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SPECIAL ISSUE: SHORT COMMUNICATION

Antidepressant-like effects of cannabidiol in a rat model of early-life stress with or without adolescent cocaine exposure

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Abstract

Background Further studies are needed to better understand the effects of potential novel antidepressants, such as cannabidiol, for the treatment of psychiatric disorders during adolescence. In this context, we evaluated in a rodent model of early-life stress (a single 24-h episode of maternal deprivation, PND 9), the antidepressant-like effects of adolescent cannabidiol alone and/or in combination with adolescent cocaine exposure (given the described beneficial effects of cannabidiol on reducing cocaine effects).

Methods Maternally deprived Sprague-Dawley male rats were treated in adolescence with cannabidiol (with or without concomitant cocaine) and exposed to a battery of behavioral tests (forced-swim, novelty-suppressed feeding, open field, sucrose preference) across time. Putative enduring molecular correlates (CB receptors, BDNF) were evaluated in the hippocampus by western blot.

Results Cannabidiol exerted antidepressant- and anxiolytic-like effects in rats exposed to early-life stress. Cocaine did not alter affective-like behavior during adolescence in rats exposed to early-life stress; however, a depressive- and anxiogenic-like phenotype emerged during adulthood, and cannabidiol exerted some behavioral improvements, along with the growing literature supporting its beneficial role for reducing cocaine intake and/or reinstatement in rodents. Finally, cannabidiol did not modulate hippocampal CB receptors or BDNF proteins, and although the data raised interesting questions about the possible role of CB1 receptors on modulating the long-term effects of cocaine, future research is needed to expand these findings.

Conclusion Cannabidiol showed a promising therapeutic response in terms of ameliorating affect in a rat model of early-life stress during adolescence and up to adulthood.

Keywords Adolescence · Antidepressant · Cannabidiol · Cocaine · Maternal deprivation · Rat

Introduction

In the last years a lot of attention has been given to the potential of medicinal cannabis (i.e., mainly cannabidiol, a non-psychotomimetic compound of *Cannabis sativa*; [1]) for the treatment of a range of psychiatric disorders (e.g., [2]), although the conclusions of its efficacy are currently premature (see systematic review in [3]), and with the limitation that most studies have been performed in adults [4]. This limitation is relevant since antidepressant drugs elicit different behavioral responses and neurochemical effects in young and adults (for a general revision of the efficacy and tolerability of several antidepressants in children and adolescents see [5]; and [6] for specific age effects of cannabidiol in naïve rats). While extensive research has been done in adult rodent models, future studies are needed to better understand the effects of potential novel antidepressants, such as cannabidiol, during adolescence. To do so, we utilized a rodent model of early-life stress, known to modify brain developmental trajectories and capable of inducing behavioral and neurochemical alterations during adulthood, which applied a single episode (24 h) of early maternal deprivation on PND 9 (see recent studies from our group using this model in [7, 8]) to evaluate the immediate and/or persistent antidepressant-like potential of cannabidiol

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exposure during adolescence through a battery of behavioral tests (forced-swim, novelty-suppressed feeding, open field, sucrose preference). We hypothesized that cannabidiol will induce an antidepressant-like effect in rats exposed to early-life stress as did in non-stressed naïve rats [6].

Moreover, we recently demonstrated that the accumulation of early-life stress (maternal separation combined with adolescent cocaine exposure) could anticipate the negative behavioral outcome induced by either factor alone during adulthood to the adolescent period [8]. In this context, several studies have described the potential beneficial effects of cannabidiol on reducing drug intake and/or relapse prevention (see some of the many recent studies, e.g., [9], and specifically for cocaine [10, 11]). Thus, we evaluated the effects of cannabidiol on improving the emergence of negative affect induced by stress accumulation (maternal deprivation plus adolescent cocaine exposure) through a battery of behavioral tests (forced-swim, novelty-suppressed feeding, open field, sucrose preference). We hypothesized that cannabidiol will improve the negative impact induced by adolescent cocaine in early-life stressed rats.

