Research article

Melatonin attenuated the behavioral despair induced by acute neurogenic stress through blockade of N-methyl D-aspartate receptors in mice

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ABSTRACT

It has been well documented that administration of melatonin could reveal antidepressant-like effect in rodents. However, the protective effect of melatonin on stress-induced depression/anxiety and its underlying mechanism is yet to be understood. In this regard, in the current study, acute foot-shock stress (FSS) was used to evaluate the antidepressant-like effect of melatonin on neurogenic stress-induced depression in mice. Behavioral evaluation was done by using the forced swimming test (FST) and Open-field test (OFT). Melatonin, MK-801, and ketamine (NMDA receptor antagonists), and NMDA (NMDA receptor agonist) were used to elucidate any association between melatonin and NMDA pathway in behavioral despair induced by acute-FSS. Applying acute-FSS to mice significantly induced depressant-like behavior in FST without any significant impact on locomotor activity in the OFT. We observed that melatonin (dose-dependently) significantly improved the depressant-like effect of FSS, but it did not impact the locomotion in animals. Acute injection of MK-801 at sub-effective doses (0.01 mg/kg) or ketamine (0.1 mg/kg) potentiated the antidepressant-like effect of a sub-effective dose of melatonin. However, the sub-effective dose of NMDA (30 mg/kg) abolished the protective effect of melatonin on the behavioral profile of stressed animals. Our results could reflect the antidepressant-like effect of melatonin on neurogenic stress-induced depressive behaviors in mice. Also, our results showed that NMDA receptors could be involved in the antidepressant-like effect of melatonin.

1. Introduction

The occurrence of depressive disorders is associated with several etiologies and exposure to stressful events (Greenberg et al., 2002; Kessler et al., 2009), which could induce a variety of pathological changes in the brain and resulting in behavioral changes such as depressive-like behaviors (Pittenger and Duman, 2008). Previous studies indicated a strong relation between facing to acute stress and the initiation of depression (Hammen, 2005). It has been shown that exposure to stressful life events could play a pivotal role in the occurrence of behavioral impairments. However, it's not clear whether acute stress contributes to depression development, and there is no practical way of controlling acute stress-induced mood disorders.

Melatonin synthesizes during the physiologic condition in the pineal gland and could be involved in the synchronization of the circadian rhythms of physiological functions. The linkage between pineal melatonin and depressive disorders has been investigated by several studies at both clinical and animal levels (Kaminski-Hartenthaler et al., 2015; Lewy et al., 1998; Hickie and Rogers, 2011; Lewy et al., 1998). Pre-clinical researches have revealed that both acute and chronic injections of melatonin could induce antidepressant-like effects in rodents (Mantovani et al., 2003; Micale et al., 2006). Although the antidepressant-like effect of melatonin has been documented in depressive disorders, its protective effect on behavioral impairments induced by acute stress- and the possible underlying mechanism has not been fully understood (Genario et al., 2019). However, a few studies have pointed out that melatonin might inhibit N-methyl-D-aspartate (NMDA) receptor function (Dilek et al., 2010; Mantovani et al., 2003).

The pivotal role of NMDA receptors in the pathophysiology of mood and affective disorders has been confirmed by several lines of research (Manji et al., 2001). The antidepressant-like effects of NMDA receptor antagonists were reported in a number of preclinical and clinical studies

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([Berman et al., 2000](#); [Haj-Mirzaian et al., 2015a](#); [Papp and Moryl, 1994](#)). Also, it has been demonstrated that acute stress could significantly impact on glutamatergic system and activate NMDA receptor, which resulting in behavioral impairments ([Nowak et al., 1995](#); [Yuen et al., 2009](#)).

In the current research, we investigated the possible antidepressant-like effect of melatonin in acute stress induced depressive-like behaviors. In this regard, we used inescapable acute foot-shock stress (FSS) to induce depressive-like activity in mice ([Haj-Mirzaian et al., 2014](#); [Shanks et al., 1991](#)); and we assessed whether glutamatergic system is involved in the antidepressant-like effect of melatonin by administrations of NMDA receptor agonists and antagonists.

2. Material and methods

2.1. Animals

Male NMRI weighing 27 ± 3 g (Naval Medical Research Institue, aged 7–8 weeks, n = 105) mice obtained from Pasteur Institue. Animals were kept in groups of five to six and given free access to food and water in rooms with a daily light/dark cycle and a standard temperature (22 ± 1 °C). All behavioral tasks were performed at 10:00 and 14:00. Both practices have been carried out in compliance with the NIH Guide to the Care and Use of Laboratory Animals and the law on the safety of animals used for research purposes (Directive 2010/63/EU) and approved by the Institutional Guidelines for the Care and Use of Animals (Department of Pharmacology, School of Medicine, TUMS). Each study group consisted of 6–8 mice and each mouse was used only once during each behavioral task.

2.2. Foot-shock stress

We used a picket box (30 × 30 × 40cm high) with a steel-rod floor to induce acute FSS (29 parallel rods, 0.3 cm in diameter, one cm apart, Coulbourn Instruments). Inescapable foot-shock (3-mA scrambled shock stimulation, 50-Hz) was transmitted to the ground grid via a scrambler ([Haj-Mirzaian et al., 2014](#), 2016; [Madden et al., 1977](#)). Animals have been subjected to intermittent foot-shock for thirty minutes (five sec of shock period was followed with 30 s of resting period). Immediately after induction of stress, mouse was removed from the box and then preparing for testing with other behavioral measurements (FST or OFT).

2.3. Forced swimming test (FST) for antidepressant screening (acute stress)

Forced swimming test (FST) as a reliable and reproducible behavioral experiment was used to assess potential antidepressant-like outcomes of applied treatments. In this respect, mice were put in an open cylinder-shaped flask (10 cm in diameter, 25 cm in height) containing 19 cm of water at 23–25 °C. The animals were allowed to swim for 6 min. A professional analyst, who was blind to the treatment groups, studied the animals' struggling behavior. The criterion for handling animals as immobile included avoiding fighting and remaining immobile and floating in the water. After an adaptation time of 2 min, the length of immobility was evaluated in the last 4 min of the experiment ([Cryan and Holmes, 2005](#); [Porsolt et al., 1977](#)).

2.4. Open field test (OFT)

Both ambulatory and locomotor activity of mice have been tested by using OFT to ensure that improvements in the period of immobility in the FST did not emerge as a result of differences that exist in motor function ([Kulesskaya and Voikar, 2014](#)). The unit wooden box 50 cm × 50 cm × 30cm was used. The field of the arena was split into 16 equal squares. Every mouse was gently positioned on the center square (25 cm × 25 cm) and the activities were captured by the camera for 5 min and evaluated by Ethovision software version 8 (Noldus, Netherlands) and the total distance moved (horizontal activity) and the number of rearings (vertical activity) were counted.

