certain instances, the use of alternative species to the rat, such as the mouse or hamster is desirable in preclinical evaluations due to cost, space, amount of compound required and genomic similarities. Over 40 different strains of genetically-modified mice have been identified as demonstrating phenotypes related to depression or antidepressant action [1], therefore, developing animal models of depression in mice is

advantageous. In addition, receptor families such as the tachykinin NK1 and serotonin 5-HT_{1B} receptors, which have potential relevance to antidepressant activity, exhibit reduced homology between the human and the rat/mouse receptor [10, 14]. In such cases, the antidepressant activity of compounds acting at these receptors must be assessed in models developed in species with greater homology to human receptor pharmacology such as the hamster, gerbil and guinea pig. Acute stress-based models of depression such as the forced swim test, tail suspension test, learned helplessness and neonatal vocalization have been successfully adapted for the mouse [2, 13, 16], gerbil [19] and guinea pig [4, 15]. However, there is a paucity of tests validated in alternate rodent species to the rat that respond to chronic antidepressant treatment.

Although some studies have examined behavioral responses of OB in mice [11, 20] and hamsters [12] few have validated the model in these species by investigating the effect of antidepressant treatment. The goal of the present study was to assess the behavioral response of bulbectomized rats, mice and hamsters in the open field arena test. Where possible, the choice of strain was comparable with previous studies [7, 8, 11, 20]. The effect of chronic imipramine treatment on OB-related hyperactivity in each of the three species was also determined.

Materials and Methods

Animals

Experiments were conducted on male Sprague Dawley rats (weight at start of experiment 220–270 g; Harlan-Olac, UK), Golden Syrian hamsters (80–100 g; Harlan-Olac, UK) and C57/Bl6j mice (25–35 g; Bantin & Kingman, UK). All animals were housed singly in a plastic bottomed cage (45 × 25 × 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 08:00 to 20:00 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 (OB) surgery

Bilateral OB was preformed on rats and mice anesthetized with chloral hydrate (375 and 750 mg/kg intraperitoneal (*ip*) respectively; Merck, Germany) or on hamsters anesthetized with sodium thiopentone (100 mg/kg, *ip*; Ciba-Geigy, UK). The procedure was essentially as previously outlined for rats [18] and mice [11, 20]. In brief, the head was shaven and a midline sagittal incision was made in the skin overlying the skull. Two burr holes of 2 mm diameter were drilled into the skull of the rat, 5 mm rostral to bregma and 2 mm lateral to the midline. In contrast, for both mice and hamsters a single burr hole of 2 mm was drilled 2 mm rostral to bregma and on the midline. In all cases, 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 hole(s) were then plugged with a hemostatic sponge to control bleeding. Sham-operated animals were treated in the same manner but the bulbs were left intact. All animals were allowed 14 days to recover following surgery and handled daily throughout in order to reduce aggressiveness that would otherwise arise. Lesions were verified upon completion of the study and animals were eliminated from the analysis if the bulbs were not completely removed or if damage extended to the frontal cortex.

Drug treatment

Following recovery from surgery, animals were randomly assigned to either drug or vehicle treatment (0.89% NaCl) groups. The tricyclic antidepressant (TCA) imipramine (Sigma, UK) was administered to rats at 10 mg/kg/day subcutaneously (*sc*) for 2 weeks. Mice received vehicle or imipramine, by the *ip* route at 40 mg/kg/day for 5 weeks. Hamsters received vehicle or imipramine (10 mg/kg/day, *sc*) for 2 weeks. For each experiment the dose and route employed were determined by prior studies where body weight and nocturnal home cage activity were employed as tolerability indices.

Open field test

The open field consisted of a white circular base (75 cm in diameter) surrounded by an aluminium wall 60 cm high. On the experimental day, each animal was placed singly into the open field apparatus (lux

100–200) and locomotor activity was assessed using an electronic video tracking system (Noldus EthoVision Version 3). Automated behavioral tracking systems such as EthoVision, provide an objective measure of behavioral output [9], allowing for comparisons to be made between studies. Locomotor activity (distance travelled: cm) was monitored in minute intervals for the entire duration of the test period (5 min). Locomotor activity on the perimeter of the arena and central area of the arena were determined to assess thigmotactic and exploratory behavior respectively. The central arena was defined as the area within the open field 10 cm from the outside wall (diameter 55 cm). Performance in the open field was assessed 24 h following imipramine administration in order to minimize acute behavioral effects of the drug.