As for the possible neuroplastic and neuroprotective mechanisms involved in the therapeutic effects of cannabidiol in psychiatric disorders [12], some key targets emerge as relevant in the hippocampus [13]. For example, some studies evaluated the regulation of cannabinoid receptors not only by cannabidiol administration (e.g., [14],), but also as a consequence of adolescent cocaine alone (e.g., [15]), or by the combination of early-life stress and adolescent cocaine exposure (see [16]). Also, cannabidiol induced rapid and sustained antidepressant-like effects in rodents through increased BDNF signaling [17], and increased BDNF acts as an essential common mechanism of action of several antidepressants (see recent study by [18]). Therefore, this study evaluated cannabinoid receptors (CB1 and CB2) and BDNF protein regulation by western blot following the described experimental procedures in the hippocampus as they might likely be involved in the negative impact induced by cocaine and/or the potential antidepressant-like effects exerted by cannabidiol.

Experimental procedures

Early-life maternal deprivation

Sprague-Dawley rats bred in the animal facility at the University of the Balearic Islands were housed (22 °C, 70% humidity, 12-h light/dark cycle, lights on at 8:00 AM) with *ad libitum* access to food and water. Experimental procedures followed the ARRIVE [19] and standard ethical guidelines (EU Directive 2010/63/EU; Spanish Royal Decree 53/2013). Eight litters were exposed to a single episode of maternal deprivation (24 h, PND 9–10; see [7, 8] for more details and earlier references within). Pups were weighted on PND 9 and PND 10 and were kept in their home cage with no nutritional supplements, while the mother was placed in a different cage in the same room. Rats were weaned at PND 22 and only male rats were selected for this study (n = 51; Fig. 1a), since female rats were reserved for a different experimental procedure. Thus, sex could not be evaluated as a biological variable in the present study.

Behavioral testing, pharmacological treatments and neurochemical analyses

Basal rates of immobility were determined for all rats in the forced-swim test prior to any pharmacological treatments (so groups could be balanced by immobility; [20]). After handling (PND 23–24), rats were individually placed in water tanks (41 cm high × 32 cm diameter, water at 25 ± 1 °C, 25 cm depth) for 15 min (pre-test, PND 25) and 24 h later for 5 min (test, PND 26). Test sessions were recorded and later analyzed (Behavioral Tracker software, CA, USA) to separate rats in four experimental groups: Veh-Sal, $n = 12$; CBD-Sal, $n = 13$; Veh-COC, $n = 13$; CBD-COC, $n = 13$ (Fig. 1a). Rats received (i.p.) cannabidiol (CBD: 10 mg/kg; dose selected from [6]) or its vehicle (Veh: 1 ml/kg of 1:1 DMSO:saline), followed by (3 h later) a daily injection of cocaine (COC: 15 mg/kg; dose and window of adolescence selected from [8, 20–22]) or saline (Sal: 0.9% NaCl, 1 ml/kg), for 7 days during adolescence (PND 33–39). The forced-swim test (5-min videotaped sessions) was repeated throughout treatment on PND 33, 36 and 40 (Fig. 1a). Then, rats were food deprived for 48 h before exposure to the novelty-suppressed feeding test on PND 43 (see [8]), since the test requires motivation for food (Fig. 1a). The 5-min test was performed in a wall-enclosed square arena (60 × 60 cm × 40 cm of height) with three food pellets in the center. Sessions were videotaped to later blindly analyze exploratory time (s), latency to center (s) and feeding time (s). Then, rats were single housed to evaluate 1% sucrose preference (vs. water) individually with the two-bottle choice test (PND 46–47, Fig. 1) as described [8]. Rats were left undisturbed until adulthood when they were challenged on PND 71 with cocaine (15 mg/kg, i.p.) and exposed to the forced-swim test 45 min later [20], to explore the long-term effects of a prior history of adolescent cocaine exposure on behavior. The forced-swim test (5-min videotaped sessions) were repeated across time on PND 78 and 100 (Fig. 1a). In between these tests, rats were exposed to the open field test (60 × 60 cm × 40 cm of height) for 5 min on PND 82 to evaluate exploratory-like behavior in an anxiogenic-like environment [20]. Videos were blindly analyzed with a specific software (Smart

