Effect of Low-Intensity Forced Exercise on Hyperemotionality in an Olfactory Bulbectomization Rat Model of Depression

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Abstract:

Exercise therapy has attracted attention as a treatment for depression and has been implemented worldwide. The effects of exercise therapy differ according to the method, frequency, and intensity of exercise, and there is no clear view on the mechanism of action. Olfactory bulbectomization (OBX) is an animal model of depression-like behavior in which surgical removal of the olfactory bulb damages the frontal neurotransmitter system. In this study, we compared the behavior of OBX rats subjected to low-intensity forced exercise with that of rats exposed to antidepressant treatment. Forty male Wistar rats were divided into four groups: Sham, OBX-Saline (Sal), OBX-Imipramine (Imi), and OBX-Exercise (Exe). In the OBX rats, both olfactory bulbs were removed by suction via two holes in the skull. Two weeks after surgery, rats in the Sham, OBX-Sal and OBX-Exe groups were injected intraperitoneally with saline and rats in the OBX-Imi group were injected with imipramine once a daily for 14 days. After the injection, rats in the OBX-Exe group received forced exercise for 30 minutes while rats in the other groups spent 30 minutes in a fixed non-rotated wheel once a day for a total of 14 days .We then evaluated hyperemotionality (HE), performance on the

Y-maze test (YMT) and open-field test (OFT) on day 15. We found that low-intensity forced exercise tend to suppress HE total scores compared with OBX-Sal but there are not significantly difference among almost all groups in OFT and YMT. Therefore, Low-intensity forced exercise may improve depression-like behavior in OBX rats, and additional experiments based on this may reveal its mechanism.

Keywords: Depression, Exercise therapy, Olfactory bulbectomization, Hyperemotionality, Low-intensity, Forced exercise.

Footnotes

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Background:

Depression is a major mental disorder with a lifetime prevalence of 6%–15% [1]. It has also been shown to cause significant challenges in terms of physical health and social engagement [1].

Although pharmacotherapy and psychotherapy are common treatment approaches, exercise therapy for depression has recently attracted attention. Although several meta-analyses have identified antidepressant effects of exercise therapy [2] [3], few general concepts have emerged regarding the optimal parameters required to induce neural activity related to antidepressant properties.

Although many studies have indicated that the greatest effects of exercise therapy for depression are obtained with moderate intensity exercise [4], there have also been reports of significant improvements with lower intensity exercise [5]. Clinical studies [6] have shown that the beneficial effects of exercise vary with the exercise parameters, including exercise intensity, duration, and type.

Although many researchers have attempted to develop animal models of depression, [3][7] various details about the symptoms and pathophysiology of depression remain unclear, challenging the construction of accurate depression models. In olfactory bulbectomized rats, removal of both olfactory bulbs induces behavioral, neurochemical, neuroendocrine, and neuroimmunological changes that are similar to the clinical manifestations of depression. This model is known to have excellent face validity [8]. Removal of the olfactory bulb has been shown to cause atrophy and ventricular enlargement in the cortex, hippocampus, caudate nucleus, and amygdala, suggesting a similarity between these structural changes in the brain and those seen in depressed patients [9].

The most striking behaviors caused by olfactory bulbectomization (OBX) are aggressive behavior and increased activity. Similar behaviors are also seen in the clinical responses to antidepressants [8] [10] when they are administered acutely instead of chronically. Therefore, OBX in rats is considered to be an excellent experimental model of depression for predicting the effects of antidepressants in humans.

Several studies have investigated the effects of exercise on depressive-like behavior in OBX rats in terms of both voluntary exercise [11] [12] and forced exercise [13] of various durations, frequencies, and intensities. However, no studies have examined the effects of low-intensity forced wheel running on depressive-like behavior in OBX rats.

To address this, we subjected OBX rats to low-intensity forced exercise, and examined changes in body weight and depressive-like behavior using hyperemotionality (HE) scores, performance on the open field test (OFT) and Y-maze test (YMT) . We chose to examine the antidepressant effects of low-intensity forced exercise because we anticipated that the results would be more easily generalizable to elderly people and patients with depression complicated by a physical disorder who cannot perform high-intensity exercise.