Statistical analysis

Analyses were performed using a two-way ANOVA with lesion (Sham and OB) and drug treatment (Imipramine and Vehicle) as factors using a GB-STAT (Version 8) statistical package. Comparisons were made between Sham + vehicle *vs.* OB + vehicle, Sham + vehicle *vs.* Sham + imipramine and OB + vehicle *vs.* OB + imipramine using a Student Newman Keuls (SNK) *post-hoc* comparison test where appropriate. Data were deemed significant when $p < 0.05$.

Results

Rat

ANOVA of distance travelled showed an effect of OB [$F(1, 28) = 5.37$ $p = 0.029$] and imipramine [$F(1, 28) = 6.99$ $p = 0.014$]. *Post-hoc* comparisons revealed that OB rats exhibited hyperactivity in the open field arena compared to sham-operated controls ($p < 0.05$). Chronic imipramine administration attenuated the OB-induced hyperactivity when compared to their vehicle-treated counterparts ($p < 0.05$) (Fig. 1). ANOVA of distance travelled in the outer perimeter of the arena showed an effect of OB [$F(1, 28) = 5.41$ $p = 0.029$] and imipramine [$F(1, 28) = 7.79$ $p = 0.010$]. *Post-hoc* comparisons revealed that OB increased distance travelled when compared to sham-operated controls ($p < 0.05$) (Tab. 1). Chronic imipramine treatment attenuated the OB-related increase in distance travelled in the outer perimeter when compared to their vehicle-treated counterparts ($p < 0.05$). ANOVA of distance travelled in the inner arena showed an effect of imipramine [$F(1, 28) = 6.15$ $p = 0.020$]. *Post-hoc* comparisons revealed no significant differences between the groups (Tab. 1).

Mouse

ANOVA of the distance travelled showed an effect of OB [$F(1, 27) = 31.44$ $p < 0.001$] imipramine [$F(1, 27) = 7.70$ $p = 0.011$] and OB x imipramine interaction [$F(1, 27) = 6.16$ $p = 0.021$]. *Post-hoc* analysis revealed an increase in distance travelled in OB mice upon exposure to the open field when compared to sham-operated controls ($p < 0.01$). Chronic imipramine administration attenuated the OB-induced hyperactivity following 5 weeks of treatment when compared to their vehicle-treated counterparts ($p < 0.01$) (Fig. 1). ANOVA of distance travelled in the outer perimeter showed an effect of OB [$F(1, 27) = 22.28$ $p < 0.001$] imipramine [$F(1, 27) = 6.53$ $p = 0.017$] and OB x imipramine interaction [$F(1, 27) = 5.36$ $p = 0.030$]. *Post-hoc* comparisons revealed that OB induced an increase in distance travelled when compared to sham-operated controls ($p < 0.01$). Chronic imipramine treatment attenuated the OB-related increase in dis-

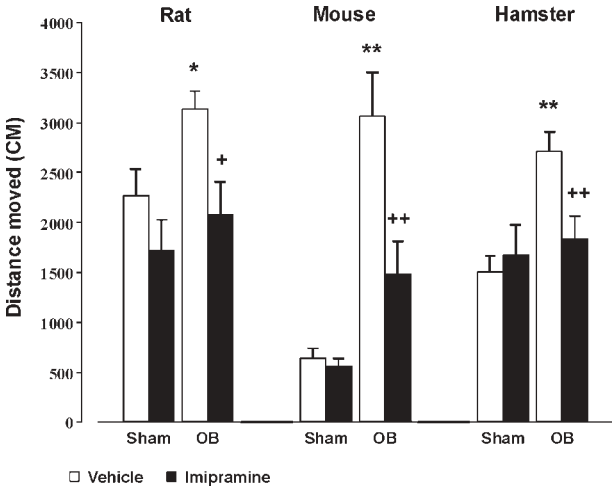

Fig. 1. Chronic imipramine treatment attenuates OB-induced increase in distance travelled (cm) in the rat (n = 7–8), mouse (n = 6–8) and hamster (n = 5–6) during exposure to a 5-min open field test. Data are expressed as the mean ± SEM. * $p < 0.05$, ** $p < 0.01$ compared to vehicle-treated sham-operated controls. $^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$ compared to vehicle-treated OB

Tab. 1. Effect of olfactory bulbectomy and chronic imipramine treatment on distance travelled in the perimeter and central arena of the open field

