Differences in pharmacological activities of Δ^9 -THC and CBD on repetitive behaviours in juvenile and young adult mice may impact decision making for Tourette syndrome

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Abstract

Background and Purpose

Medicinal cannabis is in increasing use by patients with Tourette syndrome, a neuropsychiatric disorder that affects about 1% of the general population and has a childhood onset. However, the pharmacological effects of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and cannabidiol (CBD) have not been systematically screened or compared between juvenile and young adult rodents in a model of Tourette syndrome.

Experimental Approach

In rodents, the administration of 2,5-dimethoxy-4-iodoamphetamine (DOI) increases head twitch response (HTR) and ear scratch response (ESR), and has been proposed as an animal model useful to respectively study motor tics and premonitory urges associated with tic disorders.

Key Results

Comparing the potency of Δ^9 -THC to inhibit DOI-induced repetitive behaviours, the rank order was ESR > grooming > HTR vs. ESR = grooming > HTR in young adult vs. juvenile mice. Δ^9 -THC (5 mg/kg) induced severe adverse effects in the form of cataleptic behaviour in control mice and significantly increased ESR in juvenile mice. To the best of our knowledge, the pharmacological effects of CBD have not been studied in models of Tourette syndrome. In juvenile mice, CBD had no effect on DOI-induced ESR and grooming behaviour. CBD alone induced side-effects, significantly increasing the frequency of HTR in juvenile and young adult mice.

Conclusions and Implications

Δ^9 -THC efficaciously reverses peripheral but not central motor tics. Δ^9 -THC may reduce ambulatory movements and evoke premonitory urges in some paediatric patients. The small 'therapeutic window' in juveniles suggests that CBD may not effectively treat motor tics in children and may even exacerbate tics in a population of patients with Tourette syndrome.

Abbreviations: Δ^9 -tetrahydrocannabinol (Δ^9 -THC); cannabidiol (CBD); 2,5-dimethoxy-4-iodoamphetamine (DOI); head twitch response (HTR); ear scratch response (ESR); obsessive compulsive disorder (OCD); central nervous system (CNS); peripheral nervous system (PNS).

Bullet point summary

What is already known

- The effects of Δ^9 -THC and CBD on patients with Tourette syndrome have barely been explored.

What this study adds

- Different effects of Δ^9 -THC and of CBD on tic/urge-like behaviours in juveniles vs. young adults.
- Side effects of Δ^9 -THC and CBD on tic/urge-like behaviours in juveniles vs. young adults.

Clinical significance

- Young adult patients bothered with urges may benefit from a relatively low dose of Δ^9 -THC.
- Young adult patients with caudally located motor tics, which are distributed below the level of neck/head, may benefit from treatment with CBD.

Keywords. Tourette syndrome, tic disorder, motor tic, premonitory urge, 2,5-dimethoxy-4-iodoamphetamine (DOI), Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and cannabidiol (CBD), side-effect.

Introduction

Tourette syndrome is a highly prevalent neurodevelopmental disease, part of a spectrum of tic disorders, with a childhood-onset that affects about 1% of the general population with a 3:1 male to female ratio (reviewed by (Greydanus & Tullio, 2020; McNaught & Mink, 2011)). About 80% of the patients report a feeling of premonitory urges, described as a build-up of tension as if to scratch an itch or sneeze, followed by relief after the tic occurs; this build-up can sometimes cause more distress than the tics themselves (reviewed by (Cavanna, Black, Hallett, & Voon, 2017; McNaught & Mink, 2011)). These premonitory urges are more common in patients with Tourette syndrome who also have obsessive compulsive disorder (OCD) (Cavanna et al., 2017). Moderate to severe forms of motor tics can be painful and forceful, and may require drug treatment (Greydanus & Tullio, 2020; McNaught & Mink, 2011).

The aetiology of Tourette syndrome is poorly understood. Models used for the study of Tourette syndrome are mainly based on pharmacological modulation of stereotypical behaviours with substances that affect the regulation of motor behaviour and lead to repetitive behaviours. In rodents, stereotypical behaviour has been observed after administration of 2,5-dimethoxy-4-iodoamphetamine (DOI), a highly potent agonist of the serotonin 5-HT_{2A} and 5-HT_{2C} receptors with about 10-20 fold lower affinity to the 5-HT_{2B} receptor (reviewed by (Canal & Morgan, 2012)). When DOI is systematically administered to mice, it increases the frequency of typical stereotypical behaviours including: head twitch response (HTR) (Canal, Booth, & Morgan, 2013; Dursun & Handley, 1996; Rojas-Corrales, Gibert-Rahola, & Mico, 2007), grooming behaviour (Tikhonova, Kulikov, & Kulikov, 2011) and ear scratch response (ESR) (Darmani, 2001), resembling motor tics in the form of sudden jerky movements of the head and neck, repetitive behaviours, and possibly scratch responses to premonitory urges, respectively (Klimkeit, Rinehart, May, & Bradshaw, 2017; Nespoli, Rizzo, Boeckers, Hengerer, & Ludolph, 2016). Grooming behaviour may also represent compulsive behaviour which is associated with obsessive-compulsive disorder (OCD) (Canal & Morgan, 2012; Taylor, Rajbhandari, Berridge, & Aldridge, 2010). In rodent, systematic administration of DOI is assumed to immediately affect serotonergic pathways in the central nervous system (CNS) (Hawkins et al., 2002) but not to control motor activity through neuromuscular junctions in the periphery, unlike in some invertebrates (reviewed by (Bacqué-Cazenave et al., 2020; Wu & Cooper, 2012)). Therefore, integrating pharmacological and neurological views, we may assume that HTR results from descending signalling from the serotonergic pathways to muscles that control neck and head movements. As these muscles are directly innervated by the CNS, we may refer HTR as a ‘central motor tic’. While grooming and ESR are repetitive behaviours

that requires final responses of the peripheral muscles, suggesting that descending signalling from serotonergic CNS pathways activates the peripheral nervous system (PNS), therefore we may refer to grooming behaviour and ESR as ‘peripheral motor tics’, i.e. caudally located motor tics that are distributed below the level of neck/head.