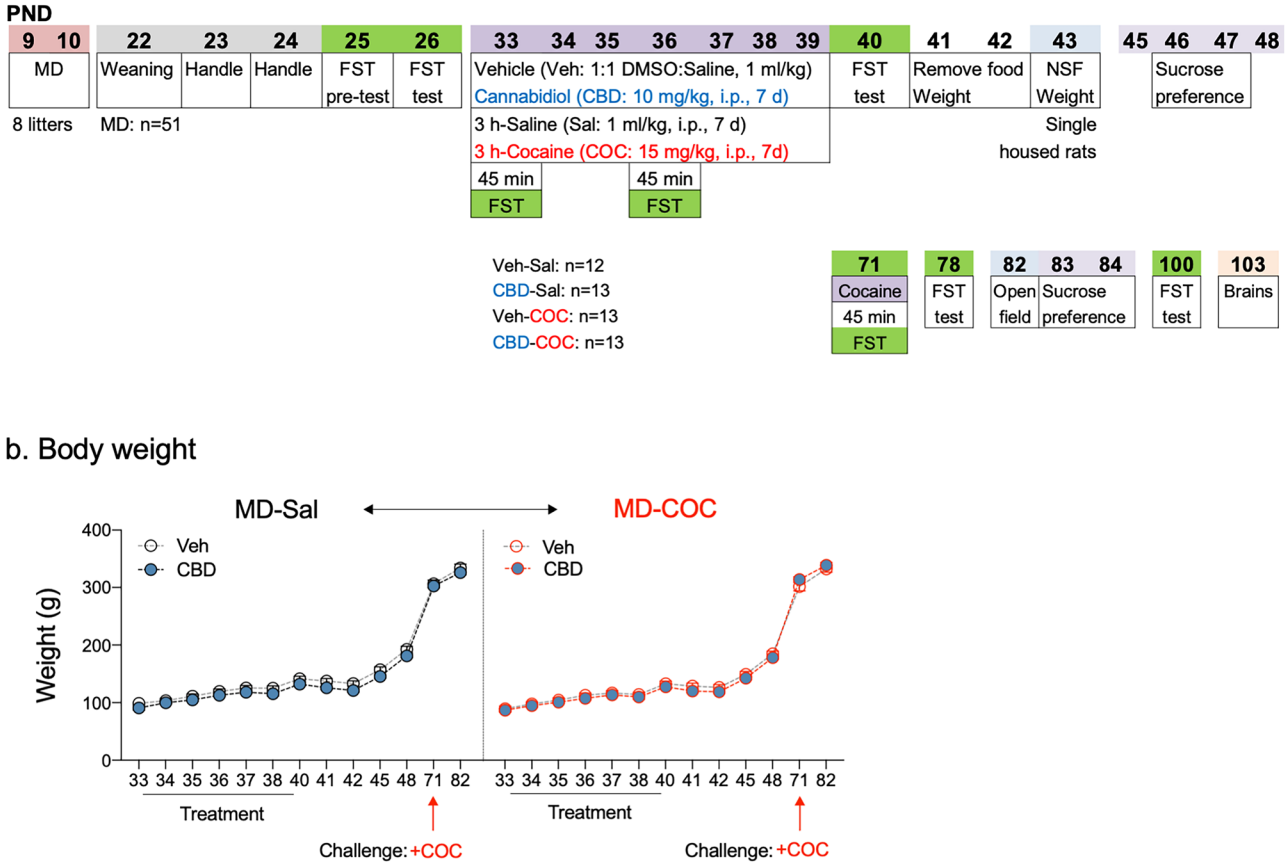


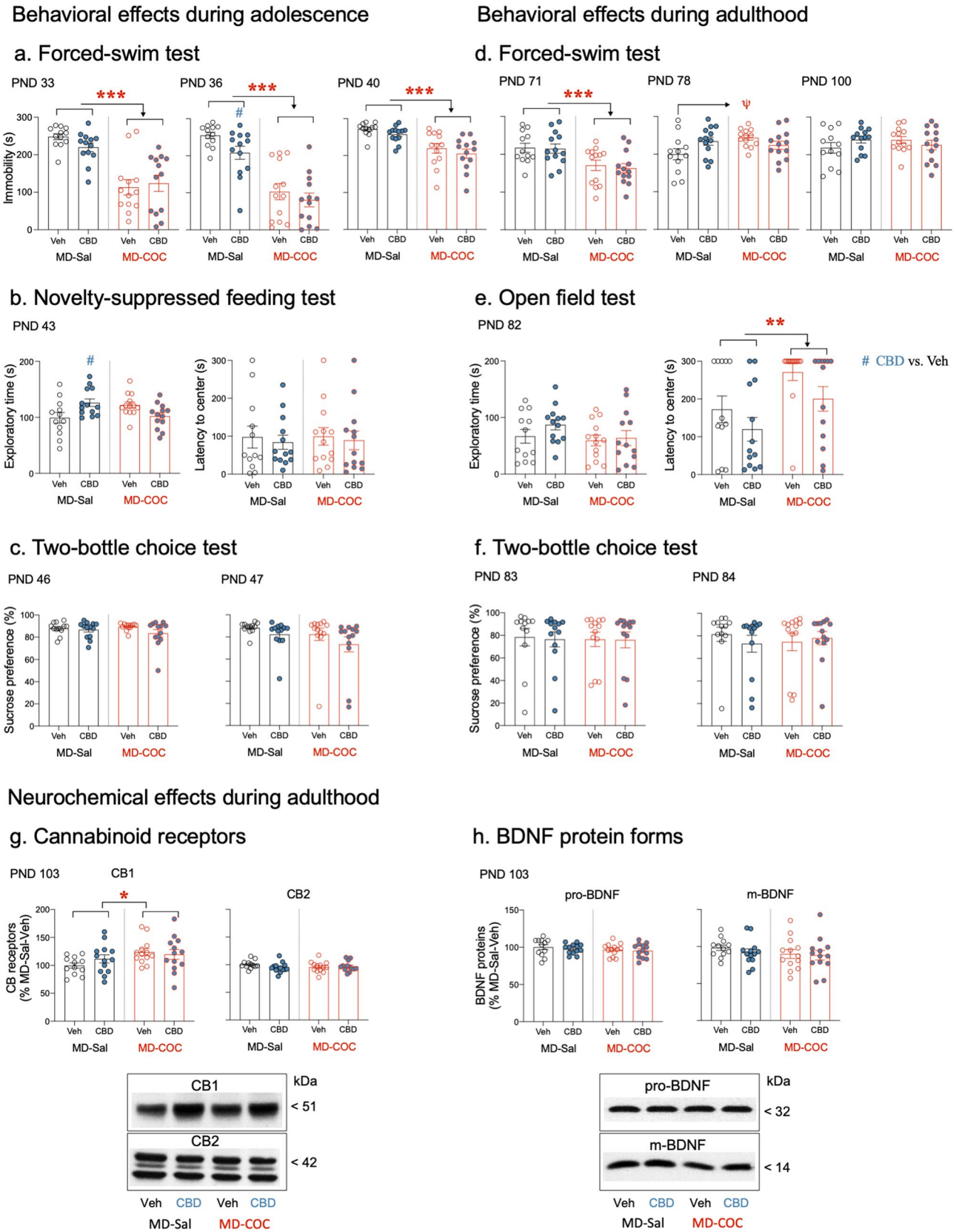
Fig. 1 **a** Experimental design. *COC* cocaine, *DMSO* dimethyl sulfoxide, *FST* forced-swim test, *MD* maternal deprivation, *NSF* novelty-suppressed feeding test, *PND* post-natal day, *Veh* vehicle. **b** Body weight (g) across days (PND 33–82). Data represent mean ± SEM of body weight (g). A three-way repeated measures ANOVA did not detect any significant changes

