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Research report

Activation of cannabinoid receptors elicits antidepressant-like effects in a mouse model of social isolation stress

Arvin Haj-Mirzaian[a,b,1], Hossein Amini-Khoei[c,d,1], Arya Haj-Mirzaian[a,b], Shayan Amiri[a,b], Maria Ghesmati[e], Mazyar Zahir[a,b], Hamed Shafaroodi[f], Ahmad Reza Dehpour[a,b,*]

[a] Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box, 13145-784, Tehran, Iran
[c] Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
[d] Department of Physiology and Pharmacology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
[e] Department of Microbiology, Islamic Azad University of Lahijan, Lahijan, Iran
[f] Department of Pharmacology and Toxicology, Pharmaceutical Sciences Branch and Pharmaceutical Sciences Research Center, Islamic Azad University, Tehran, Iran

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ABSTRACT

Social isolation stress (SIS) paradigm is a chronic stress procedure able to induce profound behavioral and neurochemical changes in rodents and evokes depressive and anxiety-like behaviors. Recent studies demonstrated that the cannabinoid system plays a key role in behavioral abnormalities such as depression through different pathways; however, there is no evidence showing a relation between SIS and the cannabinoid system. This study investigated the role of the cannabinoid system in depressive-like behavior and anxiety-like behavior of IC animals. For this purpose, NMRI mice were treated with WIN55, 212-2 (non-selective cannabinoid receptor agonist) and AM-251 (cannabinoid receptor type 1 antagonist) and AM-630 (cannabinoid receptor type 2 antagonist). We found that behavioral abnormality followed by SIS was mitigated after administration of WIN55, 212-2. Also, depressive-like effects induced by SIS were significantly increased following administration of AM-251 and AM-630. Co-administration of cannabinoid receptor antagonists (AM-251 and AM-630), significantly reversed the antidepressant effect of WIN55, 212-2 in IC animals. Our findings suggest that the cannabinoid system is involved in depressive-like behaviors induced by SIS. We showed that activation of cannabinoid receptors (type 1 and 2) could mitigate depression-like behavior induced by SIS in a mouse model.

1. Introduction

Evidences from clinical and pre-clinical studies have demonstrated that early life exposure to environmental and social stressors plays a pivotal role in the development of psychiatric disorders such as depression (Lupien et al., 2009; Pechtel and Pizzagalli, 2011). In this regards, it has been shown that social isolation stress (SIS) paradigm is a chronic stress procedure able to induce profound behavioral and neurochemical changes in rodents and evokes depressive and anxiety-like behaviors (Fone and Porkess, 2008; Weiss et al., 2004; Nestler and Hyman, 2010; Koob et al., 1989). Glutaminergic system, nitrergic system, hypothalamic-pituitary-adrenal (HPA) axis are pathways known to mediate the impacts of SIS on psychopathologies like depression (Weiss et al., 2004; Haj-Mirzaian et al., 2015; Amiri et al., 2014).

The cannabinoid system comprises the cannabinoid receptors (CB1 and CB2 receptors) and represents an important neuromodulator in the central nervous system (CNS) (Devane et al., 1992; Dinh et al., 2004; Gong et al., 2006; Matsuda et al., 1990; Sugiura et al., 1995). Recent studies demonstrated that the cannabinoid system exerts its antidepressant effects through different pathways including the modulation of HPA axis function (Weidenfeld et al., 1994), regulating the release of neurotransmitters (Domenici et al., 2006; Takahashi and Castillo, 2006) and the modulation of neuroinflammation (Walter and Stella, 2004).

Although, antithesis evidence has shown different effects for cannabinoids on behavior, overall, effects of cannabinoids depend on the doses and time of administration as well as experimental

* Corresponding author at: Experimental Medicine Research Center, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran.
E-mail addresses: dehpour@yahoo.com, dehpoura@sina.tums.ac.ir (A.R. Dehpour).
[1] Please note that the first two authors are considered as the first authors.

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conditions (Rodriguez Bambico et al., 2009; Witkin et al., 2005); however, the exact mechanisms modulated by the cannabinoid system are still unknown. Therefore, further investigations are needed to clarify the mechanisms underlying the antidepressant-like effects of these pharmacological agents targeting the cannabinoid system. Previous reports have determined that cannabinoid receptor (CBR) agonists can reverse the depressive state in animals exposed to chronic mild stress; however, there is no evidence showing that cannabinoid agonists have beneficial effects in depression following SIS paradigm (Segev et al., 2014).

Considering the above-mentioned points, we tried to demonstrate the impact of the cannabinoid system on depressive-like behaviors induced by SIS. The aim of our study is to investigate the effects of drugs acting on CB1 and CB2 receptors (CB1R and CB2R) on depressive-like behavior in SIS mice using WIN55,212-2 (non-specific agonist) and AM630 (CB2R antagonist) and AM251 (CB1R antagonist). For this purpose, we used SIS paradigm as a chronic stress model. Behavioral experiments to measure depressive-like behavior including forced swimming test (FST), splash test and open field-test (OFT) were used in order to verify our hypothesis.

2. Materials and methods

2.1. Animals and housing conditions

Male NMRI mice (Pasteur Institute, Tehran, Iran) weighing 10–12 g on postnatal day (PND) 21–24 were used in this study (animals used in this study were at adolescence period) (Spinelli et al., 2013). Animals were housed under standard laboratory conditions as temperature at 22 ± 2C, humidity at 50, 12-h light–dark cycle, and free access to food and water ad-lib for a period of 28 days in two opposing conditions: social condition (SC) or isolated condition (IC). Socially conditioned mice were located (6 per cage) in Plexiglas boxes (25 cm × 25 cm × 15 cm) and IC animals were placed individually in Plexiglas boxes (24 cm × 17 cm × 12 cm) (Amiri et al., 2015). The cages of IC animals were cleaned weekly by a same experimenter to diminish handling and social interaction. All experiments were conducted during the period between 10:00 a.m. and 02:00 p.m. Each mouse was used only once in each tests. Each experimental group contained 6–8 animals. All experiments were carried out in accordance with National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication #80-23) and institutional guidelines for animal care and use (Department of Pharmacology, School of medicine, Tehran University of Medical Sciences).

2.2. Drugs

AM-251 (CB1R antagonist), AM-630 (CB2R antagonist) and WIN55, 212-2 (non-selective agonist of CBR) were purchased from Sigma (Sigma, St Louis, MO, USA). AM-251 and AM-630 were dissolved in saline and WIN55, 212-2 dissolved in dimethyl sulfoxide (DMSO) and further diluted with 5% Tween-80 and 90% saline (0.9% NaCl). Final DMSO concentration was 5%. Injections were carried out through intraperitoneal (i.p.) route in a constant volume of 5 ml/kg body weight. DMSO, Tween-80 and saline solutions were used as vehicle (Vehicle: 1:1:18 of DMSO, Tween-80 and saline, respectively).

AM-251 (0.2 and 0.5 mg/kg) and AM-630 (0.2 and 0.5 mg/kg) were administrated 30 min before behavioral tests (Kruk-Slomka et al., 2015; Ostadhadi et al., 2016) and WIN55, 212-2 (1, 3 and 5 mg/kg) was administered 60 min prior to the behavioral tests (Bambico et al., 2007). Doses and time of administrations of each drug were chosen based on our pilot study and pervious published reports (Haj-Mirzaian et al., 2016a; Haj-Mirzaian et al., 2016c; Weiss et al., 2004).