2.5. Drugs

Melatonin, Dizocilpine (known as MK-801, a selective NMDA receptor antagonists), ketamine (a selective NMDA receptor antagonists), and NMDA (a selective NMDA receptor agonist). MK-801 and NMDA were administered 45 min and ketamine was injected 30 min before behavioral tasks ([Chamanara et al., 2019](#); [Haj-Mirzaian et al., 2015b](#); [Sadaghiani et al., 2011](#)). In this study, in order to evaluate the dose- and time-response of melatonin, we administrated melatonin in different dose and different time intervals. All drugs were purchased from Sigma Aldrich, USA. All drugs were dissolved in saline and administered intraperitoneally (i.p.) in a constant volume of 5 ml/kg body weight.
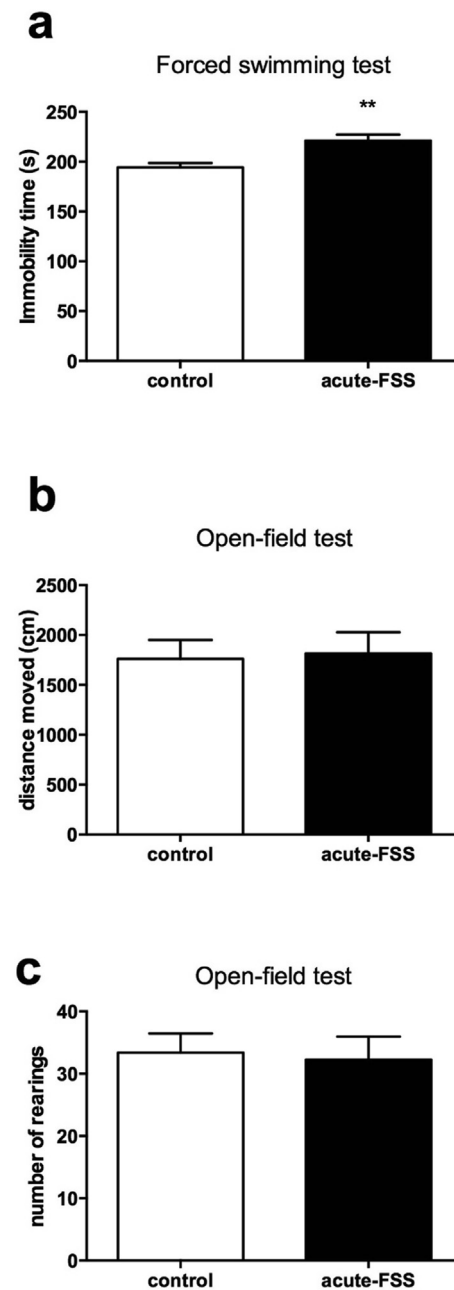
Figure 1. Effect of acute-FSS on animals' behavior. Immobility time in FST (a), distance moved in OFT (b) and number of rearings in OFT (c). Date presented as the mean ± S.E.M.; **$p < 0.01$ compared with control (non-stressed) group.
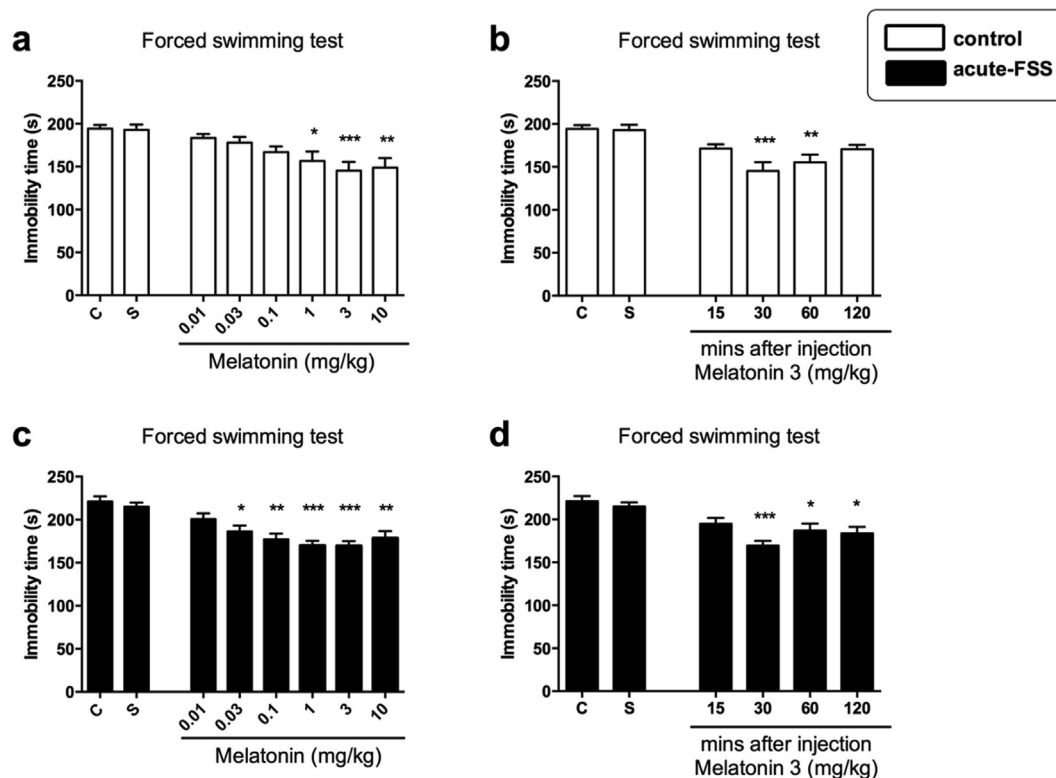
Figure 2. Effect of melatonin on the duration of immobility in FST after treating animals with melatonin based on time and dose of injection. Immobility of non-stressed (a and b) and stressed (c and d) mice in FST. Date presented as the mean ± S.E.M and were analyzed using one-way ANOVA followed by tukey's post hoc test; *p < 0.05, **p < 0.01, and ***p < 0.001 compared with saline treated (S) group in each figure.

2.6. Experimental procedure

In the first step, we evaluated the effect of acute stress induction on behavioral changes by assessing locomotor activity of animals in OFT and immobility time in FST. Then, we have performed pharmacological dose-response and time-course study for the antidepressant-like effect of melatonin. In dose-response study, melatonin at the doses of 10, 3, 1, 0.1, 0.03, and 0.01 mg/kg (i.p.) were administered 30 min before behavioral tests to control and stressed animals. In stressed groups, animals were placed in FSS box immediately after melatonin injection and behavioral tests were accompanied 30 min after stress induction. In time-course study, melatonin 3 mg/kg (i.p.) was injected 15, 30, 60, and 120 min before behavioral tests to control and stressed groups. So, we obtained the effective dose and the best time of injection of melatonin for normal and stressed mice. Saline at the dose of 5 ml/kg was injected as vehicle in control groups to rule out the placebo effect.

