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Co-administration of cannabidiol and ketamine induces antidepressant-like effects devoid of hyperlocomotor side-effects

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ABSTRACT

Background and Purpose: Although useful as a rapid-acting antidepressant drug, ketamine is known to induce psychotomimetic effects, which may interfere with its therapeutic use. Cannabidiol (CBD) is a non-psychostimulant compound from *Cannabis sativa*, which has shown promising antidepressant effects without inducing hyperlocomotion. AMPA receptor activation is involved in the antidepressant effect induced by ketamine, but its relevance for the effects of CBD is not known. Moreover, given that CBD has antipsychotic and antidepressant properties, it is unknown whether adding CBD to ketamine could potentiate the antidepressant properties of ketamine while also attenuating its psychostimulant effects.

Experimental approach: S-Ketamine (2.5, 3, 5, 10, 30 mg/kg) and cannabidiol (3, 10, 30 mg/kg) were administered alone or in combination to male Swiss mice. Independent groups received NBQX (AMPA receptor antagonist) 5 min before administration of CBD or S-ketamine. The antidepressant-like effect was assessed in the forced swimming test (FST), and the open field test (OFT) evaluated the psychostimulant effect.

Key results: CBD induced significant dose-dependent antidepressant effects without causing hyperlocomotion in the OFT. S-ketamine produced an antidepressant effect associated with hyperlocomotion in the higher dose. NBQX inhibited the antidepressant effect of both ketamine and CBD. Pretreatment with CBD (10 mg/kg) attenuated the ketamine-induced hyperlocomotion while preserving its antidepressant effect.

Conclusion: AND IMPLICATIONS: Similar to ketamine, the antidepressant-like effect elicited by CBD involves AMPA receptor activation. Additionally, CBD prevents the hyperlocomotion induced by S-ketamine without affecting its antidepressant-like effect. Our findings suggest that CBD and ketamine's combined administration can be a promising therapeutic strategy for achieving an appropriate antidepressant effect without unwanted side-effects.

This article is part of the special issue on 'Cannabinoids'.

1. Introduction

Major depressive disorder (MDD) is a chronic and recurrent mental disorder causing reduced quality of life, medical morbidity, mortality, and social-economic losses (Kessler and Bromet, 2013). The World Health Organization estimates that by 2030, depression will be the leading cause of disability worldwide (WHO, 2018). Despite proven efficacy (Bauer et al., 2015; Cipriani et al., 2011), most clinically available antidepressants have two significant limitations: delayed onset of the therapeutic effect (2–4 weeks) and no satisfactory response in a considerable number of patients (Dupuy et al., 2011; Souery et al., 2006). These facts highlight the need for new pharmacological

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; FST, forced swimming test; mTOR, mechanistic target of rapamycin; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium salt; NMDA, N-methyl-D-aspartate; OFT, open field test; SEM, standard error of the mean.

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treatments with different mechanisms of action and better clinical outcomes. In this context, several studies point to using low doses of ketamine as a new antidepressant strategy with an improved therapeutic profile (Aleksandrova et al., 2017; Berman et al., 2000; Bobo et al., 2016).

Ketamine was primarily developed and used as a dissociative anaesthetic in clinical practice (Gjessing, 1968). Berman and colleagues later showed that, at subanesthetic doses, ketamine induces an antidepressant effect with a new clinical response pattern: rapid and sustained (Berman et al., 2000). Such an effect was also observed in animals exposed to different models to study depression neurobiology and treatment, thus allowing the investigation of ketamine mechanisms of action (Willner, 2019). The primary mechanism responsible for the fast antidepressant effect of ketamine seems to involve the disinhibition of the glutamatergic neurotransmission that ultimately leads to AMPA receptor activation in the forebrain (Koike et al., 2011; Koike and Chaki, 2014; S Maeng et al., 2008).

Ketamine is presented as a racemic mixture [(R,S)-ketamine] of equal amounts of R-ketamine and S-ketamine (esketamine). S-ketamine is four times more potent on NMDA receptors than its isomer and more likely to induce psychomimetic effects (Bahji et al., 2021). S-ketamine has been more extensively studied in humans and The Federal Drug Administration (FDA) recently approved its use in patients with treatment-resistant depression (Wajs, 2020). Despite the effectiveness of S-ketamine, its broader use is limited by undesired side effects, such as psychotic symptoms and increased liability for abuse and dependence, which can be observed even in therapeutic doses (Sanacora and Schatzberg, 2015; Molero et al., 2018; Bahji et al., 2021; Chang et al., 2019). In animal models, the psychostimulant effects of ketamine and its enantiomers can be observed as increased locomotion in the open-field test and impaired prepulse-inhibition (Brakatselos et al., 2020; Chang et al., 2019). The neurochemical bases of such effects are not entirely clear (Irifune et al., 1991; Neill et al., 2010), and it is also not known if they are essential to mediate ketamine antidepressant effects (Ballard and Zarate., 2020).

Previous evidence reported that cannabidiol (CBD), the main non-psychostimulant effect of Cannabis sativa, attenuates the hyperlocomotion and PPI deficits induced by high doses of ketamine (Moreira e Guimaraes, 2005) and can prevent the psychotomimetic effects of other drugs such as THC and amphetamine in both humans and laboratory animals (Karniol et al., 1974; Moreira and Guimarães, 2005; Vann et al., 2008). Moreover, we recently demonstrated that CBD promotes fast and sustained antidepressant effects, without inducing hyperlocomotion (Sales et al., 2018a,b). The fast antidepressant effects of CBD have been confirmed in different animal models (El-Alfy et al., 2010; Linge et al., 2016; Silote et al., 2019; Zanelati et al., 2010) and its mechanism of action seems to involve the facilitation of glutamate neurotransmission in the prefrontal (Linge et al., 2016) and the BDNF-TrkB pathway (Sartim et al., 2018), as also described for ketamine. Although both mechanisms are associated with AMPA receptor activation, there is no evidence that CBD binds to these receptors or that the antidepressant properties of CBD are dependent on AMPA receptor activation. CBD does not induce a psychomimetic effect or holds potential for abuse/dependence (Silote et al., 2019) and its coadministration with ketamine could unravel important therapeutic potential to favor antidepressant effects without unwanted dissociative side-effects.