2.3. Experiment design and treatments

In the first part of study, the effects of SIS on depressive-like behaviors were investigated using previously validated behavioral tests including forced swimming test (FST), open-field test (OFT) and splash test. On the next step, the possible effects of CBR agonist/antagonists on IC mice were assessed. To do this, mice (IC and SC) were treated with the sub-effective doses of AM-630 (0.2 and 0.5 mg/kg, i.p., 30 min prior to the tests), AM-251 (0.2 and 0.5 mg/kg, i.p., 30 min prior to the tests) and WIN55, 212-2 (1, 3 mg/kg and 5 mg/kg, i.p., 60 min prior to the tests). After administration of drugs, behaviors of animals were evaluated using aforementioned behavioral tests. To exclude the possible impact of saline and vehicle administrations, mice were treated with 5 ml/kg physiological saline as well as vehicle before carrying out behavioral tests.

2.4. Open-field test (OFT)

Just before the FST, the locomotor activity of animals was evaluated using the open-field test (Haj-Mirzaian et al., 2016a; Kaster et al., 2005; Haj-Mirzaian et al., 2016b), in order to rule out the possibility that changes in duration of immobility are not the result of modifications in motor activity. OFT was used to elucidate the locomotor activity in response to SIS (Walsh and Cummins, 1976). The apparatus of OFT was made of white opaque Plexiglas (50 cm × 50 cm × 30 cm) and was faintly illuminated. Each animal was placed gently on the central zone (30 cm × 30 cm) and behaviors were recorded using a camera for a 5 min period and were analyzed by Ethovision software version 8 (Noldus, Netherlands). The surface of the apparatus was cleaned with 70% ethanol after each experiment. Each animal was used in only one experiment. The distance moved and the numbers of rearings were evaluated.

2.5. Forced swimming test (FST)

FST was carried out by using the method of Porsolt et al. (Porsolt et al., 1977a; Porsolt et al., 1977b; Haj-Mirzaian et al., 2016c). Mice were separately placed in an open cylinder-shaped flask (diameter 10 cm, height 25 cm), containing 19 cm water at 23 ± 1 °C. Mice were allowed to swim for 6 min and the period of immobility was recorded throughout the last 4 min of the test. Each mouse was considered immobile when it ceased struggling and stayed floating motionless in the water, making only those movements necessary to keep its head above water.

2.6. Splash test

This test was used to evaluate self-care and motivational behaviors. In this test, grooming activity time of mice, which can be considered as an indirect measure of palatable solution intake, was measured. A 10% sucrose solution was squirted on the dorsal coat of animals in their home cage and mice were videotaped for 5 min. The total grooming activity time was recorded for 5 min after the sucrose vaporization (Detanico et al., 2009). Grooming activity consists of nose/face grooming, head washing and body grooming.

2.7. Statistical analysis

Comparisons between the groups were assessed using *t*-test and one-way ANOVA followed by Tukey's post hoc test using GraphPad Prism 6 software (San Diego, CA, USA). *P* values less than 0.05 were considered statistically significant.

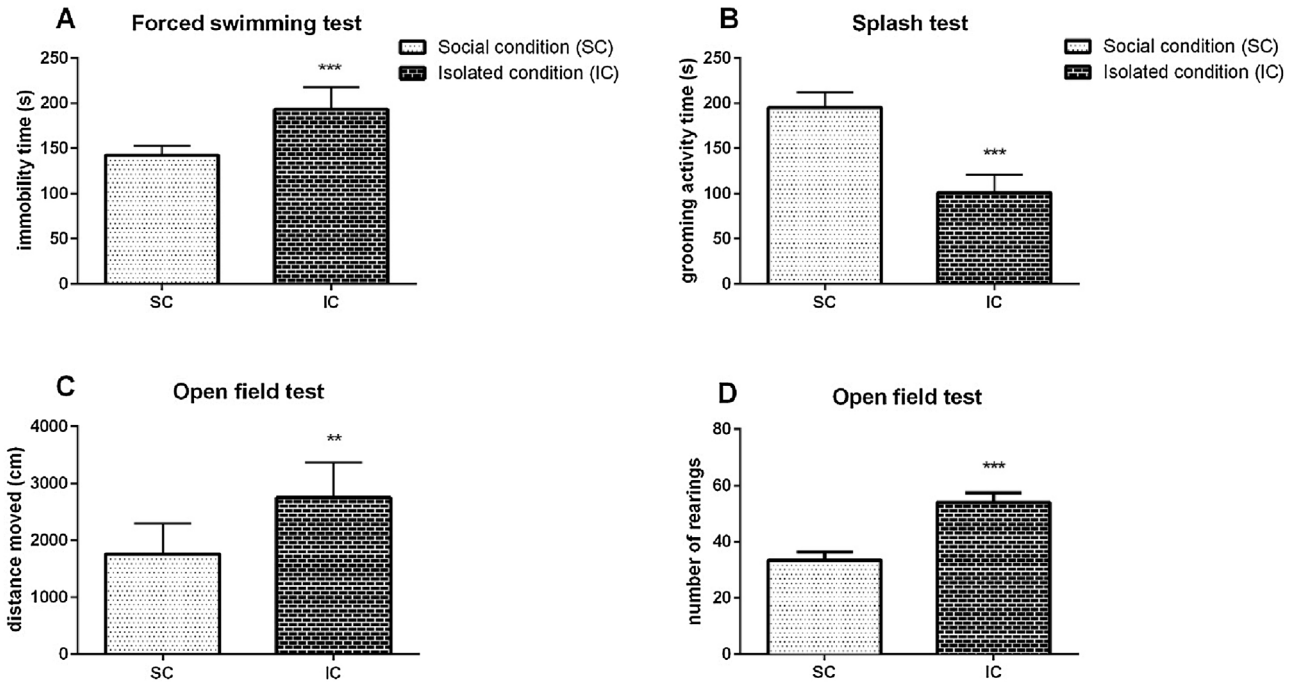

Fig. 1. Effects of SIS on behavioral despair in the FST (A), self-care behavior in the splash test (B) and locomotor activity in the OFT (C and D). Values are expressed as mean ± S.E.M. from 6 to 8 animals and were analyzed using *t*-test. $^{**}P<0.01$ and $^{***}P<0.001$ IC (isolated condition) and SC (social condition) animals were compared.

3. Result

3.1. Effect of social isolation stress on depressive- like behaviors

Results obtained from the *t*-test analysis showed that SIS induced depressive-like behaviors in FST and splash test. SIS significantly increased the immobility time in the FST ($t=4.967$, $df=12$, $P<0.001$, Fig. 1A) and significantly decreased the grooming activity time in splash test ($t=9.890$, $df=13$, $P<0.001$, Fig. 1B) when compared to SC control animals. In the open-field test, IC animals showed higher locomotor activity, including total distance moved ($t=3.442$, $df=14$, $P<0.01$, Fig. 1C) and number of rearings ($t=4.467$, $df=14$, $P<0.001$, Fig. 1D) in comparison with SC mice.

3.2. Effect of WIN55, 212-2 on depressive-like behaviors

Administration of WIN55, 212-2 (a non-selective CB1R/CB2R agonist) reversed depressant-like effect of SIS in IC animals, while had no effects on SC mice.

