Received: 2 July 2020 | Revised: 20 October 2020 | Accepted: 22 October 2020

DOI: 10.1111/bph.15302

RESEARCH PAPER

Different responses of repetitive behaviours in juvenile and young adult mice to Δ^9-tetrahydrocannabinol and cannabidiol may affect decision making for Tourette syndrome

Victoria Gorberg | Peter McCaffery | Sharon Anavi-Goffer

Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK

Correspondence
Dr Sharon Anavi-Goffer, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, UK.
Email: sharon.anavi-goffer@abdn.ac.uk

Funding information
Tourette Association of America: Research Grant Award to SAG & PM; University of Aberdeen: The Elphinstone Scholarship for Ph. D. students

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: 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 versus ESR = grooming > HTR in young adult versus juvenile mice. Δ^9-THC (5 mg·kg^{-1}) induced severe adverse effects in the form of cataleptic behaviour in control mice and significantly increased ESR in juveniles. The pharmacological effects of CBD have not been studied in models of Tourette syndrome. In juveniles, CBD had no effect on DOI-induced ESR and grooming behaviours. CBD alone induced side effects, significantly increasing the frequency of HTR in juveniles and young adults.

Conclusion 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.

KEYWORDS

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

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

1 | 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 (see 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 (see 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, Black, Hallett, & Voon, 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 for the **5-HT$_{2B}$** receptor (see Canal & Morgan, 2012). When DOI is systemically 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 OCD (Canal & Morgan, 2012; Taylor, Rajbhandari, Berridge, & Aldridge, 2010). In rodents, systemic administration of DOI is assumed to immediately affect 5-HT pathways in the CNS (Hawkins et al., 2002) but not to control motor activity through neuromuscular junctions in the periphery, unlike in some invertebrates (see 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 5-HT 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". As grooming and ESR are repetitive behaviours that require final responses of the peripheral muscles, suggesting that descending signalling from 5-HT pathways in the CNS activates the peripheral nervous system (PNS), we may refer to grooming behaviour and ESR as "peripheral motor tics," that is, caudally located motor tics that are distributed below the level of neck and head.

Clinical evidence for the involvement of the 5-HT system in Tourette syndrome has been recently reviewed (Augustine & Singer, 2019). Selective 5-HT reuptake inhibitors that increase 5-HT concentration in the synapse can induce or exacerbate tics in some patients (Paramlall & Tyagi, 2020; Rua & Damásio, 2014). In contrast, the antipsychotic drug **pimozide** (Orap®), a potent dopamine **D$_2$** 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 syndrome 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, Gibert-Rahola, & Mico, 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). **Δ^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).

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

What is the clinical significance

- Young adult patients affected by urges may benefit from a relatively low dose of Δ^9-THC.
- Young adult patients with motor tics below the neck/head level, may benefit from CBD treatment.

However, the effects of CBD, a non-psychoactive cannabinoid of the plant *C. sativa*, have 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 adult rodents has not been preclinically studied before giving medical cannabis or 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, and 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 effect of induction of repetitive behaviours. Differences 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.

2 | METHODS

2.1 | Animals

All animal care and experimental procedures were approved by the Institutional Animal Use and Care Committees of Ariel University and Tel-Aviv University. Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the British Journal of Pharmacology (Lilley et al., 2020). C57BL/6J mice (OlaHsd substrain) were 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 the supporting information. The mice were housed six to eight per cage in a temperature-controlled room (22–24°C) on a 12-h light–dark cycle. Water and food were available ad libitum.

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, and wounds). Housing was designed such that for each set of experiments (dose range), mice were housed in the same cage. Matching cage environment reduced variation, enabling a reduction in 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 measured before drug injection on the experimental day (Figures S1 and S2).

2.2 | Drug treatments

DOI (1 mg·kg^{-1}) 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, and 5 mg·kg^{-1}; n = 3 for each dose, data not shown). Our results have confirmed that DOI (1 mg·kg^{-1}) produces submaximal, 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, and 5 mg·kg^{-1}) and CBD (1, 5, and 10 mg·kg^{-1}) 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 μl·g^{-1}.

2.3 | Measurement of HTR, ESR, and grooming behaviours

Mice, four to eight in a 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 conditions of 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 pretreatment time was based on 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; and (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^{-1}) or saline was administered, and the mouse was immediately placed in the middle of a clear glass cage 30 × 40 × 30 cm. Five minutes after placing the mouse into the cage, the number of HTR, ESR, and grooming behaviours was counted for 15 min in 3-min 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 are as follows: (1) DOI is systemically administered, 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 pretreatment 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 "5-HT theory of tic causation," high 5-HT 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 5-HT-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 5-HT and dopamine signalling (illustrated by Augustine & Singer, 2019). This may explain why the results of pretreatment in the DOI model with pimozide (Dursun & Handley, 1996) and Δ^9-THC (Darmani, 2001) have been translated to the clinic, that is, 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 CSF, 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) and, therefore, vocal-like tics cannot be detected in this model system.

