

Original Investigation — PSPH-D-19-00371-R1

Decreased sensitivity in adolescent versus adult rats to the antidepressant-like effects of cannabidiol

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Short title: Cannabidiol effects in adolescent and adult rats

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Declarations of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgments

We would like to thank Drs. Huda Akil and Stanley J. Watson (University of Michigan, Ann Arbor, MI, USA) who kindly provided Ki-67 antibody.

Role of funding source

The authors disclosed receipt of the following financial support for the research, authorship and/or publication of this article. This study was funded by Delegación del Gobierno para el Plan Nacional sobre Drogas (grant 2016/002, Ministerio de Sanidad, Servicios Sociales e Igualdad, Spain) and by Fundación Alicia Koplowitz to MJG-F. MJG-F is a member of RETICS-RTA (RD16/0017/0010; Instituto de Salud Carlos III, MINECO/FEDER). The program “TECH” from project “TALENT PLUS Construint Salut, Generant Valor” (IdISBa, GOIB) supports CB-H’s salary.

Abstract

Rationale Cannabidiol is a non-psychoactive phytocannabinoid with great therapeutic potential in diverse psychiatric disorders, however its antidepressant potential has been mainly ascertained in adult rats. *Objectives* To compare the antidepressant-like response induced by cannabidiol in adolescent and adult rats and the possible parallel modulation of hippocampal neurogenesis. *Methods* Male Sprague-Dawley rats were repeatedly treated with cannabidiol (3, 10 and 30 mg/kg) or vehicle (1 ml/kg) during adolescence (postnatal days, PND 27-33) or adulthood (PND 141-147) and exposed to 3 consecutive tests (forced-swim, open field, two-bottle choice) that quantified behavioral despair, anxiety- and sucrose intake respectively. *Results* Cannabidiol induced differential effects depending on the age and dose administered, with a decreased sensitivity observed in adolescent rats: (1) cannabidiol (30 mg/kg) decreased body weight only in adult rats; (2) cannabidiol ameliorated behavioral despair in adolescent and adult rats, but with a different dose sensitivity (10 vs. 30 mg/kg), and with a different extent (2 vs. 21 days post-treatment); (3) cannabidiol did not modulate anxiety-like behavior at any dose tested in adolescent or adult rats; and (4) cannabidiol increased sucrose intake in adult rats. *Conclusions* Our findings support the notion that cannabidiol exerts antidepressant- and anorexigenic-like effects in adult rats, and demonstrate a decreased potential when administered in adolescent rats. Moreover, since cannabidiol did not modulate hippocampal neurogenesis (cell proliferation and early neuronal survival) in adolescent or adult rats, the results revealed potential antidepressant-like effects induced by cannabidiol without the need of regulating hippocampal neurogenesis.

Keywords

Age; Antidepressant; Cannabidiol; Rat; Hippocampus; Neurogenesis

Introduction

Adolescence is a vulnerable period for the onset of depression (Burke et al. 1990), while there are similarities in the clinical presentation of adolescent and adult depression, there are differences in the biological correlates (Toenders et al. 2019) as well as in the responses to pharmacological treatment (Kaufman et al. 2001). Several studies have proven the effectiveness of different classes of antidepressant drugs in the treatment of depression in adults (e.g., Charney et al. 1998, Cipriani et al. 2016), but the efficacy of antidepressants during adolescence is controversial (see a network meta-analysis comparing the efficacy and safety of different types of antidepressants in children and adolescents; Cipriani et al. 2016). For example, selective serotonin reuptake inhibitor-type antidepressants are efficacious, but tricyclic antidepressants have no or limited efficacy in treating adolescent patients (see Cipriani et al. 2016). This is an important issue because treatment with antidepressants that have marginal efficacy can lead to serious consequences (i.e., increased risk of suicidal thinking and behavior in some children and adolescent with major depressive disorder), as labeled with a black box warning in all antidepressants' prescriptions (see details in Cipriani et al. 2016). Therefore, given the limited options for a safe treatment of major depressive disorder in children and adolescents, and since antidepressants are not well studied in this population, further research on putative new targets and/or interventions are needed.

In this context, the possible role of cannabis and/or cannabinoid system in improving or worsening the symptoms associated with psychiatric pathologies has been a topic of several studies (e.g., Haney and Evins 2016). In fact, adolescence is a period of great sensitivity during brain development in which changes in the normal functioning of the endocannabinoid system signaling could potentially lead to altered developmental trajectories of neural circuits governing emotional behaviors (Meyer et al. 2018). One drug that seems to play an important neuroprotective role in neuropsychiatric disorders by modulating endocannabinoids is

cannabidiol, a fitocannabinoid non psychomimetic found in *Cannabis sativa* (see revision at Campos et al. 2017). Prior studies in rodents have proven a beneficial role for cannabidiol on ameliorating negative affect, such as ameliorating behavioral despair as measured in the forced-swim test (see revision in Silote et al. 2019), inducing anxiolytic-like effects, normally observed in rodents exposed to a prior stressor (see revision in de Mello Schier et al. 2014), or producing prohedonic-like responses (Shoval et al. 2016; Linge et al. 2016). However, these studies have been mainly centered in adult rodents and it is not currently known whether cannabidiol would produce differential antidepressant-like responses in adolescent rats. Therefore, the first part of the study aimed at comparing cannabidiol potential antidepressant-like effects in adolescent and adult rats. This is of great relevance given the FDA approval of cannabidiol (Epidiolex, pure cannabidiol) for the treatment of infantile epileptic encephalopathies and further studies on its effects in adolescence are needed.