Materials and Methods:

Animals:

The experiment was conducted using male Wistar rats (6 weeks of age) purchased from CLEA Japan, Inc. The rats were divided into two or three cages and kept in an environment with free access to food and water for two weeks before surgery. The animal room was controlled at a temperature of 22 ± 1 ° C for 24 hours per day and a 12-hour light/dark cycle (Lights on at 8:00 a.m.).

Ethical Approval:

The research protocol followed the National Institute of Health Guide for the Management and Use of Laboratory Animals and was approved by the Fukuoka University Ethical Review Board on Animal Testing (Approval Number 1809061).

Operation:

At 8 weeks of age, the rats were anesthetized with a mixed anesthetic (0.4 mg/kg medetomidine, 2.0 mg/kg midazolam, and 2.5 mg/kg butorphanol). We used a suction to remove both olfactory bulbs through 2 holes in the skull (Front & Back: + 6 mm; Interior: + 1 mm from bregma). Following surgery, rats were housed in single cages (75.5 cm x 21 cm x 17 cm) for 14 days to enable maturation of the OBX model. We operated on and used a total of 29 rats in the experiment. On the first day after the 14-day recovery period, we measured HE scores. This time point was termed day 1. The success of olfactory bulbectomization in all OBX rats was confirmed by autopsy after HE test on day 15. We performed sham surgery in a total of 11 rats. In the sham surgeries, the rats were anesthetized, the skin of the head was incised, and then the incisions were sutured.

Measurement of Hyperemotionality:

We measured behavioral changes in OBX rats using a HE scoring procedure, referred to in previous studies [14)]. Responses to the following stimuli were scored : A) Attack, presenting a rod 4–5 cm in front of the snout; B) Startle, blowing air on the dorsum using a 5-ml syringe; C) Struggle, handling the animal with a gloved hand; D) Fight, gently pinching the tail with mosquito forceps; E) Vocalization, evaluating the degree of squeaking during each above procedure. With the exception of E), the responses were graded as follows: 0, no reaction; 1, slight; 2, moderate; 3, marked; 4, extreme. Stimulus E) was graded as follows: 0, no vocalization; 1, occasional; 2, marked. The sum of these scores became the HE score. Within each group, all of the rats were assessed alternately on the same day by two observers. Each test procedure took a total of 5 min. Only rats that exhibited a HE Total score > 10 on day 1 were selected for further study in the forced running wheel and drug administration experiments. HE scores were measured before drug administration on day 1 and day 15.

Experimental protocol and grouping:

On day 1, we measured HE scores for all rats. Only OBX rats with a HE total score > 10 were included in the following experiments and divided into 3 groups so that their HE total scores were even.

Four groups are composed of Sham (administration of saline and no exercise, n = 11), OBX-Sal（administration of saline and no exercise, n = 9）, OBX-Exe (administration of saline and low-intensity forced exercise, n = 10), and OBX-Imi (administration of Imipramine and no exercise, n = 10). Starting on day 1, rats in the Sham, OBX-Sal and OBX-Exe groups were injected intraperitoneally with saline and rats in the OBX-Imi group were injected with imipramine (10 mg/kg)[15] once a daily for 14 days. After the injection, rats in the OBX-Exe group received forced exercise for 30 minutes while rats in the other groups spent 30 minutes in a fixed non-rotated wheel once a day for a total of 14 days .We purchased imipramine, which is a tricyclic antidepressant, from Sigma-Aldrich Japan.The body weights of each rat were recorded before daily administration. On day 15, we measured HE scores and conducted OFT and YMT for all rats (Fig. 1).

Forced Exercise Protocol:

We purchased a 2-wheel motor running wheel (FWS − 3002 model, dimensions 425 x 450 x 400 cm) from MELQUEST Co., Ltd. Starting on day 1, rats in the forced exercise group exercised for 30 minutes on the motor running wheel once a day for 14 days. The exercise protocol consisted of 10 min of running (start at 5 m/min and gradually increase to 8 m/min), 2 min rest, 10 min of running (start at 5 m/min and gradually increase to 8 m/min), 2 min rest, and 10 min of running (start at 5 m/min and gradually increase to 8 m/min).