2.4 | Data and statistical analysis

All data are expressed as means ± SEM. $P < 0.05$ was considered to show statistical significance. Behavioural data were analysed with GraphPad Prism versions 7 and 8 (GraphPad, San Diego, CA). Line curves of HTR, ESR, and grooming behaviours were analysed by two-way 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 ($P < 0.05$, to comply with current *BJP* guidelines).

In the presence of DOI, the % frequency of HTR, ESR, and grooming behaviours was calculated from average values after 15 min, relative to basal behaviour (vehicle + saline group), as follows: ((DOI − basal) − (drug − basal))/(DOI − basal) * 100 and, in the absence of DOI as follows: (drug − basal)/(basal) * 100.

Similarly, ID_{50} ($mg{\cdot}kg^{-1}$) and 95% confidence interval (CI; asymmetrical CI more accurate) of drug inhibition of DOI-induced grooming and ESR after 15 min were 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 ($P < 0.05$ was considered statistically significant). These data were analysed by a dose-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. ID_{50} values were considered statistically different only if the 95% CI did not overlap (GraphPad Prism version 8). Our analysis complies with *BJP*'s recommendations and requirements on experimental design and analysis using animals (Curtis et al., 2018; McGrath & Lilley, 2015), apart from one exploratory experiment ($n = 4$).

2.5 | Materials

(R)(−)-DOI hydrochloride (CAS 82864-02-6), DMSO, and Kolliphor® EL were from Sigma-Aldrich (Rehovot, Israel). CBD (99%) was from AMRI (USA), and Δ^9-THC (98%) was kindly provided by Prof. Mechoulam (The Hebrew University, Israel).

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos et al., 2019; Alexander, Fabbro et al., 2019).

3 | RESULTS

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

In juvenile male mice, Δ^9-THC significantly decreased the DOI-induced HTR (Figure 1a). Compared with the effect of DOI alone after 15 min, the HTR in the presence of Δ^9-THC (5 $mg{\cdot}kg^{-1}$) was reduced by 34%. in juvenile male mice. In an exploratory study ($n = 4$), CBD (5 and 10 $mg{\cdot}kg^{-1}$) also decreased the HTR induced by DOI by 21% and 11% respectively (Figure 1b).

In young adult male mice, Δ^9-THC (0.2, 1, and 5 $mg{\cdot}kg^{-1}$) produced a significant decrease of DOI-induced HTR (Figure 1c). Compared with the effect of DOI (15 min), the HTR in the presence of Δ^9-THC (5 $mg{\cdot}kg^{-1}$) was decreased by 68%. CBD (5 and 10 $mg{\cdot}kg^{-1}$) also produced a significant decrease of DOI-induced HTR, by 26% and 20% respectively (Figure 1d). Increasing the dose of CBD to 10 $mg{\cdot}kg^{-1}$ did not result in additional inhibition of HTR, either in juvenile or young adult male mice.

3.2 | 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, that is, in the absence of DOI. Δ^9-THC (5 $mg{\cdot}kg^{-1}$) induced cataleptic behaviour

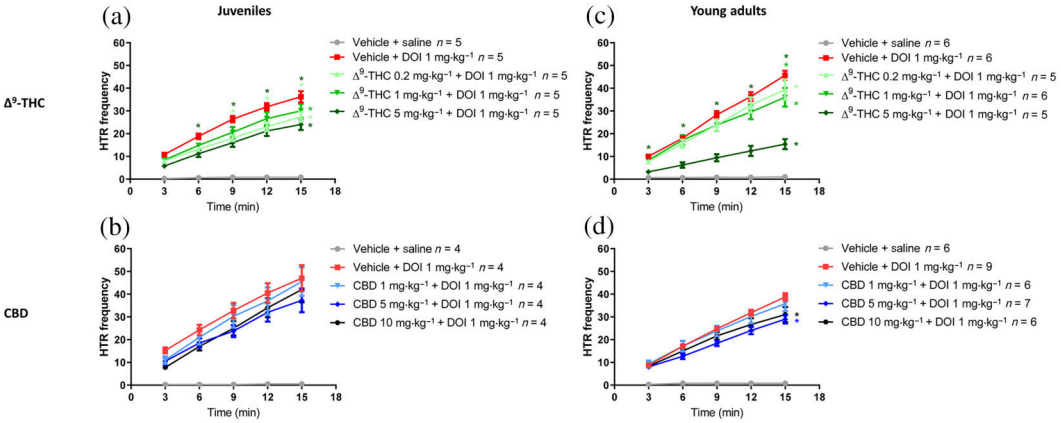

FIGURE 1 Effect of Δ^9-THC and CBD on head twitch response (HTR) in juvenile and young adult mice in the presence of DOI. In (a) and (c), the effects of Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}) in juvenile (a) and young adult (c) mice in the presence of DOI (1 mg·kg^{-1}). In (b) and (d), the effects of CBD (1, 5, and 10 mg·kg^{-1}) in juvenile (b) and young adult (d) mice in the presence of DOI (1 mg·kg^{-1}). Data shown are means ± SEM; *n* represents the number of animals in each group. The experiment was independently repeated a number of times according to the lowest *n* number. **P* < 0.05, for each time point, significantly different from vehicle + DOI group at that dose; **P* < 0.05, at the side of the graph, significantly different from the DOI only group, summary data; two-way ANOVA followed by Bonferroni's test

(immobility) in juvenile and young adult male mice, which started about 30 min after injection (i.p.) and continued for about 1–2 h. 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 was observed with Δ^9-THC (0.2 and 1 mg·kg^{-1}) in juveniles and young adults.