The mechanism of action by which cannabidiol exerts its effects involves multiple targets (recently revised in Silote et al. 2019). For instance, although it shows low affinity for CB1 and CB2 receptors, cannabidiol could act as an antagonist on CB1 (Thomas et al. 2007), probably ascribed to its capacity to act as a negative allosteric modulator at these receptors (Laprairie et al. 2015; Martinez-Pinilla et al. 2017). Moreover, since cannabidiol inhibits the enzymatic degradation and uptake of anandamide (Bisogno et al. 2001), it indirectly increases anandamide levels, which facilitates endocannabinoid signaling through CB1 and CB2 receptors (Pertwee 2008). In addition to the modulation of the endocannabinoid system, cannabidiol can regulate the serotonergic system by directly binding to 5-HT1A receptors (Russo et al. 2005) as well as other transmitter systems and targets (see Silote et al. 2019). For example, one such neuroplasticity target could be adult hippocampal neurogenesis, which is regulated by antidepressant drugs (Dranovsky and Hen 2006) and cannabinoids in general (Prenderville et al. 2015), and by cannabidiol in particular (Wolf et al. 2010). In this context,

this study evaluated cannabidiol regulation of adult hippocampal neurogenesis in adolescent and adult rats.

Materials and methods

Animals

A total of 110 male Sprague-Dawley rats bred in the animal facility at the University of the Balearic Islands were used in this study. Depending on the age and/or weight, rats were initially housed in groups of 2 to 4. The housing used standard cages in precise environmental conditions (22 °C, 70% humidity, and 12 h light/dark cycle, lights on at 8:00 AM) with *ad libitum* access to a standard diet and tap water. All animal experiments complied with the ARRIVE guidelines (McGrath and Lilley 2015), and were carried out in accordance with the EU Directive 2010/63/EU of the European Parliament and of the Council. Following the Spanish Royal Decree 53/2013, the Local Bioethical Committee (University of the Balearic Islands) and the regional Government (Conselleria Medi Ambient, Agricultura i Pesca, Direcció General Agricultura i Ramaderia, Govern de les Illes Balears) approved all experimental procedures. All efforts were made to minimize the number of rats used and their suffering. All procedures were performed during the light period.

Cannabidiol treatment

For this study, a total of 75 adolescent (PND 26 at the beginning of the experiment) or 35 adult (around PND 140 at the beginning of the experiment) male rats were used in two separate sets of experiments per age for evaluating on one hand, the effects of cannabidiol as a potential antidepressant agent (44 adolescent and 21 adult rats in total, see Fig. 1a and 1c), and on the other hand, the possible parallel regulation of hippocampal neurogenesis when behavior was altered (31 adolescent and 14 adult rats in total, see Fig. 1b and 1d). Notably,

hippocampal neurogenesis was also evaluated in rats exposed to behavior (PND 48 or PND 175, see Fig. 1a and 1c). All rats were handled for 1 day (PND 26 or PND 140) prior to any drug treatment. Rats were exposed to a daily dose of cannabidiol (3, 10 or 30 mg/kg, i.p.) (purity \geq 98%; THC Pharm, Germany) for 7 days (PND 27 to 33 or PND 141 to 147) or vehicle (1 ml/kg of a mixture of saline, 0.9% NaCl, and DMSO, 1:1 proportion or 1 ml/kg of DMSO; control groups). The doses of cannabidiol were selected based on prior studies performed in rodents (e.g., Silote et al. 2019). Rats were weighted daily throughout drug treatment.

Behavioral tests

Cannabidiol potential antidepressant-like properties were evaluated by 3 consecutive experimental tests (i.e., forced-swim test, open field, sucrose preference) that measure changes in behavioral despair, anxiety-like behavior and hedonic-like responses respectively in a subgroup of adolescent or adult rats (see Fig. 1a and 1c). The forced-swim test, which is a widely accepted experimental tool used to screen drugs for antidepressant activity in a stress situation, consisted of a 15 min pre-test on PND 34 or PND 148 and a 5-min test on PND 35 or PND 149 (this session was videotaped). Each rat was placed in an individual tank (41 cm high x 32 cm diameter), with water to a depth of 25 cm, and at 25 ± 1 °C (see for further details García-Cabrerizo et al. 2015). Videos were then analyzed (blind to the experimental groups) to evaluate the potential antidepressant-like effects (i.e., changes in the time spent immobile) of cannabidiol administration in adolescent or adult rats (Behavioral Tracker software, CA, USA). In an attempt to evaluate the temporal course of the effects observed, a modified approach to the forced-swim test was then followed (i.e., re-exposure to the test on PND 47 or PND 161, 168 and 174), until improvements on behavior in comparison to their respective control groups were vanished (see Fig. 1a and 1c; see similar

procedures followed in García-Cabrerizo and García-Fuster, 2019a). Although repetition of the same test might induce a learning dimension and/or lead to the development of tolerance to the test performance, the same conditions were followed for all rats (i.e., to compare the progression of treated rats vs. their respective controls).

Then, on PND 37 or PND 151 rats were placed for 5 min in an open field, a wall-enclosed square arena (60 x 60 cm and 40 cm of height) to evaluate potential effects on anxiety-like behavior (i.e., changes in exploratory behavior, Hernández-Hernández et al. 2018). At the beginning of the session, rats were placed in the center of the arena and left undisturbed to freely move and explore the field. The test sessions were performed and recorded under housing illumination conditions. Videos were then analyzed (blind to the experimental groups) using a digital video tracking system (Smart Video Tracking software, Version 3.0.03, Panlab SL, Barcelona, Spain) to calculate exploratory time (i.e., time spent active), distance travelled, time spent inactive, latency to center and time spent in center (see García-Cabrerizo and García-Fuster 2019a).

Right after the open field test, rats were single housed (PND 37 or PND 151, Fig. 1a and 1c) in order to evaluate their individual amount of sucrose intake in a two-bottle choice test (PND 40-41 or PND 154-155) (see further details in García-Cabrerizo and García-Fuster 2019b). To avoid bias towards any side of the cage, bottles were placed in alternate positions each day and were weighted daily to evaluate sucrose intake (g) during 48 h (PND 40 and PND 41 or PND 154 and PND 155).

Tissue collection

Rat were sacrificed at the indicated times (PND 35 or 149 for rats with no behavioral testing and PND 48 or PND 175 for rats exposed to behavioral testing, see Fig. 1) and their brains removed. For each animal, the left half-brain was quickly frozen in -30 °C isopentane and

stored at -80 °C until 30 µm sections were cryostat cut and slide-mounted throughout the entire hippocampal extent (-1.72 to -6.80 mm from Bregma). Hippocampal sections were stored at -80 °C until the content of cell genesis markers (i.e., Ki-67 for cell proliferation and NeuroD for early neuronal survival; García-Fuster et al. 2010, 2011) were evaluated by immunohistochemical analysis.

