Chapter 17

The Olfactory Bulbectomised Mouse

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Abstract

Removal of the olfactory bulbs from the rodent induces neuronal reorganisation and the expression of behavioural, neurochemical, neuroendocrine and immune changes that resemble those observed in major depressive disorder. As such this model is widely used to examine the neurobiological substrates that may underlie the pathophysiology of depression and screen antidepressant agents. One of the most consistent changes observed in the olfactory bulbectomised (OB) mouse model is hyperactivity on exposure to a novel stressful environment. This behavioural response is attenuated selectively by chronic, but not acute, antidepressant treatment. This chapter provides a detailed protocol on the establishment of the OB mouse model and assessment of OB-related increase in locomotor activity in the open field test. Experimental variables which may impact on the results will be presented in addition to a short troubleshooting guide.

Key words: Olfactory bulbectomy, Depression, Antidepressants, Mouse, Mouse model of depression

1. Background and Historical Overview

Numerous attempts and approaches have been employed to develop animal models of major depressive disorder; however, due to the complex nature of the condition, no one model encompasses all of the hallmarks of the disorder or is without shortcomings. Models that most closely resemble the human condition attempt to fulfil criteria of construct, face and predictive validity. Evaluating models in terms of these criteria has revealed that the OB rodent possesses the highest degree of validity when compared against developmental and genetic models of predisposition to depression (1). Developed over 35 year ago by Cairncross and colleagues (2, 3), the OB rodent is a well-recognised and reproducible model of depression and antidepressant activity. Removal of the olfactory bulbs induces behavioural, neurotransmitter, neuroendocrine and immune changes resembling those reported in depressed patients (for reviews see (4, 5)). In addition to the strong

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face validity for this model, the OB model displays one of the best portfolios in terms of predictive validity of antidepressant activity following chronic administration. The behavioural and physiological alterations displayed cannot be explained due to the loss of smell (anosmia) alone (6, 7) and are believed to result from compensatory neuronal reorganisation in cortical-hippocampal-amygdaloid circuits following removal of the bulbs (5). The olfactory bulbs are integrally connected with the limbic system, particularly the amygdala, structural and functional alteration has been reported in both the depressed patient (8–10) and in the OB model (11–13). Neuronal degeneration and remodelling occurs in the cortex, amygdala, hippocampus, raphe nuclei and locus coeruleus following bulbectomy, effects reversed by chronic antidepressant treatment (14–18). The resultant neurochemical changes and dysinhibition of the amygdala have been proposed to underlie many of the behavioural changes in the model (12, 19–22). Furthermore, cognitive impairment in depression has been correlated with reduced hippocampal volume and cell density (23–25), alterations in which have also been observed in the OB model (11, 15). Hence, removal of the olfactory bulbs in the rodent adversely affects the homeostatic regulation of impulse traffic within cortical and limbic system structures, mimicking functional alterations that occur in the depressed state.

Correlating with symptoms observed in the human situation, behavioural changes reported in the OB rodent include anhedonia (6, 26–29), decreased social behaviour (30–32), deficits in learning and memory (7, 15, 21, 33–35), reduced sexual behaviour (36, 37) and impaired reactivity to stressful environments (38–42). Although predominantly assessed in the rat, an increasing number of studies have examined behavioural changes following bulbectomy in mice. It should be cautioned that data obtained in rats should not be over extrapolated to mice (43); however, to date similar behavioural changes have been demonstrated in both species following bulbectomy. Table 1 presents the current behavioural changes reported in OB mice. An overview of the more commonly used tests and paradigms employed to evaluate behavioural changes in the OB mouse model is presented forthwith. Particular emphasis is placed on assessment of activity in a novel open field area, the most widely evaluated behaviour in the model.

Alterations in learning and memory following bulbectomy have been evaluated in several behavioural paradigms (passive avoidance, novel object recognition, T-maze, Y-maze and morris water maze) (Table 1). Overall, OB mice exhibit cognitive impairments and deficits in spatial memory (34, 35, 44–48). Both acute and chronic antidepressant administration reverse the behavioural deficit in passive avoidance in rats (4, 5); however, considerably less studies have examined the response to antidepressants in OB mice. Chronic administration of the tricyclic antidepressants amitriptyline