Clinical evidence for the involvement of the serotonergic system in Tourette syndrome has been recently reviewed (Augustine & Singer, 2018). Selective serotonin receptor inhibitors that increase serotonin concentration in the synapse can induce/exacerbate tics in some patients (Paramlall & Tyagi, 2020; Rua & Damásio, 2014). In contrast, the antipsychotic drug pimozide (Orap®), a potent D₂ receptor antagonist and a 5-HT_{2A} receptor antagonist, which is used as a second line of treatment for moderate-severe motor tics in patients with Tourette and reduces 70-80% of the tics in patients (Greydanus & Tullio, 2020; McNaught & Mink, 2011), fully reverses the DOI-induced HTR (Dursun & Handley, 1996). Therefore, the model of DOI-induced stereotypical behaviours in rodents might be useful to screen the pharmacological effects of drugs on motor tics, as well as their side effects (Canal & Morgan, 2012; Dursun & Handley, 1996; Rojas-Corrales et al., 2007).

Limited evidence suggests the involvement of the cannabinoid system in Tourette syndrome (Abi-Jaoude, Chen, Cheung, Bhikram, & Sandor, 2017; Artukoglu & Bloch, 2019; Milosev, Psathakis, Szejko, Jakubovski, & Muller-Vahl, 2019). Delta-9-tetrahydrocannabinol (Δ^9 -THC), the major psychoactive cannabinoid of *Cannabis sativa* (Pertwee et al., 2010), reduces motor tics in a small number of adult patients (Muller-Vahl et al., 2003) and in two case studies of adolescent patients (Hasan et al., 2010; Jakubovski & Muller-Vahl, 2017) with Tourette syndrome. Additional case studies have reported the therapeutic effects of Δ^9 -THC plus cannabidiol (CBD) extracts (Sativex® or other sources) in reducing the frequency of motor/vocal tics and premonitory urges (Jakubovski & Muller-Vahl, 2017; Kanaan, Jakubovski, & Muller-Vahl, 2017; Pichler, Kawohl, Seifritz, & Roser, 2019; Trainor, Evans, & Bird, 2016). In line with this clinical evidence, Δ^9 -THC significantly reduces the frequency of DOI-induced HTR and ESR in mice (Darmani, 2001).

However, the effects of CBD, a non-psychoactive cannabinoid of the plant *Cannabis sativa*, has not been studied in models of Tourette syndrome. Although the actions of Δ^9 -THC and CBD appear to be safe when applied to the adult, there is little known about their action when applied to children, the time when Tourette syndrome first appears. A systemic drug screening, to determine the possibility of adverse effects and to compare drug efficacy between juvenile and adults rodents has not been pre-clinically studied before administering medical

cannabis/cannabinoids to patients (pure Δ^9 -THC, pure CBD, high- Δ^9 -THC or high-CBD extracts). Therefore, it is important to investigate in the same mouse the pharmacological activities of a drug on DOI-induced repetitive behaviours (HTR, grooming, ESR) which appear simultaneously. A systemic screening enables a potency test of a selected drug to inhibit different repetitive behaviours. In the same study, screening drugs in the absence of DOI can predict the potential of an adverse effects of induction of repetitive behaviours. Difference in pharmacological effects and drug potency on repetitive behaviours in the presence and absence of DOI may reflect the 'therapeutic window' of the drugs in patients.

Materials Methods

Animals

C57BL/6J mice (OlaHsd sub-strain) purchased from Envigo, Israel. The experiments were performed in 3-week-old (unweaned, juvenile) and 6-week-old (pubertal, young adult) male mice. The number of mice in each group is described in figure legends. Data analyses of body weights are provided in supplementary material. The mice were housed 6-8/cage in a temperature-controlled room (22-24°C) on a 12h light-dark cycle. Water and food were available ad libitum. All experiments were approved by the Institutional Animal Use and Care Committees of Ariel University and Tel-Aviv University.

Mice were transferred in unequal numbers. All mice were included in the experiments unless their development was atypical (e.g. eyes did not open, a problem with the tail or ears, wounds etc.). Housing was designed such that for each set of experiment (dose range) mice were housed in the same cage. Matching cage environment reduced variation, enabling to reduce the number of animals. This housing design habituated the mice to the same environment, for at least 10 days before the experiment day, reducing the variability. Body weights were taken before drug injection on the experimental day (Figures S1 and S2).

Drugs

(R)(-)-DOI hydrochloride (CAS 82864-02-6), DMSO and Kolliphor[®] EL were from Sigma-Aldrich (Rehovot, Israel). CBD (99%) was from AMRI (USA), Δ^9 -THC (98%) was kindly provided by Prof Mechoulam (The Hebrew University, Israel). DOI (1 mg/kg) was dissolved in saline. The dose of DOI was selected after preliminary dose-response experiments in our laboratory in juvenile and young adult mice (0.5, 1, 2.5, 5 mg/kg; n=3 for each dose, data not shown). Our results have confirmed that DOI (1 mg/kg) produces sub-maximal, or maximal and significant effects, in juvenile and young adult C57BL/6J mice, similar to previous reports (Canal & Morgan, 2012). Δ^9 -THC (0.2, 1, 5 mg/kg) and CBD (1, 5, 10 mg/kg) were dissolved in vehicle made of 0.6:1:1.84 DMSO: Kolliphor[®] EL:saline. The drugs were freshly prepared, aliquoted and stored at -20°C for up to 3 months. Each aliquot was discarded after one use. Drugs were injected intraperitoneally (i.p.). All injections were performed in a volume of 10 μ l/g.

Measurement of head twitch response (HTR), ear scratch response (ESR) and grooming behaviour

Mice, 4-8 in home cage, were habituated to the experimental environment for 60 min. In each experiment, the mice were injected in random order. Each set of experiments, consisting of appropriate controls with varying concentrations of a particular cannabinoid, with or without DOI (as described in each figure legend), was performed in a single day. The experimental person was semi-blind to the study: (1) Each mouse was randomly injected (i.p.) with the tested drug or with vehicle 60 min before the exposure to DOI or saline and tail marked. The drug pre-treatment time was decided according to previous studies (Egashira et al., 2007; Navarro et al., 1993); (2) Doses were randomised in each set of experiments (according to figure legends); (3) Mice were tested in a random order, which reflects a random order of tested doses; (4) After injecting all the mice in the injection room and leaving them for 60 min in their cage, the person tested the mice according to the order as marked on their tails in the experimental room.

A second injection (i.p.) of DOI (1 mg/kg) or saline was administered and the mouse was immediately placed in the middle of a clear glass cage 30 X 40 X 30 cm. Five minutes after placing the mouse into the cage, the number of HTR, ESR and grooming behaviours were counted for 15 minutes in three minutes intervals, in the same mouse. HTR was counted every time the mouse had a head twitch, as described previously (Darmani, 2001). Shakes and other voluntary head movements were not counted. ESR was counted each time the mouse scratched itself with its hind limbs, similar to methods previously described (Darmani, 2001) with a modification in the counting method as explained below. Self-grooming was counted each time the mouse groomed any body part with its forelimbs, hind limbs or licked and cleaned the tail and nails. A counting method was applied in which a new ESR or grooming action was added to the total counts only if the mouse moved with all four paws since the previous action.