Immunohistochemical analysis

Hippocampal recent cell proliferation (all dividing cells within a cell cycle) was labeled with Ki-67 (Cameron and McKay 2001) and early neuronal survival was labeled with NeuroD (Lee, 1997). As previously described in detail (e.g., García-Fuster et al. 2010, 2011), each cell genesis labeling was performed on 3 slides per animal containing 8 hippocampal tissue sections (24 sections total; every 8th section throughout the entire extent of the hippocampus). Sections were post-fixed in 4% paraformaldehyde followed by several steps such as antigen retrieval, blocking in peroxidase solution and BSA (with 1% serum and 0.05% Triton X-100), overnight incubation with rabbit polyclonal anti-Ki-67 (1:40000; kindly provided by Drs. Huda Akil and Stanley J. Watson, University of Michigan) or goat polyclonal anti-NeuroD (1:25000; Santa Cruz Biotechnology), and 1 h incubation with biotinylated anti-rabbit or anti-goat secondary antibody 1:1000 (Vector Laboratories). An Avidin/Biotin complex (Vectastain Elite ABC kit; Vectors Laboratories) and the chromogen 3,3'-diaminobenzidine (DAB) (with nickel chloride for NeuroD) were used for visualization of the antibody bound. Finally, tissue was counterstained with cresyl violet (only for Ki-67 detection), dehydrated in graded alcohols, immersed in xylene and cover-slipped with Permount®. The number of immunostained positive cells in the dentate gyrus was quantified (by a blind experimenter to the treatment groups) with a Leica DMR light microscope (63X objective lens) in all sections following a modified unbiased stereological procedure (see

García-Fuster et al. 2010, 2011). To correct for possible different areas of analysis among different treatment groups (Amrein et al. 2011, García-Cabrerizo et al. 2015, García-Cabrerizo and García-Fuster 2016), the overall number of Ki-67+ or NeuroD+ cells counted for each animal was divided by the hippocampal area quantified (mm^2 , as measured in a densitometer, GS-800 Imaging Calibrated Densitometer, BioRad).

Statistical analyses

Data were analyzed with GraphPad Prism, Version 6 (GraphPad Software, Inc.). Results are expressed as mean values \pm standard error of the mean (SEM). Changes in body weight (g) or in behavioral despair (i.e., time spent immobile in the forced-swim test) were evaluated by two-way repeated measures ANOVAs followed by a multiple comparisons test when appropriate (e.g., Sidak's), in which Treatment (vehicle vs. cannabidiol) and Age (PND; as indicated in Fig. 1) were used as independent variables. Changes in anxiety-like behavior, sucrose intake, and brain markers (i.e., Ki-67, NeuroD) were evaluated at the indicated times (see Fig. 1) by one-way ANOVAs followed by Sidak's multiple comparison tests (i.e., experimental procedures with C and 3 and 10 mg/kg of cannabidiol in adolescent rats or C and 10 and 30 mg/kg of cannabidiol in adult rats) or Student's *t*-test (i.e., experimental procedures with C and 30 mg/kg of cannabidiol in adolescent rats, see Fig. 1a and 1b). The level of significance was set at $p \leq 0.05$.

Results

Cannabidiol effects on body weight in adolescent and adult rats

Rat's weight gain did not vary throughout the duration of cannabidiol treatment in adolescence (PND 27-33): doses 3 and 10 mg/kg (no effect of Treatment: $F_{2,46} = 0.397$, $p = 0.675$, and no Treatment x PND interaction: $F_{12,276} = 0.510$, $p = 0.908$; Fig. 2a) and dose 30

mg/kg (no effect of Treatment: $F_{1,24} = 0.001$, $p = 0.991$, but a significant Treatment x PND interaction: $F_{6,144} = 22.62$, $p < 0.001$, although no *post-hoc* comparisons rendered significant; Fig. 2b).

When cannabidiol was administered in adult rats, although no overall effect of Treatment was observed ($F_{2,28} = 2.360$, $p = 0.113$) there was a significant Treatment x PND interaction ($F_{12,168} = 5.075$, $p < 0.001$), and multiple *post-hoc* comparisons revealed a smaller body weight following 30 mg/kg of cannabidiol as observed on PND 146 and 147 ($*p < 0.05$ vs. control-treated rats; see Fig. 2c).

Cannabidiol effects on behavioral despair in adolescent and adult rats

The effects of cannabidiol (lower doses tested of 3 and 10 mg/kg) on the time spent immobile (sec) in the forced swim test in adolescent rats (expressed as percent change vs. the respective control group) were analyzed by a two-way ANOVA repeated measures as follows: effect of PND (35 vs. 47: $F_{1,25} = 4.46$, $p = 0.045$), effect of Treatment (C vs. CBD: $F_{2,25} = 3.05$, $p = 0.065$), Treatment x PND interaction ($F_{2,25} = 1.48$, $p = 0.248$). When evaluating the effects of Treatment at each time window of adolescence (PND 35 and PND 47), Sidak's multiple comparisons test revealed that the dose of 10 mg/kg of cannabidiol decreased immobility on PND 35 ($-25 \pm 9\%$, $*p < 0.05$ vs. C), suggesting a potential antidepressant-like effect for cannabidiol (dose of 10 mg/kg) when administered repeatedly in adolescent rats as observed 2 days post-treatment under the stress of a force-swim test (see Fig. 3a). The decreased immobility paralleled an increase in the time spent climbing (data not shown). The effects of a higher dose of cannabidiol (30 mg/kg) on immobility were assessed in a different experiment performed in adolescent rats (see Fig. 3b). A two-way ANOVA repeated measures revealed no effect of PND (PND 35 vs. 47: $F_{1,14} = 0.05$, $p = 0.830$), Treatment (C vs. CBD: $F_{1,14} = 0.80$, $p = 0.387$) nor a Treatment x PND interaction ($F_{1,14} = 0.06$, $p = 0.803$).