We selected this routine according to previous studies [16)], which have used this protocol to induce low-intensity exercise. However, the total driving distance of the running wheel (193.2 m) was not always equal to the total exercise distance because the rats sometimes stopped running.

Open Field Test:

We assessed spontaneous movement and anxiety-like behavior on day 15 by placing individual rats in a dimly lit OFT chamber (90 cm x 90 cm). The brightness was set to 25 lux at the center of the field and 15 lux at the perimeter. We recorded the total distance each rat traveled through the arena for 5 minutes. Data were collected and analyzed using SMART v 3.0 (Panlab Co.).

Y-Maze Test:

On day 15, we evaluated short-term memory in OBX rats. The Y-maze was made of grey-painted wood. Each arm was 35 cm long, 30 cm high, and 11 cm wide. The three arms converged in the middle area of the maze to form an equilateral triangle with sides 11 cm long. The brightness of the light in the maze was adjusted so that it was 7.0 Lux in the center and tip of each arm. In each 5-minute session, a rat was placed at the end of one arm and allowed to move freely within the maze while we visually recorded locomotion. To examine how many times the rats alternated arms (alternation), we measured the number of times the rat left the current arm and entered a different arm 3 times in a row and divided the sum by the total number of times that the rat entered any arm minus 1. The result was then multiplied by 100 to obtain a value that we used as an index of spatial working memory or short-term memory [17)].

Statistical Analysis

We conducted an ANOVA and the Tukey-kramer honestly significant difference test for each procedure using JMP 12.2 software. The results are expressed as the mean ± standard error of the mean. Significance was set as $P < 0.05$.

Results:

Body Weight:

We found that the average body weight gain rate on day 1 compared with that on the day of OBX operation (Fig.1) was significantly lower in the OBX groups compared with the Sham group (Fig. 2 (a)).

At the average weight gain rate on Day 14 compared with that on Day 1, there were no significant differences among all groups. Among them, OBX-Imi group was not significantly lower than OBX-Sal group (Fig. 2(b)).

Hyperemotionality Score:

All the 29 operated rats exhibited a HE score > 10 on day 1. The average HE scores for each group were as follows: Sham 7.0 ± 0.7, OBX-Sal 13.8 ± 0.46, OBX-Exe 12.9 ± 0.62, OBX-Imi 11.6 ± 0.3 (Mean ± S.E.M.). The HE total scores on day 1 were significantly higher in all the OBX groups compared with those in the Sham group. The HE total scores on day 15 were significantly higher in the OBX-Sal group compared with those in the Sham group. The HE total scores on day 15 OBX-Exe and OBX-Imi groups were equal but were not significantly different from that of the Sham and OBX-

Sal groups. The HE total scores of OBX-Exe on day 15 tended to be lower than that of OBX-Sal group (P=0.0636).

Open Field Test:

In the OFT, the total travel distance was significantly longer in the OBX-Exe group compared with the other groups (Figure 4(a)). We found no significant differences among all groups in the percentage of time spent (Figure 4(b)) and the percentage of the distance travelled (Figure 4(c)) in the corner of the arena.

Y-Maze Test:

We found no significant differences in total displacement (Figure 5(a)) and in the rate of alternation (Figure 5(b)) among all groups.

Discussion:

OBX is an animal model of depression that has been shown to have relative facial and predictive validity in rats [18]. This model produces a wide range of behavioral, neurochemical, endocrine, and immunological changes similar to those observed in depressed patients [8] [18]. Behavioral changes induced by OBX, such as short-term memory impairment [19], hyper-locomotion in the OFT [18], and HE [20], are considered to reflect a depressive state [14] [20] and are ameliorated by clinically effective antidepressants.

In contrast with hyper-locomotion, there is no apparent habituation of HE responses following repeated testing [20] [21] [22]. Therefore, OBX-induced HE responses are useful for evaluating antidepressant-like effects in a time-of-day-dependent manner.