One-way ANOVA analysis showed that administration of WIN55, 212-2 significantly decreased immobility time in the FST ($F(3, 24)=1.193$, $P<0.001$, Fig. 2B) and increased grooming activity time in the splash test ($F(3, 24)=0.2696$, $P<0.001$, Fig. 2D) of IC mice when compared to saline-treated IC mice. Tukey's analysis showed that WIN55, 212-2 at doses of 3 and 5 mg/kg i.p. has significant effects on FST ($P<0.001$) and splash test ($P<0.01$). Also, administration of WIN55, 212-2 at 1 mg/kg/i.p. showed no effect on FST ($P>0.05$) and splash test ($P>0.05$) of IC animals. In addition, there was no significant effect between non-treated IC and vehicle-treated IC ($P>0.05$). Administration of aforementioned drug at any dose had no significant effects on IC mice in both distance moved ($F(3, 28)=0.3453$, $P>0.05$, Fig. 2F) and number of rearings ($F(3, 28)=0.4109$, $P>0.05$, Fig. 2H) as measured in the OFT.

Post- test analysis showed that administration of WIN55, 212-2 at higher dose (5 mg/kg) exerted a significant effect on immobility time of the FST ($F(3, 24)=1.526$, $P>0.05$ Fig. 2A) in SC animals. One-way ANOVA analysis showed that injection of WIN55, 212-2 had no significant effect on the grooming activity time in the splash test ($F(3, 28)=1.600$, $P>0.05$ Fig. 2C), locomotor activity ($F(3, 28)=0.4010$, $P>0.05$ Fig. 2E) and vertical activity ($F(3, 28)=0.8368$, $P>0.05$ Fig. 2G) of the OFT in SC-treated animals when compared to the saline-treated SC group. Tukey's analysis revealed that administration of WIN55, 212-2 at 5 mg/kg/i.p. significantly decreased immobility time in SC animals when compare with saline-treated animals ($P<0.05$); however, no significant effect was seen after administration of 1 and 3 mg/kg in the FST ($P>0.05$). Also, administration of WIN55, 212-2 at doses of 3 and 5 mg/kg/i.p. had not any effects on FST ($P>0.05$), splash test ($P>0.05$) and OFT ($P>0.05$) in SC animals when compare to saline-treated animals. Results obtained by comparing the non-treated IC and vehicle-treated IC animals showed no significant differences between these groups ($P>0.05$).

3.3. Blockade of CB1R and CB2R increases depressive-like behavior

One-way ANOVA analysis revealed that administration of AM-251 in IC mice increased immobility time in the FST in comparison with saline-treated IC mice ($F(2, 18)=0.6922$, $P<0.01$, Fig. 3B). Also, AM-251 decreased grooming activity time in the splash test ($F(2, 18)=3.522$, $P<0.05$, Fig. 3D). On the other hand, locomotor activity ($F(2, 21)=0.3344$, $P>0.05$ Fig. 2F) and vertical activity ($F(2, 21)=2.392$, $P>0.05$ Fig. 3H) were not changed in the OFT after administration of AM251. Tukey's analysis showed that administration of AM251 at 0.5 mg/kg i.p. showed significant effect on FST ($P<0.01$) and splash test ($P<0.05$) of IC animals when compared with saline-treated IC mice. However, administration of lower dose of AM-251 (0.2 mg/kg/i.p.) in IC animals did not alter the immobility, grooming times, distance moved and the number of rearings in comparison to saline-treated IC group ($P>0.05$). Also, results revealed no significant differences between non-treated and vehicle-treated IC animals ($P>0.05$).

After injection of AM-251 in SC mice, one-way ANOVA analysis demonstrated no differences between drug-treated SC animals and saline-treated SC animals in FST ($F(2, 18)=0.3537$, $P>0.05$,

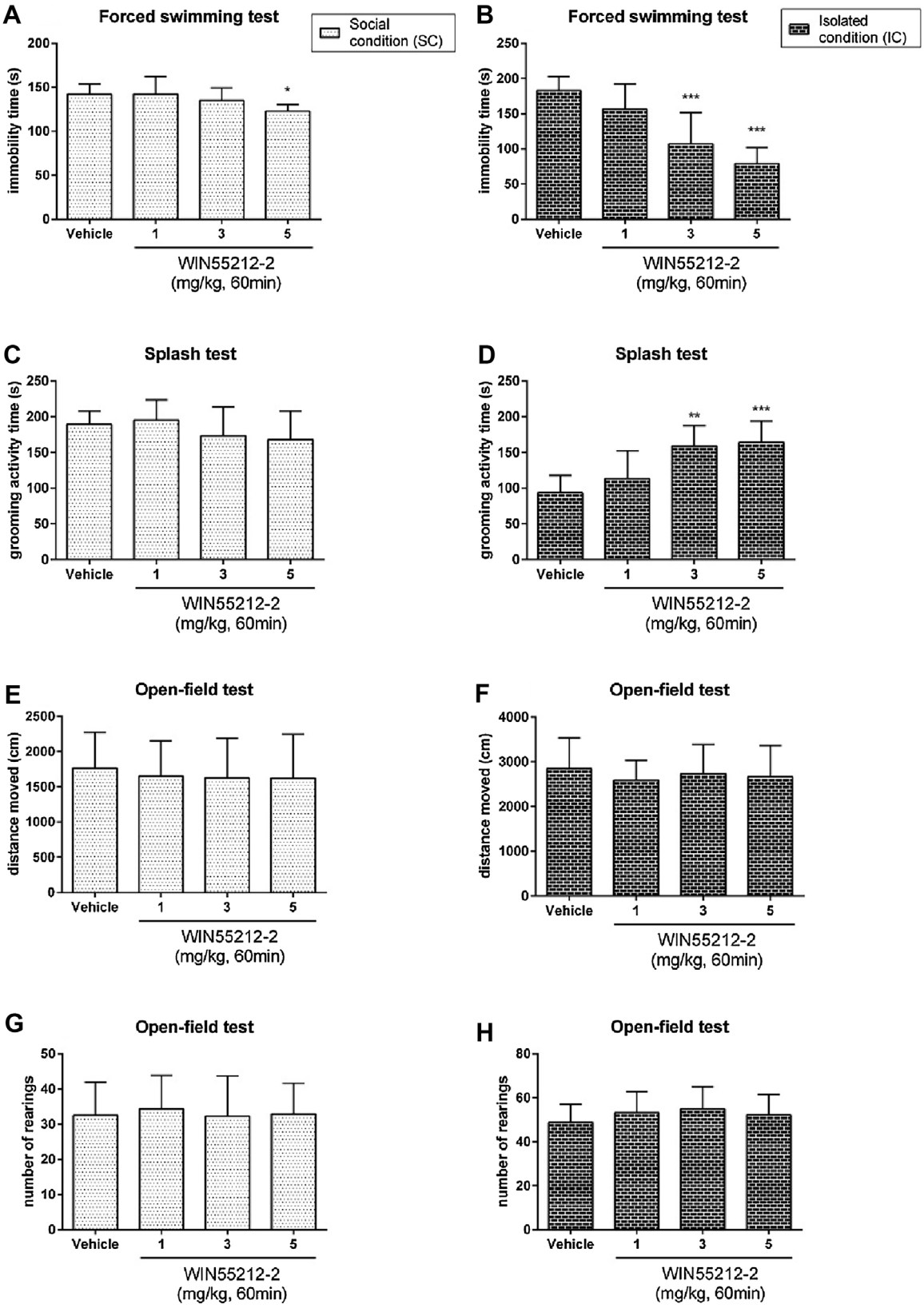

Fig. 2. Effects of WIN55, 212-2 (1, 3 and 5 mg/kg) on IC mice (B, D, F and H) and SC mice (A, C, E and G); despair behavior in the FST (A and B), self-care behavior in the splash test (C and D), distance moved in the OFT (E and F) and number of rearings in the OFT (G and H). Values are expressed as mean ± S.E.M. from 6 to 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, drug-treated mice from each group were compared with corresponding vehicle-treated animals.