In adult rats, the effects of cannabidiol (doses tested of 10 and 30 mg/kg) on immobility in the forced swim test were analyzed by a two-way ANOVA repeated measures, which revealed an effect of PND ($F_{3,51} = 4.80, p = 0.005$), an effect of Treatment ($F_{2,17} = 6.60, p = 0.008$), but no Treatment x PND interaction ($F_{6,51} = 1.66, p = 0.151$). When evaluating the effects of Treatment at each time window tested (PND 149, 161, 168 and 174), Sidak's multiple comparisons test revealed that 30 mg/kg of cannabidiol significantly decreased immobility up to 21 days post-treatment (PND 149: $-24 \pm 6\%$, $***p < 0.001$; PND 161: $-19 \pm 6\%$, $*p < 0.05$; PND 168: $-20 \pm 6\%$, $**p < 0.01$) when compared to control-treated rats, suggesting a sustained antidepressant-like effect when administered repeatedly in adult rats exposed to the stress of a force-swim test (see Fig. 3c).

Cannabidiol effects on anxiety-like behavior in adolescent and adult rats

When evaluating the effects of adolescent cannabidiol treatment (lower doses tested of 3 and 10 mg/kg) on anxiety-like behavior (PND 37), as measured in the open field, no significant results were observed by one-way ANOVAs for any of the parameters analyzed (see Fig. 4a; data expressed as percent change vs. the respective control group): overall distanced travelled ($F_{2,25} = 0.26, p = 0.772$), time spent inactive ($F_{2,25} = 0.17, p = 0.848$), or time spent in center ($F_{2,23} = 1.35, p = 0.875$). Similarly, the dose of 30 mg/kg of cannabidiol did not alter any of the parameters analyzed in the open field test in adolescent rats (see Fig. 4b): overall distanced travelled ($t = 0.14, df = 14, p = 0.893$), time spent inactive ($t = 0.53, df = 14, p = 0.606$), or time spent in center ($t = 1.35, df = 14, p = 0.199$).

Comparably, in adult rats cannabidiol (doses of 10 and 30 mg/kg) did not modulate anxiety-like behavior in the open field test (see Fig. 4c) as measured by overall distanced travelled ($F_{2,17} = 0.47, p = 0.631$), time spent inactive ($F_{2,18} = 0.17, p = 0.847$), or time spent in center ($F_{2,18} = 0.81, p = 0.459$).

Cannabidiol effects on sucrose intake in adolescent and adult rats

Adolescent cannabidiol treatment did not modify the amount of sucrose consumed (g) during 48 h (PND 40-41; data expressed as percent change vs. the respective control group): doses of 3 and 10 mg/kg ($F_{2,24} = 1.02$, $p = 0.377$, see Fig. 5a), and dose of 30 mg/kg ($t = 0.98$, $df = 13$, $p = 0.346$, see Fig. 5b). Conversely, cannabidiol increased the amount of sucrose intake following repeated cannabidiol administration in adult rats ($F_{2,18} = 4.05$, $p = 0.035$, Fig. 5c). Sidak's multiple comparisons test revealed that cannabidiol significantly increased sucrose consumption when administered at the highest dose tested (30 mg/kg: $+52 \pm 20\%$, $*p < 0.05$) when compared to control rats, and as observed 7 to 8 days post-treatment (see Fig. 5c).

Cannabidiol effects on hippocampal neurogenesis in adolescent and adult rats

When evaluating the effects of adolescent (PND 27-33) cannabidiol administration (doses of 3 and 10 mg/kg) on hippocampal neurogenesis markers, the results (data expressed as percent change vs. control group) revealed no significant changes on cell proliferation (Ki-67+ cells/area; $F_{2,18} = 0.45$, $p = 0.648$; see Fig. 6a,c) or early neuronal survival (NeuroD+ cells/area; $F_{2,18} = 1.76$, $p = 0.200$; see Fig. 6e,g) as measured 2 days post-treatment on PND 35 (when the antidepressant-like effect was observed, see Fig. 3) and compared to control rats. When adolescent rats were treated with a higher dose of cannabidiol (30 mg/kg) the results show no changes on Ki-67+ ($t = 0.23$, $df = 8$, $p = 0.821$) or NeuroD+ ($t = 1.44$, $df = 8$, $p = 0.189$) cells/area on PND 35 (see Fig. 6a,c and 6e,g). Moreover, 15 days post-treatment (PND 48), no changes were observed in Ki-67 ($t = 0.36$, $df = 16$, $p = 0.722$; Fig. 6a,c) or NeuroD ($t = 1.57$, $df = 16$, $p = 0.136$; Fig. 6e,g) for rats treated with the dose of 10 mg/kg of cannabidiol (i.e., dose that induced the antidepressant-like effects in adolescence).

Similarly, when evaluating the effects of adult cannabidiol administration (PND 141-147) on hippocampal neurogenesis markers, the results revealed no significant changes on cell proliferation (Ki-67+ cells/area; $F_{2,11} = 0.27$, $p = 0.766$; see Fig. 6b,d) or early neuronal survival (NeuroD+ cells/area; $F_{2,11} = 0.13$, $p = 0.877$; see Fig. 6f,h) as measured 2 days post-treatment on PND 149 (when the antidepressant-like effect observed was more robust, see Fig. 3) and compared to control rats. Moreover, 28 days post-treatment (PND 175), no changes were observed in Ki-67 ($t = 0.42$, $df = 12$, $p = 0.423$; Fig. 6b,d) or NeuroD ($t = 1.47$, $df = 12$, $p = 0.166$; Fig. 6 f,h) for rats treated with the dose of 30 mg/kg of cannabidiol (i.e., dose that induced the antidepressant-like effects in adulthood).

