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Acute ghrelin administration reverses depressive-like behavior induced by bilateral olfactory bulbectomy in mice

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ABSTRACT

This study aims to examine the antidepressant-like action of Ghrelin (Ghr), a hormone synthesized predominantly by gastrointestinal endocrine cells and released during periods of negative energy balance, in two behavioral models: tail suspension test (TST), a predictive model of antidepressant activity, and the olfactory bulbectomy (OB), an established animal model of depression. The reduction in the immobility time in the TST was the parameter used to assess antidepressant-like effect of Ghr. The depressive-like behavior in olfactory bulbectomized mice was inferred through the increase in the immobility time in the TST and the hyperlocomotor activity in the open-field test. Ghr produced antidepressant-like effect in TST (0.3 nmol/μl, i.c.v.), and reversed OB-induced depressive-like behavior. In conclusion, these results provide clear evidence that an acute administration of ghrelin produce antidepressant-like effect in the TST and OB.

1. Introduction

Ghrelin (Ghr) is a hormone discovered based on its ability to stimulate growth hormone (GH) release by activating the GH secretagogue receptor (GHSR1a). This receptor is found at highest concentrations in the pituitary and hypothalamus [12]. Ghrelin acts primarily at these sites stimulating GH release and food intake to regulate energy homeostasis and body weight [23,43]. Abundant expression of GHSR1a in the hypothalamus highlights its important role in energy metabolism, nevertheless a high GHSR1a expression is also found in extra-hypothalamic neuronal populations [10,51].

An increasing number of articles were published during the past decade showing advances in the knowledge about the hypothalamic peptides that regulate feeding behavior. Recent work has begun to draw connections between hypothalamic feeding peptides (i.e. MCH, NPY, CART, etc.) and depression. Many of them have been shown not only to regulate feeding but also alter reward mechanisms, which suggest that they could have effects on anhedonia related symptoms and also have been implicated in depression and anxiety-like behaviors [1]. Recent studies have suggested that key metabolic signals such as Ghr may interact with CNS circuits to regulate reward and mood [15–17,48]. Lutter et al. [27] investigated the potential role of Ghr in the development of depressive symptoms induced by chronic stress in a model of restricted food intake and have shown that Ghr is effective against depressive-like symptoms induced by chronic stress, suggesting that this peptide participates in the regulation of mood.

Depression is a disorder that is projected to become the second biggest contributor to the global burden of disease and disability for the year 2020 [34]. Several brain regions as limbic cortex, hippocampus, amygdale, and anterior cingulated cortex are been implicated in the pathophysiology of the depression. Recent neuroimaging studies have demonstrated selective structural changes across various limbic and nonlimbic circuits in the brains of depressed patients, and post-mortem morphometric studies have revealed decreased neuron densities in several brain structures, supporting the idea that major depression may be related to impairments of structural plasticity [9]. There is evidence that depression can lead to reductions of the volume not only of the hippocampal formation, but also of the amygdale [24,25].

Abbreviations: ANOVA, analysis of variance; Ghr, ghrelin; GH, growth hormone; OB, olfactory bulbectomy; OFT, open field test; TST, tail suspension test.

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The bilateral olfactory bulbectomy (OB) in rodents is an animal model that appears to fulfill many of the necessary criteria for a depression model. There is an overlap between abnormalities in animals with OB and the changes observed in patients with major depression, such as: hyperactive responses in a novel and stressful environment [14,29,52], increased aggressiveness [31,37] and poor performance in passive avoidance task [3].

The possible effects of Ghr on depressive-like symptoms and its role as an alternative therapeutic tool for the treatment of depression have not been clarified yet. Thus, this study aims, to examine the antidepressant-like action of Ghr using the tail suspension test (TST, a test predictive of antidepressant activity), and to investigate the possibility that Ghr could reverse some of the depressive-like behaviors induced by OB in female mice.

2. Materials and methods

2.1. Animals

Adult female mice (Albino Swiss-SWR/J (q)) with an initial body weight of ≅25 g were used in this study. The colony room was maintained under controlled temperature (20 ± 2 °C) and light (12 h light, 12 h dark), with access to water and food ad libitum.

All procedures performed were conducted in accordance to the guidelines of the Institutional Committee of Laboratory Animal Care and Use, School of Medical Sciences, National University of Córdoba. Different animals were used for each behavioral test.

2.2. Drugs and treatment

Ghrelin (Neosystem, France) was dissolved in saline, divided into aliquots and kept at −20 °C until the day of the experiment. The peptide was infused acutely into the lateral ventricle (i.c.v.) 15 min before the tail suspension (TST) or open-field test (OFT).