		S + V	S + IMI	OB + V	OB + IMI
Rat	Perimeter	2120 ± 277	1683 ± 316	3102 ± 176*	1991 ± 300^{+}
	Central Arena	51 ± 19	32 ± 11	69 ± 9	23 ± 12
Mouse	Perimeter	547 ± 82	482 ± 76	2452 ± 422**	1134 ± 226^{++}
	Central Arena	97 ± 21	76 ± 15	601 ± 60**	362 ± 108^{+}
Hamster	Perimeter	1173 ± 165	1417 ± 246	2356 ± 180**	1540 ± 224^{+}
	Central Arena	248 ± 76	265 ± 57	312 ± 69	277 ± 58

Rats and hamsters were administered imipramine (10 mg/kg per day) for 2 weeks. Mice were administered imipramine (40 mg/kg per day) for 5 weeks. Data expressed as the mean distance travelled (cm) ± SEM over the 5-min test period. S – sham-operated control; OB – olfactory bulbectomy, V – vehicle, IMI – imipramine. N = 5–8 per group. * $p < 0.05$, ** $p < 0.01$ compared to vehicle-treated sham-operated controls. $^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$ compared to vehicle-treated OB

tance travelled in the outer arena when compared to their vehicle-treated counterparts ($p < 0.01$) (Tab. 1). ANOVA of distance travelled in the inner arena showed an effect of OB [$F(1, 27) = 43.86$ $p < 0.001$] and imipramine [$F(1, 27) = 4.74$ $p = 0.039$]. *Post-hoc* analysis revealed that chronic imipramine treatment attenuated the OB-induced increase in distance travelled in the inner arena when compared to their vehicle-treated counterparts ($p < 0.05$) (Tab. 1).

Hamster

ANOVA of the distance travelled showed an effect of OB [$F(1, 21) = 9.36$ $p = 0.007$] and an OB × imipramine interaction [$F(1, 21) = 5.41$ $p = 0.032$]. *Post-hoc* comparisons revealed an increase in distance travelled of OB hamsters in the open field arena when compared to sham-operated controls ($p < 0.01$). Chronic imipramine treatment attenuated the OB-related hyperactivity in hamsters ($p < 0.01$) when compared to their vehicle-treated counterparts (Fig. 1). ANOVA of distance travelled in the outer perimeter showed an effect of OB [$F(1, 21) = 9.27$ $p = 0.007$] and an OB × imipramine interaction [$F(1, 21) = 6.09$ $p = 0.024$]. *Post-hoc* comparisons revealed that OB induced an increase in distance travelled when compared to sham-operated controls ($p < 0.01$). Chronic imipramine treatment attenuated the OB-induced hyperactivity when compared to vehicle-treated counterparts ($p < 0.05$). ANOVA of distance travelled in the inner arena showed no effect of OB, imipramine or an interaction effect (Tab. 1).

Discussion

The data presented in this study confirm previous findings of OB-induced hyperactivity in the rat [3, 7, 8] and mouse [20] in the open field test, and extend these to include the hamster. This is the first study to demonstrate that OB-related hyperactivity generalizes to various rodent species and that such hyperactivity is sensitive to chronic imipramine administration.

OB rats and hamsters exhibited comparable increases (~180%) in activity over the 5-min test period when compared to their sham-operated counterparts. The extent of the increase in activity in the OB rat is similar to that reported in other studies [4, 20]. Hyperactivity in both the OB rat and hamster was attributed to an increase in activity in the perimeter of the test arena as opposed to increased activity throughout the open field arena. This pattern of hyperactivity was attenuated following chronic imipramine treatment in both rats and hamsters. Mice displayed the greatest OB-induced increase in activity (320%) when compared to sham-operated controls indicating a more pronounced response in this species under similar test conditions. In contrast to the rat and hamster, OB-induced hyperactivity in the mouse can be attributed to both increased activity in both perimeter and central zones of the open field arena. Increased exploration of the central zone of the test arena suggests a pattern of reduced defensive behaviour [17] which may stem from neurobiological changes in brain areas associated with defensive behaviors such as the

amygdala. The large increase in locomotor activity in the OB mouse and potentially the strain of mouse used [5, 6] may account for the increased treatment time required for the antidepressant response to develop (5 weeks) when compared to the rat and hamster (2 weeks).

In conclusion, the present results demonstrate that the OB model of depression and antidepressant action generalizes to rat, mouse and hamster rodent species. The additional characterization of the model in mice creates opportunities for the use of transgenic mice to explore neurobiological substrates underlying antidepressant action in the model. Moreover, development of the model in hamsters should facilitate research on new antidepressant therapies in those species with greater pharmacological homology to the human.

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