Table 1
Behavioural changes observed in the OB mouse model

Behavioural test	Strain	OB-induced effect	References
Open field	C57BL/6	Increase in locomotor activity	(40–42, 48, 49, 52–54)
	DBA	Increase in locomotor activity	(49)
	Swiss	No effect	(47)
Saccharine/sucrose preference	C57BL/6	Reduction in saccharine/sucrose preference (anhedonia)	(28, 29, 64)
Elevated plus maze	ddY	No effect	(15)
Forced swim test	Swiss	Decreased duration of immobility	(47)
Hole-board test	ICR	Increased frequency of head dips No effect on locomotor activity	(57, 58)
Interspecies aggression	Swiss	Reduced aggressive behaviour	(32, 47)
Passive avoidance	C57BL/6	Impaired passive avoidance	(42, 49)
	ddY	Impaired passive avoidance	(15, 35, 46)
	DBA	No effect	(49)
Active avoidance	C57BL/6	Impaired active avoidance	(49)
	DBA	No effect	(49)
Novel object test	C57BL/6	Increased time exploring novel object	(41)
	ddY	Fail to discriminate between new and old object	(45, 50)
T-maze	C57BL/6	No preference for novel arm	(41)
Y-maze	ddY	Reduced spontaneous alternation	(45, 50)
Morris water maze	C57BL/6	Impaired spatial memory	(51)
	Swiss	Impaired spatial memory	(47)
	NMRI	Impaired spatial memory	(34, 65)
Maternal behaviour	ddY	Impaired maternal behaviour	(64, 66)

and imipramine, and the atypical antidepressant trazodone, attenuates OB-induced impairments in passive avoidance in C57BL/6 mice (49); however, a subsequent study failed to demonstrate an effect of amitriptyline (42) on this behavioural response. Similarly, reports of both the effectiveness (42) and ineffectiveness (48) of chronic treatment with the selective serotonin re-uptake inhibitor citalopram, in reversing the passive avoidance deficits in OB mice, have been presented. However, passive avoidance deficits

in DBA mice following bulbectomy were not attenuated by antidepressant treatment (49), highlighting that background strain may influence behavioural responding of OB mice. Impaired learning and memory in the model are accompanied by a loss of cholinergic neurons (15, 45, 46, 50) and increased brain β-amyloid (34, 51); therefore, the OB mouse also provides a useful experimental model for examining dementia associated with Alzheimer's disease. Acute and chronic treatment with cholinesterase inhibitors, muscarinic agonists (15) and potential cognitive enhancers (34, 45, 46, 50) ameliorates the memory impairments exhibited by OB mice.

On exposure to a novel, open field environment, OB mice exhibit behavioural hyperactivity (40–42, 48, 49, 52–54). This characteristic behavioural hallmark of the model has been proposed to exemplify psychomotor agitated depression (36). OB-induced hyperactivity is dependent on a number of factors, including the shape, size and aversiveness of the open field area (see experimental variables) and is associated with stress-induced behaviours such as thigmotaxis (time spent and activity along the walls of the arena) and defecation. A detailed protocol for evaluating OB-related hyperactivity in the open field test has been provided below. Based on behavioural observations in OB rats, the nature of this hyperactivity has been attributed to an inability to mount appropriate stress or defensive responses (38, 39, 55). The inability to habituate and inhibit behaviour in novel stressful environments or situations has also demonstrated in other acute stress regimes such as the elevated plus maze, T-maze, passive avoidance and Vogel's conflict test (38, 41, 56). Similarly, increases in emotionality and impulsivity have been demonstrated in OB mice exposed to the hole-board test (57, 58). OB-induced hyperactivity in the open field is the only behaviour currently known to selectively respond to chronic, but not acute, treatment with antidepressants. The necessity for repeated administration to correct this behavioural aberration mimics the clinical time-course of antidepressant action and distinguishes the OB model from many other simulations of depression and tests of antidepressant action. As such, attenuation of this behavioural response in OB rats is widely used as a means to screen potential new antidepressant agents. In comparison, there has not been extensive validation of pharmacologically diverse types of antidepressant drugs in mice using the OB model. Chronic treatment of OB mice with the antidepressants amitriptyline, imipramine, trazodone and citalopram reverses the hyperactivity observed on exposure to the open test (40, 42, 48, 49). Extending the characterisation of the model in mice has enabled genetically modified animals to be examined and therefore the study of neural mechanisms that subserve emotional responses. OB mice with targeted deletion of the tac 1 gene, which encodes for the neuropeptide substance P, do not exhibit behavioural hyperactivity in the open field test (54) or decreased preference for saccharine solution

(measure of anhedonia) (28). Thus, substance P neurotransmission mediates, at least in part, the behavioural responses observed in the OB mouse model.

2. Experimental Procedures

2.1. Animals and Housing

Mice may be housed in groups or individually, usually in plastic bottom cages containing wood shavings as bedding. The animals should be maintained in standard housing conditions of constant temperature (20 ± 2°C) and standard lighting (e.g. 12:12 h light–dark). Food and water should be available ad libitum. At least 7 days should be allowed for animals to acclimatise following any changes to environmental conditions prior to behavioural testing or surgery. The most widely assessed behavioural parameter in the OB model is hyperactivity on exposure to a novel environment. As such it is advisable that animals are tested in the open field test or equivalent prior to surgery to ensure comparable baseline activity between mice assigned to sham and OB groups.

2.2. OB and Sham Surgery

All procedures should be carried out under appropriate ethical approval and comply with national and international regulations regarding the use of animals for research purposes.