We also investigated the possible role of glutamatergic system in behavioral despair induced by FSS. Therefore, different doses of NMDA receptors antagonists including ketamine (3, 1, and 0.1 mg/kg, i.p.) and MK-801 (0.1, 0.05, and 0.01 mg/kg, i.p.) were administered 30 and 45 min before behavioral tests, respectively. Also, NMDA (a NMDA receptor agonist, 150, 75, 30 mg/kg, i.p.) was administered 45 min before behavioral tests to control and stressed animals (Haj-Mirzaian et al., 2015b; Sadaghiani et al., 2011).

In the last part, we tried to evaluate the possible role of NMDA receptors in mediating the antidepressant-like effect of melatonin. To reach this goal, subeffective doses of ketamine and MK-801 were co-administered with the subeffective dose of melatonin in stressed/non-stressed animals. In order to strengthen our results, we also evaluated

the effect NMDA receptor agonist pre-treatment on antidepressant-like effect of effective dose of melatonin. The subeffective or effective doses of each drug were obtained from the last parts of the experiments.

2.7. Statistical analysis

In the current study, comparison between the groups was analyzed by one-way ANOVA followed by tukey's post hoc test and *t*-test using the Graph-pad prism software (version 9). P ˂0.05 was considered statistically significant. In addition, the power of each analysis was calculated by using G*power software (ver.3.1.7, Franz Faul, Universitat Kiel, Germany). All analysis in this study reached α error of 0.05 and power (1-β) of 0.8 at least.

2.8. Ethics

Our study was in accordance with the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (HHS publication 85-23, 1985), legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU). In addition, current study was approved by Department of Medical Ethics, AJA University of Medical Science Ethical, Iran (IR.AJAUMS.REC.1399.1112).