In this study, we used weight gain and HE scores on the first day after recovery from surgery (day 1) to confirm whether the OBX model had been achieved. We observed significant weight loss and an increase in HE scores in the OBX groups compared with the Sham group, indicating that we successfully attained the OBX model.

The HE total scores of OBX-Exe and OBX-Imi groups on day 15 were not significantly different from those of Sham group. Considering that only the OBX-Sal group maintained higher total HE scores than that of the Sham group on day 15, those reduced HE scores of the OBX-Exe and OBX-Imi groups suggest that there is a possibility that the antidepressant action is on the way. Furthermore, the fact that only the total HE scores in the OBX-Exe group on day 15 tended to be lower than that of the OBX-Sal group may suggest that low-intensity forced exercise has an antidepressant effect. Although simple comparisons are difficult due to differences in the intensity, frequency, methods of forced exercise and evaluation methods, the duration of the forced exercise in the previous study[13) 16)] for OBX rats was 28 days, so a longer period of low-intensity forced exercise may make it significant difference. These results suggest that the effects of low-intensity forced exercise on depression can be assessed by changing the HE scores, a relatively simple method for evaluating OBX. Therefore, additional research could help elucidate the mechanism of action of exercise therapy, antidepressants, and depression itself in OBX rats.

There was a trend toward weight loss in the administration of imipramine group versus the administration of placebo group over time in the previous study [23)], and noted side effects of imipramine. OBX-Imi group was not significant lower in body weight gain rate at 14 days than that of OBX-Sal group in this study, which may be due in part to a shorter duration than in the previous study [23].

OBX-Exe group travelled a significantly longer distance in the OFT than the other groups on day 15. Although OBX rats are known to be hyperactive in the OFT [15], in contrast with the present study, previous studies have shown that treadmill exercise resulted in decreased activity in OBX rats [13].

Previous studies have reported that increased time spent in the inner zone or decreased time spent in the outer zone of the OFT indicates increased anxiety relief or exploration [24]. There are not significantly difference among all groups in the percentage of time and travel distance spent in the center of the OFT in the present results, so we could not conclude whether increased behavior was associated with anxiety-like behavior. It is possible that the activity levels in the other groups had decreased due to muscle weakness because they did not have the opportunity to exercise.

In previous studies, improvements in spatial working memory and short-term memory following wheel-running exercise were associated with increased expression of Brain-derived neurotrophic factor (BDNF) in the hippocampus [25) 26)]. This mechanism may be involved in the phenomenon which was not significantly difference in the present study. In this study, we did not know whether low-intensity forced exercise improves spatial memory or short-term memory.

This present study had several limitations. First, the assessment of OBX-induced changes in HE may have been subject to inter-rater differences because the raters were not blinded to group identity. Secondly, many results of this study do not show a significant difference or tendency from the OBX-Sal group, which is a comparative control group of antidepressants effects, and there may be problems in this protcol such as a small sample size, a short experimental period, experimental circumstance and strain of rats. Further, we did not examine physiological and structural changes associated with exercise-induced effects on OBX rats. Finally, as noted in previous studies [18)], there is an individual habituation problem in behavioral analysis, so we assessed the effects of exercise by performing OFT and YMT, which are known to cause changes in OBX rats only on day 15. In the present study, the results obtained by OFT and YMT were not significantly different, so they were considered with reference to the results of previous studies. Further comparative examination on day 1 is necessary in order to catch the clear tendency based on the result of this study.

Conclusion:

Our data indicate that low-intensity forced exercise may suppress OBX-induced HE and that it may have similar antidepressant effects. Further, our data indicate that the OBX model of depression might have validity as an animal model of depression that responds not only to antidepressants but also to exercise therapy. We plan to conduct further research to elucidate the mechanisms underlying the effect of low-intensity forced exercise on depression.

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Legends for Figures:

Fig. 1: The experimental procedure

Abbreviations: OBX for olfactory bulbectomization, HE for hyperemotionality, OFT for open field test, YMT for Y-maze test.