Compared with the control group, Δ^9-THC (5 mg·kg^{-1}) produced a significant decrease of basal HTR in healthy juvenile (Figure 2a) and young adult male mice (Figure 2c). In juveniles, compared with the basal HTR of the control group, Δ^9-THC (5 mg·kg^{-1}) reduced HTR frequency to 50% of basal values. Similar results were found in young adult male mice. In the presence of Δ^9-THC (5 mg·kg^{-1}), the basal HTR frequency was decreased by 83%.

Surprisingly, CBD alone significantly increased the frequency of HTR. Compared with the control group, CBD (1 and 5 mg·kg^{-1}) increased HTR in healthy juvenile male mice (Figure 2b) to 390% and

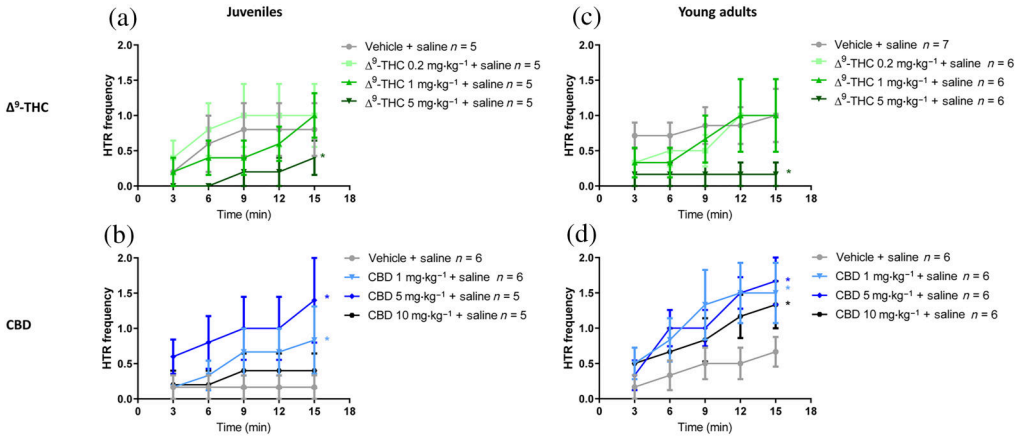

FIGURE 2 Effect of Δ^9-THC and CBD on head twitch response (HTR) in juvenile and young adult mice in the absence of DOI. In (a) and (c), the effects of Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}) in juvenile (a) and young adult (c) mice in the presence of saline injection (instead of DOI injection). In both juvenile and young adult mice, in the absence of DOI, Δ^9-THC at a dose of 5 mg·kg^{-1} induced a significant cataleptic behaviour (a, c, dark green). In (b) and (d), the effects of CBD (1, 5, and 10 mg·kg^{-1}) 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^{-1} significantly increased HTR in both young adult and juvenile mice (c, d) and CBD of 10 mg·kg^{-1} also significantly increased HTR in young adult mice (d). Data shown are means ± SEM; *n* represents the number of animals in each group. The experiment was independently repeated a number of times according to the lowest *n* number. **P* < 0.05, for each time point, significantly different from control (vehicle + saline) group at that dose; **P* < 0.05, at the side of the graph, significantly different from control (vehicle + saline) group, summary data; two-way ANOVA followed by Bonferroni's test

724% of basal HTR respectively (Figure 2b). Similar results were found in healthy young adult male mice. CBD (1, 5, and 10 mg·kg^{-1}) increasing HTR (Figure 2d) to 124%, 149%, and 99% of basal values respectively.

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

In juvenile male mice, Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}) dose-dependently reduced DOI-induced self-grooming behaviour (Figure 3a). At 15 min, the effect of DOI was inhibited by 99% in the presence of Δ^9-THC (5 mg·kg^{-1}). The ID_{50} and 95% CI of Δ^9-THC inhibition of DOI-induced grooming in juvenile mice are shown in Figure 3c.

In young adult male mice, Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}) dose-dependently reduced DOI-induced grooming behaviour (Figure 3d). The effect of DOI (15 min) was inhibited by 113%, in the presence of Δ^9-THC (5 mg·kg^{-1}). This means that this dose of Δ^9-THC inhibited DOI-induced grooming behaviour below the basal grooming behaviour of young adult male mice. This effect is likely to be due to the observed cataleptic behaviour at this dose. The corresponding ID_{50} value, with 95% CI, before constraining the bottom of the curve to zero is shown in Figure 3f and after constraining, is shown in Table 1. These ID_{50} values were not significantly different from the ID_{50} of Δ^9-THC inhibition of DOI-induced grooming in juvenile mice before (as above) or after constraining (Table 1).