3. Results

3.1. Behavior impairments induced by acute-FSS

We have evaluated the impact of acute-FSS on behavioral despair using FST. Results from *t*-test analysis revealed that acute-FSS could significantly increase the duration of immobility of animals in FST (*p* < 0.01). Our results showed that acute-FSS could not change the horizontal
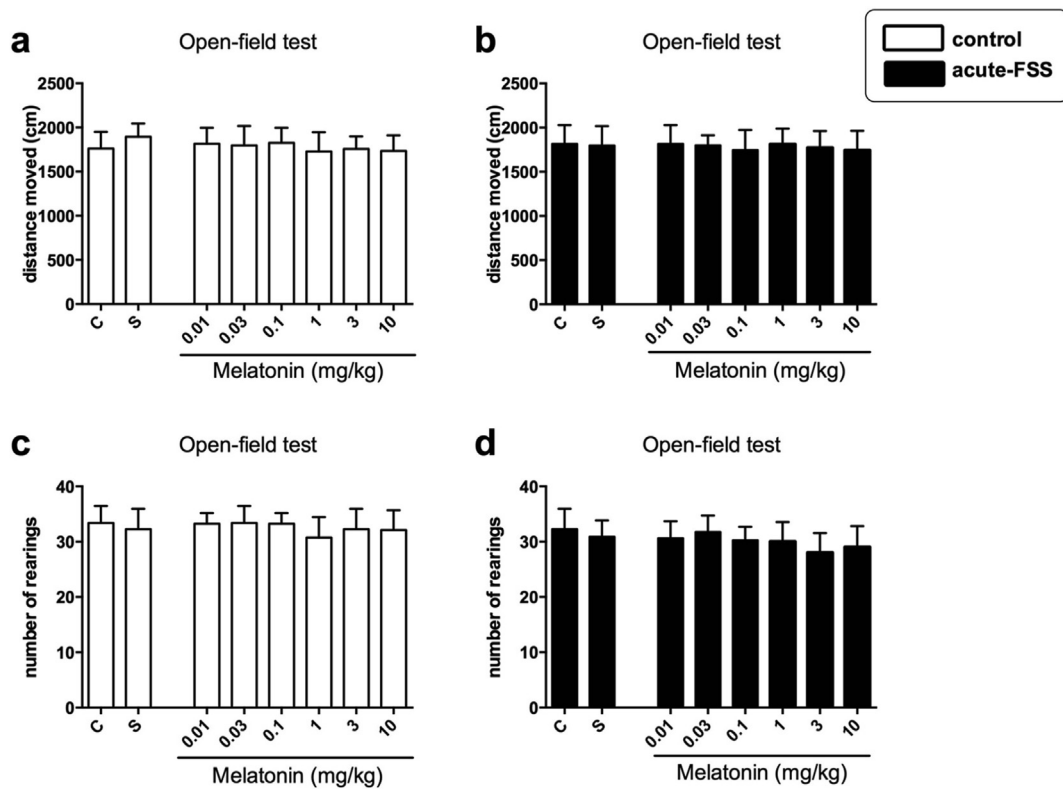
Figure 3. Effect of different doses of melatonin on locomotion behavior in OFT: Total distance moved of non-stressed (a) and stressed (b) mice; and number of rearings of non-stressed (c) and stressed (d) animals in OFT. Date presented as the mean ± S.E.M and were analyzed using one-way ANOVA followed by tukey's post hoc test.
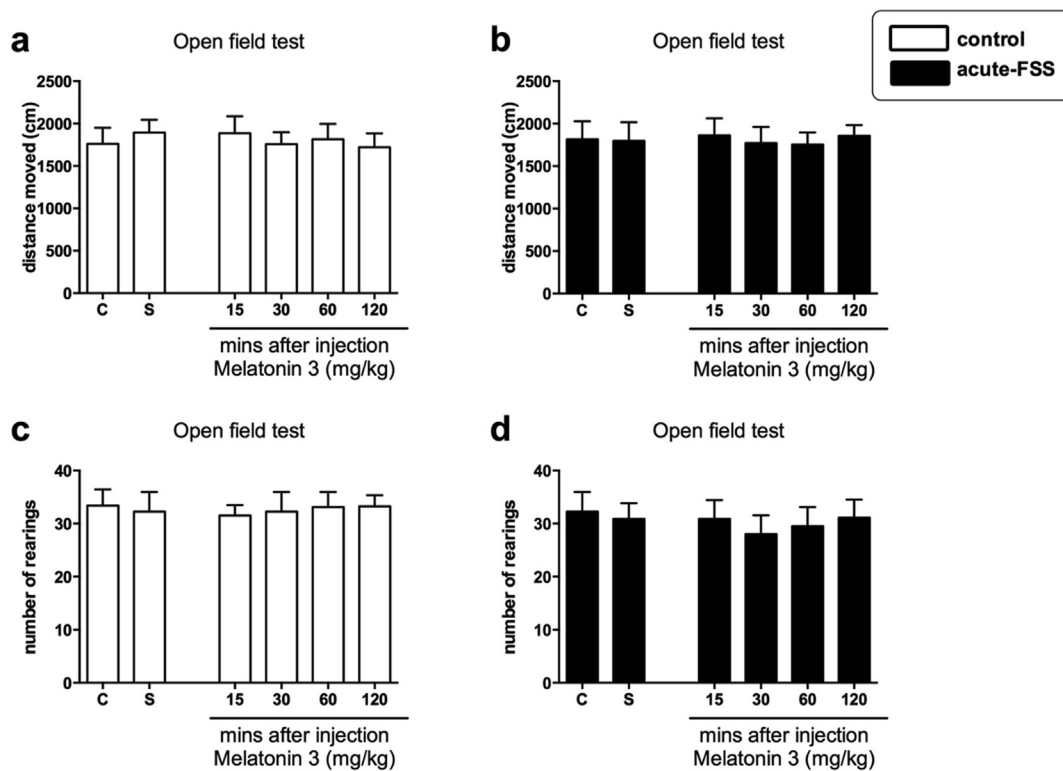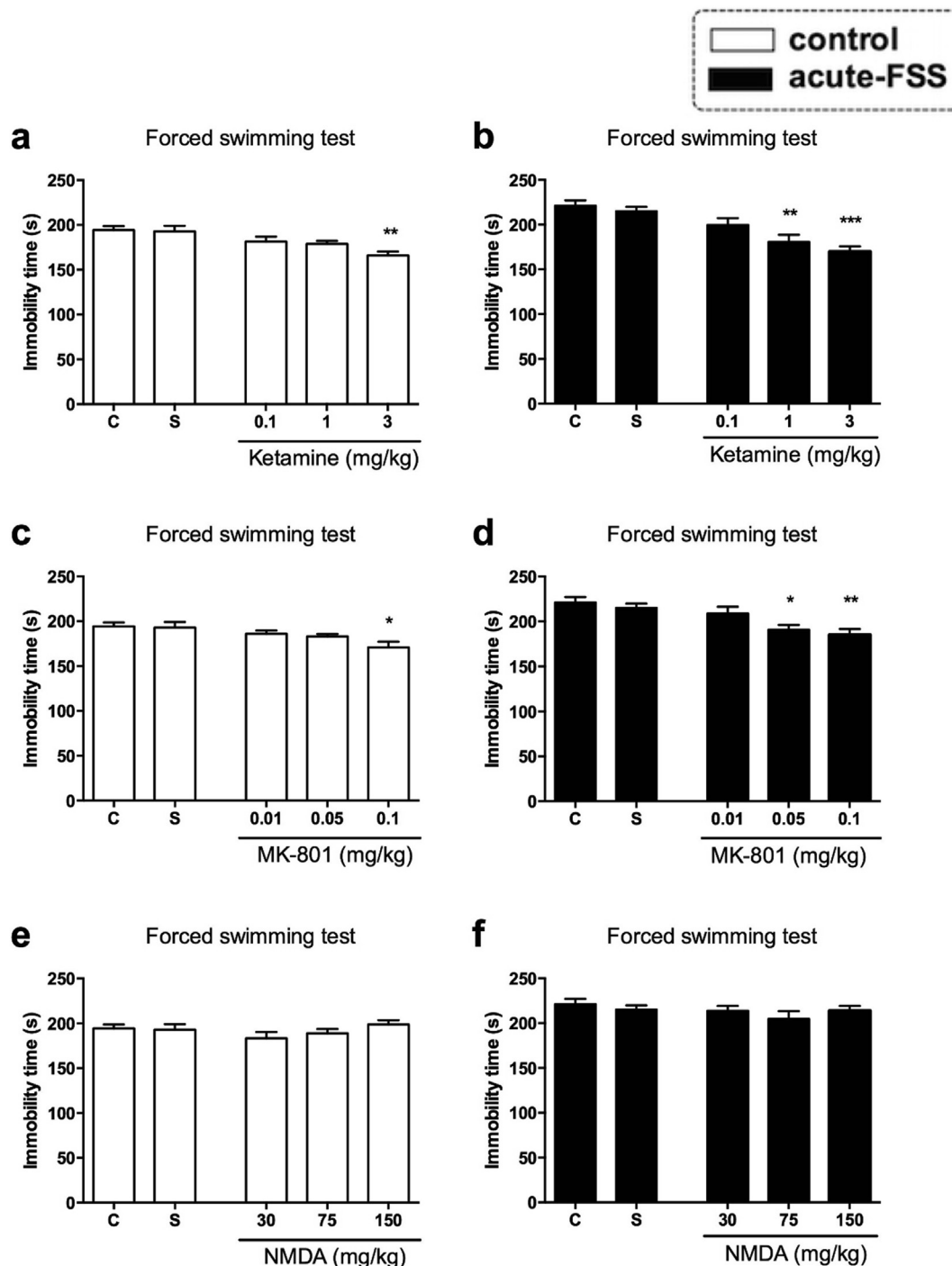