Model limitations: (1) DOI is systematically administrated, affecting different brain regions not necessarily causing tics (Canal & Morgan, 2012; Egashira et al., 2012; Hawkins et al., 2002); (2) Drugs are tested in a pre-treatment regime (Canal & Morgan, 2012; Canal et al., 2010; Darmani, 2001). Cannabinoids are much less potent and require at least 30-60 min in the body to produce effects, while DOI is a very potent drug producing its effects within 5 min. However, according to the 'serotonin theory of tic causation', high serotonin levels dysregulate dopamine release, causing a hyper-responsive spike-dependent dopamine-release, leading to tics (Wong et al., 2008). According to this view, DOI may mimic a burst of high serotonin-like level leading to hyper-responsive, spike-dependent, dopamine-release. It is possible that pretreated-drugs may occupy their respective receptors and their signalling prevents the effects of downstream descending serotonin and dopamine signalling (illustrated by (Augustine &

Singer, 2018)). This may explain why the results of pre-treatment in the DOI model with pimozide (Dursun & Handley, 1996) and Δ^9 -THC (Darmani, 2001) have been translated to the clinic, i.e. after appearance of symptoms, but provide successful therapeutic treatments only for some patients with Tourette syndrome (McNaught & Mink, 2011; Muller-Vahl et al., 2003). Moreover, in the cerebrospinal fluid, elevated endocannabinoid levels, of anandamide and 2-arachidonoylglycerol, were found in 20 adult patients with Tourette syndrome (Muller-Vahl et al., 2020). This justifies the future investigation of the possible mechanisms involved in the behaviour associated with DOI treatment and to develop cannabinoid-based therapies; (3) There are species differences, specifically that systemic administration of DOI to humans does not induce motor tics but causes hallucinations (Canal & Morgan, 2012) and (4) although Tourette syndrome consists of at least one vocal tic (McNaught & Mink, 2011), mice mainly emit ultrasonic vocals (USVs) that disappear around postnatal day 17 (subject to genetic background), moreover, systemic administration of DOI further reduces USVs in rodents (Winslow & Insel, 1991), therefore, vocal-like tics cannot be detected in this model system.

Statistical analysis

All data are expressed as a mean $\pm$ SEM. $P < 0.05$ considered statistically significant. Behavioural data were analysed with GraphPad Prism version 7 and 8 (GraphPad, San Diego, CA). Line curves of HTR, ESR and grooming behaviours were analysed by two-way analyses of variance (ANOVA) followed by Bonferroni *post hoc* test. Bar graphs of body weight were analysed with one-way ANOVA. *Post hoc* tests were run only if F achieved was significant as indicated below ($P < 0.05$, in line with current BJP guidelines).

In the presence of DOI, the % Frequency of HTR, ESR and grooming behaviour was calculated from average values after 15 min, relative to basal behaviour (vehicle + saline group), as follows: $((\text{DOI-basal}) - (\text{drug-basal})) / (\text{DOI-basal}) * 100$ and, in the absence of DOI as follows: $(\text{Drug-basal}) / (\text{basal}) * 100$.

Similarly, ID_{50} (mg/kg) and 95% confidence interval (CI; asymmetrical CI more accurate) of drug inhibition of DOI-induced grooming and ESR after 15 min was obtained by calculating % Frequency at each dose, for each mouse, relative to the average of basal frequency (vehicle + saline group). Gaussian distribution of the data was tested with the D'Agostino-Pearson omnibus (K2) test (GraphPad Prism version 8), $P > 0.05$ was considered the threshold to pass the normality test. Data that did not pass the normality test were tested with a Kruskal-Wallis test ($P < 0.05$ considered statistically significant). These data were analysed by a dose vs. response curve analysis before and after setting the bottom of the curve

to zero. Constraint reflects the subtraction of basal frequency recommended by GraphPad Prism. ID₅₀ were considered statistically different only if the 95% CI did not overlap (GraphPad Prism version 8).

The manuscript complies with BJP's recommendations and requirements on experimental design and analysis using animals (McGrath JC & Lilley E. Implementing guidelines on reporting research using animals (ARRIVE etc.): new requirements for publication in BJP. *Br J Pharmacology* 172 3189–3193, 2015; Curtis MJ, Alexander S, Cirino G, Docherty JR, George CH, Giembycz MA, Hoyer D, Insel PA, Izzo AA, Ji Y, MacEwan DA, Sobey CG, Stanford SC, Teixeira MM, Wonnacott S, Ahluwalia A. Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. *Br J Pharmacology* 175 987–993, 2018), apart from one exploratory experiment (n=4) which complies because it is called an exploratory experiment and the results are not described as 'significant'.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

Results

Effects of Δ^9 -THC and CBD on DOI-induced HTR in male mice

In juvenile male mice, Δ^9 -THC produced a significant decrease ($P < 0.05$) of DOI-induced HTR (Figure 1A; Dose: $F_{2,1,40}=47.9$, $P < 0.05$, 0.2 mg/kg; $F_{2,1,40}=14.8$, $P < 0.05$, 1 mg/kg; $F_{2,1,40}=69.8$, $P < 0.05$, 5 mg/kg). Compared with the effect of DOI after 15 min (36.2 ± 2.4), the HTR in the presence of Δ^9 -THC (5 mg/kg) was 24.0 ± 2.4 , resulting in 34% inhibition of DOI-induced HTR in juvenile male mice. CBD produced a decrease of HTR (Figure 1B, these results should be referred as an exploratory study ($n=4$)). Compared with the effect of DOI after 15 min (47.0 ± 11.4), the HTR in the presence of CBD (5, 10 mg/kg) was 37.2 ± 10.4 and 42.0 ± 11.3 , resulting in 21% and 11% inhibition of DOI-induced HTR in juvenile male mice, respectively.