2.3. Experimental procedure

Two experiments were performed:

The first set of experiments were performed to analyze if an acute i.c.v. administration of Ghr modifies the animals performance in the TST and in the open-field test in relation to those infused with saline. The animals were cannulated and 7 days after recovery, mice received different doses of Ghr (0.03, 0.3 and 3.0 nmol/μl) or saline (control) 15 min before the test in order to evaluate its antidepressant-like action.

The second set of experiments was performed in order to evaluate the possibility that Ghr could reverse some of the symptoms of depression induced by OB. The animals were divided into two groups:

a) Sham group (without OB): mice received the same surgery procedure that the bylbectomized animals, except that the olfactory bulbs were not removed.
b) Bulbectomized group (OB): animals were subjected to the surgery procedure for ablation of the olfactory bulbs.

Both groups were treated i.c.v. with only infusion of saline (sham-vehicle and OB-vehicle), Ghr 0.3 nmol/μl (sham-Ghr 0.3 and OB-Ghr 0.3) or Ghr 3.0 nmol/μl (sham-Ghr 3.0 and OB-Ghr 3.0). These doses were selected from a dose-response curve carried out in the first set of experiments.

For the surgery, the animals were anesthetized using a combination of 55 mg/kg ketamine HCl (Vetanarcol König: Laboratorios König S.A, Argentina) and 11 mg/kg xylazine (Kensol König: Laboratorios König S.A, Argentina). After surgery, the animals were housed in individual cages.

The occurrence of estrous cycle was evaluated daily by vaginal smears to females of all the experimental groups, between day 16 after OB surgery and the final of the treatment. All the behavioral tests were conducted during the diestrus phase (approximately seven days after i.c.v. surgery).

2.4. Olfactory bulbectomy surgery (OB)

Midsagittal incision was given on the skull and the skin was retracted. The soft tissues overlying the skull were removed. The landmarks of the skull, bregma and lambda, were identified and the skull was oriented such that both points were positioned in horizontal level. After clearing the underlying fascia, a burr-hole 2 mm in diameter was drilled through the skull 8 mm anterior to the bregma and 2 mm to either side of the midline. While the olfactory bulbs were removed by suction, care was taken to avoid the damage to frontal cortex. Then the burr-holes were filled with haemostatic sponge in order to control the bleeding. Sham-operated rats were treated in the same way, but the olfactory bulbs were left undisturbed. Furthermore, the i.c.v. cannulation was performed as given below.

2.5. Intracerebroventricular surgery

Thirsty days after OB surgery, mice were placed in a stereotaxic apparatus and subjected to i.c.v. surgery. Cannulae were implanted in a lateral ventricle using a steel guide cannula, according to the methods described by [33]. The coordinates relative to bregma were: anterior 0.2 mm, lateral 1.0 mm and vertical 2.8 mm. Cannulae were fixed to the skull surface with dental acrylic cement. Seven days after surgery, animals were injected with ghrelin or saline (control) using a 10 μl Hamilton syringe connected by Pe-10 polyethylene tubing to a 30-gauge needle extending it 0.75 mm beyond the guide cannula. Each infusion of 1 μl was delivered over a 1 min period.

2.6. Tail suspension test (TST)

The TST has become one of the most widely used models for assessing antidepressant-like activity in mice. The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. The total time of immobility induced by the test was measured according to the method described by Steru et al. [39] and performed 15 min after i.c.v. saline or Ghr administration. Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor with an adhesive tape placed at approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Mice were considered immobile only when they hung passively and completely motionless. The immobility time was recorded by an observer blind to the drug treatment [2,28].

2.7. Open-field test (OFT)

Mice were individually placed in a wood box (40 cm × 60 cm × 50 cm) with the floor of the arena divided into 12 equal squares. The number of squares crossed with all paws (crossing), was registered during a period of 6 min. The floor of the open-field apparatus was cleaned with ethanol 10% between tests.