Figure 4. Effect of melatonin (when was administered in different time manners) on locomotion behavior in OFT: Total distance moved of non-stressed (a) and stressed (b) mice; and number of rearings of non-stressed (c) and stressed (d) animals in OFT. Date presented as the mean ± S.E.M and were analyzed using one-way ANOVA followed by tukey's post hoc test.
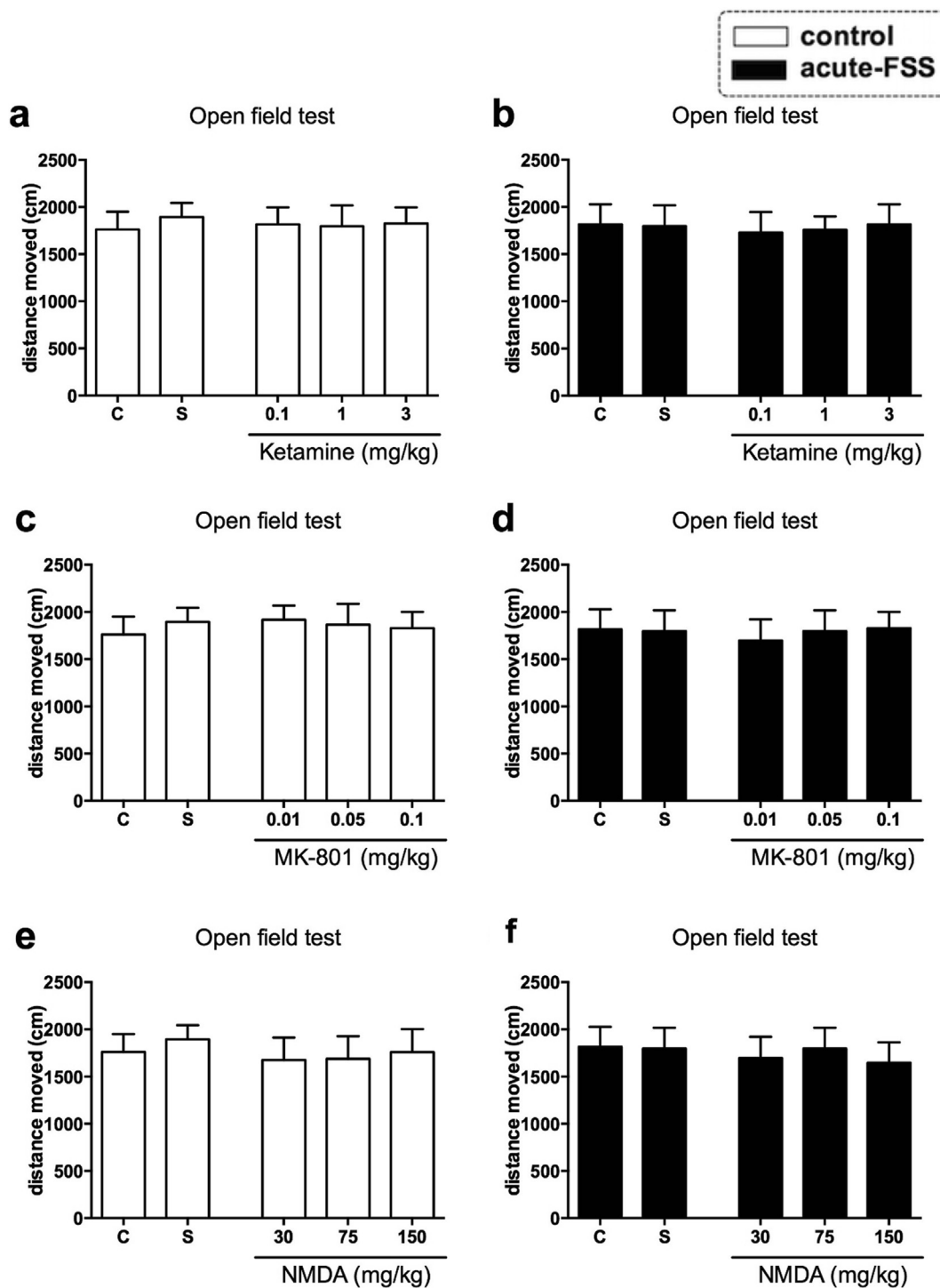
Figure 5. Effect of NMDA antagonists (ketamine and MK-801) and agonist (NMDA) on the duration of immobility in FST: Effect of acute administration of ketamine (0.1, 1, and 3 mg/kg, i.p., 30 min before test) on the duration of immobility in non-stressed (a) and stressed (b) animals. Effect of acute administration of MK-801 (0.01, 0.05, and 0.1 mg/kg, i.p., 45 min before test) on the duration of immobility in non-stressed (c) and stressed (d) animals. Effect of acute administration of NMDA (30, 75, and 150 mg/kg, i.p., 45 min before test) on the duration of immobility in non-stressed (e) and stressed (f) animals. Date presented as the mean $\pm$ S.E.M and were analyzed using one-way ANOVA followed by tukey's post hoc test; $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared with saline treated (S) group.

(total distance moved) ($p > 0.05$) and vertical (number of rearings) ($p > 0.05$) locomotor activity in animals (Figure 1b and 1c).

3.2. Effect of acute melatonin on depressive-like behaviors induced by acute FSS

We have performed a dose-response and time course study to demonstrate the best dose and time of injection of melatonin for its

protective effect on coping behavior of non-stressed and stressed animals in the FST. One-way ANOVA analysis showed significant differences between treated groups for different doses and times of administration of melatonin in non-stressed ($F_{(7, 56)} = 5.66$, $p < 0.001$, Figure 2a) ($F_{(5, 42)} = 7.79$, $p < 0.001$, Figure 2b) and stressed ($F_{(7, 53)} = 8.891$, $p < 0.001$, Figure 2c) ($F_{(5, 39)} = 8.01$, $p < 0.001$, Figure 2d) animals. In non-stressed rats, Tukey's post hoc analysis showed that acute administration of melatonin (30 min before the behavioral test) at the doses of 1, 3, and
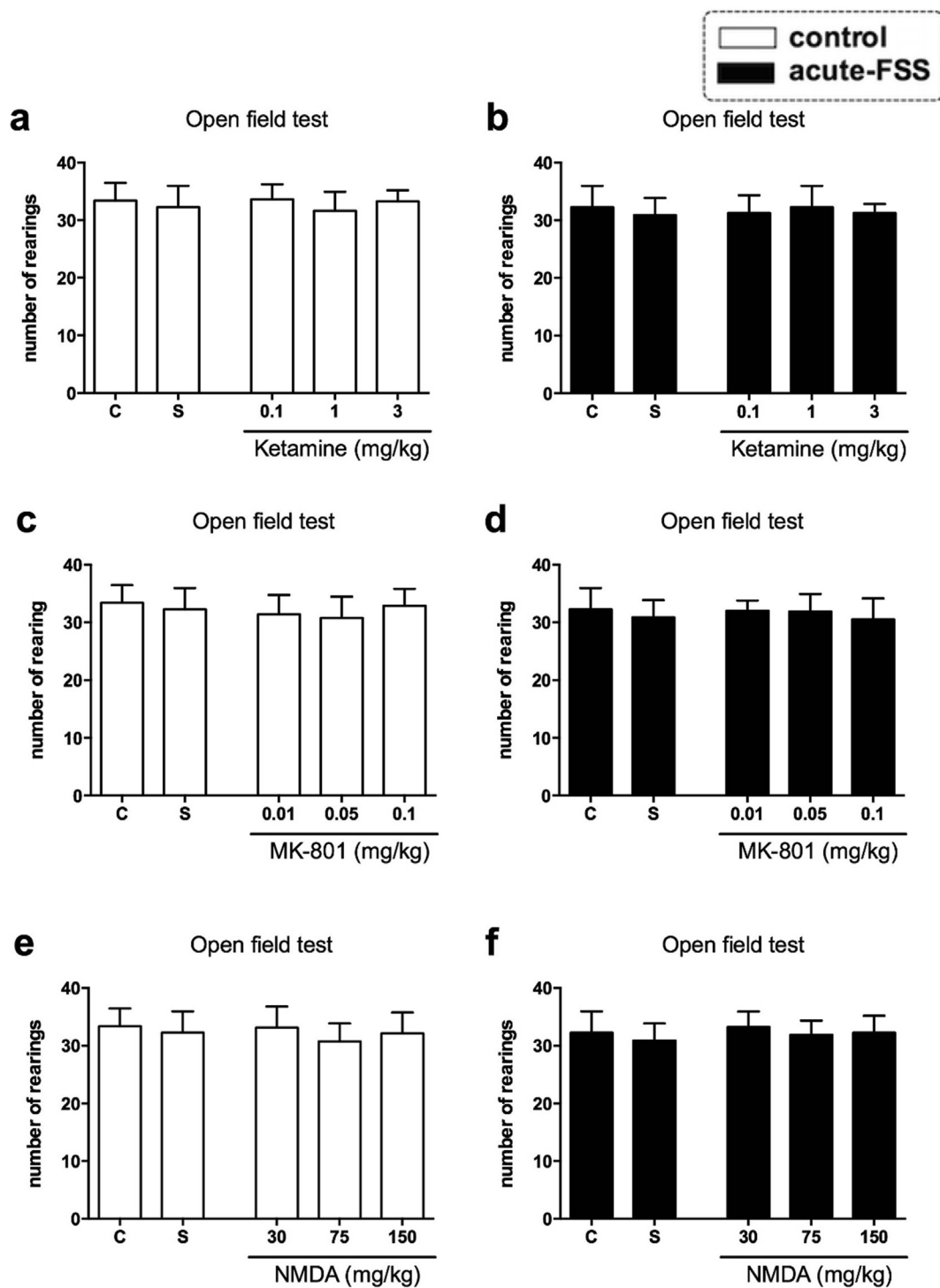
Figure 6. Effect of NMDA antagonists (ketamine and MK-801) and agonist (NMDA) on total distance moved in OFT: Effect of acute administration of ketamine (0.1, 1, and 3 mg/kg, i.p., 30 min before test) on total distance moved in non-stressed (a) and stressed (b) animals. Effect of acute administration of MK-801 (0.01, 0.05, and 0.1 mg/kg, i.p., 45 min before test) on total distance moved in non-stressed (c) and stressed (d) animals. Effect of acute administration of NMDA (30, 75, and 150 mg/kg, i.p., 45 min before test) on total distance moved in non-stressed (e) and stressed (f) animals. Date presented as the mean ± S.E.M and were analyzed using one-way ANOVA followed by tukey's post hoc test.