Discussion

The main results presented in this study suggest that cannabidiol has the potential to induce differential behavioral effects depending on the age and dose administered, with a decreased sensitivity observed in adolescent rats. For instance, cannabidiol (30 mg/kg) decreased body weight, an effect only observed for adult rats and that emerged following 6 days of treatment. While cannabidiol decreased immobility in the forced swim test both in adolescent and adult rats, it did so with different dose sensitivity, 10 vs. 30 mg/kg, and with different extent (effects observed up to 2 vs. 21 days post-treatment). Moreover, cannabidiol did not modulate anxiety-like behavior, as measured in the open field, at any dose tested in adolescent or adult rats, but increased sucrose intake exclusively in adult rats. Lastly, cannabidiol did not modulate hippocampal neurogenesis (i.e., cell proliferation and early neuronal survival) in adolescent or adult rats.

Acute cannabidiol did not affect body weight in adolescent or adult rats. However, the effects of repeated administration of cannabidiol observed on body weight were age-dependent, while no effects were observed in adolescent rats, it reduced body weight in adult

rats. In this context, a recent study showed that repeated cannabidiol administration during adolescence (0.5, 1 or 5 mg/kg from PND 30-60) did not alter body weight and did not promote motor or metabolic side effects in rodents (Peres et al. 2018), in line with the present results, and suggesting cannabidiol, both acutely and/or repeatedly, seems to be well tolerated during adolescence. Moreover, cannabidiol (15 and 30 mg/kg, oral administration during 34 days, PND 21-55) did not alter body weight in mice (Carvalho et al. 2018). Interestingly, when revising the current literature exploring the effects of cannabidiol in adult rats, some prior studies suggested that while acute cannabidiol did not affect body weight (de Moraes et al. 2018), its repeated treatment decreased body weight in rodents (e.g., Ignatowska-Jankowska et al. 2011, Santiago et al. 2019), an effect that was prevented by a CB₂ receptor antagonist (Ignatowska-Jankowska et al. 2011). There is a broad literature suggesting a potential role for endocannabinoids in controlling body weight by regulating circuits that coordinate energy homeostasis (e.g., Di Marzo et al. 2001, Di Marzo and Matias 2005, Horn et al. 2018), mainly through drugs that function as an antagonist or inverse agonist over CB₁ receptors (e.g., rimonabant, Colombo et al. 1998, Horn et al. 2018). In addition to modulating the endocannabinoid system, cannabidiol also signals through other type of receptors, such as for example 5-HT_{1A} (Campos and Guimarães 2008), associated with the control of body weight (Scopinho et al. 2011). These results support the existing literature regarding the anorexigenic-like effects of cannabidiol in adult rats, and extend it, by demonstrating a reduced sensitivity to these effects in adolescent rats.

The potential expected beneficial effects of cannabidiol on regulating different aspects of emotional-like behavior were evaluated by several consecutive tests that induce distress to the animals (i.e., forced-swim test, open field test) or assess preference for sucrose intake (two-bottle choice test) and quantify behavioral despair, anxiety-like behavior and anhedonia respectively. The results showed that cannabidiol improved behavioral despair in rats at both

ages evaluated but with a different dose sensitivity (10 vs. 30 mg/kg), and with a different duration (effects observed up to 2 vs. 21 days post-treatment) for adolescent and adult rats respectively. Prior studies in regards to this topic, mainly centered in adult rodents, have proven a role for cannabidiol on ameliorating behavioral despair when animals are exposed to the stressor of the forced-swim test. The first study evaluated the effects induced by acute systemic cannabidiol administration in mice (Zanelati et al. 2010) was followed by several others (e.g., El-Alfy et al. 2010, Reus et al. 2011, Schiavon et al. 2016) that have established that both acute and/or chronic cannabidiol treatment is an effective antidepressant, at the range of doses of 3-30 mg/kg in mice and 30-60 mg/kg in rats (see revision in Silote et al. 2019). To our knowledge this is the first study to compare the antidepressant-like potential of cannabidiol in rats at different ages during development, proving a decreased response in adolescent rats. This adds up to the existing literature suggesting a lack of efficacy of certain antidepressants when given to children or adolescents (Cipriani et al. 2016) and could be caused by age-related variations in the maturation rates of brain neurotransmitters systems involved in the response (i.e., the serotonergic nervous system matures earlier than the noradrenergic system, see Kozisek et al. 2008 for differential antidepressant-like response in adolescence; the endocannabinoid system has a crucial role during brain development, see Viveros et al. 2012) or in the elimination rates of the drug (i.e., lower doses of the antidepressant desipramine were needed in adolescent rats to obtain the same brain desipramine concentrations as adults due to slower elimination rates, see Kozisek et al. 2007). In this context, the present results denoted different dosing regimens (10 mg/kg vs. 30 mg/kg) in adolescent vs. adult rats to achieve an antidepressant-like effect in the forced-swim test 2 days post-treatment. Moreover, the present results showed that cannabidiol beneficial effects lasted up to 21 days post-treatment in adult rats, suggesting a long-term and sustained antidepressant-like effect. While most of the prior studies have been centered in proving the

fast-acting antidepressant-like induced by cannabidiol (e.g., Linge et al. 2016, see revision in Silote et al. 2019), a recent study also showed that the effects of cannabidiol lasted for up to a week after a single injection, both in mice and rats (Sales et al. 2019), that together with the current data support a promising therapeutic profile for cannabidiol as a long-lasting antidepressant when administered in adulthood.

Additionally, cannabidiol has been shown to induce anxiolytic-like effects in rodents (see revision in de Mello Schier et al. 2014), among multiple examples from the literature, following sleep disruption (Hsiao et al. 2012), a prior foot shock (Rock et al. 2017) or the induction of neuropathic pain (de Gregorio et al. 2019). Therefore, the lack of cannabidiol significant effects on modulating anxiety-like behavior, as measured by different features in the open field test, in adolescent or adult rats might be explained by the fact that cannabidiol might require the presence of a specific stressor to induce anxiolytic-like effects or that other behavioral tests more specific to measure changes in anxiety-like behavior (i.e., elevated plus maze, novelty-suppressed feeding) are needed to capture cannabidiol anxiolytic-like effects. Alternatively, the regimen of cannabidiol used in this study may have led to the development of tolerance to the anxiolytic effects of the drug, thus inducing a decreased sensitivity observed as a lack of effects in the open field. Finally, the fact that the open field test was performed 4 days after the last treatment dose administered, and/or that rats were previously exposed to 2 sessions in the forced-swim test in 2 consecutive days, might justify the present negative outcome, in disagreement with some prior literature supporting an anxiolytic-like role for cannabidiol.