3.4 | 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, and 5 mg·kg^{-1}) 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^{-1}) induced cataleptic behaviour, as discussed earlier, there was no effect on basal grooming behaviour in juvenile mice. In young adult male mice, Δ^9-THC (5 mg·kg^{-1}) produced a significant decrease of grooming behaviour (Figure 4c) which might be attributed to the cataleptic behaviour observed at this dose.

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

In juvenile male mice, CBD (1, 5, and 10 mg·kg^{-1}) 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^{-1}) produced a significant decrease of DOI-induced grooming behaviour which was not dose-related (Figure 3e), resulting in a 26–27% inhibition of DOI-induced grooming behaviour. In the absence of DOI, CBD (1 and 5 mg·kg^{-1}) had no significant effect on basal grooming behaviour. However, CBD (10 mg·kg^{-1}) significantly decreased grooming behaviour, resulting in

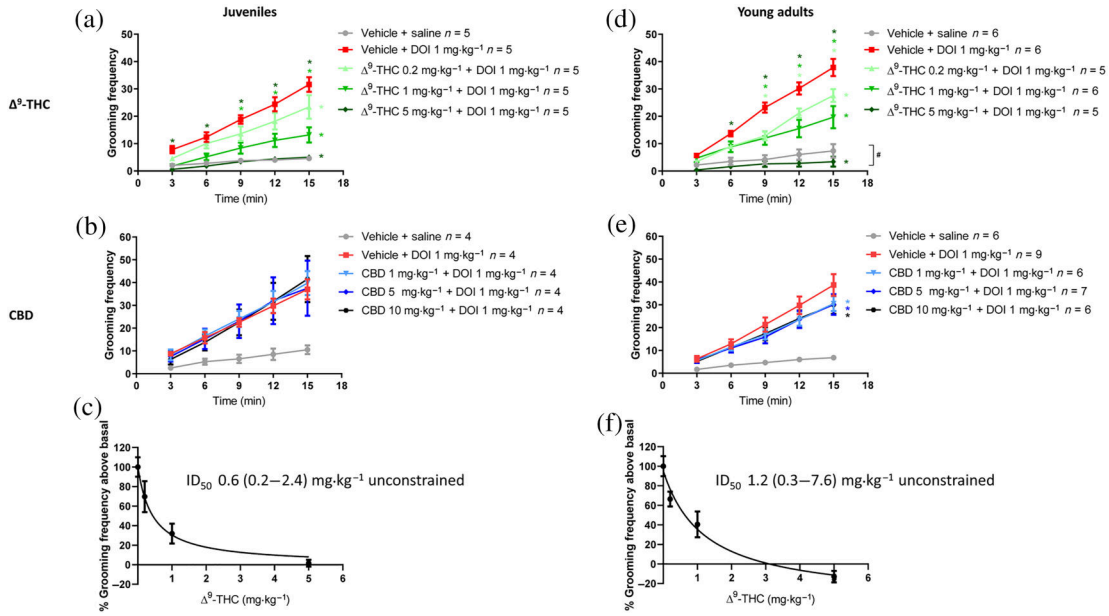

FIGURE 3 Effect of Δ^9-THC and CBD on grooming behaviour in juvenile and young adult mice in the presence of DOI. In (a) and (d), the effects of Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}) in juvenile (a) and young adult (d) mice in the presence of DOI (1 mg·kg^{-1}). In (b) and (e), the effects of CBD (1, 5, and 10 mg·kg^{-1}) in juvenile (b) and young adult (e) mice in the presence of DOI (1 mg·kg^{-1}). Data shown are means ± SEM; *n* represents the number of animals in each group. The experiment was independently repeated a number of times according to the lowest *n* number. In (c) and (f), dose versus response graphs of % grooming frequency, in the absence of Δ^9-THC (0 mg·kg^{-1}, 100%) or presence of Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}), in juvenile (c) and young adult (f) DOI-induced mice. ID_{50} (95% confidence interval) (CI) (mg·kg^{-1}) are presented (no constraint). $^{\#}P < 0.05$ significantly different from control (vehicle + saline) group; $^{*}P < 0.05$, for each time point, significantly different from vehicle + DOI group at that dose; $^{*}P < 0.05$, at the side of the graph, significantly different from the DOI only group, summary data; two-way ANOVA followed by Bonferroni's test

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

	Juvenile male mice (n = 5)	Young adult male mice (n = 5–6)
Δ^9-THC	ID_{50} (mg·kg^{-1}) (95% CI)	ID_{50} (mg·kg^{-1}) (95% CI)
HTR	5 mg·kg^{-1} produced 34% inhibition	5 mg·kg^{-1} produced 68% inhibition 1 mg·kg^{-1} 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

Note: In this study, cannabinoids were injected 60 min before (R)(−)-DOI into C57BL/6J mice. The ID_{50} (mg·kg^{-1}) 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).
Abbreviation: ND, not determined.