Animals were tested in the OFT on day 0, under basal conditions (preoperative period) and day 14 after OB surgery (postoperative period). Aproximatelly, on the day 37, after i.c.v. surgery in the diestrus phase, the animals were again exposed to the open-field test (post-treatment period); in order to assess the locomotor activity as previously described [28,35] (see Fig. 1). In this last evaluation

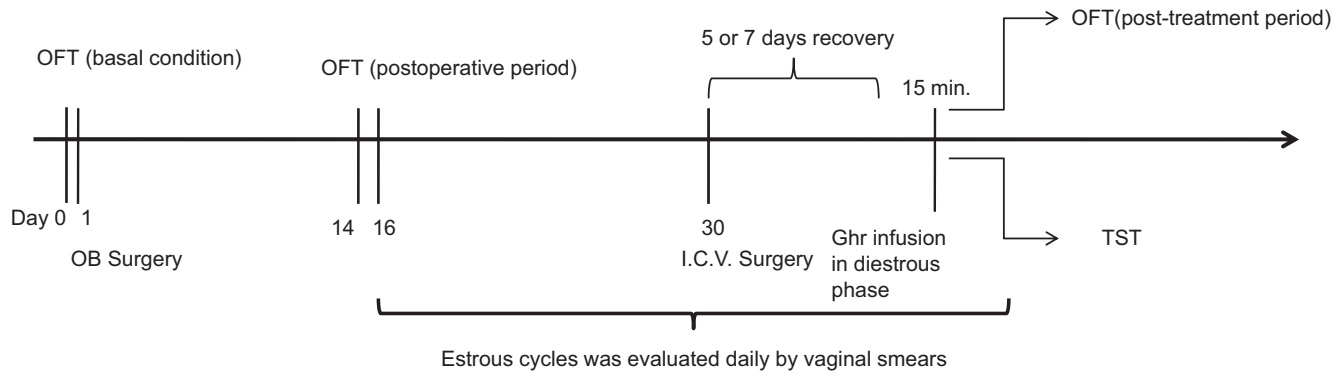

Fig. 1. Experimental protocol in the second set. The animals were divided into two groups: without OB group (sham) and bulbectomized group (OB). Animals were tested in the open-field test (OFT) on day 0, under basal conditions (preoperative period) and day 14 after OB surgery (postoperative period). On the day 30, i.c.v. surgery was carried. After recovery, in the diestrus phase, one animals group were again exposed to the open-field test (post-treatment period) 15 min after of Ghr infusion and other group were submitted to tail suspension test (TST) 15 min after Ghr infusion. Between day 16 and the final of the treatment, the occurrence of estrous cycle was evaluated daily by vaginal smears to females of all the experimental groups.

the locomotor activity was recorded after 15 min of Ghr infusion. It is well known that in the OB model, the increased exploration in an enclosed arena such as the open-field apparatus is one of the earliest and most widely accepted index of behavior [20,45].

2.8. Histology

After the behavioral test, mice were anesthetized with chloral hydrate and subjected to a cardiac perfusion with paraformaldehyde (4%) and their brains were removed. The OB lesions were verified. Frontal sections were cut with a cryostat (Leica, Germany), and the injection size was localized. Only results obtained from animals in which the tips of the cannulas were placed into i.c.v. and showed complete ablations of olfactory bulbs were considered.

2.9. Statistical analysis

In the first set of experiments results are given as the mean ± SEM and were analyzed by one-way ANOVA (dose-response curve). LSD test for post hoc comparison was performed when appropriate.

In the second set of experiments, data are expressed as mean ± SEM and evaluated with two-way ANOVA and LSD post hoc test were applied. All p values ≤ 0.05 were considered as statistically significant.

3. Results

3.1. First set of experiments

3.1.1. Effect of ghrelin on immobility time in the TST and activity in the open-field test

In the TST, the immobility time was: saline: 192.14 ± 13.62; Ghr 0.03 nmol/μl: 189.33 ± 8.25; Ghr 0.3 nmol/μl: 148.88 ± 11.89; Ghr 3.0 nmol/μl: 174.75 ± 9.54; (n = 7–9).

Ghrelin 0.3 nmol/μl significantly decreased the immobility time in the TST as compared to the control group treated with saline (Fig. 2). The percentage of reduction in immobility time was approximately 22%. The One-way ANOVA revealed a significant effect of Ghr treatment in the TST ($F(3, 28) = 3.15$; $p < 0.05$).

Ghrelin administration did not cause any change in the locomotor activity of mice as compared to saline control group (Fig. 3) (saline: 64.29 ± 12.62; Ghr 0.03 nmol/μl: 67.80 ± 2.29; Ghr 0.3 nmol/μl: 43.29 ± 7.76; Ghr 3.0 nmol/μl: 71.75 ± 8.27; n = 7–9), ($F(3, 30) = 0.72$; $p > 0.05$).