Finally, the results of the sucrose preference test revealed a potential prohedonic-like effect induced by cannabidiol when administered repeatedly (30 mg/kg) in adult rats and observed up to 7- and 8-days post-treatment. Similarly, 30 mg/kg of cannabidiol (oral administration by food pellet), but not the dose of 15 or 45 mg/kg, induced a prohedonic-like

effect in adult Wistar-Kyoto rats in the sucrose preference test (Shoval et al. 2016). Comparably, repeated, but not acute, cannabidiol (50 mg/kg, 7 d, i.p.) induced a prohedonic effect in male C57BL6 mice (with an olfactory bulbectomy), in the sucrose preference test (Linge et al. 2016). The observed increase in sucrose consumption might be a way to balance energy homeostasis (i.e., decreased body weight observed in adult rats following repeated intake of 30 mg/kg). Thus far, the behavioral results evaluating cannabidiol effects suggest a great potential for cannabidiol as a long-lasting antidepressant when administered in adulthood while prove a decreased potential in adolescence, in line with the expected differences observed for other pharmacological treatments in adolescents (Kaufman et al. 2001, Cipriani et al. 2016).

Given the accepted regulation of adult hippocampal neurogenesis by antidepressant drugs (Dranovsky and Hen 2006), and by cannabinoids (Prenderville et al. 2015), this study evaluated whether there was a parallel regulation between the antidepressant-like effect observed 2 days post-treatment in rats and hippocampal neurogenesis regulation. Surprisingly, the results showed that cannabidiol did not modulate hippocampal neurogenesis, as evaluated by a marker of recent cell proliferation (Ki-67) and a marker of early neuronal survival (NeuroD) in adolescent or adult rats. Most of the literature supports a role for cannabidiol on inducing hippocampal neurogenesis (Wolf et al. 2010), in a dose-dependent fashion (Luján et al. 2018). However, one study suggested a negative impact of cannabidiol on neurogenesis at high doses (i.e., increased neurogenesis following 3 mg/kg but decreased with 30 mg/kg, see Cheng et al. 2014). In summary, most of the experiments relied on murine models exposed to a prior stress and were performed during adulthood (see revision in Campos et al. 2017). For example, cannabidiol induced an anxiolytic effect in chronically stressed mice in association with the regulation of hippocampal neurogenesis (Fogaça et al. 2018). In support of the present results, a recent study showed a dissociation

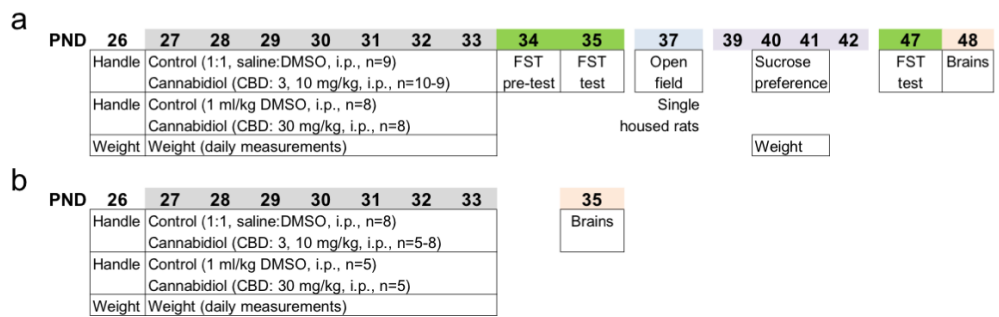
between the regulation of emotional behavior and the proliferative effects induced by repeated cannabidiol (Schiavon et al. 2016), suggesting, together with the present results that the antidepressant-like effects of cannabidiol might occur independently of adult neurogenesis in naïve animals. Thus, the potential mechanism by which cannabidiol induced its antidepressant- and/or prohedonic-like effects might involve increases of anandamide levels through the inhibition of its uptake or metabolism (Gorzalka and Hill 2011, Micale et al. 2013) and/or the activation of 5-HT_{1A} receptors (Carr and Lucki 2011, Linge et al. 2016) and deserves further studies.

To conclude, our findings support the notion that cannabidiol exerts antidepressant- and anorexigenic-like effects in adult rats, and prove a decreased potential when administered in adolescent rats. Moreover, the results reveal that the potential antidepressant-like effects induced by cannabidiol are observed without the need of regulating hippocampal neurogenesis. These results indicate clear differences in the effects cannabidiol exert in adolescent vs. adult rats suggesting the need for further studies to elucidate the mechanisms behind the observed decreased sensitivity in adolescence.

Figure Legends

Fig. 1. Experimental design. (a-b) Study I: Cannabidiol effects in adolescent rats. (c-d) Study II: Cannabidiol effects on adult rats. FST: forced swim test; PND: postnatal day.

Study I: Cannabidiol effects in adolescent rats



Study II: Cannabidiol effects in adult rats

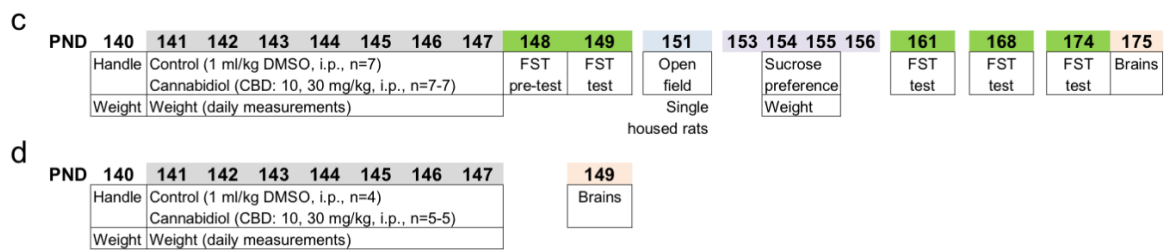


Fig. 2. Cannabidiol effects on body weight in adolescent (a-b) or adult (c) rats: an anorexigenic-like effect observed in adult rats. Doses tested: 3, 10 and 30 mg/kg of cannabidiol, i.p., 7 days. Data represents mean \pm SEM of body weight (g). Two-way repeated measures ANOVAs followed by Sidak's multiple comparisons tests: $*p < 0.05$ when compared to control rats. CBD: cannabidiol; PND: postnatal day.