Therefore, this work investigated the participation of AMPA receptors on CBD effects and the consequences induced by the combined administration of CBD and ketamine. Furthermore, we aimed to provide evidence for a translational rationale for CBD use as add-on therapy in ketamine administration in depressed patients.

2. Methods

2.1. Drugs

The following drugs were used in the experiments: cannabidiol (CBD, Pratti-Donaduzzi, Brazil) at the doses of 3, 10, and 30 mg/kg (Sartim et al., 2018); S-Ketamine (dextrocetamine hydrochloride, Cristália, Brazil) at the doses of 2.5, 3, 5, 10 and 30 mg/kg (Fukumoto et al., 2016); NBQX (sodium salt, Cayman Chemical Company), at the dose of 10 mg/kg (Fukumoto et al., 2016). The prototype antidepressant imipramine was used as a positive control at the dose of 30 mg/kg (Zanelati et al., 2010). CBD was dissolved in Tween 80 3% and sterile isotonic saline (Sartim et al., 2018). S-Ketamine, NBQX, and imipramine were dissolved in sterile isotonic saline (Fukumoto et al., 2016). All drugs were freshly prepared before use and protected from light. Each control group received the corresponding vehicle of the drug being tested (10 ml/kg).

2.2. Animals and housing

All the experiments were performed under the ethical principles adopted by the National Council for the Control of Animal Experimentation (CONCEA), and all efforts were made to avoid the animals' suffering. The local Ethical Committee of the University of São Paulo, Ribeirão Preto Campus, approved the experimental protocols (protocol number 19.1.625.60.4). All experiments were performed with male heterogeneous Swiss mice (8–9 weeks old, 35–40g) purchased from the animal facility of the University of São Paulo, Campus of Ribeirão Preto (Brazil). The animals were brought to the animal house associated with the Laboratory of Neuropsychopharmacology one week before the start of the experiments and kept in the following standard laboratory conditions: 10 animals per cage (41X34 × 18cm) containing 3 cm of bedding; controlled temperature (24° ±1); 12-h light/dark cycle (lights on at 6 a.m.); food (commercial rodent cow, Nuvilab – Quimtia – Paraná, Brasil) and tap water available ad libitum, except for the experiment time. Bedding in the home cage and the drinking water was changed twice a week. All behavioural experiments were carried out during the light phase, in the afternoon (01–06 p.m.).

To avoid potential false positive or negative results due to increased individual variability in baseline immobility, all animals were submitted to a pre-test session and randomized in the different treatment groups according to their immobility time in the pretest (high or low immobility, HI or LI, respectively). The average immobility of all animals in the pretest was calculated and an animal with higher immobility than the average was considered HI whereas below average was considered LI. The HI and LI animals were then randomly allocated to the different treatment groups without any exclusion. This strategy also decreased the experimental variability and allowed the antidepressant effect to be observed with a smaller number of animals/group (validation data, not shown). The order of testing was randomized throughout the experiment period to avoid circadian influences. The same mice were submitted to the open field and, immediately after, the forced swim test. A total of 341 Swiss mice were used. The sample sizes were calculated using power analysis, and the information about the numbers (n) of each experimental group is provided in the figures.

The animals were habituated to the experimental room 60 min before the beginning of the experiments to avoid any influence of movement or the new environment in the behavioural tests.

2.3. Locomotor activity (open field test)

The open-field test was used to measure the locomotor activity in animals, where both baseline activity and drug-induced changes can be quantified (Gould et al., 2009). The distance travelled during the test period was recorded as the index of the locomotor activity.

Two OFT protocols were performed (5 and 20 min duration). In the

5-min test, the aim was to assess possible drug effects in the locomotor activity that could be interpreted as false-positive results in the FST, thus the exposure to the OFT was of equal time as the forced swimming test, and immediately before FST exposure. The prolonged exposure to OFT (20 min) was performed to assess psycholocomotor effects induced by the drugs, which are more pronounced after an initial period of habituation in the OFT Kraeuter et al. (2020).

The interval between drug administration and the test was chosen based on the pharmacokinetic profile of the drugs. For ketamine, the time to reach the maximum concentration after an intraperitoneal injection is 25 min (Toki et al., 2018), whereas CBD takes 60 min to reach its maximum concentration in the brain after an intraperitoneal administration (Deiana et al., 2012). Based on this information, in the experiments where just one of these drugs was administered, we choose an interval between injection and test close to the peak of the effect (30 min for ketamine and 60 min for CBD). On the other hand, in the experiment with the combined administration of CBD and S-ketamine and OFT exposure for 20 min, we tried to find an interval of administration that could be closest to the maximum peak for both drugs (FST was performed 50 min after CBD administration and 40 min after ketamine, an intermediate period of time for both drugs).

2.3.1. Five minutes test

Animals were placed individually in the centre of a circular open-field arena (40 cm in diameter and height 35 cm) with dark bottom to contrast with the animal colour and the test was started immediately. The arena was cleaned between each test using alcohol 70% to avoid interfering with the smell of the previously tested animal. The exploratory activity was recorded for 5 min, and the total distance travelled during this time was measured by the software ANYMAZE.

2.3.2. Twenty minutes test

The open-field test (OFT) lasting 20 min was performed to assess the hyperlocomotor effect of the drugs after the initial exploratory activity has ceased (Kraeuter et al., 2020). The procedure during the test was the same as described in the previous item, with the difference that the exploratory activity was recorded for 20 min.

2.4. Forced swim test (FST)

The FST was used to evaluate the antidepressant-like effect of drugs and investigate their action mechanisms (Slattery and Cryan, 2012). The FST was performed with adaptations to the protocol described initially by Porsolt (Porsolt et al., 1977). To minimize variability in baseline immobility between animals, each mice was pre-exposed to forced swimming for 5 min (pretest session), 24 h before the test session (5 min duration). The same animal was submitted to the open-field test (OFT) and, immediately after the forced swimming test. The OFT duration was 5 min for experiments with pre-administration of antagonists and 20 min for hyperlocomotion assessment experiments. After the OFT, mice were individually placed to swim in acrylic cylinders (height 25 cm and diameter 18 cm) containing 15 cm of water at controlled temperature (24° ±1) under white light. The animals remained in the test for 5 min. The immobility time (interpreted as the time when the animal performs minimal movements required to keep the head above the water) was measured during the whole period. The test session was videotaped using a Sony digital video camera model DCR-SR47, and the analysis of immobility behaviour was performed by a trained experimenter that was blind to the treatments. The water in the cylinder was changed after each trial (Abel and Bilitzke, 1990).