2.2.1. Equipment, Materials and Setup

1. Appropriate surgical facilities and equipment are required. The surgical area should be cleaned and disinfected (e.g. 1–3% Milton disinfectant (Procter and Gamble, Ireland) or 10–70% alcohol). Surgical instruments (scalpel and retractors (Fine Science Tools, Germany)) should be sterilised prior to surgery. Setup should be in close proximity to a sink area equipped with an aspiration pump (also known as water aspirator or venturi pump, e.g. Vacu/Trol vacuum water aspirator, Spectrum Europe, the Netherlands), which efficiently generates a vacuum when connected to a standard laboratory tap. This is the most commonly used method to remove the olfactory bulbs from the olfactory cavity. This process may be facilitated by attachment of a blunt 16G hypodermic needle or fine glass pipette to the vacuum hose on the aspirator. Additional equipment required include mouse stereotactic frame (Harvard Apparatus, UK), homoeothermic blanket with temperature controller or equivalent (CMA, Sweden or Harvard Apparatus, UK), high speed micro drill with 2.1 mm burr (Fine Science Tools, Germany) and hair clippers. Surgical area may be set up similar to that depicted in Fig. 1.
2. The most common means of anaesthetising animals is the use of a combination of Xylazin (80 mg/kg; Bayer, Germany) and

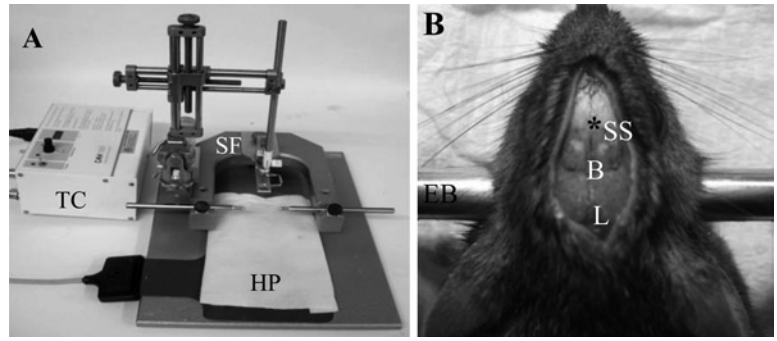

Fig. 1. (**a**) Example of a surgical setup area. *SF* stereotactic frame; *HP* heating pad; *TC* temperature controller. (**b**) Representative landmarks on the mouse skull. *Asterisk* represents proposed site for burr hole required to remove the olfactory bulbs. *B* bregma; *L* lambda; *SS* sagittal suture; *EB* ear bars.

Ketamine (100 mg/kg; Aventis Pharma, Germany), although other forms of injectable anaesthesia such as pentobarbital sodium (50 mg/kg; Sigma, Ireland) have also been used. OB surgery may be performed under inhalation anaesthesia such as Isoflurane (Isoflo (1–3% in O_2; 0.5 L/min), Abbott, Ireland) which allows for excellent control of anaesthesia and rapid recovery following surgery.

3. Additional reagents required: Betadine or Povidone (iodine) solution, sterile alcohol swabs, local anaesthetic (e.g. provocain), cotton gauze/swabs, haemostatic sponges (cut to 2 mm pieces; Dental supply companies) and/or bone wax (Fine Science Tools, Germany), antibiotic powder (e.g. Neomycin, Sigma, Ireland), sutures (3-0 Vicryl) or equivalent (e.g. Histoacryl, Aesculap Germany), sterile injectable saline (0.89% NaCl), analgesia (e.g. the nonsteroidal anti-inflammatory agent, carprofen (1.25 mg/25 μL s.c., Rimadyl, Pfizer, UK)), and hypodermic needles (25–30G).

2.2.2. Procedure

1. Remove the mouse from the cage and record body weight.
2. Anaesthetise the mouse and monitor depth of anaesthesia by toe and tail pinch.
3. Once unconscious, secure the head in the stereotactic frame using the ear bars and nose clamp. Take care not the damage the skull when mounting on the ear bars. Ensure that the head is horizontal to the stereotactic frame.
4. Place temperature probe into animal's rectum and note body temperature. The heating blanket/pad and temperature controller will act to maintain body temperature (~37°C) during surgery. Extra fabric can be placed over animal to prevent excess heat loss.