Fig. 2: Average body weight gain rate

(a) Average body weight gain rate on day 1

*: $p < 0.05$, vs. Sham

Abbreviations: OBX for olfactory bulbectomization,Exe for exercise, Sal for saline, Imi for imipramine.

Data are represented as the mean ± S.E.M.

(b) Average body weight gain rate on day 14

Abbreviations: OBX for olfactory bulbectomization, Exe for exercise, Sal for saline, Imi for imipramine.

Data are represented as the mean ± S.E.M.

Fig. 3: Total hyperemotionality scores

(a) Total HE scores on day 1

*: $p < 0.05$, vs. Sham

Abbreviations: HE for hyperemotionality,OBX for olfactory bulbectomization, Exe for exercise, Sal for saline, Imi for imipramine.

Data are represented as the mean ± S.E.M.

(b)Total HE scores on day 15

*: $p < 0.05$, vs. Sham

Abbreviations: HE for hyperemotion, OBX for olfactory bulbectomy, Exe for exercise, Sal for saline, Imi for imipramine.

Data are represented as the mean ± S.E.M.

Fig. 4: Open field test

(a) Total distance

#: $p < 0.05$, vs. Sham , OBX-Imi , OBX-Sal

Abbreviations: OBX for olfactory bulbectomization, Exe for exercise, Sal for saline, Imi for imipramine.

Data are represented as the mean ± S.E.M.

(b) Percentage of time spent in periphery of the open field

Abbreviations: OBX for olfactory bulbectomization, Exe for exercise, Sal for saline, Imi for imipramine.

Data are represented as the mean ± S.E.M.

(c) Percentage of distance traveled in periphery of the open field

Abbreviations: OBX for olfactory bulbectomization, Exe for exercise, Sal for saline, Imi for imipramine.

Data are represented as the mean ± S.E.M.

Fig. 5: Y-maze test

(a) Total Distance

Abbreviations: YM for Y-Maze, OBX for olfactory bulbectomization, Exe for exercise, Sal for saline, Imi for imipramine.

Data are represented as the mean ± S.E.M.

(b) Percentage of alternation triplets

Abbreviations: YM for Y-Maze, OBX for olfactory bulbectomization, Exe for exercise, Sal for saline, Imi for imipramine.

Data are represented as the mean ± S.E.M.