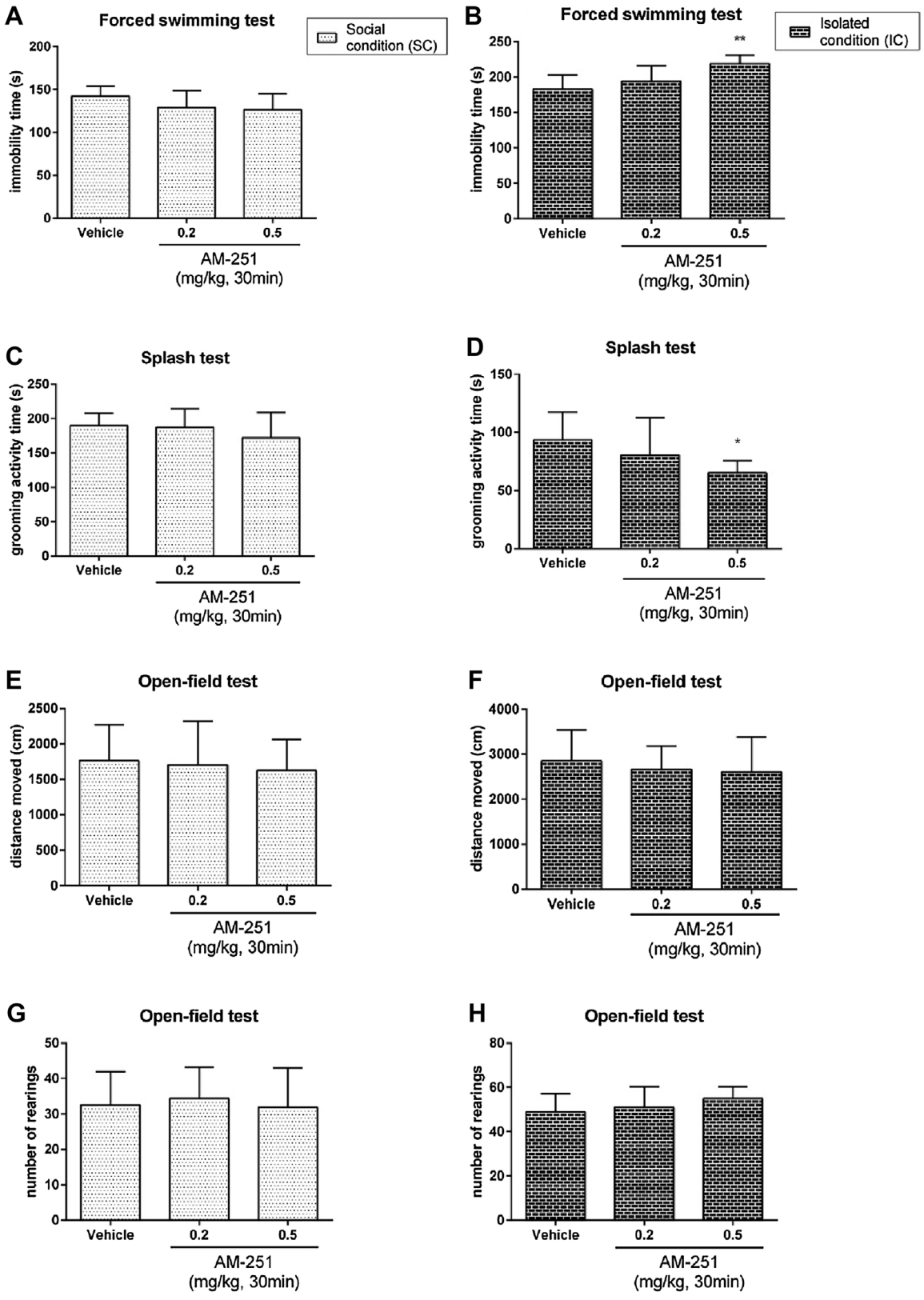

Fig. 3. Effects of AM-251 (0.2 and 0.5 mg/kg) on IC group (B, D, F and H) and SC group (A, C, E and G); despair behavior in the FST (A and B), self-care behavior in the splash test (C and D), distance moved in the OFT (E and F) and number of rearings in the OFT (G and H). Values are expressed as mean ± S.E.M. from 6 to 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{*}P < 0.05$ and $^{**}P < 0.01$, drug-treated mice from each group were compared with corresponding vehicle-treated animals.