10 mg/kg significantly reduced the immobility time when compared with saline-administered group in the FST ($p < 0.05$, $p < 0.001$, $p < 0.01$, respectively) (Figure 2a). However, lower doses of melatonin (0.01, 0.03, and 0.1 mg/kg) did not induce any antidepressant-like effect in non-stressed animals when acutely administered ($p > 0.05$, Figure 2A). The best effect on immobility time was manifested when the tests were performed 30 min after melatonin (3 mg/kg) administration in the FST ($p <$

0.001, Figure 2b), although when testing animals after 60 min of melatonin injection, lower anti-immobility effect was noticed in non-stressed animals ($p < 0.01$, Figure 2b). So, the best antidepressant-like effect of acute melatonin has been detected when the drug was administered at the dose of 3 mg/kg and 30 min before behavioral test in non-stressed animals.
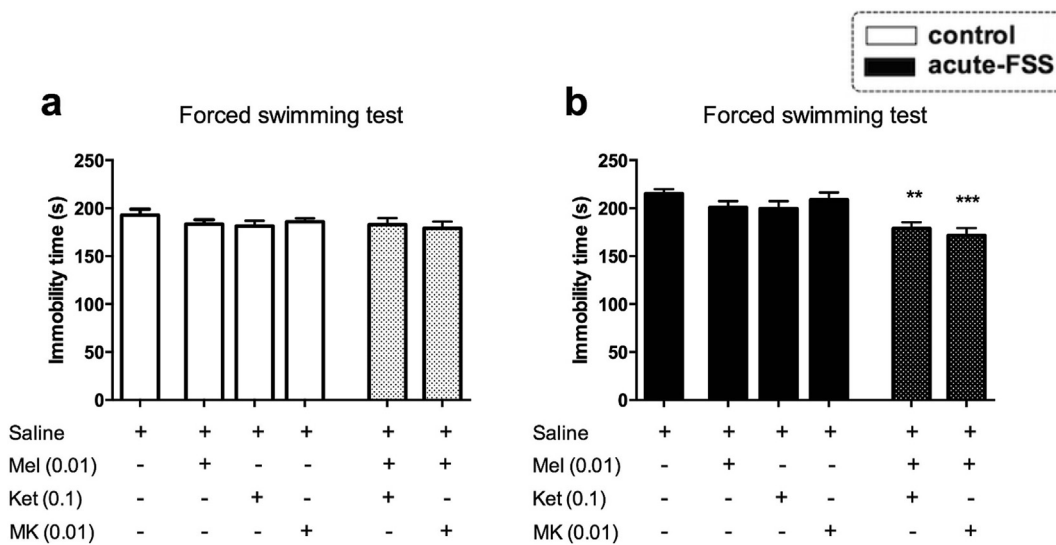
Figure 7. Effect of NMDA antagonists (ketamine and MK-801) and agonist (NMDA) on number of rearings in OFT: Effect of acute administration of ketamine (0.1, 1, and 3 mg/kg, i.p., 30 min before test) on number of rearings in non-stressed (a) and stressed (b) animals. Effect of acute administration of MK-801 (0.01, 0.05, and 0.1 mg/kg, i.p., 45 min before test) on number of rearings in non-stressed (c) and stressed (d) animals. Effect of acute administration of NMDA (30, 75, and 150 mg/kg, i.p., 45 min before test) on number of rearings in non-stressed (e) and stressed (f) animals. Values are expressed as the mean ± S.E.M. and were analyzed using one- way ANOVA followed by tukey's post hoc test; There is no significant difference when compared each group with saline treated (S) group in each figure.

In the next step, the same experiments have been conducted in stressed animals. In stressed animals, post hoc analysis have shown that melatonin treatment (30 min before the behavioral test) at the doses of 0.03, 0.1, 1, 3, and 10 mg/kg significantly decreased the immobility time in comparison with saline-administered group in the FST ($p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.001$, and $p < 0.01$, respectively; Figure 2c).