5. Apply saline to animal's eyes to prevent corneal damage.
6. Shave the superior surface of the head. Clean the area to be incised using iodine solution followed by an alcohol swab. Apply local anaesthetic to the area.
7. Analgesia may be administered prior to surgery to manage post-operative pain.
8. Make a midline longitudinal incision (2–3 cm) with a sterile scalpel blade in the scalp over the junction of the frontal and nasal bone of the skull (midway between the eyes). Retract the skin, scrape back the periosteum and dry the skull using a cotton swab.
9. Identify the sagittal suture and bregma (Fig. 1). Again ensure that the skull is parallel to the horizontal plane of the stereotactic apparatus by adjusting the nose clamp.
10. Mark the coordinates of the entry point on the surface of the skull. Coordinates should be chosen prior to surgery. For mice one hole (~2 mm diameter), 4 mm rostral to bregma (or 1 mm rostral to the sagittal suture) on the midline, is sufficient. Alternatively, mark two 1 mm holes directly above each bulb, 1 mm rostral to the sagittal suture and 1 mm lateral to midline (ML ± 1 mm). Drill the burr hole(s) at the marked entry point(s) and pierce the dura with a fine hypodermic needle.
11. For sham surgery, stop any bleeding that may have resulted, dry the skull with a cotton swab, fill burr hole with bone wax, apply antibiotic power to the area and close the skin with interrupted sutures or equivalent (proceed to point 16). Should damage to the olfactory bulbs during the procedure be suspected, it is advisable that the bulbs are removed.
12. For bulbectomy surgery, run water through the aspirator pump to create a vacuum.
13. In order to remove the olfactory bulbs, pierce the bulbs with a sterile hypodermic needle and carefully aspirate the bulbs from the individual cavities with the aid of the blunt needle or glass pipette attached to the vacuum hose. Ensure that the needle is inserted vertically, not at an angle, to avoid damage to the frontal cortex or olfactory tubercles.
14. Fill the burr hole with haemostatic sponge to stop bleeding. Bone wax may be used to seal over the burr hole(s). Ensure all bleeding has stopped prior to wound closure.
15. Apply antibiotic power to the wound and close the skin using interrupted sutures or equivalent.
16. Remove the mouse from the frame and administer 1 mL of sterile saline (i.p.) to maintain hydration.
17. Transfer the mouse to a clean warm cage until recovery from anaesthesia.

18. Re-house in new cage with fresh bedding and free access to food and water. As OB animals are rendered anosmic, it is advisable that food be placed in the base of the animals' home cage for the first 24 h in order to encourage food consumption.
19. Post-operative care: Body weight should be carefully monitored throughout the experiment as OB mice exhibit a more profound reduction in body weight following surgery when compared to sham-operated mice. Animals that lose more than 20% of their body weight in the first 24 h should be excluded. Animals should be checked for post-operative wound infection and handled regularly post surgery in order to reduce aggression that is known to develop following surgery.

2.2.3. Anticipated Results

Following OB surgery, the olfactory bulbs should be completely removed and the animal should be rendered anosmic. The most common means of verifying olfactory bulb removal is gross inspection following completion of the study. Careful examination of olfactory cavity for incomplete removal of one or both bulbs or damage to the frontal cortex following OB surgery necessitates removal of the animal from analysis. Verification that neuronal damage to the frontal cortex was not induced following removal of the olfactory bulbs may also be confirmed using Nissl (47) or thionin (32) staining and histological examination. A criterion of removal of at least two-thirds of the olfactory bulbs and lesioning of part of the olfactory nuclei has also been used as indications of successful bulbectomy. However, olfactory function can remain with relatively small bulb remnants (36) and therefore should an investigator employ such criteria it is important that anosmia is confirmed. Animals should be eliminated if damage to the olfactory bulb(s) is observed following sham surgery.

Although not routinely examined, anosmia may be confirmed following removal of the olfactory bulbs by replacing water with a bitter, scented (0.1% amyl acetate + 0.4% quinine) (32) or lithium chloride (0.12 M) (29) solution. Sham mice learn to associate the smell with the bitter unpleasant taste and avoid the solution, drinking only when plain water is presented. By contrast, if mice have been rendered anosmic, they lick both solutions in order to differentiate between water and the unpleasant solution. Anosmia may also be determined by examining the latency to approach a novel odour (e.g. vanilla extract) or find hidden food in the home cage.

2.3. OB-Induced Hyperactivity

Enhanced locomotor activity is the most commonly examined behavioural change observed in the OB mouse model, generally assessed using the open field test, although also observed in other behavioural paradigms including locomotor activity monitors (actometers), the T-maze and upon exposure to a novel home cage (41, 42) (Table 1). A comprehensive protocol relating to the open field test has been provided by Gould and colleagues in Volume I

of the Neuromethods series on Mood and Anxiety Related Phenotypes in Mice (59). As such the protocol presented below pays particular attention to amendments in the procedure required to detect OB-induced increases in locomotor activity in this test. Activity in the open field is most commonly assessed 14 days following surgery.