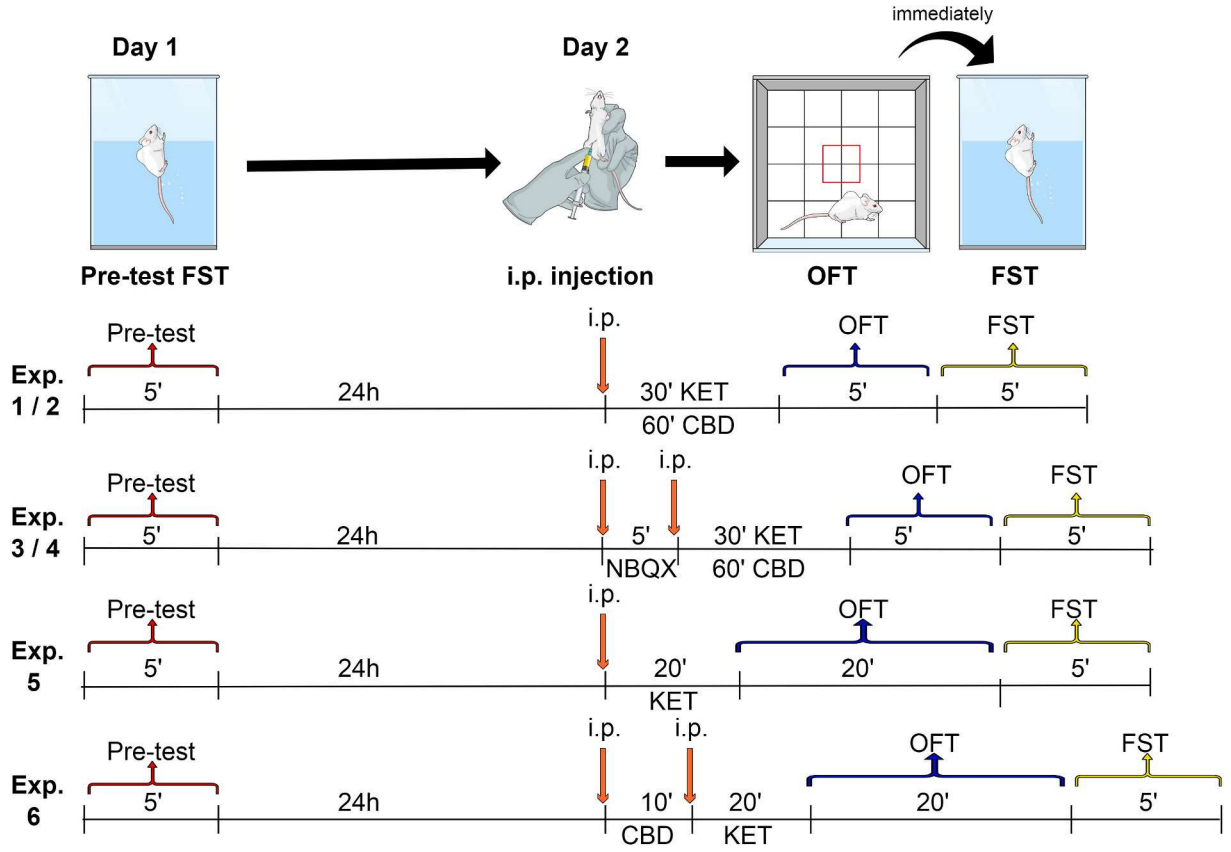

Fig. 1. Schematic representation of the experimental procedure and treatments for single or double injection.

3. Experimental design

The overall experimental design is depicted in Fig. 1.

Experiment 1: Effects of CBD in mice submitted to the forced swimming test and the open field test

To evaluate the effects induced by CBD systemically administered, 24h after the pretest mice received an intraperitoneal (i.p.) injection of vehicle or CBD (3, 10 and 30 mg/kg) and, after 60 min, were submitted to the OFT (5 min). Immediately after the OFT, the animals were submitted to the FST (5 min). An additional group, included as a positive control, received imipramine (30 mg/kg) 30 min before the test.

Experiment 2: Effects of S-Ketamine in mice submitted to the forced swimming test and the open field

To evaluate the effects induced by S-ketamine systemically administered, 24h after the pretest mice received an intraperitoneal (i.p.) injection of vehicle or S-ketamine (2.5, 5 and 10 mg/kg) and, after 30 min, were submitted to the OFT (5 min). Immediately after the OFT, the animals were submitted to the FST (5 min). An additional group, included as a positive control, received imipramine (30 mg/kg) 30 min before the test.

Experiment 3: Effects of NBQX (AMPA receptor antagonist) on CBD effects in the forced swim test and the open field test

This experiment was performed to investigate if CBD effects in the forced swim test depend on the activation of AMPA receptors. The experimental design was similar to experiment 2, but animals received an intraperitoneal injection of NBQX (10 mg/kg) or vehicle 5 min before administering CBD (10 mg/kg) or vehicle. Sixty minutes after the last injection, the animals were submitted to the OFT (5 min). Immediately after the OFT, the animals were submitted to the FST (5 min). A different animal group, included as a positive control, received an injection of the vehicle and, 5 min later, imipramine (30 mg/kg), 30 min before the test.

Experiment 4: Effects of NBQX (AMPA receptor antagonist) on S-Ketamine effects in the forced swimming test and the open field test

This experiment was performed as a control to reproduce previous findings in the literature. The mice previously submitted to the pretest received an i.p. injection of vehicle or NBQX (10 mg/kg) and, 5 min later, another i.p. injection of vehicle or S-ketamine (10 mg/kg). Thirty minutes later, the animals were individually exposed to the OFT (5 min) and, immediately after, to the FST (5 min). A different animal group was included as a positive control and received an injection of the vehicle followed, 5 min later, by imipramine (30 mg/kg), 30 min before the test.