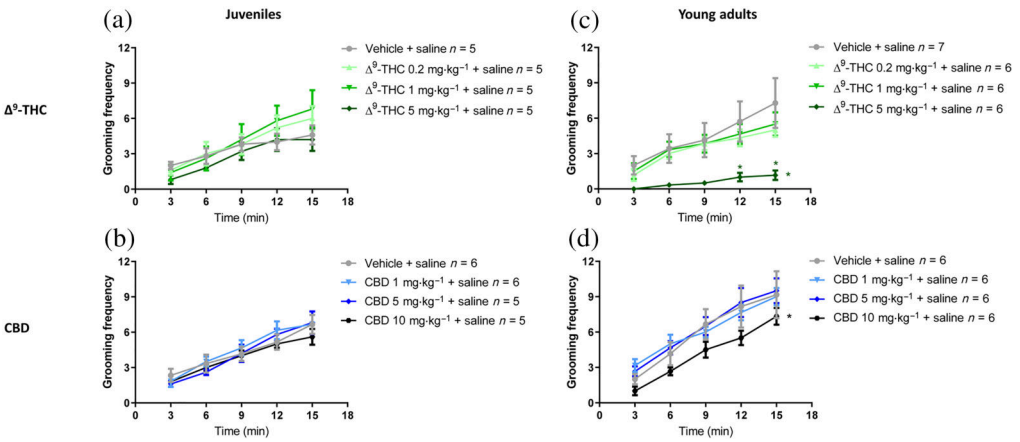

FIGURE 4 Effect of Δ^9-THC and CBD on grooming behaviour in juvenile and young adult mice in the absence of DOI. In (a) and (c), the effects of Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}) in juvenile (a) and young adult (c) mice in the presence of saline injection (instead of DOI injection). In young adult mice, in the absence of DOI, Δ^9-THC at a dose of 5 mg·kg^{-1} induced a significant cataleptic behaviour, resulting in a significant reduction of grooming behaviour (c) compared to control mice (vehicle + saline). In (b) and (d), the effects of CBD (1, 5, and 10 mg·kg^{-1}) 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^{-1} significantly decreased grooming behaviour (d) compared with control mice (vehicle + saline). Data shown are means ± SEM; n represents the number of animals in each group. The experiment was independently repeated a number of times according to the lowest n number. *P < 0.05, for each time point, significantly different from vehicle + saline group at that dose; *P < 0.05, at the side of the graph, significantly different from the vehicle + saline only group, summary data; two-way ANOVA followed by Bonferroni's test

20% inhibition of basal grooming behaviour in healthy young adult male mice (Figure 4d).

3.6 | 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, and 5 mg·kg^{-1}) dose-dependently decreased ESR (Figure 5a). At 15 min, the presence of Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}) produced 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^{-1} inhibited DOI-induced ESR by 5% below the basal ESR of juvenile male mice. The unconstrained ID_{50} of Δ^9-THC inhibition of DOI-induced grooming in juvenile male mice is shown in Figure 5c and, after constraint, in Table 1. These ID_{50} values were not significantly different.

In juvenile male mice, CBD (1, 5, and 10 mg·kg^{-1}), in the presence of DOI, had no effect on DOI-induced ESR behaviour (Figure 5b, referred as an exploratory study). In young adult male mice, CBD (1, 5, and 10 mg·kg^{-1}) significantly decreased the DOI-induced ESR at 15 min (Figure 5e) by 54%, 54%, and 42% respectively of its control value.