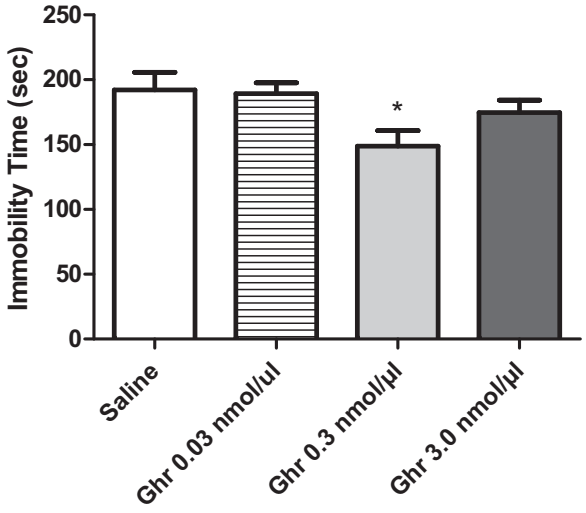

Fig. 2. Effect of intracerebroventricular ghrelin (Ghr) on the immobility time in the TST in mice. Saline: control animals infused with sterile saline solution. Mice received different doses of Ghr (0.03, 0.3 and 3.0 nmol/μl) or saline (control) 15 min before the test. Results are expressed as mean ± SEM. N = 7–12 animals in each group.

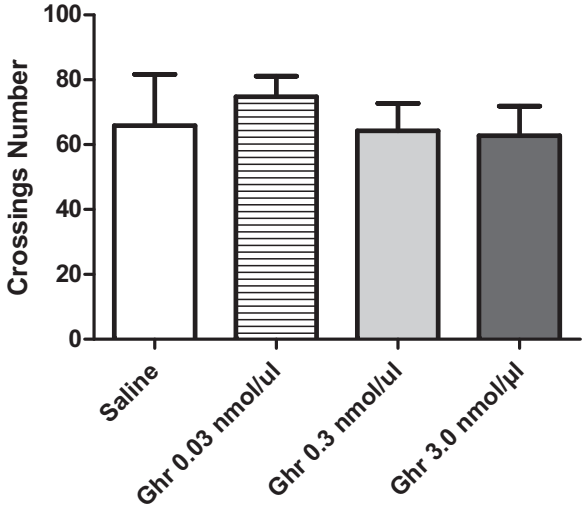

Fig. 3. Effect of intracerebroventricular ghrelin (Ghr) on the crossings number in the open-field test in mice. Saline: control animals infused with sterile saline solution. Mice received different doses of Ghr (0.03, 0.3 and 3.0 nmol/μl) or saline (control) 15 min before the test. Results are expressed as mean ± SEM. N = 7–12 animals in each group.

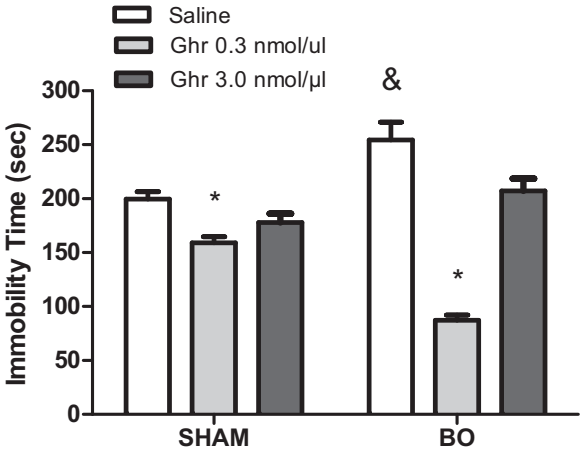

Fig. 4. Effect of ghrelin (Ghr) on the immobility time in the TST in sham and OB mice. Sham (without OB): mice received the same surgery procedure that the animals with OB, except that the olfactory bulbs were not removed. OB: bulbectomized group: Animals were subjected to the surgery procedure for ablation of the olfactory bulbs. Results are expressed as mean ± SEM. $N = 10$ animals in each group. *Significant differences comparison with sham-saline animals, $p \leq 0.05$. &Significant differences comparison with OB-saline animals, $p \leq 0.05$.