Video Tracking, Version 3.0.03, Panlab SL, Barcelona, Spain). The novelty-suppressed feeding test was not repeated in adulthood to avoid the recurrent extra stress of food depriving the rats, and since the open field is also a valid tool to explore the potential anxiolytic-like effects of drugs. Finally, rats were re-exposed to the two-bottle choice test on PND 83–84 to evaluate sucrose preference as detailed above.

On PND 103, rats were killed and western blot analyses performed in the hippocampus with anti-CB1 (1:2000 dilution, Abcam, Cat No. 23703, Cambridge, UK), anti-CB2 (1:1000 dilution, Cayman Chemical, Cat No. 101550, MI, USA) or anti-BDNF (N-20; 1:2500; Santa Cruz Biotechnology CA, USA; detects pro- and m-BDNF) primary antibodies as previously characterized in detail [15, 21]. β-actin (clone AC-15; 1:10,000; Sigma-Aldrich, MO, USA) served as a loading control since it was not altered by any treatment conditions.

Statistical analyses

Data analysis was performed with GraphPad Prism, Version 9 (GraphPad Software, CA, USA). Results are expressed as mean values ± standard error of the mean (SEM), and individual symbols for each rat are shown within bar graphs. Two- or three-way ANOVA (with or without repeated measures) were used for statistical evaluations with *post hoc* comparisons when appropriate (e.g., multiple *t* test analysis, Sidak's). Independent variables: treatment (vehicle vs. cannabidiol), post-treatment (saline vs. cocaine) and day (from PND 33 to 82). The level of significance was $p \leq 0.05$.


Behavioral effects during adolescence
a. Forced-swim test
PND 33
PND 36
PND 40
Immobility (s)
***
#
Veh CBD Veh CBD
MD-Sal MD-COC
b. Novelty-suppressed feeding test
PND 43
Exploratory time (s)
Latency to center (s)
c. Two-bottle choice test
PND 46
PND 47
Sucrose preference (%)
Behavioral effects during adulthood
d. Forced-swim test
PND 71
PND 78
PND 100
ψ
e. Open field test
PND 82
**
CBD vs. Veh
f. Two-bottle choice test
PND 83
PND 84
Neurochemical effects during adulthood
g. Cannabinoid receptors
PND 103
CB1
CB2
*
CB receptors (% MD-Sal-Veh)
kDa
< 51
< 42
h. BDNF protein forms
pro-BDNF
m-BDNF
BDNF proteins (% MD-Sal-Veh)
< 32
< 14

◂**Fig. 2** **a** Immobility (s) during adolescence in the forced-swim test during treatment (PND 33 and PND 36) and post-treatment on PND 40. **b** Exploratory time (s) and latency to center (s) in the novelty-suppressed feeding test on PND 43. **c** Sucrose preference (%) in the two-bottle choice test on PND 46–47. **d** Immobility (s) during adulthood in the forced-swim test 45 min post-cocaine exposure on PND 71, and later on PND 78 and 100. **e** Exploratory time (s) and latency to center (s) in the open field test on PND 82. **f** Sucrose preference (%) in the two-bottle choice test on PND 83–84. **g** Cannabinoid receptors (CB1 and CB2) and **h** BDNF protein forms (pro-BDNF and m-BDNF) regulation on PND 103, including representative immunoblots. Data represent mean ± SEM of each measurement for every treatment group. Symbols represent individual rat values within each experimental group. Groups of treatment (all exposed to maternal deprivation, MD): Veh-Sal, $n=12$; CBD-Sal, $n=13$; Veh-COC, $n=13$; CBD-COC, $n=13$. Rats received (i.p., 1 ml/kg) for 7 days (PND 33–39) cannabidiol (CBD: 10 mg/kg) or 1:1 DMSO:saline (Veh), followed by (3 h later) a daily injection of cocaine (COC: 15 mg/kg) or saline (Sal: 0.9% NaCl). Two-way ANOVAs followed by Sidak's multiple comparisons tests were used for statistical analysis: ***$p<0.001$, **$p<0.01$ and *$p<0.05$ when comparing the overall effect of post-treatment (cocaine vs. saline-treated groups during adolescence); #$p<0.05$ when comparing Veh-CBD vs. Veh-Sal; #$p<0.05$ when comparing Veh-COC vs. Veh-Sal