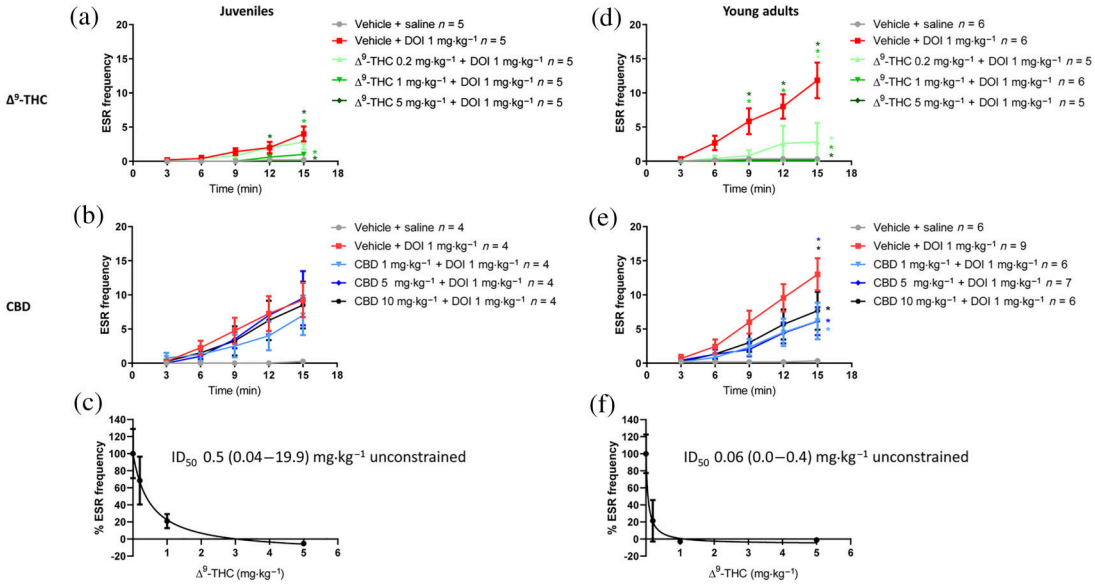

FIGURE 5 Effect of Δ^9-THC and CBD on ear scratch response (ESR) in juvenile and young adult mice in the presence of DOI. In (a) and (d), the effects of Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}) in juvenile (a) and young adult (d) mice in the presence of DOI (1 mg·kg^{-1}). In (b) and (e), the effects of CBD (1, 5, and 10 mg·kg^{-1}) in juvenile (b) and young adult (e) mice in the presence of DOI (1 mg·kg^{-1}). Data shown are means ± SEM; *n* represents the number of animals in each group. The experiment was independently repeated a number of times according to the lowest *n* number. In (c) and (f), dose versus response graphs of % ESR frequency, in the absence of Δ^9-THC (0 mg·kg^{-1}, 100%) or presence of Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}), in juvenile (c) and young adult (f) DOI-induced mice. ID_{50} (95% confidence interval) (CI) (mg·kg^{-1}) are presented (no constraint). $^*P < 0.05$, for each time point, significantly different from vehicle + DOI group at that dose; $^*P < 0.05$, at the side of the graph, significantly different from the DOI only group, summary data; two-way ANOVA followed by Bonferroni's test

In young adult male mice, Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}), in the presence of DOI, dose-dependently decreased DOI-induced ESR (Figure 5d), producing 79%, 103%, and 101% inhibition respectively. The putative ID_{50} of Δ^9-THC inhibition of DOI-induced grooming behaviour in juvenile mice is shown in Figure 5f. As 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 and, after constraint, the putative ID_{50} is presented in Table 1. These ID_{50} values were not significantly different.

3.7 | 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^{-1}) significantly increased the frequency of basal ESR (Figure 6a) compared with the control group, resulting in a 400% increase of ESR. In young adult male mice, compared with the control group, Δ^9-THC (0.2 mg·kg^{-1}) resulted in a significant, 100% decrease of basal ESR (Figure 6c).

CBD (1, 5, and 10 mg·kg^{-1}) 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^{-1} was not significantly different from that of the control group.

4 | DISCUSSION

Study of cannabinoids in juvenile and young adult model systems is essential because Δ^9-THC affects brain development and can induce psychosis (see 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 possibility that children or adults with Tourette syndrome could 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^{-1} significantly reduced basal grooming behaviour, suggesting that 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 behaviours.

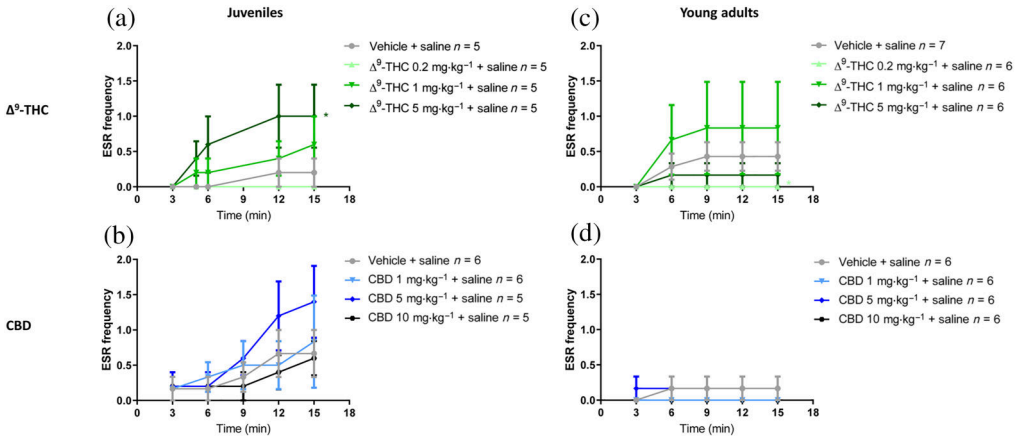

FIGURE 6 Effects of Δ^9-THC and CBD on ear scratch response (ESR) in juvenile and young adult mice in the absence of DOI. In (a) and (c), the effects of Δ^9-THC (0.2, 1, and 5 mg·kg^{-1}) in juvenile (a) and young adult (c) mice in the presence of saline injection (instead of DOI injection). In both juvenile and young adult mice, in the absence of DOI, Δ^9-THC at a dose of 5 mg·kg^{-1} induced a significant cataleptic behaviour (a, c,). In the absence of DOI, Δ^9-THC at doses of 5 mg·kg^{-1} significantly increased ESR in juvenile mice (a) while a dose of 0.2 mg·kg^{-1} significantly decreased ESR in young adult mice (c). In (b) and (d), the effects of CBD (1, 5, and 10 mg·kg^{-1}) 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 shown are means ± SEM; *n* represents the number of animals in each group. The experiment was independently repeated a number of times according to the lowest *n* number. *$P < 0.05$, at the side of the graph, significantly different from the control (vehicle + saline) group, summary data; two-way ANOVA followed by Bonferroni's test