In young adult male mice, Δ^9 -THC (0.2, 1, 5 mg/kg) produced a significant decrease ($P < 0.05$) of DOI-induced HTR (Figure 1C; Dose: $F_{2,1,45}=7.4$, $P < 0.05$, 0.2 mg/kg; $F_{2,1,50}=10.6$, $P < 0.05$, 1 mg/kg; $F_{2,1,45}=318.2$, $P < 0.05$, 5 mg/kg). Compared with the effect of DOI (15 min) (45.7 ± 2.0), the HTR in the presence of Δ^9 -THC (5 mg/kg) was 15.4 ± 2.2 , resulting in 68% inhibition of DOI-induced HTR. CBD produced a significant decrease ($P < 0.05$) of HTR (Figure 1D; Dose: $F_{2,1,70}=55.8$, $P < 0.05$, 5 mg/kg; $F_{2,1,65}=14.1$, $P < 0.05$, 10 mg/kg). Compared with the effect of DOI (15 min) (38.8 ± 1.6), the HTR in the presence of CBD (5, 10 mg/kg) was 29.0 ± 1.9 and 31 ± 3.3 , resulting in inhibition of 26% and 20% of DOI-induced HTR in young adult male mice, respectively. Increasing the dose of CBD to 10 mg/kg did not result in additional inhibition of HTR either in juvenile or young adult male mice.

Effects of Δ^9 -THC and CBD on basal HTR in male mice

The effects of Δ^9 -THC and CBD were tested on basal HTR, i.e. in the absence of DOI. Δ^9 -THC (5 mg/kg) induced cataleptic behaviour (immobility) in juvenile and young adult male mice, which started about 30 min after injection (i.p.) and continued for about 1-2 hours. Cataleptic behaviour was evident when mice were tested, but was not observed after injection of DOI (described above). No cataleptic behaviour nor effect on basal HTR were observed with Δ^9 -THC (0.2, 1 mg/kg) in juveniles and young adults.

Compared with the control group, Δ^9 -THC (5 mg/kg) produced a significant decrease ($P < 0.05$) of basal HTR in healthy juvenile (Figure 2A, Dose: $F_{2,1,40}=7.6$, $P < 0.05$) and young adult male mice (Figure 2C, $F_{2,1,55}=20.8$, $P < 0.05$). In juveniles, compared with the basal HTR

of the control group (0.8 ± 0.5), Δ^9 -THC (5 mg/kg) reduced HTR frequency to 0.4 ± 0.2 , resulting in 50% inhibition of basal HTR (1.0 ± 0.4). Similar results were found in young adult male mice with basal HTR of 1.0 ± 0.4 , and in the presence of Δ^9 -THC (5 mg/kg) the HTR frequency was 0.2 ± 0.2 , resulting in 83% inhibition of basal HTR of healthy young adult mice.

Surprisingly, CBD alone significantly increased the frequency of HTR. Compared with the control group, CBD (1, 5 mg/kg) significantly increased ($P < 0.05$) HTR in healthy juvenile male mice (Figure 2B; Dose: $F_{2,1,50}=5.0$, $P < 0.05$, 1 mg/kg; $F_{2,1,45}=16.5$, $P < 0.05$, 5 mg/kg). Compared with basal HTR of the control group (0.2 ± 0.2), CBD (1, 5 mg/kg) significantly increased HTR to 0.8 ± 0.5 and 1.4 ± 0.6 , resulting in an increase of 390% and 724% of basal HTR in juvenile male mice, respectively (Figure 2B). Similar results were found in healthy young adult male mice. CBD (1, 5, 10 mg/kg) significantly increased ($P < 0.05$) basal HTR (Figure 2D; Dose: $F_{2,1,50}=12.6$, $P < 0.05$, 1 mg/kg; $F_{2,1,50}=20.0$, $P < 0.05$, 5 mg/kg; $F_{2,1,50}=8.0$, $P < 0.05$, 10 mg/kg). Compared with the basal HTR of the control group (0.7 ± 0.2), CBD (1, 5, 10 mg/kg) significantly increased HTR to 1.5 ± 0.4 , 1.7 ± 0.3 and 1.3 ± 0.3 , resulting in an increase of 124%, 149% and 99% of basal HTR in young adult mice, respectively.

Effects of Δ^9 -THC on DOI-induced grooming behaviour

In juvenile male mice, Δ^9 -THC (0.2, 1, 5 mg/kg) dose-dependently reduced DOI-induced self-grooming behaviour (Figure 3A; Dose: $F_{2,1,40}=10.7$, $P < 0.05$, 0.2 mg/kg; $F_{2,1,40}=74.7$, $P < 0.05$, 1 mg/kg; $F_{2,1,40}=277.1$, $P < 0.05$, 5 mg/kg). Compared with the effect of DOI (15 min) (31.6 ± 2.7), the effect in the presence of Δ^9 -THC (5 mg/kg) was 5.0 ± 0.9 , resulting in 99% inhibition of DOI-induced grooming behaviour in juvenile male mice (basal grooming behaviour was 4.6 ± 0.8). The ID_{50} and 95% confidence interval (CI) of Δ^9 -THC inhibition of DOI-induced grooming in juvenile mice was 0.6 (0.2 - 2.4) mg/kg (Figure 3C, passed D'Agostino-Pearson omnibus (K2) test).

In young adult male mice, Δ^9 -THC (0.2, 1, 5 mg/kg) dose-dependently reduced DOI-induced grooming behaviour (Figure 3D; Dose: $F_{2,1,45}=40.3$, $P < 0.05$, 0.2 mg/kg; $F_{2,1,50}=42.6$, $P < 0.05$, 1 mg/kg; $F_{2,1,45}=342.3$, $P < 0.05$, 5 mg/kg). Compared with the effect of DOI (15 min) (37.8 ± 3.1), the effect in the presence of Δ^9 -THC (5 mg/kg) was 3.4 ± 1.8 , resulting in 113% inhibition of DOI-induced grooming. Frequency of basal grooming in young adult mice was 7.3 ± 2.5 . This means that Δ^9 -THC (5 mg/kg) inhibited DOI-induced grooming behaviour by 13% below the basal grooming behaviour of young adult male mice. This effect is likely to be attributed to the observed cataleptic behaviour at this dose. The Δ^9 -THC inhibition of DOI-

induced grooming in young adult mice was with an ID₅₀ (95% CI) of 1.2 (0.3 - 7.6) before constraining the bottom of the curve to zero (Figure 3F, passed D'Agostino-Pearson omnibus (K2) test) and with an ID₅₀ of 0.5 (0.2 - 1.1) mg/kg after constraining (Table 1). These ID₅₀ values were not significantly different from the ID₅₀ of Δ^9 -THC inhibition of DOI-induced grooming in juvenile mice before (as above) or after constraining with an ID₅₀ of 0.4 (0.2 - 1.0) mg/kg (Table 1).

