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Caffeine protects against memory loss induced by high and non-anxiolytic dose of cannabidiol in adult zebrafish (*Danio rerio*)

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ABSTRACT

Cannabidiol (CBD) has been investigated in a wide spectrum of clinical approaches due to its psychopharmacological properties. CBD has low affinity for cannabinoid neuroreceptors and agonistic properties to 5-HT receptors. An interaction between cannabinoid and purinergic receptor systems has been proposed. The purpose of this study is to evaluate CBD properties on memory behavioral and locomotor parameters and the effects of pre-treatment of adenosine receptor blockers on CBD impacts on memory using adult zebrafish. CBD (0.1, 0.5, 5, and 10 mg/kg) was tested in the avoidance inhibitory paradigm and anxiety task. We analyzed the effect of a long-term caffeine pre-treatment (~20 mg/L — four months). Also, acute block of adenosine receptors was performed in co-administration with CBD exposure in the memory assessment. CBD promoted an inverted U-shaped dose–response curve in the anxiety task; in the memory assessment, CBD in the dose of 5 mg/Kg promoted the strongest effects without interfering with social and aggressive behavior. Caffeine treatment was able to prevent CBD (5 mg/kg) effects on memory when CBD was given after the training session. CBD effects on memory were partially prevented by co-treatment with a specific A_{2A} adenosine receptor antagonist when given prior to or after the training session, while CBD effects after the training session were fully prevented by adenosine A_1 receptor antagonist. These results indicated that zebrafish have responses to CBD anxiolytic properties that are comparable to other animal models, and high doses changed memory retention in a way dependent on adenosine.

1. Introduction

Cannabidiol (CBD) and Delta-9-tetrahydrocannabinol (THC) are the main active compounds from *Cannabis sativa*. Much evidence suggests that CBD acts as an anticonvulsant, anxiolytic, antipsychotic, and antirheumatic (Carlini and Cunha, 1981; Hampson et al., 1998; Zuardi, 2008; Campos et al., 2013). The main proposed mechanisms of action underlying CBD properties are related to the 5HT1A receptors' activation and to the increase of endocannabinoid effects (Bisogno et al., 2001; Russo et al., 2005). It was shown that pre-treatment of rats with a 5HT1A antagonist blocked the anxiolytic-like effect promoted by injection of CBD into the bed nucleus of the *stria terminalis* in two models of anxiety (Gomes et al., 2011). The second main mechanism of action of CBD is the activation of the endocannabinoid system, proposed by the facilitation of CBD on endocannabinoid-mediated neurotransmission as a consequence of the blockage of anandamide metabolism and uptake (Bisogno et al., 2001). However, several additional biological targets of CBD have been identified (reviewed by Das et al., 2013; Devinsky et al., 2014). At low concentrations, CBD is a blocker of the equilibrative nucleoside transporter (ENT), the orphan G-protein-coupled receptor GPR55, and the transient receptor potential of melastatin type 8 (TRPM8) channel. CBD also activates the α3 and α1 glycine receptors and the transient receptor potential of ankyrin type 1 (TRPA1) channel, and has a bidirectional effect on intracellular calcium. At high concentrations, CBD activates the nuclear peroxisome proliferator-activated receptor-c and the transient receptor potential of vanilloid type 1 (TRPV1) and 2 (TRPV2) channels while also inhibiting cellular uptake and fatty acid amide hydrolase-catalyzed degradation.

Learning and memory appear to be affected by CBD through a mechanism including the endocannabinoid system (Marsicano and Lafenêtre, 2009; Campos and Guimarães, 2009; Campos et al., 2013). Endocannabinoids are released during stressful conditions, and CB1 receptor agonism impairs the acquisition of contextual fear (Marsicano et al., 2002; Hohmann and Suplita, 2006). Acute and chronic

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administration of CBD and the use of different doses reveal a variety of responses, especially in avoidance tasks (Soares et al., 2010; Cassol-Jr et al., 2010; Barichello et al., 2012; Fagherazzi et al., 2012). In rodents, CBD has a demonstrated therapeutic potential for specific cognitive impairments associated with Alzheimer's disease, probably through enhancement of endocannabinoid-mediated actions (Cheng et al., 2014).

Those complementary mechanisms of action proposed to underlie CBD action could explain some controversial results, such as the indirect agonistic effect on adenosine receptors, especially A_2 receptors, by increasing adenosine levels as a response to nucleoside transport inhibition (Carrier et al., 2006; Liou et al., 2008). Also, endocannabinoids have the same potential to block adenosine transporters as the known inhibitor of nucleoside transporters, dipyridamole (Pandolfo et al., 2011). Another interaction proposed between adenosine and cannabinoids is the existence of A_{2A}–CB1 receptor heterodimerization, which has been demonstrated by means of bioluminescence resonance energy transfer (BRET) techniques (Carriba et al., 2007; Ferré et al., 2010). However, CBD is 10 times less active on CB receptors than THC.

Despite the increased number of studies on the CBD mechanism of action from clinical and preclinical investigations, the therapeutic window for CBD is not defined. For this reason, we performed experiments with zebrafish – an animal model with well-described signaling pathways, allowing the translation of information to higher vertebrates by overcoming the intrinsic differences – in order to contribute to preclinical studies. We analyzed the effects of several doses of CBD on anxiety and memory. Considering that adenosine could have some effect on cannabinoid signaling, we analyzed the effect of long-term treatment with caffeine, a non-specific adenosine receptor antagonist. Caffeine is a known psychoactive drug with mnemonic effects credited to the non-specific antagonism of adenosine receptors (Fredholm et al., 1999; Cunha and Agostinho, 2010). We also analyzed the effect of acute specific block of adenosine receptors on CBD mnemonic properties in order to contribute to the study of a possible interaction between CBD and purinergic signaling.

2. Materials and methods

2.1. Drugs

Caffeine, ZM241385, and DPCPX were purchased from Sigma-Aldrich (USA). Tween 80 was purchased from Invitrogen (USA). CBD, approximately 99.9% pure, was kindly supplied by THC-Pharm, Frankfurt, Germany, and STI-Pharm, Brentwood, UK. All other reagents were of analytical grade.

