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Olfactory bulbectomy in mice induces alterations in exploratory behavior

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Abstract

The olfactory bulbectomy syndrome is thought to represent a rodent model for psychomotor agitated depression. While this model has been extensively characterized in rats, fewer studies have been conducted with mice. Therefore, the present study aimed at extending the characterization of the OBX-induced behavioral syndrome in mice, using tests like open field, novel object exploration, novel cage and T-maze learning. OBX mice exhibited hyperactivity in a brightly illuminated open field, and also in a novel home cage as well as in the T-maze. Furthermore, OBX mice demonstrated increased exploratory behavior in the novel object test and in the T-maze. The complex alterations described here with respect to locomotion and exploration are robust and can be achieved by relatively simple test procedures. The extended behavioral characterization of the murine OBX model may contribute in particular to the increasing need to test transgenic mice for the presence of depression-like behaviors.

Keywords: Olfactory bulbectomy; Mice; T-maze test; Depression model; Novel object test

The bilateral destruction of the olfactory bulbs creates a chronically altered brain state with complex changes of behavioral, neurochemical, neuroendocrinological and neuroimmunological parameters, many of which are comparable to those seen in patients with major depression [8,12,23]. Thus, the olfactory bulbectomy (OBX) in rodents has been proposed to represent a model for chronic psychomotor agitated depression which has also a high predictive validity [7,8,10]. The major behavioral change in this model is a hyperactive response in a brightly illuminated open field arena which is reversed almost exclusively by chronic, but not acute, antidepressant treatment [14,15]. Furthermore, bulbectomy leads in rats to different signs of anhedonia, combined with deficits in spatial learning, avoidance learning, conditioned taste aversion and food-motivated behaviors [10,14]. While the olfactory bulbectomy model has been intensely investigated in rats, fewer studies were conducted with mice. Indeed it has been cautioned not to overextrapolate the data obtained in rats to mice [8]. Apart from the characteristic hyperlocomotion, OBX induces in mice deficits in active and passive avoidance learning [9,11,19,22] and in spatial memory [1,2].

The purpose of the present study was threefold. First, we wanted to know whether in mice the characteristic OBX-induced hyperlocomotor alterations in the aversive, because brightly lit, open field are also found under other test conditions. Secondly, we investigated exploratory behaviors in bulbectomized mice, because altered exploration is a key behavioral feature in OBX rats [15]. Third, since several studies have suggested learning deficits in mice following bulbectomy [1,11,22], we tested OBX mice in a T-maze paradigm, in which the animals' natural tendency to explore novelty as well as short-term memory can be investigated. In summary, we aimed to contribute to a better behavioral characterization of the OBX model in mice.

All animal experiments were approved by the German animal welfare office of the Regierungspäsidium Karlsruhe, Germany. Adult male 3-month-old C57Bl/6N mice were

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purchased from Charles River (Sulzfeld, Germany). Prior to surgery, mice were allowed to acclimatize for 2 weeks to a reversed 12 h dark/light cycle. Animals were housed individually with access to food and water ad libitum.

Olfactory bulbectomy: Olfactory bulbectomy (OBX) was performed as described in detail elsewhere [15]. Briefly, mice were anesthetized with Xylazin 80 mg/kg bw i.p. (Bayer, Leverkusen, Germany) and Ketamin 90 mg/kg bw i.p. (Aventis Pharma, Bad Soden, Germany), diluted in 0.9% saline. The skull covering the bulbs was exposed by skin incision and a burr hole was drilled (Steel burr, No 095956, FST, Heidelberg, Germany), through which both olfactory bulbs were removed by suction with a hypodermic needle attached to a water pump. Finally, the burr hole was filled with bone wax in order to avoid further bleeding. After application of antibiotic powder (Neomycin, SIGMA, Deisenhofen, Germany), the skin was closed with Histoacryl (Aesculap AG, Tuttlingen, Germany). Sham operations were done in the same way, but with the bulbs left intact.