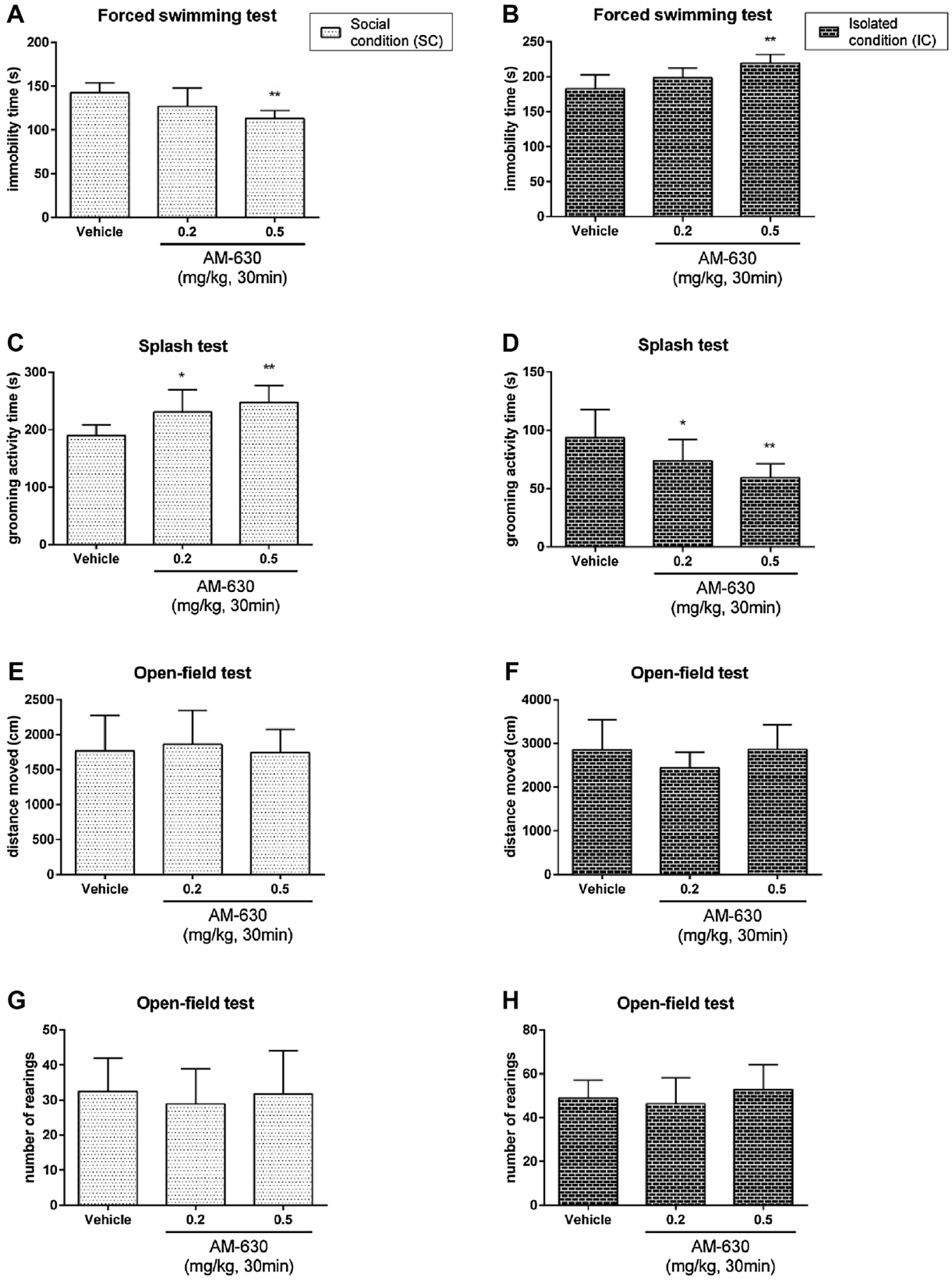

Fig. 4. Effects of AM-630 (0.2 and 0.5 mg/kg) on IC group (B, D, F and H) and SC group (A, C, E and G); despair behavior in the FST (A and B), self-care behavior in the splash test (C and D), distance moved in the OFT (E and F) and number of rearings in the OFT (G and H). Values are expressed as mean ± S.E.M. from 6 to 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{*}P < 0.05$ and $^{**}P < 0.01$, drug-treated mice from each group were compared with corresponding vehicle-treated animals.

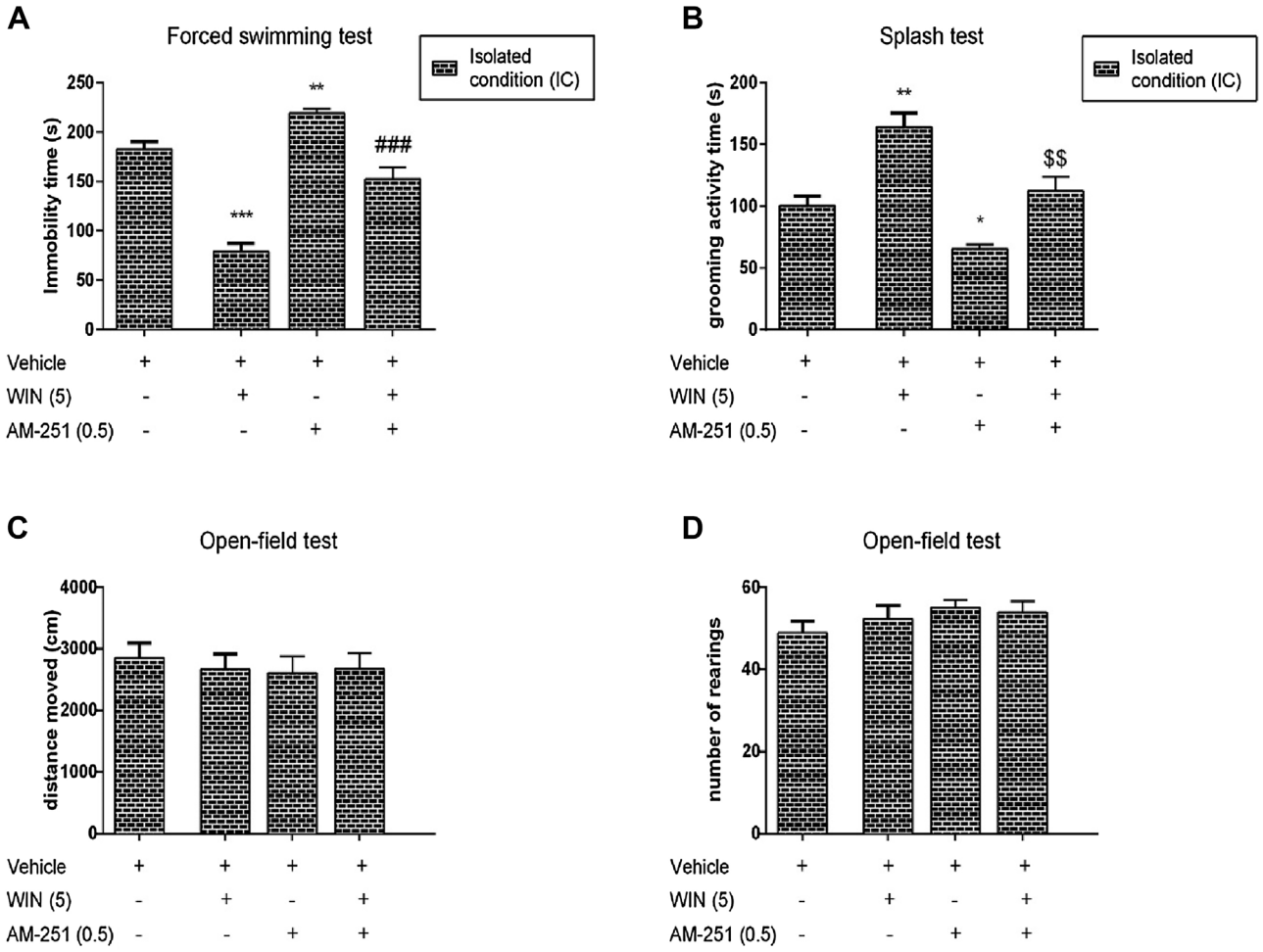

Fig. 5. Effects of co-administration of AM-251 (0.5 mg/kg) and WIN55, 212-2 (5 mg/kg) on behavioral despair in the FST (A), self-care behavior in the splash test (B), distance moved in the OFT (C) and number of rearings in the OFT (D) in IC group. Values are expressed as mean ± S.E.M. from 6 to 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{###}P<0.001$ and $^{\$\$}P<0.01$ compared with the WIN55, 212-2-treated group. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared with vehicle-treated group.

Fig. 3A), splash test (F (2, 21) = 0.9670, $P>0.05$, Fig. 3C) and OFT (distance moved (F (2, 21) = 1.463, $P>0.05$, Fig. 3E)) and number of rearings (F (2, 21) = 0.1039, $P>0.05$, Fig. 3G). In addition, no significant effect was found between non-treated and vehicle-treated SC animals ($P>0.05$).

One-way ANOVA revealed that AM-630 exerted significant effects on depressive-like behaviors of IC mice in the FST (F (2, 18) = 0.2816, $P<0.01$, Fig. 4B) and splash test (F (2, 18) = 1.604, $P<0.05$, Fig. 4D). Also, data showed that AM-630 injection had not significant effects in both locomotor activity (F (2, 21) = 1.525, $P>0.05$, Fig. 4F) and vertical activity (F (2, 21) = 0.2135, $P>0.05$, Fig. 4H) of OFT in IC animals.

Tukey's analysis revealed that injection of AM-630 significantly increased immobility time in FST at the highest dose (0.5 mg/kg) ($P<0.01$) and decreases grooming activity in splash test when used at 0.2 mg/kg ($P<0.05$) and 0.5 mg/kg ($P<0.01$); however, AM-630 failed to show any significant effects in the OFT ($P>0.05$). Also, no significant difference was found between no non-treated and vehicle-treated IC animals ($P>0.05$).

However, the results were different in SC mice. Administration of AM-630 to SC mice showed antidepressant-like effect in the FST (F (2, 18) = 1.525, $P<0.05$, Fig. 4A) and splash test (F (2, 21) = 4.929, $P<0.05$, Fig. 4C). On the other hand, no significant difference was seen after injection of AM-630 in distance moved (F (2, 21) = 0.8115, $P>0.05$, Fig. 4E) and number of rearnigs (F (2, 21) = 1.708, $P>0.05$, Fig. 4G) in OFT.