2.2. Animals

Fertilized eggs were obtained from wild-type adult zebrafish (*Danio rerio*) (Tübingen background) from the sixth generation of our breeding stock held at the Pontifícia Universidade Católica do Rio Grande do Sul. Fertilized eggs were collected and kept in maintenance water (water from reverse osmosis reconstituted with marine salt, 0.4 parts per trillion) in an incubator at 28.5 °C on a 14:10 light/dark cycle. After egg hatching, the animals were maintained in an aquarium (5 L; 27 × 17 × 12 cm [width × height × depth]) with maintenance water under biological and mechanical water filtration and aeration (7.20 mg O_2/L) and temperature controlled (28 ± 2 °C) up to four months. The density of animals per tank was adjusted over time. Animals were fed three times a day with commercial flakes (TetraMin™, NC, USA) and supplemented with live brine shrimp. We used a male and female similar distribution between experimental groups, including in memory and anxiety evaluation, as the use of male and female zebrafish irrespectively of their gender has been common (Blank et al., 2009; Gebauer et al., 2011; Manuel et al., 2014). All protocols followed Brazilian legislation and were approved by the Institutional Animal Care Committee (12/00310 — CEUA PUCRS).

2.3. Cannabidiol exposure

One week before the experiments, all the fishes were weighted to adjust the CBD dose into 10 μL per fish *via* intraperitoneal (i.p) injection. The CBD doses (0.1 0.5, 5.0, or 10 mg/kg) were freshly prepared on 2% of Tween 80 diluted in saline. The animals were anesthetized with tricaine (100 mg/L) and then treated in four separate groups: Tween/CBD 0.1, 0.5, 5.0, and 10 mg/kg. The CBD exposure was performed 1 h before the behavior and locomotor assessment and 1 h before or after the training within the memory analysis (Scheme A). The fish spent this time in constantly aired tanks. All experiments were carried out with two concomitant control groups: one receiving saline and the other receiving 2% of Tween 80 diluted in saline under the same conditions of the treated group.

2.4. Caffeine long-term pre-treatment

At three days post-fertilization, embryos were divided into two groups: the Control (CTRL) group (no caffeine added) and CAF group (with caffeine added). The CAF fishes started being treated with ~20 mg/L of caffeine (19.4 mg/L) (Capiotti et al., 2011) dissolved in water. This treatment was extended over four months, after which the memory and anxiety experiments were performed (Scheme A1 and 2). To guarantee constancy on caffeine concentration, the caffeine solution was changed each of the three days, and animal density was adjusted over time.

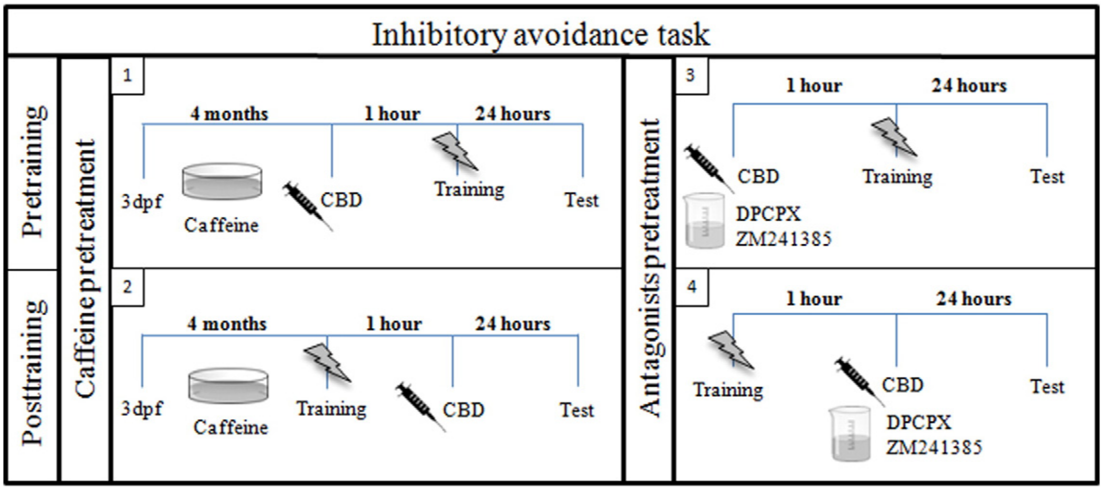
2.5. Acute block of adenosine receptor

Four month-old animals were exposed to adenosine receptor antagonist (DPCPX 6 mg/L and ZM241385 6 mg/L) for 1 h in recipient containing maintenance water (300 mL/fish). Antagonists were dissolved in DMSO until achieving the designed doses in a final DMSO concentration of 1% in the water of the animals. The treatment was concomitant to CBD injection, 1 h prior to or after the training session on the memory analysis, as indicated below (Scheme A3 and 4). Control animals received DMSO 1% in the same condition of the animals treated with antagonist blockers.

2.6. Locomotor and behavioral assessment

A curve of CBD dose (0–10 mg/kg) was used to analyze the effects of CBD on the time spent in the top area of the aquarium as a measure of anxiety behavior (Egan et al., 2009) and locomotor parameters. The animals were individually placed in a single tank (30 × 15 × 10 cm; w × h × d) virtually divided into two horizontal lines (lower and upper zones). After 30 s of habituation, the behavior was recorded by a digital camera for 5 min for posterior analysis. The parameters, total distance (m), max speed (m/s), mean speed (m/s), absolute turn angle (degrees), and the time spent in the upper zone were registered.

Aggressive behavior and social interaction were analyzed after CBD (5 mg/kg) exposure. For aggressive behavior, the fishes were placed individually in a single tank (30 × 15 × 10 cm; w × h × d), with a mirror positioned in the back of the tank forming a 22.5° angle. The tank was divided into four equal sessions from which the time spent in each zone was analyzed, with the time spent in the zone near the mirror being the sign of aggressive behavior. Additionally, the observation of aggressive behavior like biting, sprinting, and changes in color pattern was also registered. The fishes were habituated for 5 min, after which the video was recorded for 1 min (Gerlai et al., 2000). The last behavioral analysis was the social interaction in which three tanks (30 × 15 × 10 cm; w × h × d) were placed side by side, the far left being empty, the one in the middle with the test animals, and the far right with stimulus fish (Gerlai et al., 2000). After a 5 minute habituation, the social interaction was recorded for 10 min. The tank with the test fish was separated into two equal sessions, with the social



Scheme A. Experimental design of the inhibitory avoidance task. Chronic caffeine treatment: The animals were treated with caffeine in the water since 3 dpf up to four months. After this time, one group was injected with CBD 1 h before trained with the shock (1) or trained and after 1 h injected with CBD (2). Acute antagonist treatment: Four month-old animals were injected with CBD and concomitantly treated with the antagonists in the water for 1 h before training (3) or trained and received CBD 1 h after and remained in antagonist treatment for 1 h before the training (4).

interaction indicator being the time spent in the side with the stimulus fish. All the data were analyzed by the Software ANY-maze (Stoelting Co., Wood Dale, USA).