Results

Body weight

Changes in body weight (g) were monitored through the experimental procedure. A three-way repeated measures ANOVA detected a significant treatment x post-treatment x day interaction ($F_{12,564}=1.83$, $p=0.0413$), which was driven by a significant effect of day ($F_{2.12,99.53}=8219$, $p<0.0001$); i.e., normal weight gain expected from adolescence to adulthood (Fig. 1b), since no significant effects of treatment or post-treatment were observed.

Behavioral effects during adolescence and adulthood

During adolescence, the analysis of immobility (s) on PND 33 did not show a treatment x post-treatment interaction ($F_{1,47}=1.39$, $p=0.245$) nor an effect of treatment ($F_{1,47}=0.28$, $p=0.598$). However, there was a significant effect of post-treatment ($F_{1,47}=48.66$, $p<0.0001$; represented in Fig. 2a by ***), driven by the acute psychomotor effects of cocaine. These effects were repeatedly observed on PND 36 ($F_{1,47}=62.40$, $p<0.0001$; represented in Fig. 2a by ***) and on PND 40 (24 h after the last set of injections; $F_{1,47}=27.13$, $p<0.0001$; represented in Fig. 2a by ***), with no other changes detected, except for a significant effect of treatment on PND 36 ($F_{1,47}=3.822$, $p=0.05$) (Fig. 2a). A multiple *t* test analysis revealed that cannabidiol significantly decreased immobility on PND 36 (− 47 s, #$p=0.0299$ vs. vehicle-treated rats; Fig. 2a).

When evaluating exploratory time (s) in a novel environment (Fig. 2b), a two-way ANOVA showed a significant treatment x post-treatment interaction ($F_{1,47}=10.91$, $p=0.0018$), with a significant *post hoc* comparison (Sidak's); cannabidiol increased exploratory time (+27 s, #$p=0.0214$ vs. vehicle-treated rats). As for latency to center (s) or feeding time (s), the analyses did not show significant treatment x post-treatment interactions ($F_{1,47}=0.01$, $p=0.945$, Fig. 2b; and $F_{1,47}=0.38$, $p=0.543$, data not shown in graph, respectively).

Moreover, when evaluating sucrose preference in adolescence, the results showed a lack of treatment x post-treatment interaction during adolescence (Fig. 2c) on PND 46 ($F_{1,47}=1.18$, $p=0.282$) or PND 47 ($F_{1,47}=0.09$, $p=0.761$).

During adulthood, the analysis of immobility (s) on PND 71 did not show a treatment x post-treatment interaction ($F_{1,47}=0.04$, $p=0.839$), nor an effect of treatment ($F_{1,47}=0.15$, $p=0.700$). However, there was an effect of post-treatment ($F_{1,47}=15.04$, $p=0.0003$; represented in Fig. 2d by ***), driven by lower immobility scores in rats that received cocaine during adolescence (i.e., psychomotor sensitization after an acute challenge). Finally, rats were re-scored in the forced-swim test on PND 78 and PND 100 to evaluate the progression of the behavioral response. A significant treatment x post-treatment interaction was observed on PND 78 ($F_{1,47}=6.10$, $p=0.0172$), with a significant *post hoc* comparison (Sidak's); vehicle rats with a history of adolescent cocaine showed increased immobility (+44 s, ψ$p=0.046$) when compared to saline-treated rats (Fig. 2d); an effect that dissipated on PND 100 ($F_{1,47}=2.38$, $p=0.130$).