Experiment 5: Effects of S-Ketamine in mice submitted to the forced swimming test and the open field test (20 min)

To evaluate if the antidepressant-like effect of S-ketamine (3, 10, and 30 mg/kg) would be associated with hyperlocomotion, mice previously submitted to the pretest received an intraperitoneal injection of vehicle or S-ketamine (3, 10, and 30 mg/kg) 20 min before exposure to the open field test (OFT), which lasted 20 min (the distance travelled was quantified in the last 5 min). Immediately after the OFT exposure, animals were submitted to the FST (5 min).

Experiment 6: Effects of CBD and S-ketamine co-administration in mice submitted to the forced swimming test and the open field test lasting 20 min

This experiment was performed to investigate whether the pre-administration of CBD (3 and 10 mg/kg) could alter the effects induced by S-Ketamine (10 and 30 mg/kg) in the FST and OFT. The mice received an i.p. injection of vehicle or CBD (3 and 10 mg/kg) followed, 10 min later, by a second i.p. injection of vehicle or S-ketamine (10 and 30 mg/kg). Twenty minutes later, animals were individually exposed to the OFT for 20 min and immediately after the FST. The distance travelled in the OFT was quantified in the last 5 min of the test, whereas immobility time in the FST was measured for 5 min.

4. Data and statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2015). Immobility time data obtained from FST were analyzed by one-way ANOVA or two-way ANOVA followed by Dunnett's or Tukey post-hoc test least significant difference test. The comparison between the immobility of animals with and without pretest was performed by *t*-test. The distance travelled in the OFT was analyzed by one-way ANOVA followed by Dunnett's post-hoc. All results are presented as means ± SEM. The post hoc tests were only applied when ANOVA results were significant ($P < 0.05$). In the figures, the significance level was set at $p < 0.05$. The raw data are published on figshare (Sartim, 2021a,b,c,d,e,f).

5. Results

Experiment 1: Effects of CBD of mice submitted to the forced swimming test and the open field test

Exposure to the pretest increased the animals' immobility time in the forced swim test ($t = 3.552$, $df = 17$, $p < 0.05$). Treatment with CBD (10 mg/kg) and imipramine (30 mg/kg) significantly reduced the immobility time of mice when compared to the vehicle-treated group ($N = 8–12$, $F_{4,54} = 4.461$, $p < 0.05$, Fig. 2A). None of the tested doses changed the distance travelled performed by animals in the OFT ($N = 8–13$, $F_{4,55} = 0.5656$, $p > 0.05$, Fig. 2B).

Experiment 2: Effects of S-Ketamine in mice submitted to the forced swimming test and the open field

Exposure to the pretest increased the animals' immobility time in the forced swim test ($t = 3.919$, $df = 9$, $p < 0.05$), an effect attenuated by S-ketamine (2.5, 5, and 10 mg/kg) and imipramine (30 mg/kg) ($N = 5–7$, $F_{4,28} = 13.94$, $p < 0.05$, Fig. 2C). None of the tested doses changed the distance travelled performed by animals in the OFT ($N = 5–7$, $F_{4,29} = 1.147$, $p > 0.05$, Fig. 2D).

Experiment 3: Effects of NBQX on CBD effects in the forced swimming test and the open field test

Exposure to the pretest increased the animals' immobility time in the forced swim test ($t = 2.877$, $df = 12$, $p < 0.05$) compared to the group not exposed to the pretest and this effect was attenuated by Imipramine (30 mg/kg) reduced the immobility time in the FST compared to the control group ($t = 9.148$, $df = 13$, $p < 0.05$). A two-way ANOVA indicated an interaction between the first and second injection ($F_{1,26} = 7.372$, $p < 0.05$), demonstrating that CBD (10 mg/kg) was effective when administered alone (Tukey, $p < 0.05$ vs control), but not when NBQX (10 mg/kg) was preadministered ($N = 6–8$, Tukey post-test, $p > 0,05$ vs control, Fig. 3A). None of the tested doses changed the distance travelled in the OFT ($N = 6–8$, $F_{4,32} = 0.5163$, $p > 0.05$, Fig. 3B).

Experiment 4: Effects of NBQX on S-Ketamine effects in the forced swimming test and the open field test