2.7. Memory assessment task

A curve of CBD dose (0–10 mg/kg) was used to analyze the effect of CBD on the latency to cross chambers in an avoidance inhibitory task as a measure of memory retention (Blank et al., 2009). Also, the animals were evaluated for the ability of memory formation after prolonged caffeine exposure challenged by an acute CBD (5 mg/kg) administration before or after the training session (Scheme A). To address the results of specific adenosine receptors, we treated the animals with DPCPX or ZM241385, antagonists of A_1 and A_{2A} adenosine receptors, respectively, concomitantly with CBD (5 mg/kg), 1 h before or after the training session. The protocol followed Blank et al. (2009). Briefly, the animals were individually trained and tested in a tank (18 cm × 9 cm × 7 cm; w × h × d) subdivided into white and dark chambers apart from a sliding wall. In each session, the animals were gently placed in the white tank compartment while the sliding wall was closed. After 1 min of habituation and orientation, the wall was raised, allowing the fish to cross to the dark side of the tank through a 1 cm high opening. In a training session, immediately after crossing to the dark compartment, the sliding partition was closed and a pulsed electric shock of 3 ± 0.2 V administered for 5 s, after which animals were removed from the apparatus. Twenty-four hours after training, the animals were submitted to a test session that repeated the training protocol, except that no shock was administered and the sliding wall was kept open, allowing the animals to freely explore the apparatus. The latency to enter the dark compartment was measured in all sessions and the test latency was used as an index of memory retention. In the set of experiments with long-term caffeine treatment, the control group was maintained for the same period in normal water and received an injection of Tween 80 2% as a vehicle of CBD. In the set of experiments with specific adenosine receptor antagonists, the control group was exposed during the same period to DMSO 1% and received Tween 80 2% as a vehicle of CBD.

2.8. Statistical analysis

For memory assessment, a T test was used to compare latency in training *versus* latency in the test session during the inhibitory avoidance task (comparison inside the groups), while One-Way ANOVA was used to compare the latency between groups. One-Way ANOVA was also used to compare the results from the CBD dose on anxiety and locomotion. Aggressive and social interaction between CAF, CBD, and control groups was analyzed by One-Way ANOVA. The T test was used to compare weights between the control and caffeine-treated animals. The significance levels were attributed at $p < 0.05$. The multiple comparisons of means were conducted when appropriated by Tukey's test. Data are expressed as mean ± standard error of mean.

3. Results

3.1. Cannabidiol effects

The locomotor parameters evaluated (total distance [TD], mean [MS] and maximum speed [MaS], and absolute turn angle [ATA]) were not affected by CBD 0.1–10 mg/kg (Table A). In order to check if CBD could have some effect on anxiety in zebrafish, we evaluated the time spent in the upper zone of the aquarium as a marker of anxious behavior. CBD exhibited an inverted U-shaped dose–response curve on this parameter (Fig. A) ($F[4;40] = 5.996$; $p = 0.0002$). The control animals spent 25% of the total time in the upper zone, while the CBD-treated animals with 0.5 mg/kg spent 58% of the total time there. The lowest (0.1 mg/kg) and highest doses (5 and 10 mg/kg) did not alter the time in the upper zone in relation to the control group (Tween) (Fig. A). Control/saline did not differ from control/Tween (data not shown; $p = 0.8824$).

Table A
Cannabidiol effects on locomotor parameters (total distance, mean speed, maximum speed, and absolute turn angle). All results are expressed in mean ± SEM.

Parameters	Ctrl/Tween	0.1 mg/kg	0.5 mg/kg	5 mg/kg	10 mg/kg
Total distance (m)	16.49 ± 2.706	20.12 ± 5.337	10.53 ± 1.450	12.33 ± 1.012	10.59 ± 0.8632
Mean speed (m/s)	0.0430 ± 0.00269	0.0610 ± 0.01344	0.03518 ± 0.00484	0.0411 ± 0.00333	0.0333 ± 0.00226
Maximum speed (m/s)	0.8137 ± 0.1442	0.9745 ± 0.2108	0.4763 ± 0.1113	0.8088 ± 0.1487	0.4590 ± 0.1086
Absolute turn angle (°)	38078 ± 4121	41050 ± 5789	29064 ± 3230	32556 ± 3725	28509 ± 3281

In the inhibitory avoidance paradigm, no differences in the time to cross chambers were detected among the groups during the training session (Fig. B1 and B2). The vehicle Tween 80 2% did not change latencies during the memory assessment when compared to the control/saline group (training and test latencies; $p = 0.4598$, $p = 0.8936$, respectively) (data not shown). As expected, the control/Tween animals increased their latencies to cross chambers during the test session, demonstrating preserved memory (Fig. B1 and B2). CBD-treated animals had their latencies to cross chambers during the test session altered in different ways according to the dose and period of exposure to CBD (pre-training: $F[4;57] = 2.651$; $p = 0.0423$; post-training: $F[4;58] = 7.124$; $p < 0.0001$). When CBD was given prior to the training session, the lowest dose (0.1 mg/kg) did not affect memory retention (Fig. B1). The test latency in the animals treated with CBD 0.1 mg/kg was significantly higher than the training session latency when compared to the Tween group (Fig. B1). CBD 0.5 and 10 mg/kg, increased the test latency in relation to training latency, but did not reach similar results to the Tween group (Fig. B1). The dose of 5 mg/kg CBD given prior to the training session decreased the latency in the test session, affecting memory retention (Fig. B1). When CBD was given after the training session, the doses 0.1 and 10 mg/kg reduced the latency to cross chambers in the test session. This was not enough to be statistically similar to the latency in the training section, but enough to be statistically different from the Tween group (Fig. B2). CBD given at 0.5 mg/kg did not affect the memory parameter, showing similar latency to the Tween group to cross chambers in the test session. CBD given at 5 mg/kg affected the memory parameter, showing similar latency to cross chambers between the training and test sessions.

We selected one of the CBD doses, 5 mg/kg, to test the hypothesis of contribution of purinergic system on CBD effects. We performed these two kinds of behavioral assessment, aggressive and social behaviors, just on the selected dose since the rationale to do that was to evaluate if the innate behavioral aspects of zebrafish could interfere with the effects detected on the inhibitory avoidance task. Aggressive behavior and social interaction were not affected by CBD 5 mg/kg ($p = 0.2902$; $p = 0.6675$, respectively).

3.2. Caffeine long-term treatment and cannabidiol

The long-term treatment with caffeine affects the body weight gain of zebrafish ($p = 0.017$). The average weight of the control animals was 0.121 ± 0.006 g, while the average weight of the caffeine-treated animals was 0.099 ± 0.006 g at the end of the treatment (four months). Locomotor parameters were not affected by long-term treatment with caffeine (data not shown). The evaluation between groups indicated that during the training session all groups presented similar profiles of latencies to cross chambers (pre-training injected animals: $F[3;40] = 1.138$; $p = 0.3454$; post-training injected animals: $F[3;45] = 1.367$; $p = 0.2649$), suggesting no effect of long-term caffeine exposure on exploratory behavior as confirmed by locomotor evaluation. Caffeine by itself did not alter memory retention, since the latency from the test session was not different from the Tween group (Fig. C1 and C2).