Fig. 1:The experimental procedure

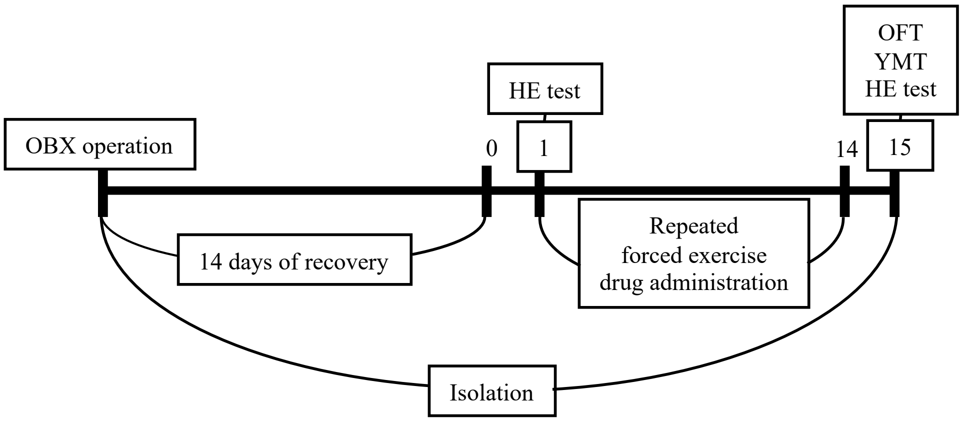


Fig. 2 :Average body weight gain rate

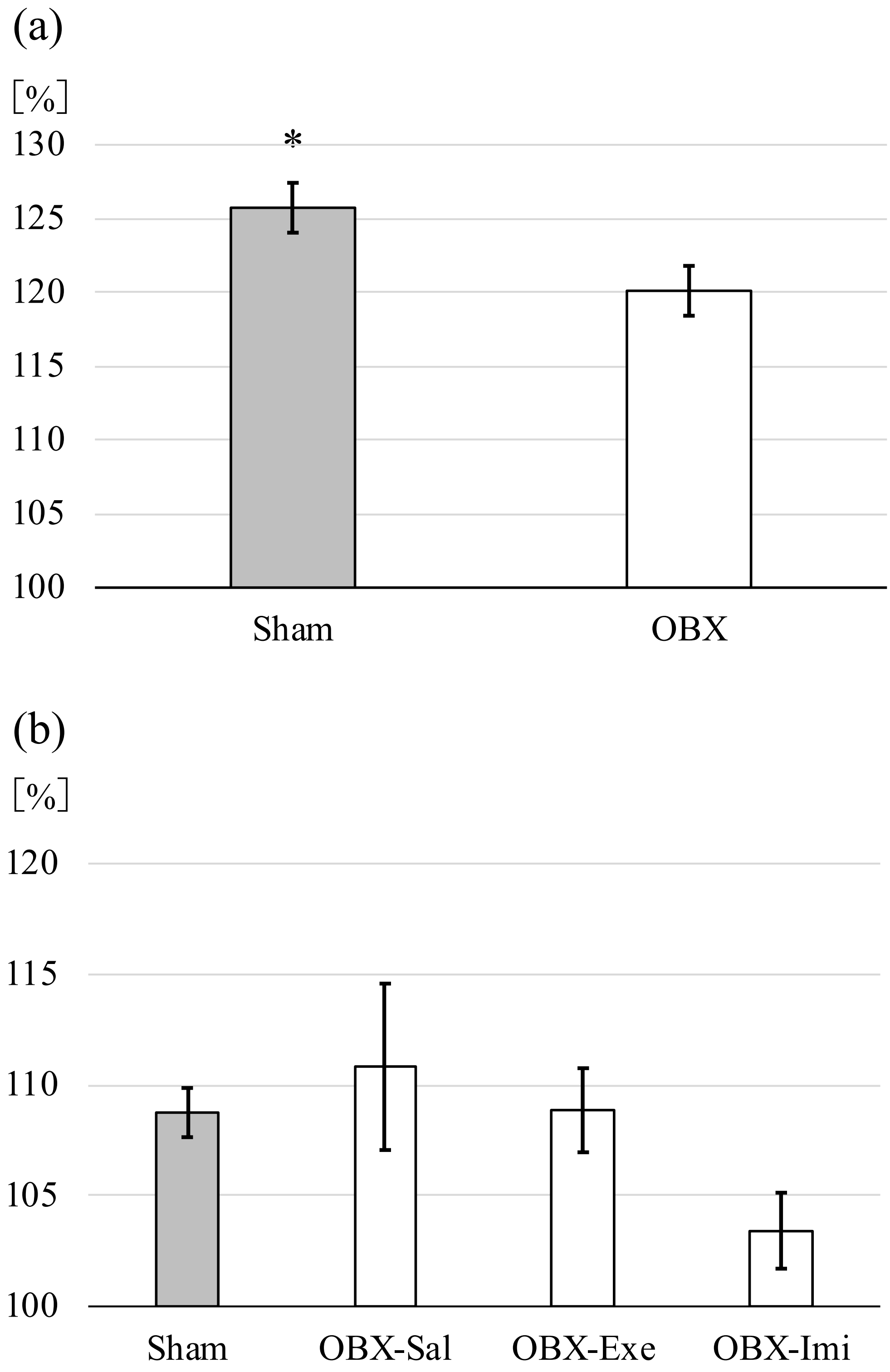
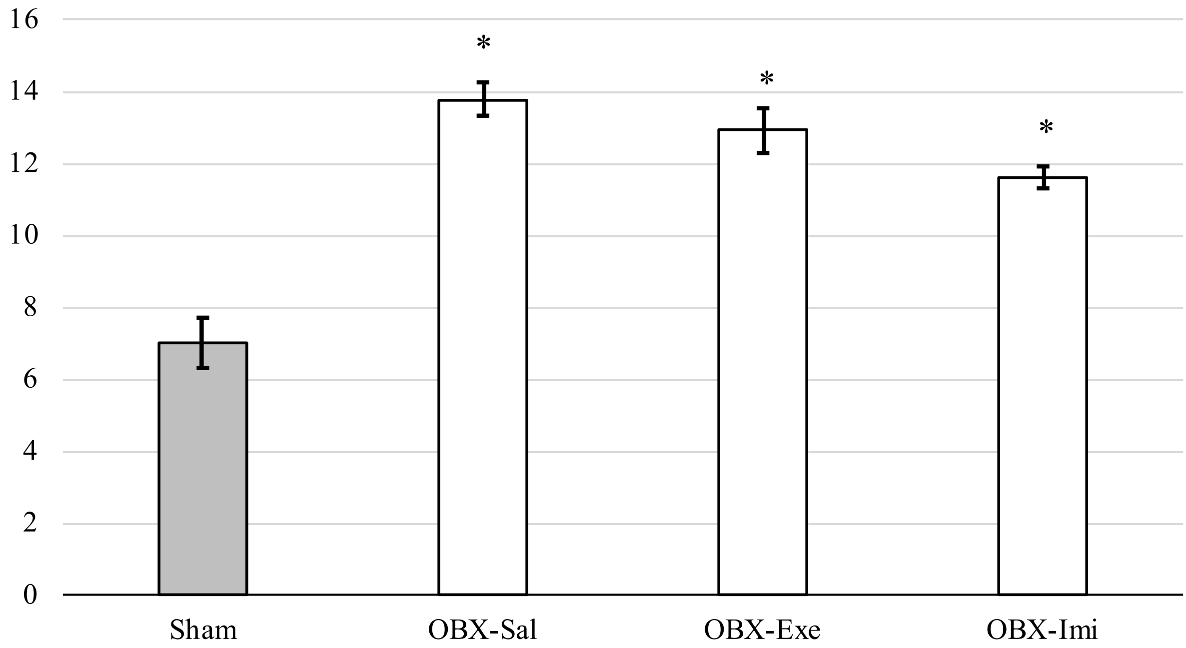