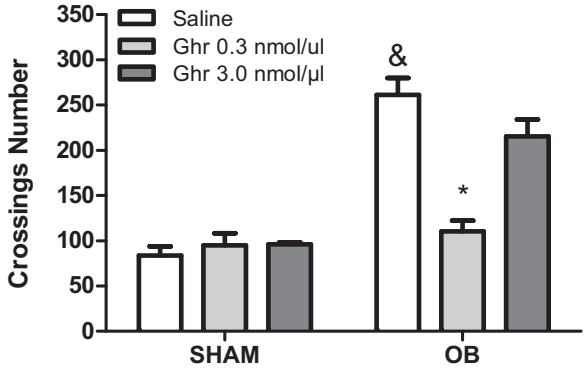

Fig. 5. Effect of ghrelin (Ghr) on crossings number in the open-field test in sham and OB mice. Sham (without OB): mice received the same surgery procedure that the animals with OB, except that the olfactory bulbs were not removed. OB: bulbectomized group: Animals were subjected to the surgery procedure for ablation of the olfactory bulbs. Results are expressed as mean ± SEM. $N = 10$ animals in each group. *Significant differences comparison with sham-saline animals, $p \leq 0.05$. &Significant differences comparison with OB-saline animals, $p \leq 0.05$.

3.2. Second set of experiments

3.2.1. Effect of ghrelin on the immobility time in the TST in sham and OB mice

In this set of experiments we studied the effects of i.c.v. Ghr administration on the immobility time in OB and sham mice. As shown in Fig. 4. OB animals treated with saline presented an increase on immobility time as compared with sham animals ($p < 0.05$). OB mice injected with Ghr 0.3 nmol/µl exhibited a decrease on immobility time ($p \leq 0.05$) in relation to animals administered with saline (OB-saline), indicating that Ghr was able to reverse the immobility response in OB mice.

Sham animals treated with Ghr 0.3 nmol/µl showed significant reductions with respect to the control animals on immobility time, confirming the evidence of the antidepressant-like effect of this compound (Fig. 4). The two-way ANOVA test revealed a significant interaction between the Ghr treatment and condition (sham and OB) ($F = 3.38$, df = 2, $p \leq 0.05$); a significant effect of the animals condition ($F = 13.12$, df = 1, $p \leq 0.05$) and a significant effect of Ghr administration ($F = 56.98$, df = 2, $p \leq 0.05$).

3.2.2. Ghrelin effect on the OFT in animal models of depression

The Ghr effect on crossing behavior observed in the open-field test in animal models of depression are shown in Fig. 5. As depicted in Fig. 4, OB animals had significantly altered locomotion activity. OB showed a significant increase on the crossings in relation to control animals (sham-saline); however, Ghr (0.3 nmol/µl) administration significantly reduced the crossings behaviors ($p \leq 0.05$).

The two-way ANOVA test revealed a significant interaction between Ghr treatment and condition (sham and OB) ($F = 6.79$, df = 2, $p \leq 0.05$); a significant effect of condition ($F = 19.85$, df = 1, $p \leq 0.05$) and a significant effect of Ghr administration ($F = 19.75$, df = 2, $p \leq 0.05$).

3.2.3. OB effects on estrous cycles

Bulbectomized animals showed a significant decrease in the number of estrus cycles during the evaluation period (≈23 days) (2.16 ± 0.19 vs. 3.35 ± 0.13 estrus cycles) and in the length of the estrous cycles observed with respect to control animals (1.06 ± 0.11 vs. 1.41 ± 0.11 days).

4. Discussion

In the present work, our results demonstrate that Ghr produced a significant antidepressant-like response in the TST and was capable of reversing the hyperactivity induced by olfactory bulbectomy. Taking into account that there are few studies that report behavioral changes induced by OB in females and that depression is more prevalent in women than in men [50], this study was carried out in female mice. We provide the first evidence that acute administration of Ghr induces an antidepressant-like effect in an animal model of depression, as the olfactory bulbectomy in female mice. Indeed, these data along with previous evidence suggesting that neural circuits involving the prefrontal cortex, hippocampus, amygdala, pallidum between other, are involved in the pathophysiology of cognitive and emotional symptoms of depression and also the fact that various of these extra hypothalamic structures present Ghr receptors seems suggest that probably neuronal circuits of the above mentioned structures could be target for the antidepressant-like response showed in this manuscript [12,24,25].

It is well known that the bilateral destruction of the olfactory bulbs results in behavioral, neurochemical, neuroendocrine, neuroimmunologic and morphological changes, which are compatible and comparable with those observed in depressive patients [38]. In general, changes induced by OB are measurable, replicable and reversed only by chronic administration of antidepressants including SSRIs (Selective Serotonin Reuptake Inhibitors) [19]. Noteworthy, a few studies in the literature have shown that the acute administration of riluzole or zinc was able to produce a rapid reversal of the hyperlocomotion activity induced by OB [32,40]. In the present study, Ghr administered acutely produced reversal of the hyperlocomotion induced by OB.