The previous long-term caffeine treatment was not able to alter CBD (5 mg/kg) effects on memory, when CBD was given prior to training in the inhibitory avoidance task, considering the small latency to cross chambers in the test session (CAF/CBD: training latency *versus* test latency $p = 0.2049$) (Fig. C1). Long-term caffeine-treated animals exhibited a protective effect against the memory disruption promoted by CBD (5 mg/kg) when CBD was given after training in the inhibitory avoidance task, as seen by the increase of the latency period in the test session (CAF/CBD: training latency *versus* test latency $p = 0.0003$) (Fig. C2).

3.3. Acute treatment with specific adenosine receptor antagonists and cannabidiol

The acute treatment with DPCPX and ZM241385 was accompanied by control/vehicle 1% DMSO. The control/DMSO/Tween had preserved memory as demonstrated by the increased latency to cross chambers in the test session in comparison to the training session in both sets of experiments (Fig. D1 and D2) (pre- and post-training sets of experiments; $p < 0.001$ and $p < 0.0001$). The decrease in latency in the test session, which means the impairment on memory, exerted by CBD 5 mg/kg given before the training session was prevented by the co-administration of DPCPX, a specific antagonist of A_1 adenosine receptors (Fig. D1). However, DPCPX effects were hard to interpret since DPCPX affected the latency to cross chambers in the training session (Fig. D1). When DPCPX was co-administered with CBD 5 mg/kg after the training session, the latency to cross chambers was increased and had similar results to the control animals (Fig. D2). This indicated that DPCPX *per se* affected the behavior during the training phase, which could affect the

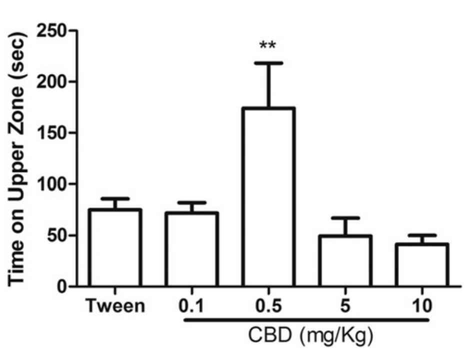


Fig. A. Time in the upper zone (seconds). CBD (0.1, 0.5, 5, and 10 mg/kg; n = 5–13). CBD was prepared on 2% of Tween 80 diluted in saline. Time spent in the upper zone was registered for 5 min by video recording in the tank diving behavioral test. Data were expressed as mean ± S.E.M. and analyzed by One-Way ANOVA. **p < 0.01 denotes a significant difference from the control (Tween alone).

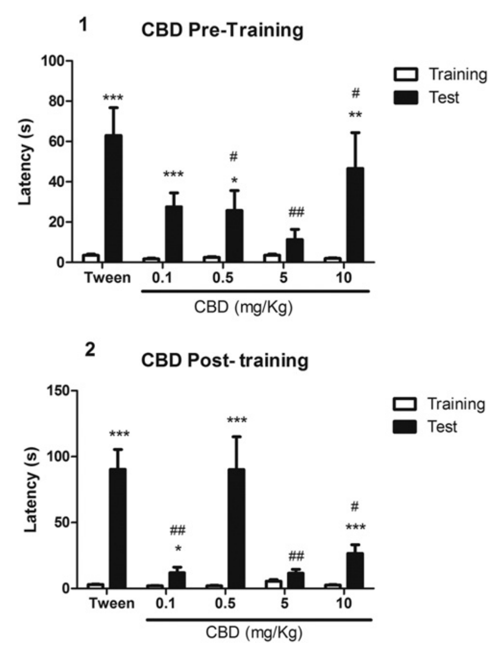


Fig. B. Effect of CBD (0.1, 0.5, 5, and 10 mg/kg; n = 10–19) on memory acquisition and consolidation in a one-trial inhibitory avoidance task (n = 9–12). CBD was prepared on 2% of Tween 80 diluted in saline. Latency (seconds) to cross to the dark compartment in the training and test sessions (1) in the animals receiving CBD 1 h prior to the training session or (2) 1 h after the training session. Data were expressed as mean ± S.E.M. and analyzed by T test (comparison inside groups) and One-Way ANOVA (comparison between groups). *p < 0.05 and ***p < 0.001 denote a significant difference from the test session to the corresponding training session (inside groups) and #p < 0.05 and ##p < 0.01 denote differences revealed by the comparison of the test latency of CBD-treated animals to the control/Tween group.

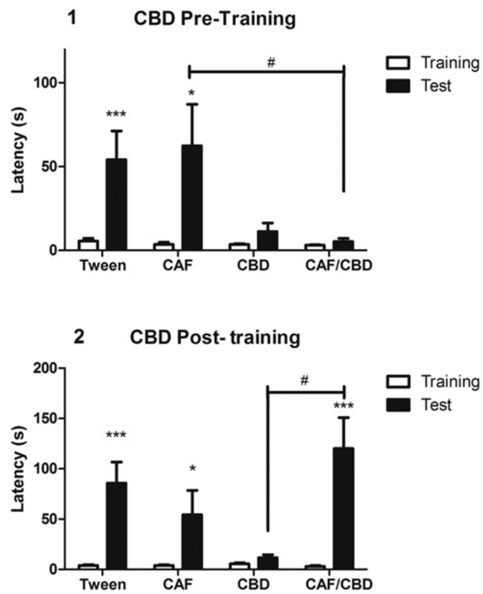


Fig. C. Long-term caffeine (20 mg/L) treatment on CBD (5 mg/kg) effected memory acquisition and consolidation in a one-trial inhibitory avoidance task (n = 9–12). Latency (seconds) to cross to the dark compartment in the training and test sessions (1) in the animals receiving CBD (5 mg/kg) 1 h prior to the training session or (2) 1 h after the training session. CBD was prepared on 2% of Tween 80 diluted in saline. Data were expressed as mean ± S.E.M. and analyzed by T test (comparison inside groups) and One-Way ANOVA (comparison between groups). *$p < 0.05$ and ***$p < 0.001$ denote a significant difference from the test session to the corresponding training session (inside groups) and #$p < 0.05$ denotes differences revealed by the comparison of the test latency between the indicated groups.