2.3.1. Equipment, Materials and Setup

1. The room used to conduct the open field test should be isolated from sound; however, if this is not possible a while noise generator (San Diego Instruments, US or equivalent) can be used. Ensure that noise generator is turned on prior to introducing animal into the test room.
2. Open field arenas have been constructed of various materials (wood, metal, plastic), in numerous shapes (circular, square, rectangle) and sizes. Our studies have found that OB-induced hyperactivity is reliably detected when assessed in a large aversive arena (e.g. circular arena (75–90 cm diameter) with aluminium walls (60 cm high) and white floor) (see experimental variables) (Fig. 2). Large arenas also allow for behaviours such as thigmotaxis (distance moved or time in outer perimeter) and anxiety-related behaviours (time in the centre zone) to be assessed. Although no consensus on whether arenas should be cleaned between test subjects has been reached, the majority of studies employ a regime of cleaning with mild detergent or disinfectant (e.g. 1% Milton disinfectant solution or 10–50% alcohol).
3. Various lighting conditions have been reported in the open field test; however, OB-induced hyperactivity is most consistently observed when testing occurs in brightly lit open field arena (Lux > 100) (see experimental variables). In general, the open field should be evenly illuminated, the light intensity recorded using a lux metre and reported in all publications.

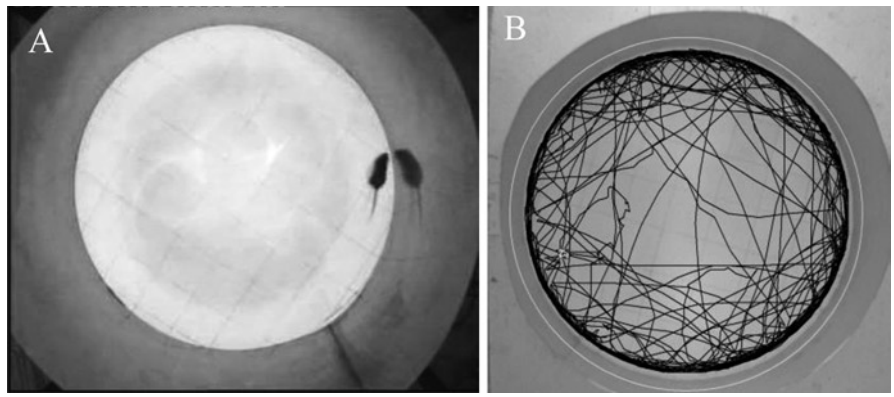

Fig. 2. Representative images of (**a**) OB mouse in the open field test and (**b**) automated video tracking of the open field test using EthoVision software (Noldus, the Netherlands).

4. Behaviour in the open field test is most commonly assessed over a period of 3–5 min although longer periods have also been examined. Manual rating of open field activity generally involves determining the number of line crosses on the base of the arena (e.g. 10 × 10 cm squares painted on base of arena) and frequency of rearing. An increasing number of studies now employ either photocell automated open field (Opto M3, Columbus, US) or video tracking software (EthoVision, Noldus, the Netherlands; Fig. 2b) to assess locomotor activity. Irrespective of the means of assessing behaviour in the open field, it is advisable that the open field test be recorded by video camera onto DVD to enable reassessment of behaviour at a later date if required.
5. Drug treatment generally commences following the establishment and confirmation of OB-induced hyperactivity (14 days post surgery) and continues for a minimum of 14 days. Chronic administration of a number of antidepressants (amitriptyline, imipramine and citalopram) has been demonstrated to attenuate OB-related increase in locomotor activity in the open field (40, 42, 48, 49). When evaluating antidepressant-like activity of novel compounds in the OB model, it is advisable that a reference antidepressant be included in the experimental design.

2.3.2. Procedure

Prior to behavioural testing, animals within an experiment should be handled in the same manner. Due to the potential for the development of OB-induced aggression (particularly in individually housed animals), our experimental protocol calls for daily handling of mice post surgery. The animals' home cage should not be changed within the last 24 h prior to open field testing. Overall these procedures act to minimise background stress that may impact on behavioural assessments.

1. As the open field test is used to determine not only locomotor activity but also behavioural responding of OB mice in a novel, stressful environment, animals need not be acclimatised to the test room, particularly if located close to the holding room and there is minimal change in lighting, temperature and humidity difference between the rooms. In instances where animals must be transported and habituated to the test room, mice should acclimatised to the room but not to the high illumination associated with the open field test, for at least an hour prior to testing.
2. Clean open field arena thoroughly with mild detergent or disinfectant prior to testing.
3. Turn on video camera, photocell and/or video tracking software. In order to easily rescore behaviour of individual animals from DVD at a later time point, it is recommended that an

identity number be placed beside the arena (out of sight of the animal) that can be seen by the video camera.

4. Place the mouse in the open field. Although the position which investigators place the mouse in the open field varies between laboratories (centre vs. perimeter), positioning along the perimeter allows for the latency to enter the centre zone to be assessed, a useful measure of anxiety-related behaviour.
5. Set the timer.
6. During the test the investigator should either leave the room or position themselves as far away from the arena and remaining as still as possible during the trial.
7. At the end of the trial the mouse should be removed from the arena and placed into a new cage.
8. The number of faecal boli should be counted (as a measure of anxiety). Other behaviours may be assessed manually from video or automatically recorded using photobeam or tracking software.
9. The arena should be thoroughly cleaned and dried before the next mouse is introduced.