However, lower doses of melatonin (0.01 mg/kg) did not show any protective effect in the behavioral profile of stressed animals ($p > 0.05$, Figure 2c). Also, in time course study, we found that the best anti-immobility effect of melatonin is detected when the tests were performed 30 min after melatonin (3 mg/kg) administration in the FST ($p < 0.001$, Figure 2D). Thus, in comparison with non-stressed groups, lower

Figure 8. Effect of NMDA antagonists and melatonin co-treatment on the duration of immobility in FST: effect of co-administration of MK-801 (0.01 mg/kg) or ketamine (0.1 mg/kg) with melatonin (0.01 mg/kg) on duration of immobility of non-stressed (a) and stressed. (b) mice in FST. Date presented as the mean ± S.E.M and were analyzed using one-way ANOVA followed by tukey's post hoc test; **$p < 0.01$ and ***$p < 0.001$ compared with saline treated group in each figure.
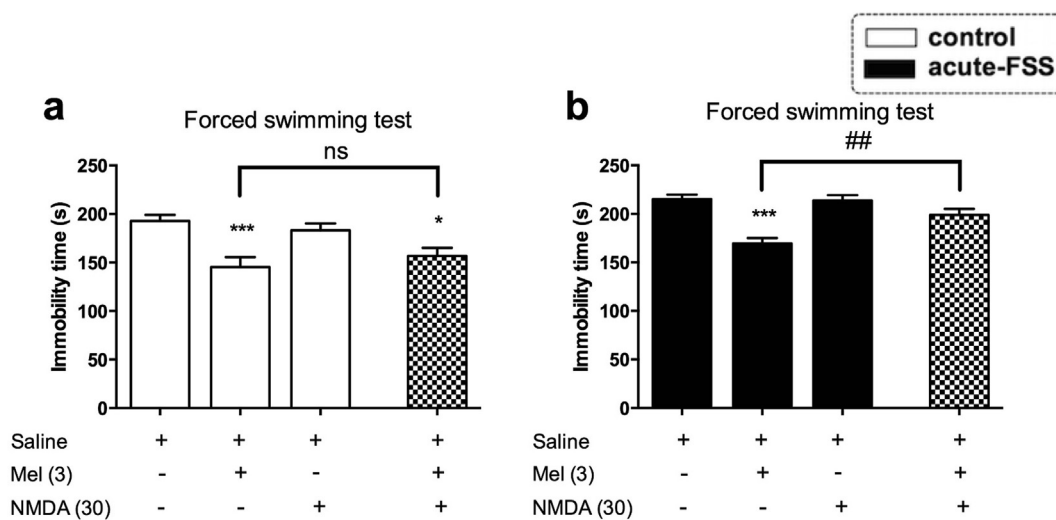
Figure 9. Effect of NMDA agonist treatment on antidepressant effect of melatonin in FST: effect of co-administration of NMDA (30 mg/kg) with melatonin (3 mg/kg) on duration of immobility of non-stressed (a) and stressed (b) mice in FST. Date presented as the mean ± S.E.M and were analyzed using one-way ANOVA followed by tukey's post hoc test; *$p < 0.05$ and ***$p < 0.001$ compared with saline treated group in each figure. ns $= p > 0.05$ and ##$p < 0.01$ compared with melatonin treated group in each figure.

doses of acute melatonin could induce significant antidepressant-like effect in stressed animals.

In order to validate our findings in the FST, we evaluated the locomotor activity of animas in the OFT. Results obtained from the one-way ANOVA revealed that there is no significant difference between different melatonin doses (Figure 3a and Figure 3c) and times of administration (Figures 3b and 3d) of melatonin in non-stressed and stressed animals for total distance moved in the OFT. Also, no significant difference was found between treated groups for different doses (Figure 4a and Figure 4c) and times of administration (Figures 4b and 4d) of melatonin in non-stressed and stressed animals for number of rearings in the OFT.

3.3. Effect NMDA antagonists and agonist treatments on behavioral despair induced by acute FSS

In this step, one-way ANOVA analysis demonstrated that there was a significant difference through treated animals with ketamine ($F(4, 35) =$

$4.431, p < 0.01$, Figure 5a) ($F(4, 32) = 9.733, p < 0.001$, Figure 5b) and MK-801 ($F(4, 35) = 3.656, p < 0.05$, Figure 5c) ($F(4, 29) = 5.782, p < 0.001$, Figure 5d), but not NMDA, in the FST for non-stressed and stressed groups, respectively.

In non-stressed animals, post hoc analysis showed that ketamine at the dose of 3 mg/kg ($p < 0.01$) (but not at 0.1 and 1 mg/kg) as well as MK-801 at the dose of 0.1 mg/kg ($p < 0.05$) (but not at 0.01 and 0.05 mg/kg) significantly reduced the immobility time of mice in FST. In stressed groups, lower doses of these drugs induce significant antidepressant-like effect. Based on tukey's analysis, ketamine at the doses of 1 and 3 mg/kg ($p < 0.01$ and $p < 0.001$) (but not at 0.1 mg/kg) and MK-801 at the doses of 0.05 and 0.1 mg/kg ($p < 0.05$ and $p < 0.01$) (but not at 0.01 mg/kg) significantly decreased the duration of immobility of animals in FST when compared with saline-treated control group. NMDA agonist treatment at the doses of 30, 75, and 150 mg kg did no alter the immobility time of non-stressed and stressed animals when compared to saline-treated groups ($p > 0.05$).

In OFT test (total distance moved as well as number of rearing), one-way ANOVA analysis demonstrated that there is no significant difference between animals treated with NMDA antagonists or agonist in both non-stressed and stressed groups ($p > 0.05$, Figures 6 and 7).

3.4. Role of NMDA receptors in the antidepressant-like effect of melatonin in stressed animals

In this step, we observed that single treatment of MK-801 (0.01 mg/kg), ketamine (0.1 mg/kg), and melatonin (0.01 mg/kg) could not impact the immobility time in both control and acute-FSS groups ($p > 0.05$, Figure 8). Also, in FST, we observed that the combination of MK-801 (0.01 mg/kg) or ketamine (0.1 mg/kg) with the sub-effective dose of melatonin (0.01 mg/kg) could not alter the behavior outcomes in control mice ($p > 0.05$, Figure 8a). Nevertheless, our results showed obtained from administration of MK-801 or ketamine concurrently with melatonin (0.01 mg/kg) in FST showed that the depressive-like behavior was significantly improved in acute-FSS groups ($p < 0.01$ and $p < 0.001$, respectively; Figure 8b). Compared with saline-injected mice, the co-injection of MK-801/ketamine with melatonin could not significantly alter the total distance moved and number of rearings in both control and acute-FSS group in OFT (data not shown).

As shown in Figure 9, NMDA (30 mg/kg) could not significantly change the duration of immobility time in control and acute-FSS animals ($p > 0.05$, Figure 9). However, co-injection of NMDA with the effective dose of melatonin (3 mg/kg) could significantly reverse the antidepressant-like effect of melatonin in acute-FSS animals in FST ($p < 0.01$, Figure 9b).