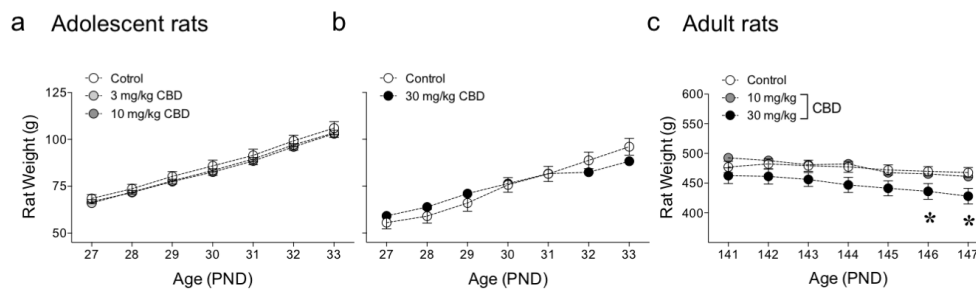


Fig. 3. Cannabidiol antidepressant-like effects in (a-b) adolescent or (c) adult rats as measured under the stress of the forced-swim test. Doses tested: 3, 10 and 30 mg/kg of cannabidiol, i.p., 7 days. Data represent mean \pm SEM of the time (sec) spent immobile and is expressed as % change vs. control rats. Two-way repeated measures ANOVAs followed by Sidak's multiple comparisons tests: *** $p < 0.001$, ** $p < 0.01$ or * $p < 0.05$ when compared to control rats. CBD: cannabidiol; PND: postnatal day.

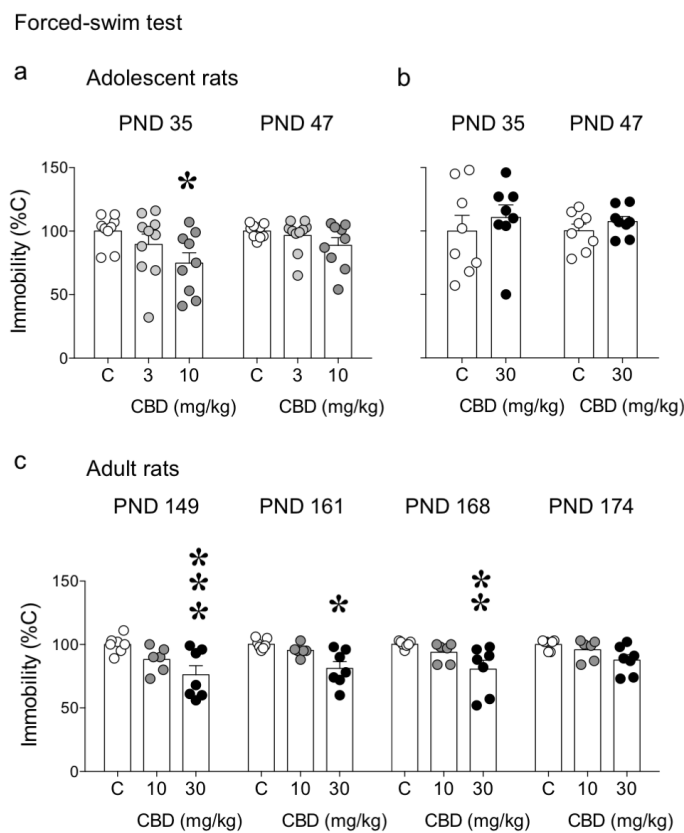


Fig. 4. Cannabidiol did not induce changes in anxiety-like behavior in (a-b) adolescent or (c) adult rats as measured in the open field test PND 37 or PND 151 respectively. Doses tested: 3, 10 and 30 mg/kg of cannabidiol, i.p., 7 days. Data represents mean \pm SEM of the distance travelled (cm), the time spent inactive (sec), and the time spent in center (sec) and is expressed as % change vs. control rats. Two-way ANOVAs did not detect any significant effects. CBD: cannabidiol; PND: postnatal day.

Open field test

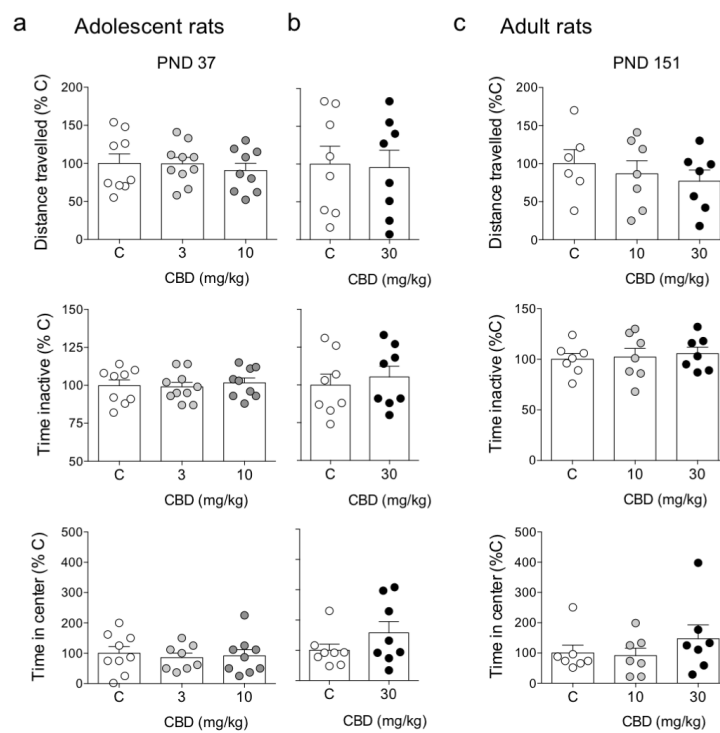


Fig. 5. Cannabidiol effects on the two-bottle choice test in adolescent (a-b) or adult (c) rats as measured by 1% sucrose intake (PND 40-41 or 154-155 respectively). Doses tested: 3, 10 and 30 mg/kg of cannabidiol, i.p., 7 days. Data represents mean \pm SEM of the amount of 1% sucrose consumed (g) during the length of the two-bottle choice test (48 h) and is expressed as % change vs. control rats. One-way ANOVAs followed by Sidak's multiple comparison tests (a, c) or Student's *t*-test (b): **p* < 0.05 when compared to age-matched control rats.