This study is relevant to clinical use of pure CBD and possibly other extracts with high CBD content, and implies 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; and (4) have a smaller "therapeutic window" in paediatric patients than in 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 a wide range of pharmacological sites (Fernandez-Ruiz et al., 2013). CBD inhibits **5-HT$_{3A}$** receptors (Yang et al., 2010) and indirectly enhances **5-HT$_{1A}$** receptor 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 (see Canal & Morgan, 2012), while antagonism of 5-HT$_3$ receptors does not reverse DOI-induced HTR (Wettstein, Host, & Hitchcock, 1999). In addition, similar to **SR141716A**, a selective **CB$_1$** receptor antagonist/inverse agonist, CBD is a high potency antagonist of CB$_1$ receptor agonists and shows inverse agonist efficacy in the mouse brain (Thomas et al., 2007). While CB$_1$ 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 μM) acts as a negative allosteric modulator of the CB$_1$ receptor (Laprairie, Bagher, Kelly, & Denovan-Wright, 2015), and at high concentrations (>10-μM), 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 CB$_1$ 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 showed that Δ^9-THC inhibited DOI-induced HTR and ESR in ICR mice which were pretreated for 20 min before treatment with (R)(±)-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^{-1} 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 5-HT release in the subregion 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^{-1} requires different mechanisms than these causing Δ^9-THC at a dose of 5 mg·kg^{-1} to induce catalepsy. The specific mechanisms are yet to be investigated.

Cataleptic behaviour was not observed after injection of DOI (1 mg·kg^{-1}). These results replicate a previous report showing that Δ^9-THC at a dose of 6 mg·kg^{-1} induced cataleptic behaviour in 6-week-old male ddY mice (Egashira et al., 2007). In the same study, Δ^9-THC-induced catalepsy was reversed by DOI (0.3 and 1 mg·kg^{-1}), suggesting that cataleptic behaviour is mainly controlled by 5-HT$_{2A}$ receptors (Egashira et al., 2007). Importantly, this points to the hypothesis that repetitive behaviours that are controlled by 5-HT$_{2A}$ receptors can be affected by cataleptic behaviour which reduces general motor activity; that is, non-ambulatory motor function (catalepsy) will block all muscle activity including that required for DOI (1 mg·kg^{-1})-induced repetitive behaviours. This is important because Δ^9-THC-induced catalepsy is reversed by SR141716A, a

selective CB_1 receptor antagonist/inverse agonist, suggesting that the CB_1 receptor mediates this effect of Δ^9-THC (Sano et al., 2008). However, Δ^9-THC is a non-selective partial agonist of CB_1/CB_2 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) $mg{\cdot}kg^{-1}$ (Darmani, 2001). In this study, a dose of 5 $mg{\cdot}kg^{-1}$ resulted in 34% inhibition of DOI-induced HTR, though a longer pretreatment time was given (Table 1). The inhibitory effect of Δ^9-THC on DOI-induced ESR appears to have a similar ID_{50} value 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) $mg{\cdot}kg^{-1}$ compared with juvenile ICR mice with an ID_{50} of 0.25 (0.22–0.3) $mg{\cdot}kg^{-1}$ (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) $mg{\cdot}kg^{-1}$. 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 reversed the SR141716A-induced ESR in adolescent ICR mice (Janoyan, Crim, & Darmani, 2002).

Grooming behaviour was not tested in the same mice by Darmani (2001), but Δ^9-THC (oral, 5 $mg{\cdot}kg^{-1}$) 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) $mg{\cdot}kg^{-1}$. This suggests a 10-fold lower potency than that required to inhibit DOI-induced ESR, in the same mice, with an ID_{50} of 0.05 (0.0–0.2) $mg{\cdot}kg^{-1}$.

4.1 | 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 $5\text{-}HT_{2A}$ receptors, 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 $mg{\cdot}kg^{-1}$) to inhibit repetitive behaviours, for example, ESR (100%) = grooming (100%) > HTR (34%) in juvenile mice. The results could 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 who are concerned more with the premonitory urges than with the tics themselves, possibly, may benefit from treatment with a relatively low dose of Δ^9-THC (0.2–0.4 mg vs. 10 $mg{\cdot}day^{-1}$ reported for adult patients, Muller-Vahl et al., 2003), reducing side effects. Our 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 subpopulations 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 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$. A daily dose of Δ^9-THC exceeding 0.4 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ 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 from 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 subpopulations of patients with Tourette syndrome may benefit from CBD as a standalone drug treatment.