Tukey's analysis showed that administration of AM-630 significantly decreased immobility time in FST at the highest dose (0.5 mg/kg) ($P<0.01$) and increased grooming activity in splash test when used at 0.2 mg/kg ($P<0.05$) and 0.5 mg/kg ($P<0.01$); however, AM-630 failed to show any significant effects in the OFT ($P>0.05$).

3.4. CBR1 and CBR2 receptor antagonists reverse the effects of CBR agonist in SIS

To further investigate the role of the cannabinoid system on depressive-like behaviors following SIS, we carried out simultaneous administration of WIN55, 212-2 (60 min before tests) with AM-251 (30 min before tests) or AM-630 (30 min before test).

Results from abovementioned experiment showed that both AM-251 and AM-630 reversed the effects of WIN55, 212-2. One-way ANOVA analysis showed that co-administration of AM-251 increased immobility time in the FST (F (3, 24) = 0.9986, $P<0.001$, Fig. 5A) and decreased grooming activity time in the splash test (F (3, 24) = 0.8833, $P<0.001$, Fig. 5B) when compared to WIN55,212-2-treated IC mice. On the other hand, no effect was detected in the distance moved of OFT results in IC (F (3, 28) = 0.06603, $P>0.05$, Fig. 5C) after administration of AM-251 plus WIN55,212-2. Also, we did not detect any significant effect in the number of rearings

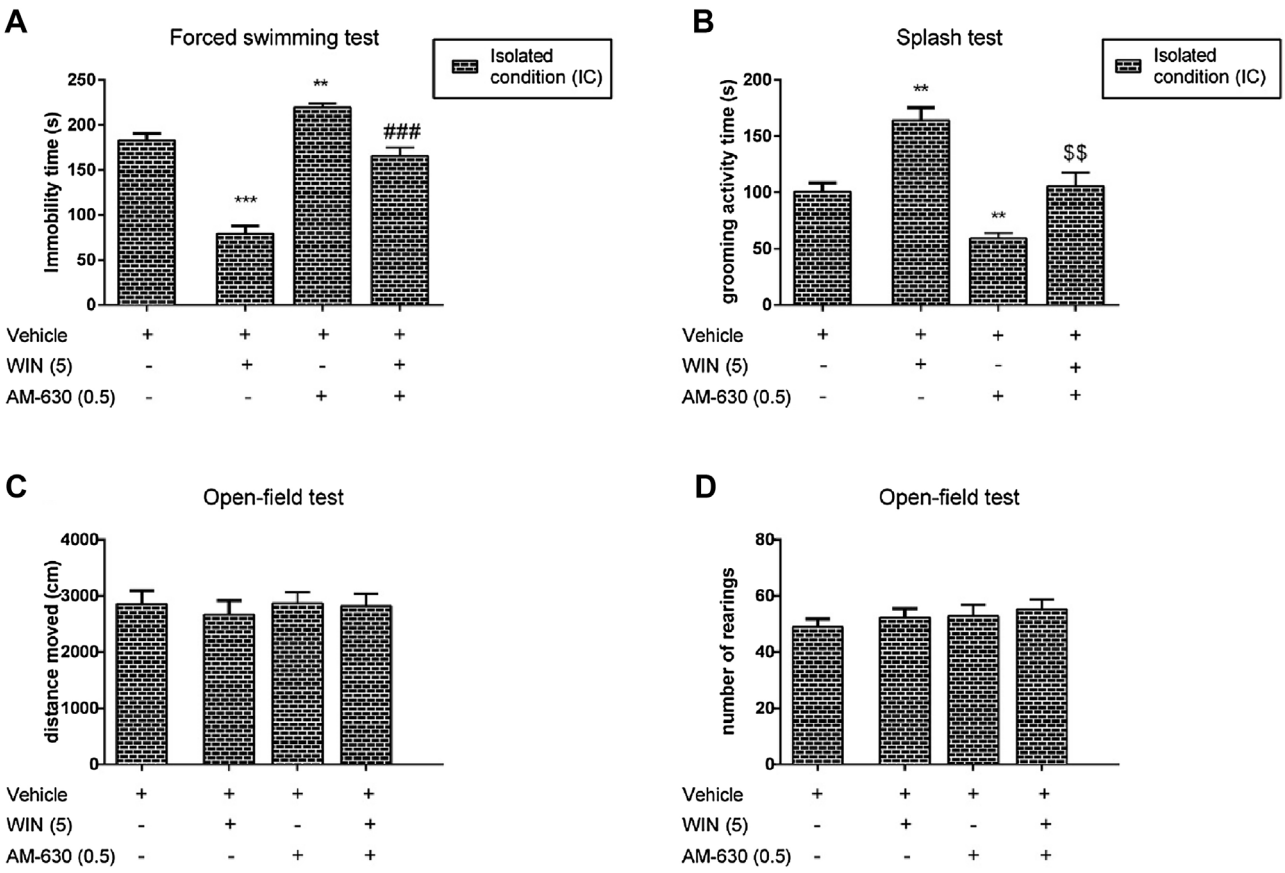

Fig. 6. Effects of co-administration of AM-630 (0.5 mg/kg) and WIN55, 212-2 (5 mg/kg) on behavioral despair in the FST (A), self-care behavior in the splash test (B), distance moved in the OFT (C) and number of rearings in the OFT (D) in IC group. Values are expressed as mean ± S.E.M. from 6 to 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{###}P < 0.001$ and $^{\$\$}P < 0.01$ compared with the WIN55, 212-2-treated group. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with vehicle-treated group.

(F (3, 28) = 0.4705, $P > 0.05$, Fig. 5D) in the OFT after co-administration of the two drugs. Result from Tukey's study showed that administration of both AM-251 and WIN55, 212-2 reversed the effects of WIN55, 212-2 in both FST ($P < 0.001$) and splash test ($P < 0.01$) in IC mice.

One-way ANOVA showed that AM-630 reversed the effects of WIN55,212-2 in the FST (F (3, 24) = 0.4947, $P < 0.001$, Fig. 6A) and splash test (F (3, 24) = 0.7111, $P < 0.001$, Fig. 6B) in IC mice. Fig. 6C shows that AM-630 and WIN55, 212-22 co-injection had no significant effects on distance moved of OFT (F (3, 24) = 0.2586, $P > 0.05$, Fig. 6C) and number of rearings (F (3, 28) = 0.1826, $P > 0.05$, Fig. 6D) in IC mice. Result obtained from Tukey's revealed that AM-630 co-injection reversed the effects of WIN55, 212-2 on the FST ($P < 0.001$) and splash test ($P < 0.01$) in IC mice.

3.5. Co-administration of CBR agonist with CBR antagonists in SC animals

To further clarify the involvement of the cannabinoid system in the pathophysiology of depression induced by SIS, we investigated the effect of co-administration of the CBR antagonists with CBR agonist in SC animals. In this regard, we injected WIN55, 212-22 (60 min before tests) prior to administration of AM-251 and AM-630 (30 min before tests) in SC mice.

One-way ANOVA analysis showed no significant difference between WIN55, 212-22-treated animals and co-administration of AM-251 with WIN55, 212-22 in the FST (F (3, 24) = 0.9888, $P > 0.05$, Fig. 7A), the splash test (F (3, 28) = 1.088, $P > 0.05$, Fig. 7B), distance moved of the OFT (F (3, 28) = 0.6489, $P > 0.05$, Fig. 7C) and number of rearings (F (3, 28) = 0.2688, $P > 0.05$, Fig. 7D) in SC mice.