Fig.3: Total hyperemotionality scores

(a)



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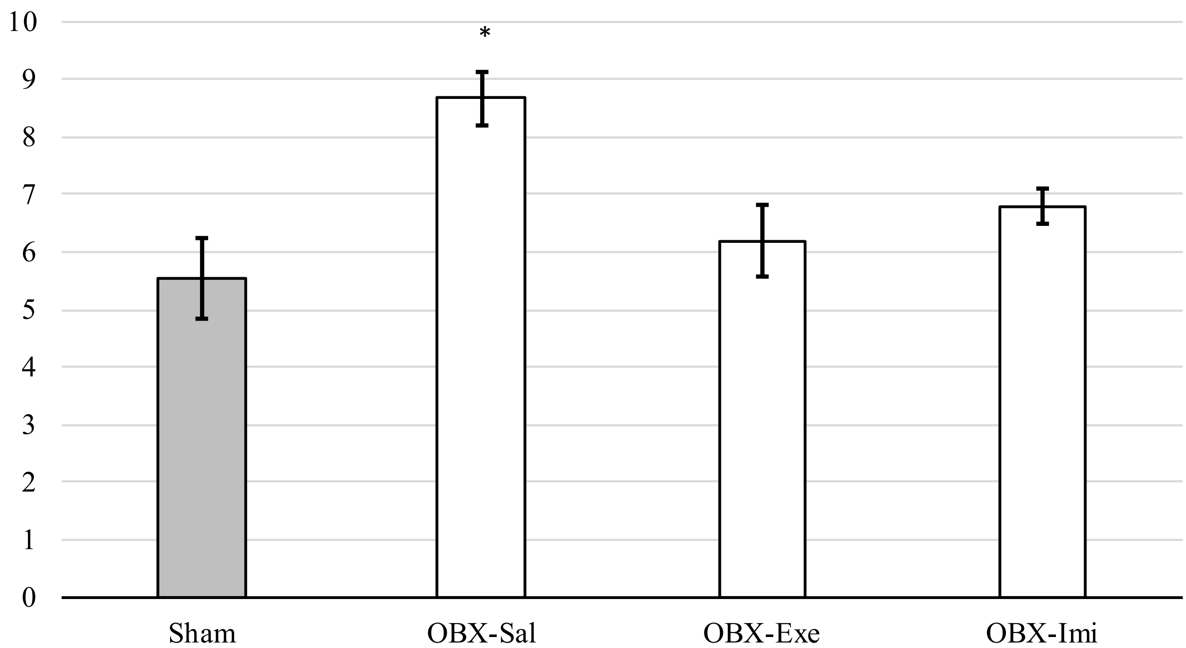
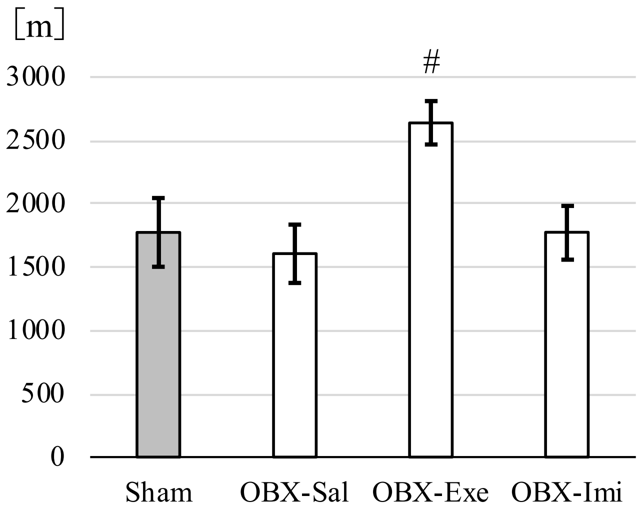