Data collected: Behaviours recorded manually are primarily the number of line crosses, frequency of rearing and number of faecal boli. Although, it is possible to determine the time spent, latency to enter and number of line crosses in the centre zone manually, these events are rarely reported in studies employing this method of evaluation. In comparison, large amounts of data can be easily and quickly generated with the use of automated systems, particular video tracking software (Fig. 2). This technology also eliminates much of the subjectivity associated with manual recording. Behavioural output from these systems generally includes distance moved (in the entire arena and in different zones), duration of time spent in and latency to enter the centre zone and frequency of rearing. In addition, data can be assessed over the entire duration of the trial or in individual time bins.

2.3.3. Data Analysis and Anticipated Results

Examining the effects of bulbectomy alone on behavioural responding (e.g. distance moved) is generally assessed using *t*-test (sham vs. OB) or repeated measures ANOVA (sham vs. OB effect over time). Two-way ANOVA are routinely used to determine effects of drugs/treatments in the model (with the factors of surgery and treatment).

Prior to surgery, no difference should be noted between groups assigned to sham or OB for any of the behavioural parameters examined, e.g. distance moved or frequency of rearing. However following bulbectomy, mice exhibit enhanced locomotor activity (number of line crosses or distance moved) in the open field

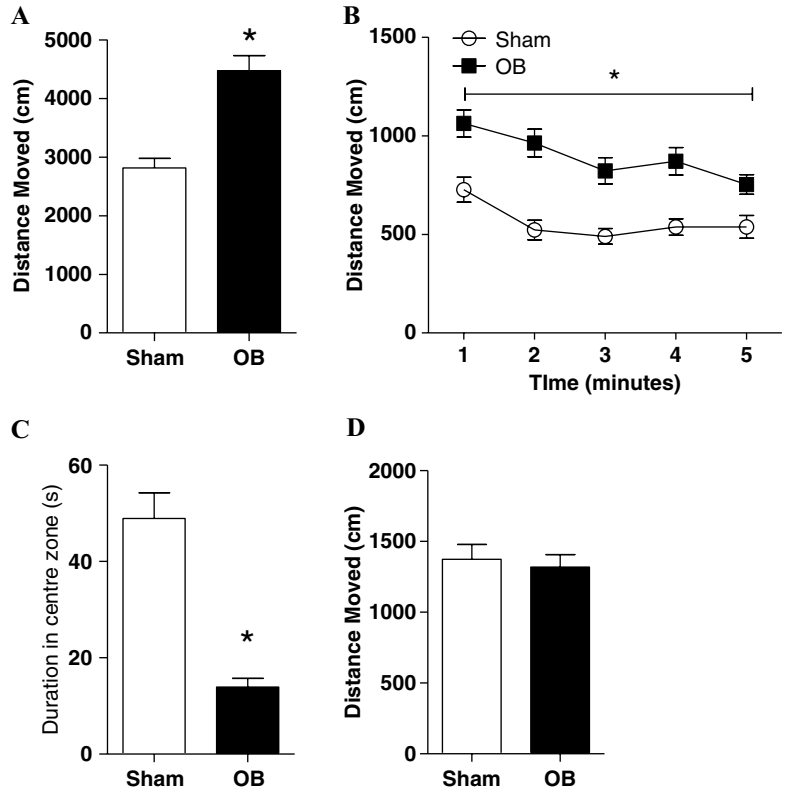

Fig. 3. Typical results that may be obtained using the open field test. (**a**, **b**) OB mice demonstrate an increase in distance moved in the open field over 5 min when compared to sham controls. (**c**) OB mice spend less time during the trial in the centre of the open field when compared to sham controls. (**d**) No significant difference in distance moved between sham and OB mice when tested in a small, dimly lit arena. *$P < 0.05$ sham vs. OB.

(Fig. 3a, b). Although this behavioural change is most commonly assessed 14 days post surgery, development of OB-induced hyperactivity may be observed at an earlier time points (e.g. 7 days) post surgery. The majority of studies assess open field activity over 3–5-min period during which time OB mice do not habituate to the test arena (Fig. 3b). The OB-related increase in distance moved is primarily associated with increased thigmotactic but not anxiolytic behaviour, as OB mice spend less time in the anxiety-related centre of the arena (Fig. 3c).

The antidepressant-like effect of compounds may be determined by their ability to attenuate OB-related increases in locomotor activity in the open field, an effect observed by traditional antidepressants (e.g. tricyclics and SSRIs) following chronic, but not acute, administration.

3. Experimental Variables

A number of experimental variables have been identified which may affect the successful outcome of both the surgery and subsequent behavioural assessment in this particular model and are discussed below.