On the other hand, one-way ANOVA analysis revealed that injection of AM-630 plus WIN55, 212-22 in SC animals does not induce any significant effect as compared to WIN55, 212-22-treated mice in FST (F (3, 24) = 0.1773, $P < 0.01$, Fig. 8A), distance moved of the OFT (F (3, 28) = 0.6352, $P > 0.05$, Fig. 8C) and number of rearings (F (3, 28) = 1.587, $P > 0.05$, Fig. 8D). However, one-way ANOVA showed that there is a significant difference between co-injection (AM-630 with WIN55, 212-22) and AM-630-treated animals in the splash test (F (3, 28) = 3.915, $P < 0.001$, Fig. 8B). Results obtained from Tukey's analysis revealed that grooming activity time after injection of AM-630 and WIN was significantly longer as compared with animals treated with WIN alone in the splash test ($P < 0.001$).

4. Discussion

The results described in the present study showed that the depressive-like behaviors induced by SIS are mitigated through the activation of CBRs. Our results provided evidence for antidepressant-like effects of WIN55, 212-22 (CB1R and CB2R non-selective agonist) on IC mice using a number of previously validated behavioral tests, which are valid in rodents. In addition, we showed that antidepressant-like effects of WIN55, 212-22 were prevented by co-administration of CB1R antagonist (AM-251) or CB2R antagonist (AM-630). We found that administration of WIN55, 212-22 in IC mice significantly decreased immobility time in the FST, and increased grooming activity time in the splash test. Furthermore, antidepressant-like effects of WIN55, 212-22 reversed following co-administration with AM-630 (CB2R antagonist) and AM251 (CB1R antagonist).

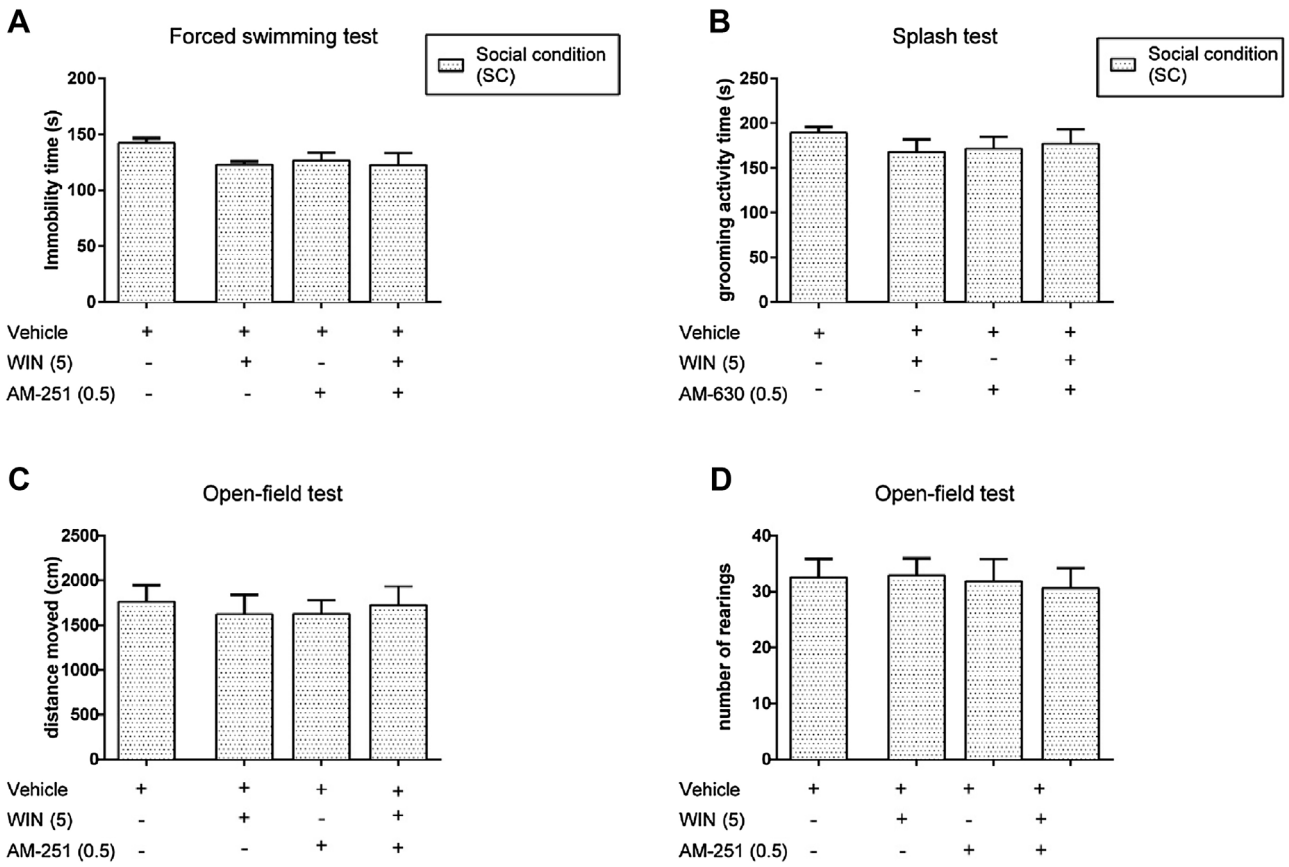

Fig. 7. Effects of co-administration of AM-251 (0.5 mg/kg) and WIN55, 212-2 (5 mg/kg) on behavioral despair in the FST (A), self-care behavior in the splash test (B), distance moved in the OFT (C) and number of rearings in the OFT (D) in SC group. Values are expressed as mean ± S.E.M. from 6 to 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test.

SIS in early life is known as a pivotal risk factor for the development of psychiatric disorders such as depression (Fone and Porkess, 2008; Weiss et al., 2004; Grippo et al., 2008). Previous studies have demonstrated that SIS provoked anxiety and depressive-like behaviors in animal models (Grippo et al., 2008). In the current study, using FST (increase in the immobility time) and splash test (decrease in grooming activity time) we showed that SIS induced depressive-like behaviors in IC animals in comparison to SC mice. Increase in immobility time in the FST reflects the depressive mood (despair behavior) in rodents (Cryan et al., 2005). Additionally, we used splash test in order to investigate self-care behavior. Decrease in grooming activity in the splash test reflects self-care difficulties in rodents (Detanico et al., 2009; Willner, 2005). Our findings showed that IC mice showed an increase in immobility time in the FST as well as a decrease in grooming activity time in the splash test. We used OFT to evaluate the effects of SIS and treatments on motor function, exploratory behavior, and to exclude possible alterations in locomotion that might affect FST (Kulesskaya and Voikar, 2014; Amiri et al., 2016). OFT was performed directly before the FST to assess ambulatory behavior and also to prove that variations which occur in motor activity did not affect the immobility time (Kaster et al., 2005).

The cannabinoid system is a neuromodulator system which classically consists of the CB1R and CB2R (Matsuda et al., 1990; Gerard et al., 1991). The cannabinoid system is involved in many physiological processes such as appetite, pain-sensation, memory and mood (Rodriguez Bambico et al., 2009; Cota et al., 2003; Marsicano et al., 2002). Previous studies have shown that stress, fear and emotions alter the expression of the CB1R in different regions of the brain such as amygdala, nucleus accumbens (NAc), hippocampus, and pre-frontal cortex (PFC) (Breivogel and Sim-Selley, 2009; Herkenham et al., 1991; Pazos et al., 2005).