Rats were exposed to the open field test (PND 82), with no significant changes in exploratory time (s) (Fig. 2e), but a significant effect of treatment ($F_{1,47}=4.07$, $p=0.0494$; cannabidiol induced an overall anxiolytic-like effect by decreasing the latency to center independent of treatment) and post-treatment ($F_{1,47}=8.56$, $p=0.0053$; cocaine induced an overall anxiogenic-like effect by increasing the latency to center independently of pre-treatment; this effect is represented in Fig. 2e by **) for latency to center (s).

Finally, no significant effects (lack of treatment x post-treatment interaction) were observed on sucrose preference during adulthood (Fig. 2f) on PND 83 ($F_{1,47}=0.01$, $p=0.914$) or PND 84 ($F_{1,47}=0.72$, $p=0.399$).

Neurochemical effects during adulthood

When evaluating CB1 receptor content, a two-way ANOVA showed no treatment x post-treatment interaction ($F_{1,47}=1.30$, $p=0.2597$), but a significant effect of post-treatment ($F_{1,47}=5.23$, $p=0.0268$; effect of adolescent cocaine exposure represented in Fig. 2g by *). However, no significant effects were observed for CB2 regulation

(Fig. 2g). Similarly, BDNF protein forms were not altered by any of the prior treatments, as observed in Fig. 2h and in the two-way ANOVAs (i.e., lack of significant treatment x post-treatment interaction for pro-BDNF: $F_{1,47}=0.04$, $p=0.845$, and m-BDNF: $F_{1,47}=0.20$, $p=0.660$).

Discussion

Cannabidiol administration during adolescence induced immediate beneficial effects on improving affective-like behavior in a rat model of early-life stress, since it induced an antidepressant-like effect (i.e., improved response under the stressor of the forced-swim test, and increased exploratory time in the novelty-suppressed feeding test). These data align with a recent study from our group performed in naïve rats [6] in which adolescent cannabidiol induced an antidepressant-like response (improved immobility in the forced-swim test), while showed no effects on ameliorating anxiety- or hedonic-like behaviors, therefore reinforcing the notion that a specific stressor might be needed (i.e., early-life stress) for cannabidiol to show its anxiolytic-like potential (e.g., see revision in [4]). Thus, the present results demonstrated a good therapeutic potential for cannabidiol during adolescence in a rat model of early-life stress, in line with its described fast antidepressant-like effects in adolescent [6] and/or adult rodents (see recent revisions in [4, 23]). Rats were then re-exposed in adulthood to a battery of tests to measure affective-like behavior (i.e., potential enduring beneficial effects of cannabidiol). While the effects in the forced-swim test vanished (see [23] for similar immediate effects of cannabidiol in adult rodents, and [4]), cannabidiol induced an anxiolytic-like response (i.e., decreased latency to center in the open field). In conclusion, so far, cannabidiol showed a promising therapeutic response in terms of ameliorating affect in a rat model of early-life stress during adolescence and up to adulthood.

In this context, the next question we tackled was to ascertain whether adolescent cannabidiol administration could be used to prevent the combined negative impact on affective-like behavior induced by early-life stress and adolescent cocaine exposure (see [8]). The results showed that adolescent cocaine exposure in early-life stressed rats reduced immobility in the forced-swim test at all time-points tested during adolescence, and independent of prior treatment (cannabidiol vs. vehicle). Although cocaine is known to elevate mood, these effects could not be attributed to an antidepressant-like response because of the increased locomotion it causes (i.e., acute and repeated psychomotor effects). It is worth remarking that the effects of cocaine were so potent that they completely overpowered any possible improvements induced by cannabidiol. Moreover, cocaine did not alter anxiety-like behavior nor it reduced sucrose preference during adolescence, suggesting, in line with prior results (see [8, 20]), that it did not cause an immediate negative impact on affect.