Effect of Δ^9 -THC on basal grooming behaviour in male mice

Compared with the control group (i.e. in the absence of DOI), Δ^9 -THC (0.2, 1, 5 mg/kg) did not produce a significant change in basal, spontaneous, self-grooming behaviour in juvenile male mice (Figure 4A). Interestingly, though Δ^9 -THC (5 mg/kg) induced cataleptic behaviour, as discussed earlier, there was no effect on basal grooming behaviour in juvenile mice. While in young adult male mice, Δ^9 -THC (5 mg/kg) produced a significant decrease ($P < 0.05$) of grooming behaviour (Figure 4C; Dose: $F_{2, 1,55}=27.3$, $P < 0.05$) which might be attributed to the cataleptic behaviour observed at this dose.

Effect of CBD on basal and DOI-induced self-grooming behaviour

In juvenile male mice, CBD (1, 5, 10 mg/kg) had no effect on grooming behaviour in the presence (Figure 3B, referred as an exploratory study) or absence (Figure 4B) of DOI. However, in young adult male mice, CBD (1, 5 and 10 mg/kg) produced a significant decrease ($P < 0.05$) of DOI-induced grooming behaviour which was to a similar extent between the doses (Figure 3E; Dose: $F_{2, 1,65}=4.6$, $P < 0.05$, 1 mg/kg; $F_{2, 1,70}=4.8$, $P < 0.05$, 5 mg/kg; $F_{2, 1,65}=4.2$, $P < 0.05$, 10 mg/kg), resulting in a 26-27% inhibition of DOI-induced grooming behaviour.

In the absence of DOI, CBD (1 and 5 mg/kg) had no significant effect on basal grooming behaviour. However, CBD (10 mg/kg) significantly decreased ($P < 0.05$) grooming behaviour, resulting in 20% inhibition of basal grooming behaviour in healthy young adult male mice (Figure 4D; Dose: $F_{2, 1,50}=7.3$, $P < 0.05$).

Effects of Δ^9 -THC and CBD on DOI-induced ESR

In juvenile male mice in the presence of DOI, Δ^9 -THC (0.2, 1, 5 mg/kg) dose-dependently decreased ESR (Figure 5A; Dose: $F_{2, 1,40}=16.2$, $P < 0.05$, 1 mg/kg; $F_{2, 1,40}=27.1$, $P < 0.05$, 5 mg/kg). Compared with the effect of DOI (15 min) (4.0 ± 1.1), the ESR of DOI in the presence of Δ^9 -THC (0.2, 1, 5 mg/kg) was 2.8 ± 1.1 , 1.0 ± 0.3 and 0.0 ± 0.0 , resulting in 32%, 79% and

105% inhibition of DOI-induced ESR, respectively (Figure 5A). This means that Δ^9 -THC at a dose of 5 mg/kg inhibited DOI-induced ESR by 5% below the basal ESR of juvenile male mice. The ID_{50} of Δ^9 -THC inhibition of DOI-induced grooming in juvenile male mice was 0.5 (0.04 - 19.9) mg/kg (Figure 5C, passed D'Agostino-Pearson omnibus (K2) test) and after constraint the ID_{50} was 0.3 (0.1 - 1.7) mg/kg (Table 1). These ID_{50} values were not significantly different.

In juvenile male mice, CBD (1, 5, 10 mg/kg), in the presence of DOI, had no effect on DOI-induced ESR behaviour (Figure 5B, referred as an exploratory study). While in young adult male mice, CBD produced a significant decrease ($P < 0.05$) of DOI-induced ESR (Figure 5E; Dose: $F_{2, 65}=10.5$, $P < 0.05$, 1 mg/kg; $F_{2, 70}=12.4$, $P < 0.05$, 5 mg/kg; $F_{2, 65}=6.2$, $P < 0.05$, 10 mg/kg). Compared with the effect of DOI (15 min) (13.0 ± 2.4), the ESR in the presence of CBD (1, 5, 10 mg/kg) was 6.2 ± 2.7 , 6.1 ± 2.0 and 7.7 ± 2.8 , resulting in 54%, 54% and 42% inhibition of DOI-induced ESR, respectively (Figure 5E).

In young adult male mice, Δ^9 -THC (0.2, 1, 5 mg/kg), in the presence of DOI, dose-dependently decreased ESR (Figure 5D; Dose: $F_{2, 45}=15.9$, $P < 0.05$, 0.2 mg/kg; $F_{2, 50}=55.7$, $P < 0.05$, 1 mg/kg; $F_{2, 45}=42.8$, $P < 0.05$, 5 mg/kg). Compared with the effect of DOI (15 min) (11.8 ± 2.6), the effect in the presence of Δ^9 -THC (0.2, 1, 5 mg/kg) was 2.8 ± 2.8 , 0.0 ± 0.0 and 0.2 ± 0.2 , resulting in 79%, 103% and 101% inhibition of DOI-induced ESR, respectively (Figure 5D). The putative ID_{50} of Δ^9 -THC inhibition of DOI-induced grooming behaviour in juvenile mice was 0.06 (0.0 - 0.4) mg/kg (Figure 5F, the results did not pass the D'Agostino-Pearson omnibus (K2) test due to an outlier. The data set was further analysed with the Kruskal-Wallis test, $P < 0.05$) and after constraint the putative ID_{50} was 0.05 (0.0 - 0.2) mg/kg (Table 1), these ID_{50} values were not significantly different.

Effect of Δ^9 -THC and CBD on basal ESR in male mice

In juvenile male mice, compared with the control group, Δ^9 -THC (5 mg/kg) significantly increased ($P < 0.05$) the frequency of basal ESR (Figure 6A; Dose: $F_{2, 40}=9.7$, $P < 0.05$), to 1.0 ± 0.4 , compared with the control group (0.2 ± 0.2), indicating a 400% increase of ESR.

In young adult male mice, compared with the control group, Δ^9 -THC (0.2 mg/kg) resulted in a significant decrease ($P < 0.05$) of basal ESR (Figure 6C; Dose: $F_{2, 55}=13.3$, $P < 0.05$), indicating a 100% decrease of basal ESR (Figure 6C).

CBD (1, 5, 10 mg/kg) had no significant effect on basal ESR frequency in juvenile (Figure 6B) or young adult (Figure 6D) male mice. The increase (100%) of basal ESR

frequency induced by CBD at a dose of 5 mg/kg was not significantly different from that of the control group.