Fig. 4:Open field test

(a)



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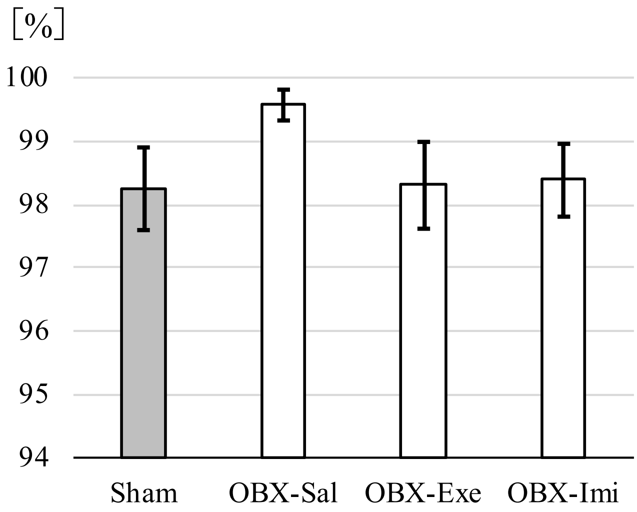

(c)

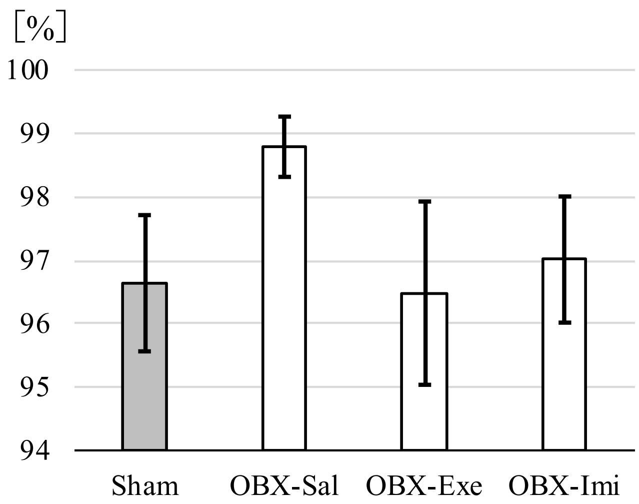

Fig. 5:Y-maze test