Since its introduction almost 20 years ago, the TST has become one of the most widely used models for assessing antidepressant-like activity in mice. The test is usually quite short, and the amount of time that animals spend immobile is recorded either manually or through an automated device [28,39]. Antidepressant treatments decrease these immobility scores and it has been postulated that immobility may be analogous to the clinical observations in depressed patients, which often lack sustained expenditure of effort reflected in a pronounced psychomotor impairments [47]. Our results shown that OB mice exhibited an increased immobility time in relation to control animals (sham), an indicative of a depressive-like behavior, and that Ghr 0.3 nmol/µl reversed that

immobility and promoted the occurrence of escape-related behavior, suggesting an antidepressant effect of this peptide. In addition, the acute administration of Ghr modified the performance of mice tested in the TST also in non-bulbectomized animals.

Our results also showed that Ghr restored the performance of bulbectomized adult female mice in the OFT. The locomotor hyperactivity in an enclosed arena such as the open-field apparatus is one of the earliest and most widely accepted indices of depressive behavior in bulbectomized rats or and mice. Our results showed that OB increased the locomotor activity (crossings) in the open-field with respect to sham animals and that acute central Ghr administration in OB mice restores this performance. This is, as far as we are aware of, the first report suggesting that Ghr reverses the hyperactivity induced by OB in mice. The hyperactivity observed in open-field environment is the most commonly assessed behavioral change in the OB model, a response which is attenuated following chronic, but not acute antidepressant treatments [23,26].

The difference in the threshold dose to induce antidepressant-like effect in TST and OFT in sham animals could probably attributed to the relative density of receptors in the brain structures involved in each behavioral paradigm.

Our findings are in line with those reported by Lutter et al. [27] in which an increase in plasmatic Ghr levels induced by chronic food restriction as well an sub-cutaneae injection of Ghr causes an anxyolitic and antidepressant-like behavior in the elevated plus maze test and in the forced swimming test, respectively. However, our results contrast with the conclusion of a recent study reported by Hansson and col., who demonstrated that chronic central Ghrelin treatment to rats increases anxiety- and depression-like behavior [11].

Several lines of evidence have suggested that psychopathological improvement of major depression is associated with a significant decrease in plasma Ghr levels [8,36,42]; however, the results obtained in humans studies are inconsistent and some publications found no difference in plasma ghrelin levels in patients with major depressive disorders [8,22]. The discrepancy may be due to differences in the dose used and/or the administration chronicity.

With respect to the mechanism involved in the reversal of the depression symptoms induced by Ghr, it is well known that olfactory bulbectomy reduces the levels of neurotransmitters such as serotonin [13,26,44] and noradrenaline, as well as enhances proinflamatory cytokines that participates in the psychopathology of depression [45]. On the other hand, it has been demonstrated that exogenous Ghr modulates release of proinflammatory cytokines, inhibiting the production of TNF-α, IL1β, IL 6 and IL 8 in human endothelial cells, which could play key roles in the psychopathology of depression [46]. In addition, decreased central monoaminergic function is an established hypothesis for the ethiopathogenesis of depression [24]. It has been demonstrated that Ghr increases noradrenergic transmission [5,7,18] that supports an antidepressant action. Thus, considering the above mentioned findings we can hypothesize that the Ghr effects reversing the symptoms of depression could probably be attributed to a decrease in proinflamatory cytokines and/or enhancement of noradrenergic transmission. Nevertheless, the results presented in this paper are preliminary to corroborate this hypothesis and further studies must be performed.

In contrast, ghrelin's suppressive effect on central release of serotonin [4], may rather suggest a depressogenic effect such as stimulatory action on the hypothalamic–pituitary–adrenal (HPA) axis [21,30,41]. The HPA axis hyperactivity is closely associated with depressive disorders [6].

In addition, our work also demonstrated that OB could modify the estrous cycles, reducing both the length and number of cycles. This evidence is in accordance with a study by Whitten and Champlin that olfactory bulbectomized mice presented a decreased number of cycles after the surgery procedure [49].

In conclusion, the present study shows that OB mice exhibited depressant-like behavior (hyperactivity and increased immobility time in TST) and that these parameters were reversed by the acute treatment with ghrelin, indicating that this compound may be effective for the treatment of depression.

Conflict of interest

The authors have not any potential conflicts of interest related to this paper.

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