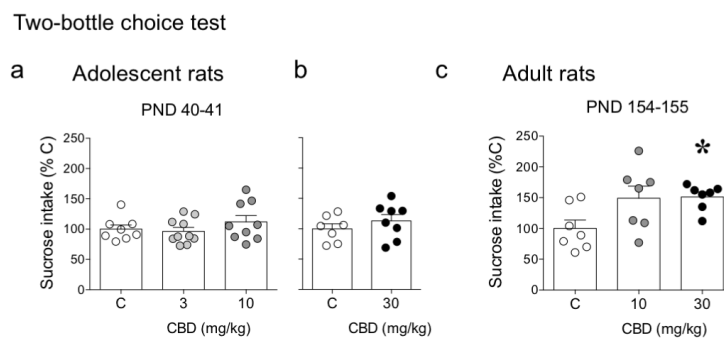
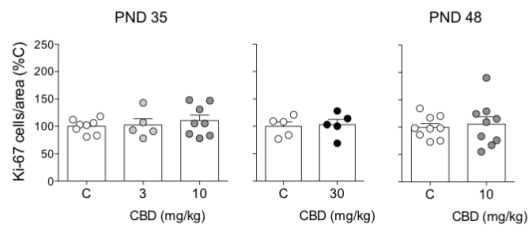


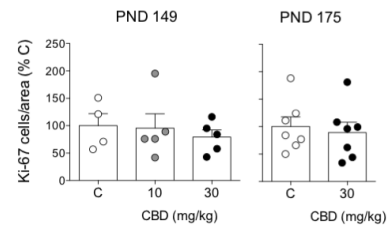
Fig. 6. Cannabidiol did not modulate cell proliferation (a-d) or early neuronal survival (e-h) in the hippocampus of adolescent (PND 35 or PND 48, a,c and e,g) or adult (PND 149 or PND 175, b,d and f,h) rats. Doses tested: 3, 10 and 30 mg/kg of cannabidiol, i.p., 7 days. (a-b) Quantitative analysis (mean \pm SEM) of Ki-67⁺ cells per area (mm²) in the left dentate gyrus and expressed as % change vs. control rats. (c,d) Representative images of Ki-67⁺ (brown labeling in the blue granular layer, DAB) taken with a light microscope using a 63X objective lens to identify individual cells. Scale bar = 30 μ m. (e-f) Quantitative analysis (mean \pm SEM) of NeuroD⁺ cells per area (mm²) in the left dentate gyrus and expressed as % change vs. control rats. (g,h) Representative images of NeuroD (dark blue labeling in the blue granular layer, DAB + nickel chloride) taken with a light microscope using a 63X objective lens to identify individual cells. Scale bar = 30 μ m. One-way ANOVAs or Student's *t*-test did not detect any significant effects.

Hippocampal neurogenesis markers

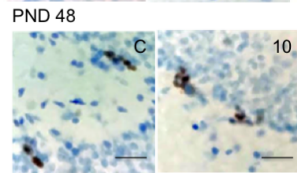
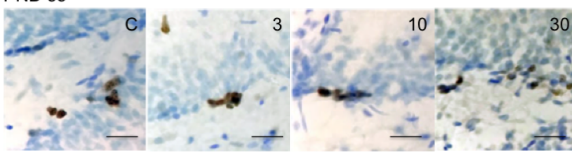
a Adolescent rats



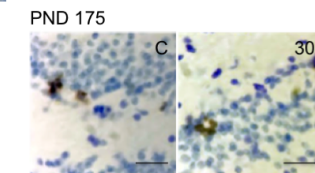
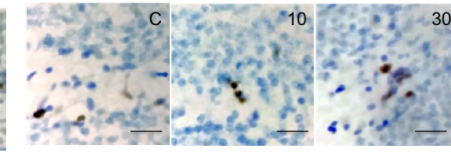
b Adult rats



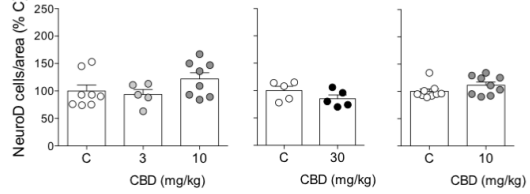
c PND 35



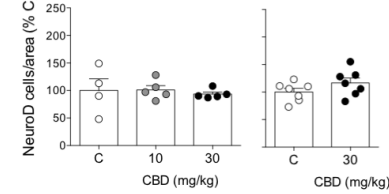
d PND 149



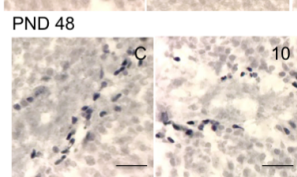
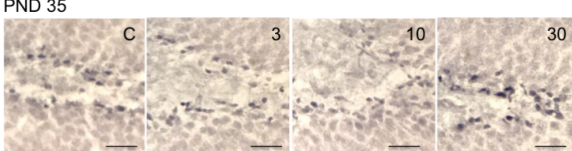
e PND 35



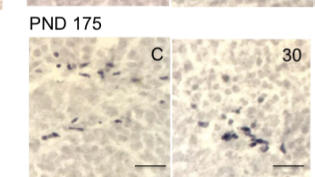
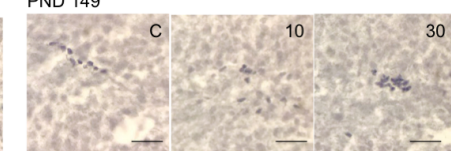
f PND 149



g PND 35



h PND 149



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