Exposure to the pretest increased the animals' immobility time in the

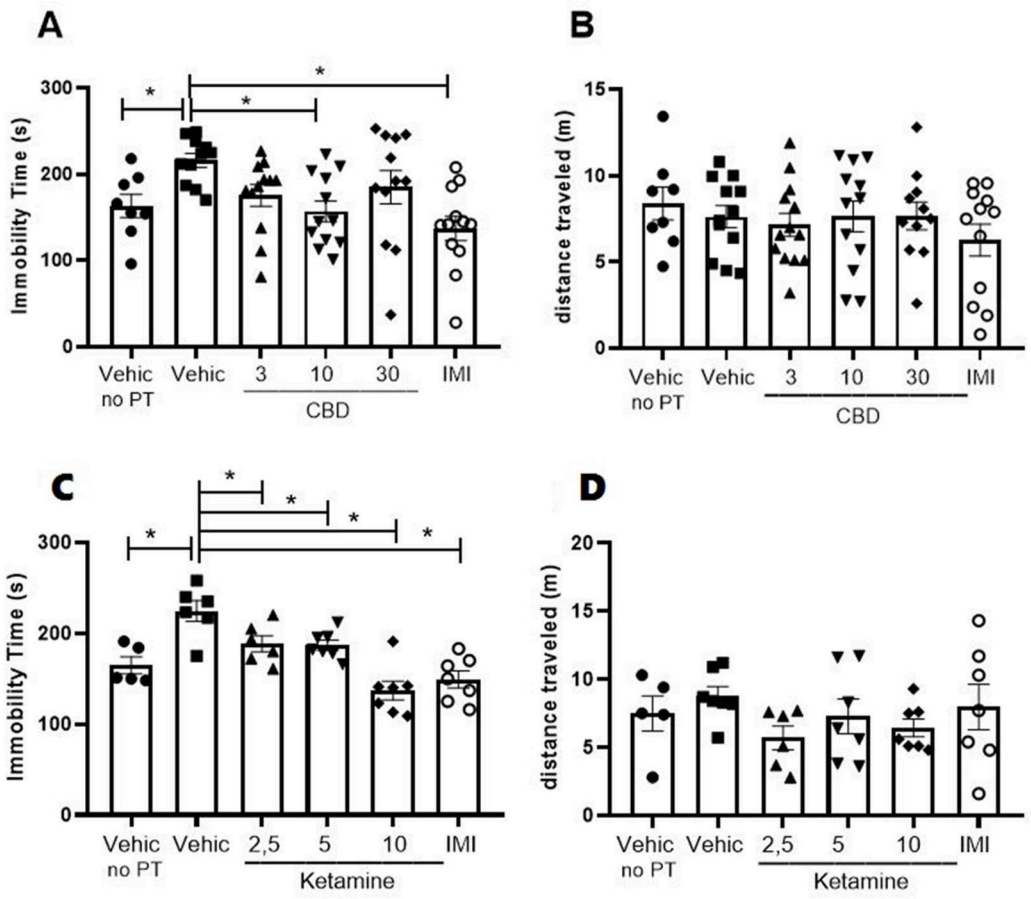

Fig. 2. CBD (3, 10, and 30 mg/kg) and S-ketamine (2.5, 5, and 10 mg/kg) effects in mice submitted to forced swimming and open field (5 min). **(A)** CBD (10 mg/kg) induced an antidepressant-like effect in the FST. Each bar represents the mean ± SEM of 8–12 mice (one-way ANOVA followed by Dunnett's test, $p < 0,05$). **(B)** CBD induced no change in the distance travelled in the OFT ($N = 8–13$, one-way ANOVA followed by Dunnett's, $p > 0,05$). **(C)** S-ketamine (2.5, 5, and 10 mg/kg) induced an antidepressant-like effect in the FST. Each bar represents the mean ± SEM of 5–7 mice (one-way ANOVA followed by Dunnett's test, $p < 0,05$). **(D)** S-ketamine induced no change in the distance travelled in the OFT ($N = 5–7$, one-way ANOVA followed by Dunnett's, $p > 0,05$).

forced swim test ($t = 6.429$, $df = 20$, $p < 0.05$) compared to the group not exposed to the pretest, which was attenuated in imipramine (30 mg/kg)-treated animals ($t = 6.897$, $df = 21$, $p < 0.05$). The two-way ANOVA indicated ketamine significantly decreased the immobility time (second injection: $F_{1,40} = 18.91$, $p < 0.05$), but no interaction between factors was observed. However, the post-test indicated that NBQX + S-ketamine group was not different from the control group (Tukey, $p > 0.05$). None of the tested doses changed the distance travelled performed by animals in the OFT ($N = 11–12$, $F4,52 = 1.795$, $p > 0.05$, Fig. 3D).

Experiment 5: Effects of S-Ketamine in mice submitted to the forced swim test and the open field test (20 min)

S-ketamine (10 and 30 mg/kg) significantly reduced the immobility time of mice submitted to FST when compared to the vehicle-treated group ($N = 11$, $F_{3,40} = 15.04$, $p < 0.05$; Dunnet's, $p < 0.05$, Fig. 4A). There was a trend for the interaction between time and treatment factors in the OFT lasting 20 min ($F9,114 = 1.920$, $p = 0.055$, $n = 9–11$). In the last 5 min of the OFT, the highest dose of KET (30 mg/kg) significantly increased the distance travelled ($N = 9–11$, $F_{3,38} = 3.505$, $p < 0.05$; Dunnet's, $p < 0.05$, Fig. 4B).

Experiment 6: Effects of CBD and S-ketamine co-administration in mice submitted to the forced swim test and the open field test lasting 20 min

Similar to their single injections, co-administration of CBD (10 mg/kg) and S-ketamine (10 and 30 mg/kg) reduced the immobility time in the FST ($N = 9–16$, $F_{7,90} = 11.16$, $p < 0.05$, Fig. 4C). In the OFT, confirming the results obtained in experiment 5, a single injection of S-ketamine (30 mg/kg) increased the distance travelled during the last 5 min tested ($N = 7–14$, $F_{7,78} = 10.26$, $p < 0.05$, Fig. 4D). Pre-administration of CBD (10 mg/kg) prevented the hyperlocomotion induced by S-ketamine (30 mg/kg).

6. Discussion

The main findings in the present work show that the antidepressant-like effects of both CBD and S-ketamine depend on AMPA receptor activation since NBQX (an AMPA receptor antagonist) prevented the decrease in immobility time induced by both drugs. This result suggests a potential common mechanism of action for CBD and S-ketamine, as described for other fast-acting antidepressant drugs (Botanas et al., 2017; Shen et al., 2019). Secondly, although pre-administration of CBD did not interfere with the antidepressant-like effect of S-ketamine, it prevented S-ketamine-induced hyperlocomotion in the OFT. These results suggest that co-administration of CBD and S-ketamine could be an attractive therapeutic strategy in treating depression, by promoting antidepressant effect while preventing the psychostimulant side-effect of S-ketamine.

The main advantages of S-ketamine over classic clinically available antidepressants are the fast and sustained effect after a single administration and the good response pattern in treatment-resistant depression (aan het Rot et al., 2010; Autry et al., 2011; Berman et al., 2000; Zarate et al., 2006). The mechanism of action involved in the S-ketamine antidepressant effect has been extensively studied and, although it directly acts as an NMDA receptor antagonist, literature data have consistently proposed that activation of AMPA receptors may be essential for its antidepressant-like effect (Koike et al., 2011; Koike and Chaki, 2014; Sungho Maeng et al., 2008; Zanos and Gould, 2018). By blocking NMDA receptors located at GABAergic interneurons in brain regions associated with mood control, such as the prefrontal cortex, S-ketamine disinhibits