Open field test: The open field test was used to evaluate locomotor activity and exploratory behavior. All mice were tested in this paradigm, because locomotor hyperactivity is the key behavioral feature of bulbectomized mice. We used a circular arena (diameter: 90 cm) with a white plastic floor surrounded by a reflecting aluminium wall, essentially as described elsewhere [13]. Light intensity was 320 Lux. Mice were placed near the sidewall and locomotion was recorded for 5 min by a video camera suspended above the center of the arena. *xy*-coordinates were analyzed by an electronic imaging system (Noldus Etho Vision 2.3). The following parameters of the recorded tracks were evaluated: "distance moved (cm)" and "time spent in center (% of total time)". The center was defined as a circular zone in the middle of the arena (diameter: 15 cm). One day before OBX surgery, basal locomotor activity of the mice was analyzed with the open field test using the same protocol as described above. After recovery of 2 weeks following the surgical procedure, locomotion was re-analyzed to monitor hyperactivity, the classical behavioral consequence of OBX.

Novel object test: The same arena and test conditions were employed as for the open field test. At the end of the open field testing period, a new and unknown object (50 ml Falcon tube, placed top down) was introduced to the center of the arena. Object exploration was assessed for 5 min, monitoring the time spent in close vicinity to the object (diameter: 15 cm) ($n = 20$ OBX; $n = 13$ sham).

Novel cage test: The novel cage test is used to investigate exploratory behavior in a new environment by measuring vertical activity. The test was performed 24 h after the novel object test. Animals were placed in another macrolon cage containing a thin layer of new bedding material as described earlier [5]. Rearings were counted for 5 min ($n = 20$ OBX; $n = 13$ sham).

T-maze: Our T-maze test assesses spatial short-term memory, analyzing the animals' ability to recognize and differentiate between a new unknown and a familiar compartment [6,16]. The T-shaped maze was made of black wood with two 20 cm long arms, which extended right-angled from a 40 cm long alley. The arms had a width of 10 cm and were surrounded by 25 cm high walls. The test consisted of two trials with an intertrial interval of 1 h, during which the animals were put back to their home cage. During an 8 min acquisition trial, one of the short arms was closed. In a 3 min retention trial, mice had access to all three arms. Number of visits and time spent in each of both short arms were assessed. Light intensity was 20 Lux. ($n = 59$ OBX; $n = 33$ Sham). *Statistics:* Data of all experiments were analyzed using one-way-ANOVA or repeated one-way-ANOVA. Statistical calculations were done by Statview 5.0 for Windows.

Two weeks after bulbectomy, OBX mice were clearly hyperactive when compared to sham-operated controls ($p \leq 0.001$; Fig. 1A). Furthermore, OBX animals demonstrated a significant increase in locomotor activity as compared to basal activity levels before the operation (49.85 m versus 40.30 m; $p \leq 0.001$, data not shown). In contrast, the control animals exhibited similar locomotor activity before and after sham operation (41.25 m versus 39.08 m). Analyses of the temporal profiles of locomotion in OBX and control mice exhibited habituation during the open field test, i.e. a gradual decrease in locomotor activity in both groups ($p \leq 0.01$, respectively; Fig. 1B). However, OBX mice were more active than the controls throughout the complete observation period, even during the last minute (Fig. 1B). Despite their locomotor hyperactivity, OBX mice spent less time than sham-operated controls in the center of the open field (9.3% versus 13.97% out of total time; $p \leq 0.05$; Fig. 1C). However, following introduction of a novel object into the center of the arena, OBX animals spent significantly more time exploring the object than the controls (8.88% versus 3.35% out of total time; $p \leq 0.05$; Fig. 1D). OBX mice also showed a significant increase in vertical activity, measured by the number of rearings in the novel cage test (78.7 versus 46; $p \leq 0.001$; data not shown). In the T-maze, OBX mice did not show differences in the number of entries into the new versus the familiar arm 1 h after training. In contrast, sham-operated mice displayed a significant preference for the new arm (Fig. 2). The hyperactivity of OBX mice was also observed in the T-maze paradigm, reflected by a significantly larger number of total entries of OBX mice compared to sham controls (14.11 versus 11.0; $p \leq 0.01$, data not shown).

The present study further characterizes the OBX-induced behavioral syndrome in mice. Under our testing conditions, OBX mice do not only exhibit hyperactivity in a brightly illuminated open field, but also in a novel home cage and a T-maze (Fig. 1A, B and Fig. 2). These results are consistent with the behavioral profile of OBX rats, which demonstrate hyperactivity in the open field test and also in the elevated plus maze [21]. This hyperactive response has been interpretated as a failure or delay to habituate to a novel situation or environment [10,15,17]. The disability to cope with novel