3.1. Surgical Procedure

As with any surgical procedure, the experience of the surgeon, aseptic technique, the ability to maintain body temperature during surgery and appropriate post-operative care will affect the success of the surgery and the recovery of the animal. OB surgery is a relatively simple procedure and often successful even for those with minimal prior surgical experience. In order to allow for full recovery prior to the nocturnal phase, during which time mice are naturally active and consume most food, it is recommended that surgery is performed during the early light phase. Although not examined directly, it is possible that mice that have not recovered completely from surgery will consume less food during this period and as such loose greater body weight. This may be particularly relevant for the OB mice as they will be rendered anosmic and possibly hypophagic as a consequence, resulting in greater body weight loss post surgery when compared to sham-operated controls. In accordance, anaesthetics that result in rapid recovery from surgery (such an inhalation anaesthetics) should be considered for this procedure. If choosing to use an injectable form of anaesthesia, nonbarbiturates are recommended due to shorter recovery period and reduced complications associated with prolonged anaesthesia. Consideration should also be paid to the appropriate coordinates for surgery and the aspirator vacuum pressure, inaccuracy of which may result in either an inability to remove the olfactory bulbs completely or damage to the frontal cortex.

3.2. Genetic Background

Bilateral olfactory bulbectomy has been performed in several mouse strains although OB-induced hyperactivity has not been assessed in all studies. The most commonly used mouse strain is that of the inbred C57BL/6, where it has been consistently demonstrated that removal of the olfactory bulbs induces hyperactivity in the open field test under several different experimental conditions, an effect reversed by repeated antidepressant treatment. (40, 41, 48, 49, 52–54). Deficits in passive and active avoidance (42, 49) and sucrose and saccharine preference (28, 29) have also been demonstrated following bulbectomy in this strain (Table 1). By contrast, although DBA mice exhibit hyperactivity in the open field test following bulbectomy that is attenuated by chronic antidepressant treatment, a deficit in passive or active avoidance was not observed in this strain of mouse (49). Removal of the olfactory bulbs from Swiss and ICR mice did not result in increased locomotor activity

in the open field (47) or hole-board test (57) respectively, although factors other than genetic background may participate in the inability to detect OB-induced hyperactivity in these strains. The effects of bulbectomy on memory, emotionality and circadian rhythm, but not locomotor activity, have been assessed in ddY, CF-1 and NMRI mice (Table 1). Careful consideration should be paid to the background strain of the mouse, particularly when working with genetically modified mice, when examining OB-induced behavioural alterations and the ability of pharmacological treatments to attenuate such alterations.

3.3. Gender

Male and female rats exhibit differential effects related to sucrose preference but not open field activity following bulbectomy (27, 39). However, no such study has been conducted in mice. It has been demonstrated that both male and female mice exhibit anhedonia following bulbectomy (28, 29); however, it remains to be determined if the magnitude of this response is comparable between the genders. OB-induced behavioural changes have predominantly been conducted in male mice and further studies are required in order to determine if gender differences exist in the OB mouse model. If examining OB-induced effects in both male and female mice, it is important that gender be recognised as a separate factor during analysis.

3.4. Age

The majority of studies using the OB model have been conducted in mice between 2 and 4 months of age (20–30 g body weight), although mice ranging from 18 days to 30 weeks old have also been used. The effect of age on behavioural responses in OB mice is largely unknown; however, age had been demonstrated to induce significant changes on OB behaviour in the rat. Older (19 month) OB rats exhibit greater locomotor activity when compared to young (9 week) rats and although young OB rats demonstrate a loss of passive avoidance and startle reflex when compared to sham-operated animals, this was not observed in older rats (60). Based on these observations, it has been proposed that examining the effects of bulbectomy in aged rodents may provide a model of geriatric depression. When comparing across and between studies, it is important to be aware that the age of the mouse may impact on the behavioural response observed in the model.

3.5. Housing Conditions

Saitoh and colleagues demonstrated that singly housed OB mice demonstrate enhanced emotional behaviour as assessed using the hole-board test but not locomotor activity when compared to group-housed counterparts (57). In addition, our studies indicate that housing conditions do not alter the development of OB-induced hyperactivity in the open field test, although the magnitude of the response was reduced in group-housed (10 per cage; 5 sham + 5 OB) when compared to singly housed mice. As such housing conditions

(single vs. group) may alter OB-induced behavioural responses. Mice are predominantly housed in groups per cage as they are social animals and exhibit stress-like behaviour when individually housed. There is a lack of detail from several studies on whether all sham, all OB or equal amounts of both are housed together following surgery. Group housing configurations may have a profound impact on behavioural assessments in the model particularly as OB mice display impaired social and aggressive behaviour (32, 47). Several studies report housing mice individually post surgery (40, 41, 52). In an attempt to reduce stress associated with singly housing, environmental enrichment may be provided and animals may be handled on a regular basis. It should be noted that environmental enrichment may alter emotional behaviour of mice in the open field test (61). Irrespective of the housing conditions employed, similar conditions should be maintained between studies and reported on research papers.