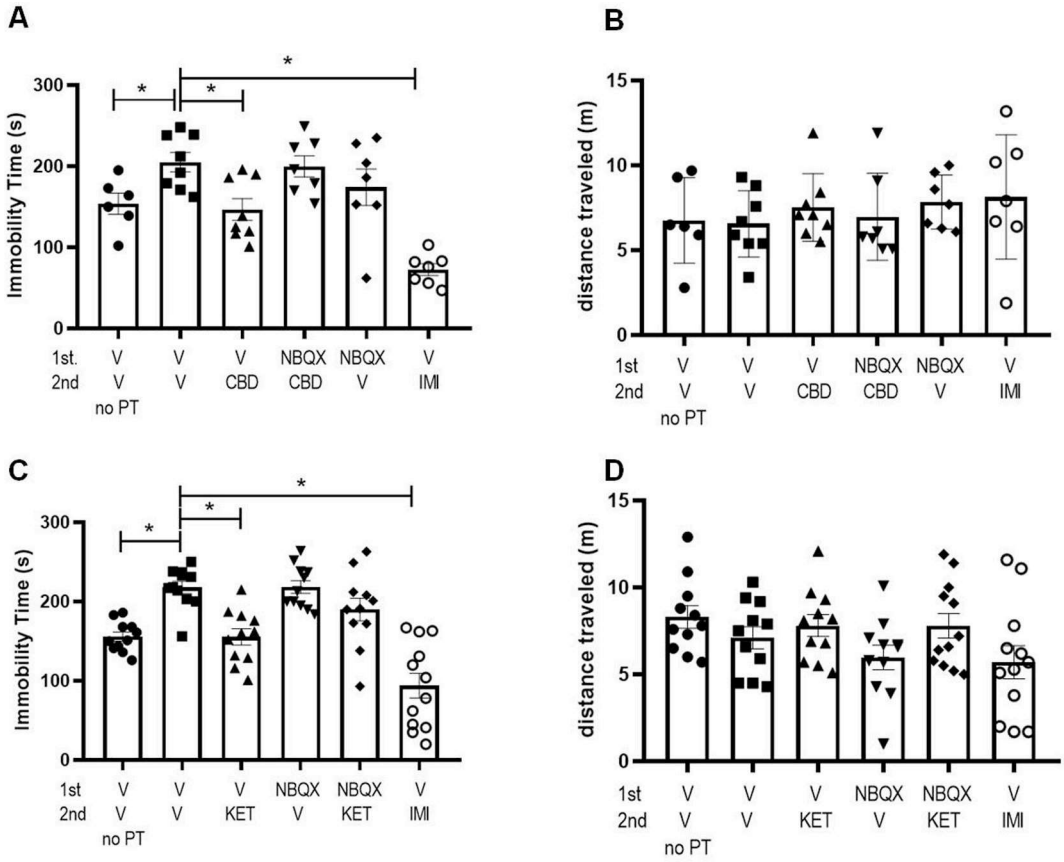

Fig. 3. Effects of pre-administration of NBQX (10 mg/kg) follow by CBD (10 mg/kg) or S-ketamine (10 mg/kg) in mice submitted to forced swimming and open field tests (5 min). **(A)** Pre-administration of NBQX prevented the antidepressant-like effect induced by CBD in the FST. Each bar represents the mean ± SEM of 6–8 mice (one-way ANOVA followed by Dunnett's test, $p < 0.05$). **(B)** NBQX and CBD induced no change in the distance travelled in the OFT, lasting 5 min, alone or combined. Each bar represents the mean ± SEM (N = 6–8, one-way ANOVA followed by Dunnett's, $p > 0.05$). **(C)** Pre-administration of NBQX prevented the antidepressant-like effect induced by S-ketamine in the FST. Each bar represents the mean ± SEM of 11–12 mice (one-way ANOVA followed by Dunnett's test, $p < 0.05$). **(D)** NBQX and S-ketamine induced no change in the distance travelled in the OFT, lasting 5 min, alone or combined. Each bar represents the mean ± SEM (N = 11–12, one-way ANOVA followed by Dunnett's, $p > 0.05$).

glutamatergic pyramidal neurons (Gould et al., 2019; Homayoun and Moghaddam, 2007). This mechanism would increase glutamate release and activate AMPA receptors (Moghaddam et al., 1997), resulting in the release of brain-derived neurotrophic factor (BDNF), activation of downstream synaptogenic signalling pathways (mTOR), and improvement of synaptic strength (Duman, 2014; Li et al., 2010; Zanos and Gould, 2018; Zhou et al., 2014).

Preclinical studies have shown that CBD induces antidepressant-like responses similar to those observed with S-ketamine, with a single administration causing a rapid and sustained effect in different animal models (Sales et al., 2018a,b). Additionally, CBD is effective in genetically modified animal models that do not respond adequately to classic antidepressants (Shbiro et al., 2019). Despite this similarity between ketamine and CBD in these models, the involvement of AMPA receptors in CBD effects has not yet been investigated. Our results show for the first time that the antidepressant-like effect of CBD, similar to ketamine, depends on the activation of AMPA receptors. They reinforce, therefore, the hypotheses that CBD may act similarly to ketamine to induce an antidepressant-like effect. It is, however, still unclear how CBD promotes AMPA receptor activation since there is no evidence to date that it binds to these receptors. However, CBD can increase glutamate release in the prefrontal cortex (Linge et al., 2016), where AMPA receptor activation is known to promote antidepressant effects (Jiménez-Sánchez et al., 2016). The primary mechanism involved in CBD effects is not known but previous evidence indicates the involvement of 5-HT1A receptors since CBD binds and activates such receptors and its antidepressant effects can be blocked by selective 5-HT1A antagonists (Zanelati et al., 2010; Sartim et al., 2016). Furthermore, direct activation of 5-HT1A receptors in the prefrontal cortex promotes a fast antidepressant effect which is mediated by AMPA receptor activation and TrkB signalling (Fukumoto et al., 2020). This hypothesis is further corroborated by our previous findings that CBD administration into the prefrontal cortex promotes antidepressant effects mediated by 5-HT1A receptor activation (Sartim et al., 2016) and that the rapid antidepressant effect of CBD is dependent on BDNF- TrkB signalling in the prefrontal cortex and hippocampus (Sales et al., 2018a,b; Sartim et al, 2016, 2018). Nevertheless, it remains to be tested if the antidepressant effect of CBD is due to the activation of AMPA receptors in the prefrontal cortex, as described for ketamine and 5-HT1A agonists (Zhou et al., 2014). Further studies are thus necessary to investigate the involvement of glutamatergic neurotransmission in CBD-induced antidepressant effects.