Then, rats were left undisturbed until adulthood (i.e., 32 days of forced abstinence), when they were challenged with acute cocaine and scored in the forced-swim test 45 min later. The results showed the expected psychomotor behavioral sensitization [24, 25], driven by the observed lower immobility scores in rats that received cocaine during adolescence, which dissipated over time. However, in line with our prior data [20], a pro-depressant-like effect (i.e., increased immobility on PND 78) and an anxiogenic-like phenotype (i.e., increased latency to center on PND 82) emerged in adulthood exclusively in rats with a history of adolescent cocaine exposure. These data, consistently with earlier results, reinforced the notion that cocaine administration during adolescence (and in particular during this specific age window; PND 33–39; [21]) produced persistent negative affect [8, 20], that could likely increase the susceptibility to develop an addictive-like phenotype (e.g., [22, 25]). Interestingly, cannabidiol improved some of these negative outcomes, along with the growing literature supporting its beneficial role for reducing cocaine intake and/or reinstatement in rodents (e.g., [10, 11, 13, 26]), but in contrast to the recent randomized placebo-controlled trial showing that cannabidiol did not reduce cocaine craving or relapse among people being treated for cocaine use disorder [27].

As for the putative molecular changes evaluated, the western blot results showed a lack of enduring effects on the regulation of cannabinoid receptors or BDNF protein forms in the hippocampus, contrarily to prior reports (e.g., [13, 17]) performed either in a different species (mice vs. rats) or following alternative experimental procedures (e.g., fast effects vs. enduring responses when the behavioral response was no longer present). Interestingly, CB1 receptor was increased in rats previously exposed to cocaine during adolescence, demonstrating some enduring effects emerging during cocaine abstinence in the hippocampus, in line with prior data showing an increased CB1 receptor functionality within hippocampus as a consequence of early-life stress and adolescent cocaine exposure [16]. Moreover, the same paradigm of adolescent cocaine exposure dysregulated the content of CB receptors by increasing CB1 and decreasing CB2 receptor proteins in rat brain during adolescence, although the changes did not persist into adulthood [15] (probably because no cocaine challenge was administered as compared to the present study). These results raise interesting questions about the possible role of CB1 receptor in modulating some of the long-term effects of cocaine in the brain. Moreover, since adolescent cannabidiol did not induce long-term neurochemical changes, it might not be the best candidate to improve the enduring molecular impact of cocaine. Finally,

although prior data suggested that during forced abstinence from repeated cocaine treatment, the duration of immobility significantly increased (similarly to the present results), as well as the mRNA hippocampal content of BDNF (among other brain regions [28]), our results did not find any significant changes on hippocampal BDNF protein regulation. In the context of these neurochemical results, the main limitations of the study are related to not having brain samples at the time-points when behavior was altered (i.e., cannabidiol's behavioral effects in adolescence), to ascertain the putative molecular mechanisms behind its actions. Therefore, future studies should evaluate hypothetical molecular correlates at the time when the behavioral responses occurred, and expand these findings to also include other brain regions such as prefrontal cortex or striatum.

In summary, cannabidiol exerted antidepressant- and anxiolytic-like effects in a rat model of early-life stress (as it was previously shown in naïve rats). Although adolescent cocaine exposure did not alter affective-like behavior during adolescence in rats exposed to early-life stress, a depressive- and anxiogenic-like phenotype emerged during adulthood, and cannabidiol showed some behavioral improvements.

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Author contributions All authors were responsible for the study concept and design. CB-H conducted the experiments with help from RG-C. CB-H and RG-C analyzed the data. MJG-F revised the data, plotted the figures and drafted the manuscript. All authors contributed and have approved the final version for publication.

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