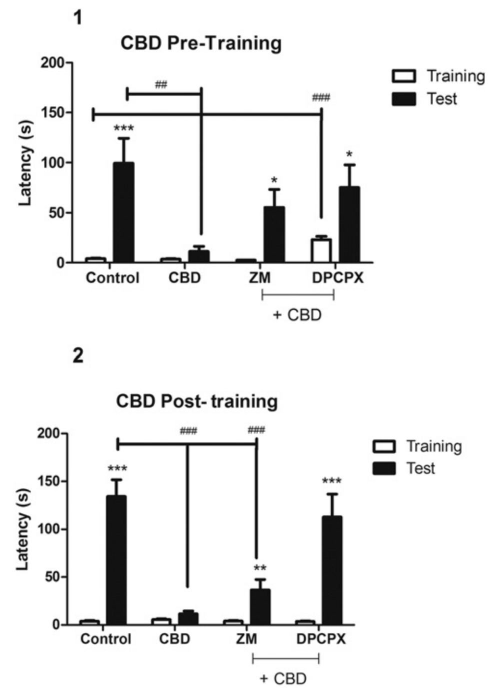


Fig. D. Effect of specific antagonists of adenosine receptors, DPCPX (6 mg/L) and ZM 241385 (6 mg/L) on CBD (5 mg/kg) effects on memory acquisition and consolidation in a one-trial inhibitory avoidance task (n = 8–11). CBD was prepared on 2% of Tween 80 diluted in saline and antagonists were prepared in 1% DMSO. Latency (seconds) to cross to the dark compartment in the training and test sessions in the animals receiving CBD (5 mg/kg) in co-administration with DPCPX or ZM 241385 (1) 1 h prior to the training session or (2) 1 h after the training session. Data were expressed as mean ± S.E.M. and analyzed by T test (comparison inside groups) and One-Way ANOVA (comparison between groups). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ denote a significant difference from the test session to the corresponding training session (inside groups) and #$p < 0.05$, ##$p < 0.01$ and ###$p < 0.001$ denote differences revealed by the comparison of the training or test latency of treated animals to the control/Tween/DMSO group.

memory formation process. Also, DPCPX was able to prevent CBD effects on memory when CBD was given after the training session.

ZM241385, a specific A_{2A} adenosine receptor antagonist, did not affect the time to cross chambers during the training session (Fig. D1 and D2). When ZM241385 was co-administered with CBD 5 mg/kg before the training session, the animals increased their latency between the training and test sessions ($p = 0.0026$). When ZM241385 was co-administered with CBD 5 mg/kg after the training session, the animals increased their latency between the training and test sessions ($p = 0.0168$), but not enough to be similar to the control latency (Fig. D2).

4. Discussion

Behavioral effects of CBD of clinical interest have been well recognized, especially concerning anxiolytic properties (Campos and Guimarães, 2009). The anxiolytic effects show an inverted U-shaped dose–response curve in several models of anxiety protocols (Campos and Guimarães, 2009; Campos et al., 2013; Schier et al., 2012). Here, for the first time, we show that zebrafish also display anxiolytic responses to CBD in an inverted U-shaped dose–response curve. While the link between anxiety decrease and CBD use has been examined in several pre-clinical and clinical studies, the safety of the CBD doses is still uncertain. Despite the well-known issue about anxiolytic properties of low-intermediary CBD doses, several studies have supported the use of a higher CBD dose as a protective substance against memory deficits induced by THC, inflammatory illness, and other memory-disruptive conditions (Cassol-Jr et al., 2010; Soares et al., 2010; Barichello et al., 2012). On this basis, the use of CBD-rich strains of *cannabis* has been encouraged, mainly through other strategies of memory assessments that are not aversive (Morgan et al., 2010; Mechoulam and Parker, 2013; Wright et al., 2013).

In general, CBD appears as an innocuous drug in memory formation or as a potential protective drug against memory disruption in several protocols, while few studies are concerned about high CBD doses (Fadda et al., 2006; Hayakawa et al., 2008; Cassol-Jr et al., 2010; Long et al., 2010; Soares et al., 2010; Barichello et al., 2012). However, endocannabinoid signaling, especially through CB1 receptors, has been shown to cause impairment of memory acquisition in a contextual fear assessment and memory extinction (Pamplona et al., 2006; De Carvalho et al., 2014). In this study, we used a dose of CBD 10 times higher than an anxiolytic one, which caused memory impairment in an avoidance task apparatus used to assess memory formation in zebrafish. In zebrafish telencephalon, long-term potentiation (LTP) has been shown to depend on glutamatergic transmission (Nam et al., 2004). The importance of glutamatergic signaling during memory acquisition and consolidation has been demonstrated in non-aversive tests like the Y-maze memory task and inhibitory avoidance in zebrafish (Blank et al., 2009; Cognato et al., 2012). Endocannabinoids have been implicated to affect neurotransmission mediated by glutamate through pre- and post-synaptic mechanisms involving CB1 receptor activation (Gerdeman and Lovinger, 2001; Huang et al., 2001), while no information is available for specific CBD effects. On the other hand, CBD is able to reduce glutamate reuptake in striatal synaptosomes of rats just in the higher doses tested (30–100 μM) by Pandolfo et al. (2011). Additionally, glutamate neurotransmission could be enhanced by CBD inhibition of nucleoside transport, increasing adenosine extracellular levels and action on facilitative A_{2A} adenosine receptors, as demonstrated in previous works (Carrier et al., 2006). Here, we present a striking effect of CBD on memory, which could be a result of a different interaction between animal species and its response to high doses of CBD in the context of aversive memory, possible through exacerbated glutamatergic activation.

These results of CBD on latency to cross chambers could not be associated with a catalepsy-like effect, since regular locomotion was always seen when animals received this high of a dose of CBD. Also, the literature shows no catalepsy effects of CBD (Long et al., 2010). However, hypnotic effects of high doses of CBD and longer sleep in humans have already been registered, while alertness was described in rats exposed to CBD (Carlini and Cunha, 1981; Murillo-Rodríguez et al., 2006). Additionally, *cannabis* cigarettes, *cannabis* extract, and analogs of THC and derivatives have been indicated to relieve pain in non-cancer types of chronic pain. The analgesic effect could influence the sensibility to the inhibitory avoidance task, but the pharmacological base seems to be related to CB receptor activation, which is a weak property of CBD, while THC seems to be responsible for the analgesic property (Bushlin et al., 2010; Ellis et al., 2009; Grotenhermen and Müller-Vahl, 2012).