4. Discussion

In this study, we showed that induction of acute neurogenic stress such as FSS could induce behavioral despair in animals and significantly increased the duration of immobility time in FST. These findings are in line with previous studies asserting that electric FSS could induce depressive-like behaviors in animals (Haj-Mirzaian et al., 2014, 2016; Ostadhadi et al., 2015). Also, our results have shown that acute FSS did not alter the locomotor activity (total distance moved and the number of rearings in OFT) of both stressed and non-stressed animals; so, it could be concluded that the depressive-like behavior of stressed animals in our study was not related to the changes of locomotor activity. Although the FST is a valid test of depressive-like behaviors, false-negative results might be observed with sedation and sickness conditions. But in this study, it seems that the behavioral impairments did not associate with locomotor activity changes in the OFT.

It is well known that stress, whether acute or chronic, involves in pathophysiology of various psychiatric disorders especially depressive disorder (Mercier et al., 2003; Weiss et al., 1981). Acute-FSS applies under an inescapable condition and animals will receive an uncontrollable stress, which has been suggested to activate/block different molecular pathways to induce behavioral changes related to the depression (Henn and Vollmayr, 2005; Vollmayr and Henn, 2003; Anisman et al., 2001).

In the current study, we have investigated the possible antidepressant-like effect of melatonin. The results presented here show that acute melatonin treatment at the doses of 1, 3, and 10 mg/kg (but not at the lower doses) induced antidepressant-like effects in non-stressed animals in the FST. We have also demonstrated that the sub-effective doses of melatonin (0.03, 0.1, 1 mg kg, which did not induce any antidepressant effect in non-stressed animals) significantly reversed stress-induced behavioral despair in stressed mice. The link between melatonin and depressive disorders such as seasonal affective disorder (SAD) has received attention (Kaminski-Hartenthaler et al., 2015; Lewy et al., 1998). The antidepressant effects of melatonin have been supported by many clinical studies (Hickie and Rogers, 2011; Lewy et al., 1998). Also, antidepressant effects of melatonin have been reported in

the FST when it was acutely administered (Mantovani et al., 2003). Confirming these data, it has been shown that S20304, a melatonin receptor agonist, produced an antidepressant-like effect in the FST in rodents (Bourin et al., 2004). Also, melatonin receptor-knockout animals have shown to possess depressive-like symptoms when compared with normal animals (Weil et al., 2006). Animals with global knock-out melatonin receptors spent more time floating in the Porsolt test (increase in the duration of immobility). Mantovani et al. have performed a dose repose study for the acute antidepressant-like effect of melatonin on tail suspension test. They found that melatonin at the doses of 0.1–30 mg/kg induce antidepressant-like effect in rodents (Mantovani et al., 2003). Also, Detanico et al. have demonstrated the protective effect of melatonin on chronic mild stress induced depressive behaviors in rodents (Detanico et al., 2009). Our results, in this study are in line with the past reports. But there are a few studies to evaluate the antidepressant-like effect of melatonin in acute stress-induced depression in animals. Although the antidepressant effect of this drug has been documented in SAD and other related diseases, its protective effect on acute stress and stress-induced behavioral changes has not been fully studied.

The role of glutamatergic system and NMDA receptors in the pathophysiology of depression is well documented (Berman et al., 2000). Also, it has been fully described that under acute stressful conditions, glutamatergic system activation might be involved in behavioral symptoms followed by stress such as depression (Ribeiro et al., 2005). Previous studies supported the antidepressant effects of NMDA receptor antagonists in both humans and animals (Berman et al., 2000; Haj-Mirzaian et al., 2015a; Papp and Moryl, 1994). According to these reports and our recent findings, we evaluated the possible antidepressant effect of ketamine and MK-801 (NMDA receptor antagonists) as well as NMDA receptor agonist in stressed and non-stressed animals. We showed that the subeffective doses of NMDA antagonists including ketamine (1 mg/kg) and MK-801 (0.05 mg/kg) significantly decreased the immobility duration in stressed animals. However, NMDA receptor agonist administration (at the doses were used in the current study) did not alter the behavioral despair of animals. The obtained results can be explained by the overactivation of glutamatergic system under acute stress condition and role of this system as an underlying mechanism of stress-induced depression in animals. These results are in accordance with the previous findings which determined the effect of these drugs in acute-stress induced depressive-like behaviors in the FST (Haj-Mirzaian et al., 2015a).

In the next step, we have performed a pharmacological study in order to illustrate the possible impact of melatonin on NMDA receptors. We have shown that co-administration of NMDA receptor antagonists with the sub-effective dose of melatonin, enhanced its antidepressant-like effect in stressed animals. On the other hand, NMDA receptor antagonists reversed the protective antidepressant effect of melatonin in stressed animals. These findings suggested that the antidepressant-like effect of melatonin in acute stress condition is partially mediated through blockade of NMDA receptors. It has been shown that NMDA receptor could have an interaction with melatonin receptors. In this regard, the possible role of NMDA receptor in the anticonvulsant effect of melatonin has been reported in past studies (Lapin et al., 1998). Dilek et al. showed the effects of melatonin on NMDA receptor subunits concentrations in the blood of ovariectomized rats (Dilek et al., 2010). Also, Mantovi et al. have demonstrated that NMDA receptor antagonists augmented the antidepressant effect of melatonin in rodents (Mantovani et al., 2003). They have shown these effects in normal non-stress condition. In this study, we showed for the first time that under stressful condition melatonin could exert its antidepressant effect through NMDA receptor blockade and modulation of glutamatergic system.

An antidepressant effect of melatonin has been well documented; but its mechanism of action especially under stress-induced behavioral deficit has not been fully studied. In this regard, we tried to find the underlying mechanisms by which melatonin exert its antidepressant

effects. According to our findings, we showed that melatonin exerts its antidepressant effect through NMDA receptor blockade; however, more studies should be performed to illustrate the exact mechanism of action of melatonin and also to find the direct effect of melatonin on NMDA receptors function.

5. Conclusion

Results of the current research demonstrated the antidepressant-like effect of melatonin on neurogenic stress-induced depressive behaviors in mice. Also, we have demonstrated that NMDA receptors are involved in antidepressant-like effect of melatonin against physical stress.

Declarations

Author contribution statement

A. Hajmirzaeyian; S. Shakyba: Performed the experiments; Wrote the paper.

M. Chamanara; R. Akhavan-Sigari: Analyzed and interpreted the data; Wrote the paper.

A. Rashidian: Contributed reagents, materials, analysis tools or data.

E. Nassireslami: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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