3.6. Handling

It is well recognised that OB rats are irritable and aggressive following surgery and that frequent handling prior to and post surgery reduces these behavioural traits (22). Similarly, unless irritability and aggressive behaviour are the behavioural outcomes of interest in the experiment, most investigators employ a regime of regular handling of mice following bulbectomy. It is unknown what direct effect handling may have on OB-behaviour other than aggression, although in naïve mice handling does not modify open field behaviour (62). Damage to the frontal cortex during surgery often results in animals remaining irritable despite extensive handling, and these animals should be removed from the analysis. The time of day, means and duration of handling may differ between laboratories; however, it is important that the handling procedure remains consistent between studies.

3.7. Open Field Size, Shape and Lighting

Behavioural hyperactivity in OB mice is most commonly assessed in highly illuminated (100–320 lux) large (50–90 cm diameter) arenas (40–42, 52, 53). Although it has been demonstrated in certain studies that OB mice exhibit hyperactivity in a dimly lit small open field areas (54), observations from both our own laboratory (Fig. 3d) and that of others (47) failed to demonstrate an effect of bulbectomy on locomotor activity when assessed under similar conditions (arena ($22 \times 40 \times 25$) with low level illumination (lux 30–35)). Large arenas also allow for anxiety-related behaviour (time spent in and latency to enter centre area) to be concurrently assessed. The open field arenas used are primarily either black or white and constructed of plastic to allow ease of cleaning. In order to increase the aversive nature of the test, several laboratories have included reflective walls into the area. Illumination is generally provided by light(s) positioned directly above the arena. It is important that the arena is evenly illumi-

nated; otherwise, the mouse will most likely spend a greater proportion of the test time in the darker parts of the arena. In general, testing is conducted without bedding in the arena. Therefore, as previously demonstrated for the rat (4, 63), the design and aversiveness of open field apparatus appears to affect behavioural responses of OB mice.

4. Troubleshooting

4.1. Incomplete Removal of the Olfactory Bulbs or Damage to the Frontal Cortex During OB Surgery

Effects such as these may result from inappropriate vacuum pressure created by the aspirator. Placing a pressure gauge indicator on the pump will allow for the recording and maintenance of an appropriate vacuum pressure to aspirate the bulbs. Damage to the frontal cortex may also occur due to incorrect surgical coordinates. It is recommended that the OB surgery is carried out on a test mouse in order to confirm appropriate coordinates and vacuum pressure required for bulb removal prior to embarking on a large scale study.

4.2. Irritability and Aggression in OB Mice

This is most commonly due to a lack of handling post surgery or damage to the frontal cortex during removal of the bulbs. As such it is recommended that animals are regularly handled prior to and following surgery. Although it remains to be examined, it is also possible that removal of the olfactory bulbs in certain strains of mice may result in enhancement of aggressive behaviour.

4.3. Inability to Detect OB-Induced Hyperactivity

This is most commonly attributed to the design of the testing apparatus. OB-induced hyperactivity is most pronounced when assessed in a large aversive (high illuminated) arena when compared to a small, low illuminated area (see experimental variables). Alternatively, a lack of effect may be due to small sample size. Although the number of animals per group varies drastically between studies, based on our observations, we recommend at least 8–10 mice per group when assessing behavioural changes in the OB model. Ensure all mice are of similar age and have been housed and handled under similar conditions. Consideration should also be paid to the strain of mice used. Olfactory bulbectomy in Swiss mice results in the characteristic impairment in spatial memory but not hyperactivity in the open field (47).

4.4. Inability to Detect Reversal of OB-Induced Hyperactivity Following Treatment

Careful consideration should be given to the strain of mouse (e.g. C57BL/6 vs. DBA), handling, housing (single vs. group) and dosing regimen (dose, frequency of administration, time tested post administration, etc.) employed. Confirm that the studies have sufficient power – a minimum of 8–10 mice per group. Effects of novel compounds should be compared to those of a reference

antidepressant (e.g. imipramine or citalopram) known to attenuate OB-induced hyperactivity. Longer treatment regimes (e.g. 4–6 weeks) may be required when examining effects of novel compounds/treatments, particularly if not monoamine based.

5. Conclusion

In conclusion, the OB mouse model is a robust, reproducible and valid animal model of depression and antidepressant activity. Taking into consideration the experimental variables, investigators with minimal prior surgical and behavioural experience can develop this model with relative ease. Characterisation of the OB model in mice continues to be evaluated in a host of behavioural paradigms, and may extend the models' utility in evaluating novel conditions and treatments. Furthermore, development of the model in mice has afforded the opportunity to examine the involvement of novel neural mechanisms involved in mediating OB-induced behavioural changes through the use of genetically modified mice.

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