Several methodological exposures to caffeine in animal and human studies contribute to the recognition of caffeine as a memory enhancer (reviewed by Cunha and Agostinho, 2010). In doses relevant to human consumption, caffeine is able to block A_1 and A_{2A} adenosine receptors, with emphasis on the latter (Fredholm et al., 1999). However, caffeine has been proposed to have a role more related to a normalizer than an improver of memory, based on its ability to prevent memory impairment induced by stress and chronic neuropathology (Cunha and Agostinho, 2010). This information appears to be more related to A_{2A} adenosine receptor than A_1 adenosine receptor antagonism (Cunha, 2008; Cognato et al., 2010). Here, we observed no alteration in memory formation in animals chronically treated with caffeine, although this treatment was able to prevent the acute CBD-induced memory disruption, when CBD was given after the training session. Long-term caffeine treatment has been associated with tolerance to locomotor effects attributed to the up-regulation or increasing in binding of A_1 adenosine (Johansson et al., 1993; Jacobson et al., 1996), while mnemonic effects are more related to A_{2A} adenosine receptor antagonism (Takahashi et al., 2008; Cunha and Agostinho, 2010). In this way, caffeine could prevent CBD effects on memory by a mechanism mediated by A_{2A} receptor block. Few studies have investigated the long-term effects of caffeine treatment on neurochemical aspects in zebrafish. In larval zebrafish, one week of caffeine treatment showed a slight and non-persistent increase of A_1 and A_{2A} adenosine receptors and BDNF mRNA expression (Capiotti et al., 2011). The ability of caffeine to prevent memory impairment was effective only when CBD was given after the training session, suggesting a strong effect of a high dose of CBD during memory acquisition, since CBD given before the training session had its effect preserved between the control and caffeine-treated animals. The effects of caffeine on memory acquisition and consolidation are complex, depending on the manner of administration, and they include different mechanisms from those reached in normal LTP (Martín and Buño, 2003; Takahashi et al., 2008; Alzoubi et al., 2013). These effects can be beyond the direct action on adenosine receptor contributing to this still unknown scenario of chronic caffeine interplay with acute CBD exposure.

The use of a specific antagonist of A_{2A} adenosine receptors, given in co-administration with CBD before or after the training session, increased latency between the training and test sessions. Meanwhile, the increased latency in the test session did not reach the profile of the control group. This result indicated a significant improvement in the index of memory retention, but not a full prevention of CBD effects. The specific block of A_1 adenosine receptor before the training session affected the latency to cross chambers during the training session. These results prevented the interpretation of the role of A_1 adenosine receptor when CBD was given before training. DPCPX (6 mg/L) used with adult zebrafish in the light/dark box paradigm decreased the time in the white compartment, while no alteration on distance traveled was registered (Stewart et al., 2011). Here, the animals delayed the time to cross chambers (from the white to black compartment), which is contradictory to the cited work, which leads us to suggest that DPCPX induced depressant locomotor effects in zebrafish, as in rodents when exposed to low doses (Florio et al., 1997). At high doses, a hyperlocomotor effect of this antagonist was registered in rodents (Florio et al., 1997; Kuzmin et al., 2006). However, when DPCPX was given after training, the latency to cross chambers in the test session increased similarly to the control group and prevented CBD effects. Since the test session occurred 24 h after animal exposure to DPCPX/CBD, the block of A_1 adenosine receptor appears to avoid CBD effects on memory consolidation. While the specific and/or integrative role of A_1 and A_{2A} adenosine receptors in the phases of memory formation is not fully elucidated, the block of the latter receptor seems to be more important in the consolidation of memory, as shown by Kopf et al. (1999). Here, no specific antagonists were able to reproduce caffeine effects, but differences between treatments (acute *versus* chronic treatment) probably contributed to these differences. We could suggest possible additive effects of both adenosine receptors on caffeine prevention of CBD, which could be reached by co-administration of both antagonists as seen in Kuzmin et al. (2006).

An animal model of cannabinoid-psychoactivity should be a living organism with a cannabinoid-induced effect or process that resembles those general biological processes in humans in as many pharmacological/behavioral parameters as possible. In this way, we showed here that zebrafish can be used as an animal model to evaluate CBD effects on anxiety-like behavior. However, the dose–response should consider a careful analysis of the particularities of this animal model, since CBD affected memory formation in higher doses. Also, we highlighted the need of studies on high CBD-content herbs and CBD alone administration in order to underlie the encouragement of CBD use on clinical treatment. Chronic exposure to caffeine appears as a protective way to avoid the non-desired effects of high doses of CBD, at least on memory consolidation, through a mechanism involving an adenosine receptor block. Meanwhile, more studies on caffeine and CBD interaction should be performed.

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References

Alzoubi, K.H., Srivareerat, M., Aleisa, A.M., Alkadhi, K.A., 2013. Chronic caffeine treatment prevents stress-induced LTP impairment: the critical role of phosphorylated CaMKII and BDNF. J. Mol. Neurosci. 49 (1), 11–20.

Barichello, T., Ceretta, R.A., Generoso, J.S., Moreira, A.P., Simões, L.R., Comim, C.M., Quevedo, J., Vilela, M.C., Zuardi, A.W., Crippa, J.A., Teixeira, A.L., 2012. Cannabidiol reduces host immune response and prevents cognitive impairments in Wistar rats submitted to pneumococcal meningitis. Eur. J. Pharmacol. 697 (1–3), 158–164.

Bisogno, T., Hanus, L., De Petrocellis, L., Tchilibon, S., Ponde, D.E., Brandi, I., Moriello, A.S., Davis, J.B., Mechoulam, R., Di Marzo, V., 2001. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br. J. Pharmacol. 134 (4), 845–852.

Blank, M., Guerim, L.D., Cordeiro, R.F., Vianna, M.R., 2009. A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. Neurobiol. Learn. Mem. 92 (4), 529–534.

Bushlin, I., Rozenfeld, R., Devi, L.A., 2010. Cannabinoid–opioid interactions during neuropathic pain and analgesia. Curr. Opin. Pharmacol. 10 (1), 80–86.

Campos, A.C., Guimarães, F.S., 2009. Activation of 5HT1A receptors mediates the anxiolytic effects of cannabidiol in a PTSD model. Behav. Pharmacol. 20, S54.

Campos, A.C., Ortega, Z., Palazuelos, J., Fogaça, M.V., Aguiar, D.C., Díaz-Alonso, J., Ortega-Gutiérrez, S., Vázquez-Villa, H., Moreira, F.A., Guzmán, M., Galve-Roperh, I., Guimarães, F.S., 2013. The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. Int. J. Neuropsychopharmacol. 16 (6), 1407–1419.

Capiotti, K.M., Menezes, F.P., Nazario, L.R., Pohlmann, J.B., de Oliveira, G.M., Fazenda, L., Bogo, M.R., Bonan, C.D., Da Silva, R.S., 2011. Early exposure to caffeine affects gene expression of adenosine receptors, DARPP-32 and BDNF without affecting sensibility and morphology of developing zebrafish (*Danio rerio*). Neurotoxicol. Teratol. 33 (6), 680–685.