Surprisingly, in the present study, the antidepressant-like effect of S-ketamine was not facilitated by CBD since the co-administration of these drugs produced effects similar to those observed after their single administration. This might reflect a floor effect due to an already maximum effect induced by S-ketamine at either 10 or 30 mg/kg. In order to test for synergistic or additive effects, isobologram analysis of dose-response effects induced by the combination of ketamine and CBD should be considered in future studies.

The abuse potential and psychotomimetic effects caused by S-ketamine are important limitations for its wide clinical use (Molero et al., 2018). In animal models, ketamine induces behavioural and cognitive

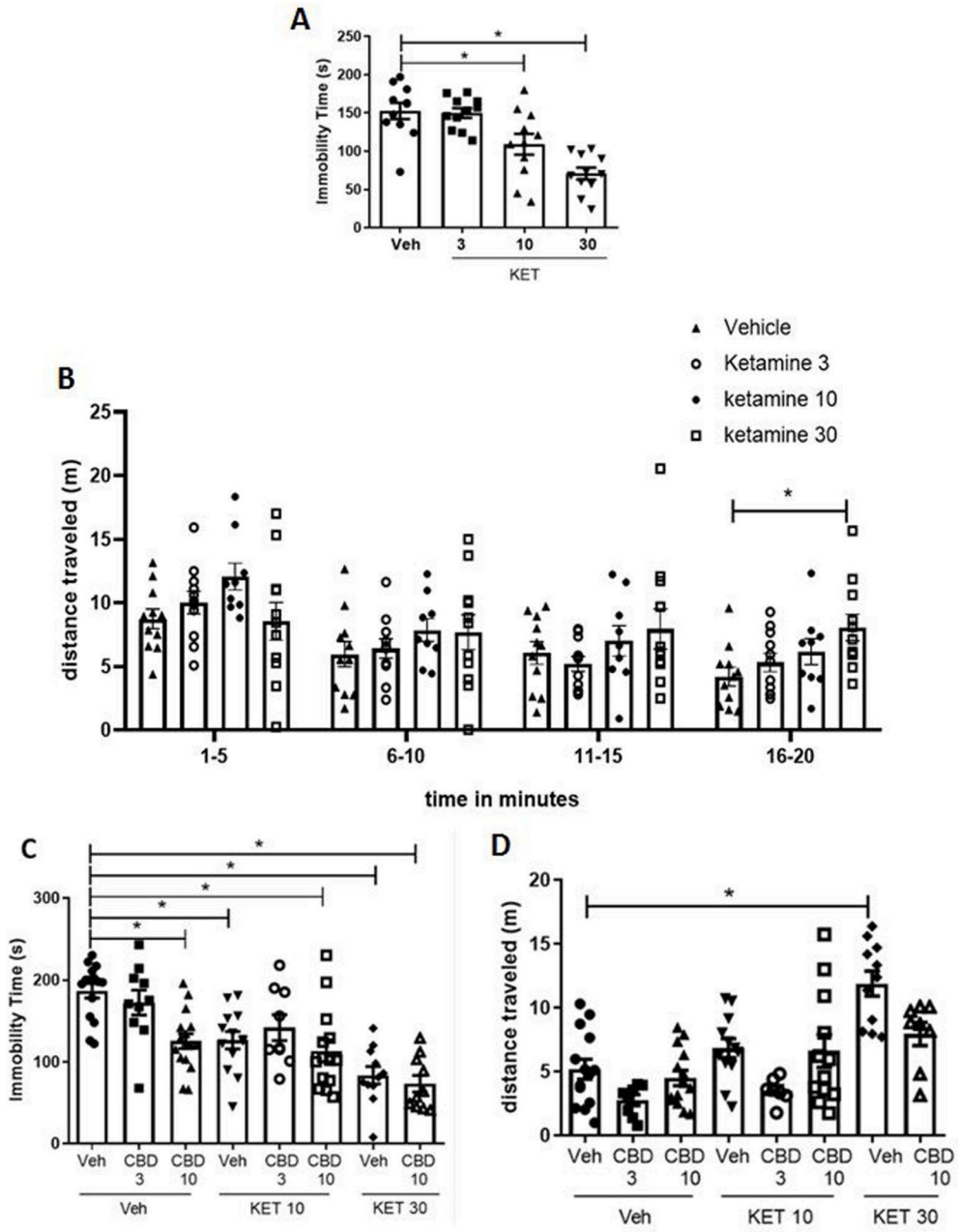

Fig. 4. Effects of S-ketamine (3, 10, and 30 mg/kg) or CBD (3 and 10 mg/kg) and S-ketamine (10 and 30 mg/kg) i.p. injection in mice submitted to forced swimming and open field test lasting 20 min. **(A)** S-ketamine (10 and 30 mg/kg) induced antidepressant-like effects in the FST. Each bar represents the mean ± SEM of 11 mice (one-way ANOVA followed by Dunnett's test, $p < 0,05$). **(B)** S-ketamine (30 mg/kg) increased the distance travelled in the OFT ($N = 9–11$, one-way ANOVA followed by Dunnett's, $p < 0,05$). **(C)** CBD (10 mg/kg) and S-ketamine (10 mg/kg and 30 mg/kg), alone or combined, induced antidepressant-like effects in the FST. Each bar represents the mean ± SEM of 9–16 mice (one-way ANOVA followed by Dunnett's test, $p < 0,05$). **(D)** S-ketamine (30 mg/kg) injected alone increased the distance travelled during the last 5 min tested in the OFT. Pre-administration of CBD (10 mg/kg) prevented the hyperlocomotion induced by S-ketamine (30 mg/kg) ($N = 7–14$, one-way ANOVA followed by Dunnett's, $p < 0,05$).

changes associated with psychotic conditions (Lin et al., 2016). These changes include cognitive and social interaction deficits, low motor coordination performance, and hyperlocomotion (Brakatselos et al., 2020; Lin et al., 2016). Besides, the neurochemical alterations promoted by ketamine in rodents are also similar to those seen in schizophrenic conditions (Shi et al., 2010). Additionally, hypoglutamatergic states induced by different NMDA receptor antagonists are widely used as animal models of schizophrenia (Neill et al., 2010). These antagonists cause a broad spectrum of behavioural changes in rodents, including hyperlocomotion, stereotypy, and cognitive impairments (Rung et al., 2005).