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Discussion

Study of cannabinoids in juvenile and young adult model systems is essential because Δ^9 -THC affects brain development, can induce psychosis (reviewed by (Anavi-Goffer & Mulder, 2009; Pertwee et al., 2010)) and because the FDA-approval of CBD (Epidiolex[®], 98% pure) for the treatment of children with certain forms of epilepsy, a neurological disease that can cause uncontrollable jerking and shaking movements, has led to the question whether children or adults with Tourette syndrome can benefit from treatment with essentially pure CBD.

Our study shows that CBD significantly reduced DOI-induced repetitive behaviours. In young adult mice, CBD was more potent than in juveniles in the inhibition of DOI-induced HTR (20-26% in young adult vs. 11-21% in juvenile mice) but, its ability to increase basal HTR frequency in young adult was lower compared to its effect in juvenile mice (99-149% in young adult vs. 390-724% in juvenile mice). In addition, CBD at a dose of 10 mg/kg significantly reduced basal grooming behaviour, suggesting CBD may reduce spontaneous peripheral repetitive behaviour in humans.

CBD at selected doses significantly increases the basal frequency of HTR, both in juvenile and young adult mice, by about 100% to about 700%, suggesting that CBD may evoke spontaneous sudden jerky movements of the head and neck in healthy children and young adults. In juvenile mice, CBD had no effect on ESR and grooming behaviour.

This study is relevant to clinical use of pure CBD, and possibly high-CBD extracts, and imply that CBD may: (1) Increase the risk to develop Tourette-like symptoms; (2) Enhance frequency in paediatric patients with existing motor tics; (3) Not effectively inhibit peripheral motor tics and premonitory urges in children with Tourette syndrome; (4) Have a smaller 'therapeutic window' in paediatric patients vs. adult patients with Tourette syndrome.

The pharmacological target that mainly contributes to CBD-induced HTR is still to be investigated. CBD is a pleotropic ligand, acting at multi-pharmacological sites (Fernandez-Ruiz et al., 2013). CBD inhibits 5-HT_{3A} (Yang et al., 2010) and indirectly enhances 5-HT_{1A} activity (because CBD does not activate 5-HT_{1A} receptors in the brainstem) (Rock et al., 2012; Russo, Burnett, Hall, & Parker, 2005). However, 8-OH-DPAT, a 5-HT_{1A} receptor agonist, inhibits DOI-induced HTR (reviewed by (Canal & Morgan, 2012)), while antagonism of 5-HT₃ receptor does not reverse DOI-induced HTR (Wettstein, Host, & Hitchcock, 1999). In addition, similar to SR141716A, a selective CB₁ receptor antagonist/inverse agonist, CBD is a high potency antagonist of CB₁ receptor agonists, and shows inverse agonist efficacy in the mouse brain (Thomas et al., 2007). While CB₁ receptor agonists inhibit DOI-induced HTR and

ESR frequencies (Darmani, 2001), SR141716A increases HTR and ESR frequencies (Darmani, Janoyan, Kumar, & Crim, 2003). But in contrast to SR141716A, CBD did not potently increase ESR (this study). In cells, CBD (below 1 μ M) acts as a negative allosteric modulator of the CB₁ receptor (Laprairie, Bagher, Kelly, & Denovan-Wright, 2015) and at a concentration > 10 μ M CBD inhibits fatty acid amide hydrolase (FAAH) (De Petrocellis et al., 2011). Thus, it is possible that CBD inhibits DOI via enhancement of 5-HT_{1A} receptor activity and via inhibition of FAAH activity, but CBD enhances DOI actions via antagonism/negative allosterism of the CB₁ receptor, thus the ratio between receptors and enzymes that respond to CBD in neurones that control HTR and ESR will determine the final effect of CBD on repetitive behaviours.

A previous study shows that Δ^9 -THC inhibits DOI-induced HTR and ESR in ICR mice which were pre-treated for 20 min before administrating (R)($\pm$)-DOI (Darmani, 2001), but this study did not investigate the effects of Δ^9 -THC on basal repetitive behaviours. In our study, Δ^9 -THC at a dose of 5 mg/kg induced cataleptic behaviour, a 'decreased motivation towards movement' also induced by cannabis (Egashira et al., 2007; Sano et al., 2008; Varvel et al., 2005), both in juvenile and young adult mice. Δ^9 -THC-induced catalepsy is mediated by decreased serotonin release in the sub-region of the ventral striatum, the nucleus accumbens, and enhanced by anti-glutamatergic drugs such as MK-801 (Sano et al., 2008). Therefore, it is possible that the inhibitory effect of Δ^9 -THC on DOI-induced repetitive behaviours at a dose lower than 5 mg/kg requires different mechanisms than these causing Δ^9 -THC at a dose of 5 mg/kg to induce catalepsy. The specific mechanisms are yet to be investigated.

Cataleptic behaviour was not observed after injection of DOI (1 mg/kg). These results replicate a previous report documenting that Δ^9 -THC at a dose of 6 mg/kg induces cataleptic behaviour in 6-week-old male ddY mice (Egashira et al., 2007). The same study reported that the Δ^9 -THC-induced catalepsy is reversed by DOI (0.3, 1 mg/kg), suggesting that cataleptic behaviour is mainly controlled by 5-HT_{2A} receptor (Egashira et al., 2007). Importantly, this points to the hypothesis that repetitive behaviours that are controlled by 5-HT_{2A} receptor can be affected by cataleptic behaviour which reduces general motor activity, i.e. nonambulatory motor function (catalepsy) will block all muscle activity including that required for DOI (1 mg/kg)-induced repetitive behaviours. This is important because Δ^9 -THC-induced catalepsy is reversed by SR141716A, a selective CB₁ receptor antagonist/inverse agonist, suggesting that the CB₁ receptor mediates this effect of Δ^9 -THC (Sano et al., 2008). However, Δ^9 -THC is a non-selective partial agonist of the CB₁/CB₂ receptors (Pertwee et al., 2010). The contribution

of each cannabinoid receptor to the inhibition by Δ^9 -THC of DOI-induced repetitive behaviours is still to be studied.