Evidence indicates that both CBR antagonists and agonists are able to produce antidepressant-like effects in different animal models of depression (Rodriguez Bambico et al., 2009). In this regard it should be noted that there is an inconsistency in the literature regarding the effect of CBR activation and/or inhibition on depression state.

In this regard, there are some studies reporting that CBR agonists decrease immobility in the FST without affecting locomotor activity in the OFT in a rat model (Hill and Gorzalka, 2005; Jiang et al., 2005). In addition, other studies showed that CBR antagonists exerted antidepressant-like effect by decreasing the immobility time without any change in locomotor activity in a mouse model (Takahashi et al., 2008; Tzavara et al., 2003). These results may be related to some fundamental differences between the rat and mice cannabinoid system. Overall, it could be concluded that both cannabinoid agonists and antagonists have antidepressant-like effect.

A study by Macri and Laviola has shown that the effects of WIN55, 212-2 on depressive-like behaviors induced by early-life stress are dose dependent in a mouse model. Indeed, administration of WIN55, 212-2 at low dose (0.5 mg/kg, intraperitoneal once daily for 3 days) has antidepressant-like effect and there is no significant effect at high dose (2 mg/kg, intraperitoneal once daily for 3 days) (Macrıı̀and Laviola, 2004). However, these data differ from the reported antidepressant- like activity of acute high doses of the CB1R antagonist Rimonabant (Tzavara et al., 2003; Shearman et al., 2003) or the inverse agonist AM-251 (Shearman et al., 2003) in mice. Our results showed that administration of WIN55, 212-2 at doses of 3 and 5 mg/kg i.p. significantly reversed depressive-

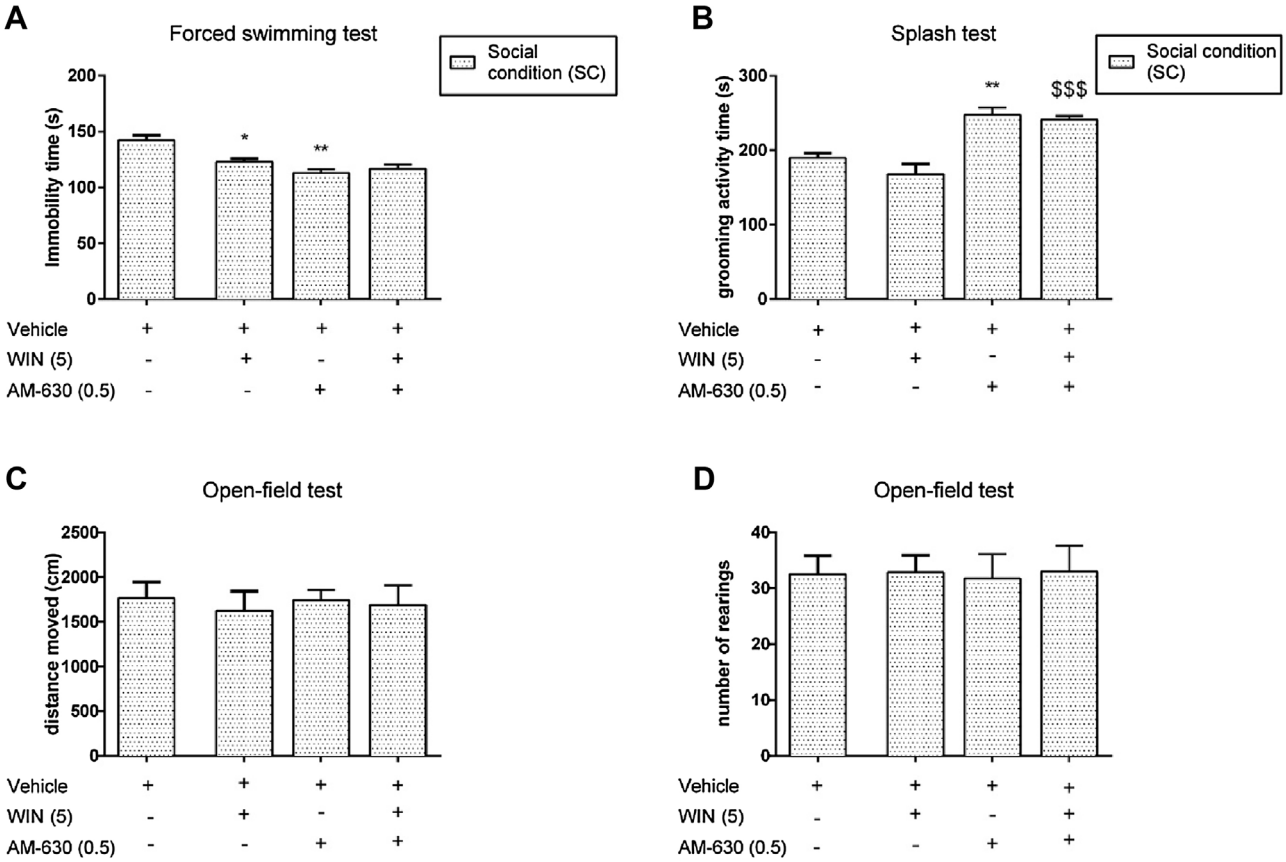

Fig. 8. Effects of co-administration of AM-630 (0.5 mg/kg) and WIN55, 212-2 (5 mg/kg) on behavioral despair in the FST (A), self-care behavior in the splash test (B), distance moved in the OFT (C) and number of rearings in the OFT (D) in SC group. Values are expressed as mean ± S.E.M. from 6 to 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{\$\$}P < 0.01$ compared with the WIN55, 212-2-treated group. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with vehicle-treated group.

like behaviors provoked by SIS as measured in the FST and splash test while dose 1 mg/kg i.p. had no significant effect in these tests. Furthermore, WIN55, 212-2 at aforementioned doses had no significant effect in the OFT. The reason of inconsistency between the results of our work with previously mentioned studies may be associated with the different animal models used (social isolation and maternal separation) and also the duration of drug administration (acute and chronic WIN55, 212-2 injection).

Data from previous studies showed that CB2R agonists have antidepressant-like effect in a rat model (Hu et al., 2009). In line with previously mentioned studies, our findings showed that WIN55, 212-2 (a non-selective agonist of CBR) elicited antidepressant-like activity in IC mice. Administration of the CB2R antagonist, similarly to the CB1R antagonist, significantly reversed the antidepressant properties of WIN.

In this study, we showed that CBR agonist (WIN55, 212-22) has antidepressant-like effect at doses of 3 mg/kg and 5 mg/kg in SC mice. This suggests that both CBRs are involved in the pathway of depression-like behavior induced by SIS in mice.

Although literature has shown that CBRs play a role in depression, little is known about the relation between SIS and the cannabinoid system (Zamberletti et al., 2012; Sciolino et al., 2010). In this study, we investigated to investigate the role of the cannabinoid system in a mouse model of SIS. Data from our study showed the possible role for the involvement of cannabinoid system in depressive-like behaviors induced by SIS. This study showed that CB1R and CB2R agonist reversed the depressive-like behaviors of SIS whereas co-administration of CB1R or CB2R selective antagonists with CBR non-selective agonist reversed the antidepressive-like effect of CBR agonist (increase in immobility time as well as decrease in grooming activity time).

5. Conclusion

Our results showed that the CBRs are involved in depressive-like behaviors induced by SIS. In this study, we showed that CB1R and CB2R are both involved in promoting antidepressant-like effects in the SIS mouse model.

Compliance with ethical standards

The authors declare no conflict of interest. Also, all applicable international and institutional guidelines for the care and use of animals were followed.

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