The open-field test is widely used to assess locomotor activity changes in laboratory animals, including drug-induced hyperlocomotion associated with psychosis or mania (Ma and Guest, 2018). In the present study, S-ketamine, at the highest dose tested (30 mg/kg) promoted motor hyperactivity in the OFT, as described previously (Moreira and Guimaraes, 2005). The effect of S-ketamine in locomotion was observed only in the last 5 min of the 20 min test. This result might indicate that the hyperlocomotion effect can be more easily observed when the novel environment's exploratory behaviour has decreased due to prolonged exposure to the apparatus (Walsh and Cummins, 1976). Although we observed a hyperlocomotor effect only with the highest dose tested of ketamine (30 mg/kg), previous data show that ketamine can promote this effect in a wide dose range (from 3 mg/kg) (Irifune et al., 1991). Such difference in results may be related to protocol difference between studies, including the absence of habituation period to the arena before the start of quantification, testing time after the injection, and ketamine preparation (racemic or S-ketamine) which might affect the peak effect of the drug (Chang et al., 2019). The mechanism by which S-ketamine-induced hyperlocomotion is thought to involve NMDA receptor blockade since this effect is also observed after the administration of other NMDA receptor antagonists such as MK-801 and phencyclidine (Irifune et al., 1995; Zanos et al., 2018). Blockade of NMDA receptors increases glutamate and dopamine release in the

prefrontal cortex, which are effects associated with the psychostimulant properties of NMDA antagonists (Moghaddam et al., 1997; Chartoff et al., 2005). These effects can be attenuated by AMPA receptor antagonist, also implying the activation of such receptors in ketamine psychostimulant effects (Al-Amin et al., 2000).

Previous evidence has demonstrated that CBD attenuates the psychotomimetic effects induced by ketamine and amphetamine in mice (Moreira and Guimaraes, 2005). Our results corroborate such findings by demonstrating that CBD prevented the hyperlocomotion induced by high dose ketamine (30 mg/kg). CBD did not induce any significant effects per se on locomotion, nor did the combination drugs in smaller doses. These results are in line with evidence indicating that CBD has antipsychotic-like properties in animals and humans (McGuire et al., 2018; Roser and S. Haussleiter, 2012) and attenuates the occurrence of behaviours associated with drug abuse and dependence (Batalla et al., 2019; Calpe-López et al., 2019; Lee et al., 2017). More recently, it has been shown that repeated CBD treatment attenuated the molecular changes associated with ketamine-induced cognitive impairments in mice (Kozela et al., 2019). None of these studies, however, investigated the antidepressant-like properties of CBD. Therefore, our findings bring an important contribution by demonstrating that CBD blocks S-ketamine-induced psycholocomotor changes while preserving its antidepressant properties. This supports further investigations on the therapeutic potential of add-on therapy with CBD to prevent S-ketamine-induced psychomimetic side-effects and the increased risk of abuse and dependence with its repeated administration in depressed patients (Park et al., 2019).

Contrary to our findings, however, a recent report described that previous CBD administration could not prevent ketamine-induced hyperlocomotion but rather facilitated it at the highest dose tested, 30 mg/kg (Brakatselos et al., 2020). Important differences in the protocols between the studies can help to explain these divergent data. In the study mentioned above, the locomotor activity was recorded for 60 min, whereas we recorded in the last 5 min of a 20 min exposure to the open field test. Another critical difference refers to ketamine itself. Brakatselos et al. (2020) tested a racemic mixture of ketamine enantiomers, whereas S-ketamine was used in the present study (Brakatselos et al., 2020). Recent studies have highlighted significant differences in the pharmacodynamics and pharmacokinetics profiles of ketamine enantiomers, resulting in different pharmacological effects (Muller et al., 2016). Altogether, the results indicate that CBD effects on S-ketamine-induced hyperlocomotion should be interpreted in the light of the protocol used and the drug preparation. It would be interesting to explore this issue further in different experimental conditions (acute vs repeated CBD treatment, different ketamine enantiomers).

It has been described in humans that CBD administration can increase some psychotomimetic effects of ketamine while decreasing others (depersonalization) (Hallak et al., 2011). However, this study was performed on healthy volunteers. It remains to be investigated if CBD could attenuate the psychotomimetic effects associated with S-ketamine administration in depressed individuals.

The innovative results of the present work suggest that the association of CBD and S-ketamine may represent an interesting clinical application in order to reduce the abuse potential of ketamine. Further studies in the area will be needed to confirm this therapeutic alternative. Some limitations related to our study must be taken into account, and additional studies may solidify our findings. In this sense, it is worth noting that only one animal model was used for each behaviour analyzed, and studies in other models, both of depression and locomotor activity, are needed to further investigate our conclusions. Furthermore, we were not able to conclude if there would be an additive effect to the antidepressant-like effect of CBD and KET in comparison to the isolated administrations due to the lack of testing subeffective doses of S-ketamine. New studies including co-administration in other animal models of depression may solidify this possibility.

7. Conclusion

Our results support the hypothesis that facilitation of AMPA-receptor-mediated neurotransmission is, similar to ketamine, crucial to the antidepressant effects of CBD. Moreover, by preventing the hyperlocomotion without interfering with the antidepressant effects of ketamine, CBD could be explored as a possible new add-on therapeutic option for depression.

Authors contribution

The main idea of this study was from Sâmia Joca, Gregers Wegener, and Ariandra Sartim. Ariandra Sartim, Sâmia Joca, and Francisco Guimarães designed the protocol of the study together. Data collection has been done by Jade Marques, Ariandra Sartim, Kennia Silveira, and Pedro Gobira. Writing the first draft of manuscripts has been done by Ariandra Sartim. All authors contributed to and have approved the final manuscript.

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