Carlini, E.A., Cunha, J.M., 1981. Hypnotic and antiepileptic effects of cannabidiol. J. Clin. Pharmacol. 21 (8–9 Suppl.), 417S–427S.

Carriba, P., Ortiz, O., Patkar, K., Justinova, Z., Stroik, J., Themann, A., Müller, C., Woods, A.S., Hope, B.T., Ciruela, F., Casadó, V., Canela, E.I., Lluis, C., Goldberg, S.R., Moratalla, R., Franco, R., Ferré, S., 2007. Striatal adenosine A_{2A} and cannabinoid CB1 receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. Neuropsychopharmacology 32 (11), 2249–2259.

Carrier, E.J., Auchampach, J.A., Hillard, C.J., 2006. Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. Proc. Natl. Acad. Sci. U. S. A. 103, 7895–7900.

Cassol-Jr, O.J., Comim, C.M., Silva, B.R., Hermani, F.V., Constantino, L.S., Felisberto, F., Petronilho, F., Hallak, J.E., De Martinis, B.S., Zuardi, A.W., Crippa, J.A., Quevedo, J., Dal-Pizzol, F., 2010. Treatment with cannabidiol reverses oxidative stress parameters, cognitive impairment and mortality in rats submitted to sepsis by cecal ligation and puncture. Brain Res. 1348, 128–138.

Cheng, D., Low, J.K., Logge, W., Garner, B., Tim, K., 2014. Chronic cannabidiol treatment improves social and object recognition in double transgenic APPswe/PS1ΔE9 mice. Psychopharmacology 231, 3009–3017.

Cognato, G.P., Agostinho, P.M., Hockemeyer, J., Müller, C.E., Souza, D.O., Cunha, R.A., 2010. Caffeine and an adenosine A(2A) receptor antagonist prevent memory impairment and synaptotoxicity in adult rats triggered by a convulsive episode in early life. J. Neurochem. 112 (2), 453–462.

Cognato, G. de P., Bortolotto, J.W., Blazina, A.R., Christoff, R.R., Lara, D.R., Vianna, M.R., Bonan, C.D., 2012. Y-Maze memory task in zebrafish (*Danio rerio*): the role of glutamatergic and cholinergic systems on the acquisition and consolidation periods. Neurobiol. Learn. Mem. 98 (4), 321–328.

Cunha, R.A., 2008. Caffeine, adenosine receptors, memory and Alzheimer's disease. Med. Clin. 131, 790–795.

Cunha, R.A., Agostinho, P.M., 2010. Chronic caffeine consumption prevents memory disturbance in different animal models of memory decline. J. Alzheimers Dis. 20 (Suppl. 1), S95–S116.

Das, R.K., Kamboj, S.K., Ramadas, M., Yogan, K., Gupta, V., Redman, E., Curran, H.V., Morgan, C.J., 2013. Cannabidiol enhances consolidation of explicit fear extinction in humans. Psychopharmacology (Berl) 226 (4), 781–792.

De Carvalho, C.R., Pamplona, F.A., Cruz, J.S., Takahashi, R.N., 2014. Endocannabinoids underlie reconsolidation of hedonic memories in Wistar rats. Psychopharmacology (Berl) 231 (7), 1417–1425.

Devinsky, O., Cilio, M.R., Cross, H., Fernandez-Ruiz, J., French, J., Hill, C., Katz, R., Di Marzo, V., Jutras-Aswad, D., Notcutt, W.G., Martinez-Orgado, J., Robson, P.J., Rohrback, B.G., Thiele, E., Whalley, B., Friedman, D., 2014. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. Epilepsia 55 (6), 791–802.

Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M.F., Elkhayat, S.I., Bartels, B.K., Tien, A.K., Tien, D.H., Mohnot, S., Beeson, E., Glasgow, E., Amri, H., Zukowska, Z., Kalueff, A.V., 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav. Brain Res. 205 (1), 38–44.

Ellis, R.J., Toperoff, W., Vaida, F., et al., 2009. Smoked medicinal cannabis for neuropathic pain in HIV: a randomized, crossover clinical trial. Neuropsychopharmacology 34 (3), 672–680.

Fadda, P., Robinson, L., Fratta, W., Pertwee, R.G., Riedel, G., 2006. Scopolamine and MK801-induced working memory deficits in rats are not reversed by CBD-rich cannabis extracts. Behav. Brain Res. 168 (2), 307–311.

Fagherazzi, E.V., Garcia, V.A., Maurmann, N., Bervanger, T., Halmenschlager, L.H., Busato, S.B., Hallak, J.E., Zuardi, A.W., Crippa, J.A., Schröder, N., 2012. Memory-rescuing effects of cannabidiol in an animal model of cognitive impairment relevant to neurodegenerative disorders. Psychopharmacology (Berl) 219 (4), 1133–1140.

Ferré, S., Lluís, C., Justinova, Z., Quiroz, C., Orru, M., Navarro, G., Canela, E.I., Franco, R., Goldberg, S.R., 2010. Adenosine–cannabinoid receptor interactions. Implications for striatal function. Br. J. Pharmacol. 160, 443–453.

Florio, C., Rosati, A.M., Traversa, U., Vertua, R., 1997. Inhibitory and excitatory effects of adenosine antagonists on spontaneous locomotor activity in mice. Life Sci. 60 (17), 1477–1486.

Fredholm, B.B., Bättig, K., Holmén, J., Nehlig, A., Zvartau, E.E., 1999. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol. Rev. 51 (1), 83–133 (Review).

Gebauer, D.L., Pagnussat, N., Piato, A.L., Schaefer, I.C., Bonan, C.D., Lara, D.R., 2011. Effects of anxiolytics in zebrafish: similarities and differences between benzodiazepines, buspirone and ethanol. Pharmacol. Biochem. Behav. 99 (3), 480–486.

Gerdeman, G., Lovinger, D.M., 2001. CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. J. Neurophysiol. 85 (1), 468–471.

Gerlai, R., Lahav, M., Guo, S., Rosenthal, A., 2000. Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. Pharmacol. Biochem. Behav. 67 (4), 773–782.

Gomes, F.V., Resstel, L.B., Guimarães, F.S., 2011. The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT1A receptors. Psychopharmacology (Berl) 213 (2–3), 465–473.

Grotenhermen, F., Müller-Vahl, K., 2012. The therapeutic potential of cannabis and cannabinoids. Dtsch. Arztebl. Int. 109 (29–30), 495–501.

Hampson, A.J., Grimaldi, M., Axelrod, J., Wink, D., 1998. Cannabidiol and (−)Delta9 tetrahydrocannabinol are neuroprotective antioxidants. Proc. Natl. Acad. Sci. U. S. A. 95 (14), 8268–8273.