In juvenile ICR mice, the inhibitory effect of Δ^9 -THC on DOI-induced HTR has been reported with an ID_{50} of 4.7 (1.53 – 14.2) mg/kg (Darmani, 2001). In this study a dose of 5 mg/kg resulted in 34% inhibition of DOI-induced HTR, though a longer pre-treatment time was given (Table 1). The inhibitory effect of Δ^9 -THC on DOI-induced ESR appears to be with a similar ID_{50} values between studies (Table 1), suggesting that strain differences may affect HTR but not ESR. Noteworthy, although juvenile 3-week-old ICR mice (18-24 g) (Darmani, 2001) have a similar weight to young adult 6-week-old C57BL/6J mice (this study), the inhibitory effect of Δ^9 -THC on DOI-induced ESR in young adult C57BL/6J was about 5 times more potent with an ID_{50} of 0.05 (0.0 - 0.2) mg/kg compared with juvenile ICR mice with an ID_{50} of 0.25 (0.22 - 0.3) mg/kg (Darmani, 2001), and about 7 times more potent compared with juvenile C57BL/6J mice with an ID_{50} of 0.3 (0.1 - 1.7) mg/kg. These differences in the potency of Δ^9 -THC between juvenile and young adult mice may reflect the developmental changes in the expression of cannabinoid receptors in brain areas that control ESR (Anavi-Goffer & Mulder, 2009). In line with this, Δ^9 -THC only partly reverses the SR141716A-induced ESR in adolescent ICR mice (Janoyan, Crim, & Darmani, 2002).

Grooming behaviour was not tested in the same mice by Darmani (Darmani, 2001), but Δ^9 -THC (oral, 5 mg/kg) inhibits spontaneous grooming in male rats (Navarro et al., 1993). In this study, the inhibitory effect of Δ^9 -THC on DOI-induced grooming behaviour of young adult mice was with an ID_{50} of 0.5 (0.2 - 1.1) mg/kg. This suggests a 10 fold lower potency than this required to inhibit DOI-induced ESR, in the same mice, with an ID_{50} of 0.05 (0.0 - 0.2) mg/kg.

Implications of the results

Increased doses of Δ^9 -THC may inhibit motor tics because it causes a loss of voluntary motion which results in a fixed posture (results of this study, (Sano et al., 2008)). Comparing the potency of Δ^9 -THC to inhibit repetitive behaviours that are evoked by stimulation of 5-HT_{2A} receptor, the rank order of potency is ESR > grooming > HTR in young adult male mice, while in juvenile mice the rank order of potency is ESR = grooming > HTR, suggesting that ESR is pharmacologically regulated during development, affecting cannabinoid potency. A similar conclusion can be obtained by comparing the efficacy of Δ^9 -THC (5 mg/kg) to inhibit repetitive behaviours e.g. ESR (100 %) = grooming (100 %) > HTR (34 %) in juvenile mice. The results possibly imply that Δ^9 -THC may have a lower effect on reducing the frequency of motor tics in children with Tourette syndrome and predict that Δ^9 -THC may evoke premonitory urges in

some paediatric patients. Adult patients bothered with urges more than the tics themselves, possibly, may benefit from treatment with a relatively low dose of Δ^9 -THC (0.2-0.4 mg vs. 10 mg per day reported for adult patients (Muller-Vahl et al., 2003)), reducing side effects. The results imply that Δ^9 -THC should not be considered as a first line treatment to alleviate tics in patients with head/neck tics. In sub-populations of adult patients, up to 50% reduction of head/neck tic frequency may be predicted with clinical doses of Δ^9 -THC up to 0.25 mg/kg/day. A daily dose of Δ^9 -THC exceeding 0.4 mg/kg/day to further alleviate head/neck tics may result in adverse-effects including increased anxiety (Hines et al., 2020).

CBD increases the frequency of spontaneous HTR in mice, suggesting that pure CBD may evoke central motor tics in healthy children, and paediatric patients with Tourette syndrome may even experience an increase in the frequency of sudden head shaking. Similarly, adult patients with central motor tics may not benefit of treatment with pure CBD. However, adult patients who mainly have peripheral repetitive behaviours may benefit from treatment with pure CBD. Further studies in animal models for Tourette syndrome will provide better insights into which sub-populations of patients with Tourette syndrome may benefit from CBD as a standalone drug treatment.

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Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Natural Products Research, Design and Analysis, and Animal Experimentation, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

Authors contribution

SAG and PM were the PIs and co-mentored VG. SAG conceived and designed the research. VG (Ph.D. student) was the main contributor to this research, performed experiments, analysed and graphed data. SAG, VG and PM wrote the manuscript.

Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

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Table 1. Comparison of the effects of Δ^9 -THC on DOI-induced repetitive behaviours

Δ^9 -THC	Juvenile male mice (n=5)	Young adult male mice (n=5-6)
	ID ₅₀ (mg/kg) (95% CI)	ID ₅₀ (mg/kg) (95% CI)
HTR	5 mg/kg produced 34% inhibition	5 mg/kg produced 68% inhibition 1 mg/kg produced 22% inhibition
Grooming	0.4 (0.2 - 1.0)	0.5 (0.2 - 1.1)
ESR	0.3 (0.1 - 1.7)	0.05 (0.0 - 0.2) (putative)
Darmani (2001)		
HTR	4.7 (1.53 – 14.2)	ND
Grooming	ND	ND
ESR	0.25 (0.22 - 0.3)	ND

In this study, cannabinoids were injected 60 min before (R)(-)-DOI into C57BL/6J mice. The ID₅₀ (mg/kg) 95% confidence interval (95% CI) was obtained from frequency values after 15 min, and after subtracting the basal frequency and constraining the bottom of the dose-response curve to zero (GraphPad Prism version 8). The inhibition of DOI-induced HTR by Δ^9 -THC could not be fitted on a dose-response curve. In Darmani (2001), Δ^9 -THC was injected 20 min before (R)(±)-DOI into 3-week-old ICR mice (18-24 g). Interestingly, the weight of these mice corresponds to that of 6-week-old C57BL/6J mice. Basal frequency and self-grooming behaviours were not investigated by Darmani (2001). ND, not determined.

Figure 1

Effect of Δ^9 -THC and CBD on head twitch response (HTR) in juvenile and young adult mice in the presence of DOI. In A and C, the effects of Δ^9 -THC (0.2, 1, 5 mg/kg) in juvenile (A) and young adult (C) mice in the presence of DOI (1 mg/kg). In B and D, the effects of CBD (1, 5, 10 mg/kg) in juvenile (B) and young adult (D) mice in the presence of DOI (1 mg/kg). Data represent mean $\pm$ SEM. *n* represent the number of animals in each group. The experiment was independently repeated a number of times according to the lowest *n* number. Two-way ANOVA analysis of variance followed by Bonferroni's test for multiple comparisons was performed by GraphPad Prism 7 or 8. **P* < 0.05 significantly different as indicated. Asterisks aside the graph are *p* value summary vs. DOI group. Asterisks along the DOI curve are *p* values of multiple comparisons at each dose vs. vehicle + DOI group at the same dose.