Hayakawa, K., Mishima, K., Hazekawa, M., Sano, K., Irie, K., Orito, K., Egawa, T., Kitamura, Y., Uchida, N., Nishimura, R., Egashira, N., Iwasaki, K., Fujiwara, M., 2008. Cannabidiol potentiates pharmacological effects of Delta(9)-tetrahydrocannabinol via CB(1) receptor-dependent mechanism. Brain Res. 1188, 157–164.

Hohmann, A.G., Suplita, R.L., 2006. Endocannabinoid mechanisms of pain modulation. AAPS J. 8 (4), E693–E708.

Huang, C.C., Lo, S.W., Hsu, K.S., 2001. Presynaptic mechanisms underlying cannabinoid inhibition of excitatory synaptic transmission in rat striatal neurons. J. Physiol. 532 (Pt 3), 731–748.

Jacobson, K.A., von Lubitz, D.K., Daly, J.W., Fredholm, B.B., 1996. Adenosine receptor ligands: differences with acute versus chronic treatment. Trends Pharmacol. Sci. 17 (3), 108–113.

Johansson, B., Ahlberg, S., van der Ploeg, I., Brené, S., Lindefors, N., Persson, H., Fredholm, B.B., 1993. Effect of long term caffeine treatment on A_1 and A_2 adenosine receptor binding and on mRNA levels in rat brain. Naunyn Schmiedeberg's Arch. Pharmacol. 347, 407–414.

Kopf, S.R., Melani, A., Pedata, F., Pepeu, G., 1999. Adenosine and memory storage: effect of A(1) and A(2) receptor antagonists. Psychopharmacology (Berl) 146 (2), 214–219.

Kuzmin, A., Johansson, B., Gimenez, L., Ogren, S.O., Fredholm, B.B., 2006. Combination of adenosine A_1 and A_{2A} receptor blocking agents induces caffeine-like locomotor stimulation in mice. Eur. Neuropsychopharmacol. 16 (2), 129–136.

Liou, G.I., Auchampach, J.A., Hillard, C.J., Zhu, G., Yousufzai, B., Mian, S., Khan, S., Khalifa, Y., 2008. Mediation of cannabidiol anti-inflammation in the retina by equilibrative nucleoside transporter and A_{2A} adenosine receptor. Invest. Ophthalmol. Vis. Sci. 49 (12), 5526–5531.

Long, L.E., Chesworth, R., Huang, X.F., McGregor, I.S., Arnold, J.C., Karl, T., 2010. A behavioural comparison of acute and chronic Delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. Int. J. Neuropsychopharmacol. 13 (7), 861–876.

Manuel, R., Gorissen, M., Roca, C.P., Zethof, J., van de Vis, H., Flik, G., van den Bos, R., 2014. Inhibitory avoidance learning in zebrafish (*Danio rerio*): effects of shock intensity and unraveling differences in task performance. Zebrafish 11 (4), 341–352.

Marsicano, G., Lafenêtre, P., 2009. Roles of the endocannabinoid system in learning and memory. Curr. Top. Behav. Neurosci. 1, 201–230.

Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., et al., 2002. The endogenous cannabinoid system controls extinction of aversive memories. Nature 418, 530–534.

Martín, E.D., Buño, W., 2003. Caffeine-mediated presynaptic long-term potentiation in hippocampal CA1 pyramidal neurons. J. Neurophysiol. 89 (6), 3029–3038.

Mechoulam, R., Parker, L., 2013. Towards a better cannabis drug. Br. J. Pharmacol. 170 (7), 1363–1364.

Morgan, C.J., Schafer, G., Freeman, T.P., Curran, H.V., 2010. Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study: naturalistic study [corrected]. Br. J. Psychiatry 197 (4), 285–290 (Erratum in: Br J Psychiatry. 2010, 197:416).

Murillo-Rodríguez, E., Millán-Aldaco, D., Palomero-Rivero, M., Mechoulam, R., Drucker-Colín, R., 2006. Cannabidiol, a constituent of *Cannabis sativa*, modulates sleep in rats. FEBS Lett. 580 (18), 4337–4345.

Nam, R.H., Kim, W., Li, C.J., 2004. NMDA receptor-dependent long-term potentiation in the telencephalon of the zebrafish. Neurosci. Lett. 370, 248–251.

Pamplona, F.A., Prediger, R.D., Pandolfo, P., Takahashi, R.N., 2006. The cannabinoid receptor agonist WIN 55,212-2 facilitates the extinction of contextual fear memory and spatial memory in rats. Psychopharmacology (Berl) 188 (4), 641–649 (Nov).

Pandolfo, P., Silveirinha, V., Santos-Rodrigues, A., Venance, L., Ledent, C., Takahashi, R.N., Cunha, R.A., Köfalvi, A., 2011. Cannabinoids inhibit the synaptic uptake of adenosine and dopamine in the rat and mouse striatum. Eur. J. Pharmacol. 655, 38–45.

Russo, E.B., Burnett, A., Hall, B., Parker, K.K., 2005. Agonistic properties of cannabidiol at 5-HT1a receptors. Neurochem. Res. 30 (8), 1037–1043.

Schier, A.R., Ribeiro, N.P., Silva, A.C., Hallak, J.E., Crippa, J.A., Nardi, A.E., Zuardi, A.W., 2012. Cannabidiol, a *Cannabis sativa* constituent, as an anxiolytic drug. Rev. Bras. Psiquiatr. 34 (Suppl. 1), S104–S110.

Soares, V. de P., Campos, A.C., Bortoli, V.C., Zangrossi Jr., H., Guimarães, F.S., Zuardi, A.W., 2010. Intra-dorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors. Behav. Brain Res. 213 (2), 225–229.

Stewart, A., Maximino, C., Brito, T.M., Herculano, A.M., Gouveia Jr., A., Morato, S., Cachat, J.M., Gaikwad, S., Elegante, M.F., Hart, P.C., Kalueff, A.V., 2011. Neurophenotyping of adult zebrafish using the light/dark box paradigm. In: Kalueff, A.V., Cachat, J.M. (Eds.), Zebrafish Neurobehavioral Protocols vol. 51. Humana Press, New York, pp. 157–168.

Takahashi, R.N., Pamplona, F.A., Prediger, R.D., 2008. Adenosine receptor antagonists for cognitive dysfunction: a review of animal studies. Front. Biosci. 13, 2614–2632.

Wright Jr., M.J., Vandewater, S.A., Taffe, M.A., 2013. Cannabidiol attenuates deficits of visuospatial associative memory induced by Δ(9) tetrahydrocannabinol. Br. J. Pharmacol. 170 (7), 1365–1373.

Zuardi, A.W., 2008. Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. Rev. Bras. Psiquiatr. 30, 271–280.