Figure 2

Effect of Δ^9 -THC and CBD on head twitch response (HTR) in juvenile and young adult mice in the absence of DOI. In A and C, the effects of Δ^9 -THC (0.2, 1, 5 mg/kg) in juvenile (A) and young adult (C) mice in the presence of saline injection (instead of DOI injection). In both juvenile and young adult mice, in the absence of DOI, Δ^9 -THC at a dose of 5 mg/kg induced a significant cataleptic behaviour (A, C, dark green). In B and D, the effects of CBD (1, 5, 10 mg/kg) in juvenile (B) and young adult (D) mice in the presence of saline injection. Importantly, in the absence of DOI, CBD at doses of 1 and 5 mg/kg significantly increased HTR in both young adult and juvenile mice (C, D) and CBD 10 mg/kg also significantly increased HTR in young adult mice (D). Data represent mean $\pm$ SEM. n represent the number of animals in each group. The experiment was independently repeated a number of times according to the lowest n number. Two-way ANOVA analysis of variance followed by Bonferroni's test for multiple comparisons was performed by GraphPad Prism 7 or 8. * $P < 0.05$ significantly different as indicated. Asterisks aside the graph are p value summary vs. control (vehicle + saline) group. Asterisks along the curve are p values of multiple comparisons at each dose vs. control (vehicle + saline) group at the same dose.

Figure 3

Effect of Δ^9 -THC and CBD on grooming behaviour in juvenile and young adult mice in the presence of DOI. In A and D, the effects of Δ^9 -THC (0.2, 1, 5 mg/kg) in juvenile (A) and young adult (D) mice in the presence of DOI (1 mg/kg). In B and E, the effects of CBD (1, 5, 10 mg/kg) in juvenile (B) and young adult (E) mice in the presence of DOI (1 mg/kg). Data represent mean $\pm$ SEM. *n* represent the number of animals in each group. The experiment was independently repeated a number of times according to the lowest *n* number. Two-way ANOVA analysis of variance followed by Bonferroni's test for multiple comparisons was performed by GraphPad Prism 7 or 8. In C and F, dose vs. response graphs of % Grooming frequency, in the absence of Δ^9 -THC (0 mg/kg, 100%) or presence of Δ^9 -THC (0.2, 1, 5 mg/kg), in juvenile (C) and young adult (F) DOI-induced mice. ID₅₀ (95% confidence interval) (CI) (mg/kg) are presented (no constrain). #*P* < 0.05 significantly different from control (vehicle + saline) group. **P* < 0.05 significantly different as indicated. Asterisks aside the graph are *p* value summary vs. DOI group. Asterisks along the curve are *p* values of multiple comparisons at each dose vs. vehicle + DOI group at the same dose.

Figure 4

Effect of Δ^9 -THC and CBD on grooming behaviour in juvenile and young adult mice in the absence of DOI. In A and C, the effects of Δ^9 -THC (0.2, 1, 5 mg/kg) in juvenile (A) and young adult (C) mice in the presence of saline injection (instead of DOI injection). In young adult mice, in the absence of DOI, Δ^9 -THC at a dose of 5 mg/kg induced a significant cataleptic behaviour, resulting in a significant reduction of grooming behaviour (C, dark green) compare to control mice (vehicle + saline, grey). In B and D, the effects of CBD (1, 5, 10 mg/kg) in juvenile (B) and young adult (D) mice in the presence of saline injection. In young adult mice, in the absence of DOI, CBD at a dose of 10 mg/kg significantly decreased grooming behaviour (D, dark green) compare to control mice (vehicle + saline, grey). Data represent mean $\pm$ SEM. n represent the number of animals in each group. The experiment was independently repeated a number of times according to the lowest n number. Two-way ANOVA analysis of variance followed by Bonferroni's test for multiple comparisons was performed by GraphPad Prism 7 or 8. * $P < 0.05$ significantly different as indicated. Asterisks aside the graph are p value summary vs. control (vehicle + saline) group. Asterisks along the curve are p values of multiple comparisons at each dose vs. control (vehicle + saline) group at the same dose.

Figure 5

Effect of Δ^9 -THC and CBD on ear scratch response (ESR) in juvenile and young adult mice in the presence of DOI. In A and D, the effects of Δ^9 -THC (0.2, 1, 5 mg/kg) in juvenile (A) and young adult (D) mice in the presence of DOI (1 mg/kg). In B and E, the effects of CBD (1, 5, 10 mg/kg) in juvenile (B) and young adult (E) mice in the presence of DOI (1 mg/kg). Data represent mean $\pm$ SEM. *n* represent the number of animals in each group. The experiment was independently repeated a number of times according to the lowest *n* number. Two-way ANOVA analysis of variance followed by Bonferroni's test for multiple comparisons was performed by GraphPad Prism 7 or 8. In C and F, dose vs. response graphs of % ESR frequency, in the absence of Δ^9 -THC (0 mg/kg, 100%) or presence of Δ^9 -THC (0.2, 1, 5 mg/kg), in juvenile (C) and young adult (F) DOI-induced mice. ID₅₀ (95% confidence interval) (CI) (mg/kg) are presented (no constrain). **P* < 0.05 significantly different as indicated. Asterisks aside the graph are *p* value summary vs. DOI group. Asterisks along the curve are *p* values of multiple comparisons at each dose vs. vehicle + DOI group at the same dose.

Figure 6

Effects of Δ^9 -THC and CBD on ear scratch response (ESR) in juvenile and young adult mice in the absence of DOI. In A and C, the effects of Δ^9 -THC (0.2, 1, 5 mg/kg) in juvenile (A) and young adult (C) mice in the presence of saline injection (instead of DOI injection). In both juvenile and young adult mice, in the absence of DOI, Δ^9 -THC at a dose of 5 mg/kg induced a significant cataleptic behaviour (A, C, dark green). In the absence of DOI, Δ^9 -THC at doses of 5 mg/kg significantly increased ESR in juvenile mice (A) while a dose of 0.2 mg/kg significantly decreased ESR in young adult mice (C). In B and D, the effects of CBD (1, 5, 10 mg/kg) in juvenile (B) and young adult (D) mice in the presence of saline injection. CBD had no effect on ESR in healthy young adult mice. Data represent mean $\pm$ SEM. n represent the number of animals in each group. The experiment was independently repeated a number of times according to the lowest n number. Two-way ANOVA analysis of variance followed by Bonferroni's test for multiple comparisons was performed by GraphPad Prism 7 or 8. * $P < 0.05$ significantly different as indicated. Asterisks aside the graph are p value summary vs. control (vehicle + saline) group.