ACKNOWLEDGEMENTS

Our special thanks to Professor Roger G. Pertwee, University of Aberdeen, UK, for critical comments. This study was supported by a Research Grant Award from the Tourette Association of America (S.A.G. and P.M.). V.G. was supported by The Elphinstone Scholarship for Ph.D. students, University of Aberdeen.

AUTHOR CONTRIBUTIONS

S.A.G. and P.M. were the PIs and co-mentored V.G. S.A.G. conceived and designed the research. V.G. (Ph.D. student) was the main contributor to this research, performed experiments, and analysed and graphed data. S.A.G., V.G., and P.M. wrote the manuscript.

CONFLICT OF INTEREST

S.A.G. is a member of the National Committee for Tourette syndrome, Tourette Syndrome Association of Israel (TSAI) and a member of the International Consortium for Medical Cannabis and Related Drugs for Tic Disorders, Tourette Association of America (TAA). S.A.G. has filed patent applications. The authors V.G. and P.M. have no financial/non-financial interests.

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 Design & Analysis, and Animal Experimentation, and as recommended by funding agencies, publishers, and other organisations engaged with supporting research.

DATA AVAILABILITY STATEMENT

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.

REFERENCES

Abi-Jaoude, E., Chen, L., Cheung, P., Bhikram, T., & Sandor, P. (2017). Preliminary evidence on cannabis effectiveness and tolerability for adults with Tourette syndrome. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *29*(4), 391–400. https://doi.org/10.1176/appi.neuropsych.16110310

Alexander, S. P. H., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., … Collaborators, C. (2019). The Concise Guide to PHARMACOLOGY 2019/20: G protein-coupled receptors. *British Journal of Pharmacology*, *176*(S1), S21–S141. https://doi.org/10.1111/bph.14748

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., … CGTP Collaborators. (2019). THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Enzymes. *British Journal of Pharmacology*, *176*, S297–S396. https://doi.org/10.1111/bph.14752

Anavi-Goffer, S., & Mulder, J. (2009). The polarised life of the endocannabinoid system in CNS development. *Chembiochem*, *10*(10), 1591–1598. https://doi.org/10.1002/cbic.200800827

Artukoglu, B. B., & Bloch, M. H. (2019). The potential of cannabinoid-based treatments in Tourette syndrome. *CNS Drugs*, *33*(5), 417–430. https://doi.org/10.1007/s40263-019-00627-1

Augustine, F., & Singer, H. S. (2019). Merging the pathophysiology and pharmacotherapy of tics. *Tremor and Other Hyperkinetic Movements (N Y)*, *8*, 595. https://doi.org/10.7916/D8H14JTX

Bacqué-Cazenave, J., Bharatiya, R., Barrière, G., Delbecque, J. P., Bouguiyoud, N., Di Giovanni, G., … De Deurwaerdère, P. (2020). Serotonin in animal cognition and behavior. *International Journal of Molecular Sciences*, *21*(5), 1649. https://doi.org/10.3390/ijms21051649

Canal, C. E., Booth, R. G., & Morgan, D. (2013). Support for 5-HT2C receptor functional selectivity in vivo utilizing structurally diverse, selective 5-HT2C receptor ligands and the 2,5-dimethoxy-4-iodoamphetamine elicited head-twitch response model. *Neuropharmacology*, *70*, 112–121. https://doi.org/10.1016/j.neuropharm.2013.01.007

Canal, C. E., & Morgan, D. (2012). Head-twitch response in rodents induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine: A comprehensive history, a re-evaluation of mechanisms, and its utility as a model. *Drug Testing and Analysis*, *4*(7–8), 556–576. https://doi.org/10.1002/dta.1333

Canal, C. E., Olaghere da Silva, U. B., Gresch, P. J., Watt, E. E., Sanders-Bush, E., & Airey, D. C. (2010). The serotonin 2C receptor potently modulates the head-twitch response in mice induced by a phenethylamine hallucinogen. *Psychopharmacology*, *209*(2), 163–174. https://doi.org/10.1007/s00213-010-1784-0

Cavanna, A. E., Black, K. J., Hallett, M., & Voon, V. (2017). Neurobiology of the premonitory urge in Tourette's syndrome: Pathophysiology and treatment implications. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *29*(2), 95–104. https://doi.org/10.1176/appi.neuropsych.16070141

Curtis, M. J., Alexander, S., Cirino, G., Docherty, J. R., George, C. H., Giembycz, M. A., … Ahluwalia, A. (2018). Experimental design and analysis and their reporting II: Updated and simplified guidance for authors and peer reviewers. *British Journal of Pharmacology.*, *175*, 987–993.

Darmani, N. A. (2001). Cannabinoids of diverse structure inhibit two DOI-induced 5-HT_{2A} receptor-mediated behaviors in mice. *Pharmacology, Biochemistry, and Behavior*, *68*(2), 311–317. doi: S0091-3057(00)00477-9 [pii]

Darmani, N. A., Janoyan, J. J., Kumar, N., & Crim, J. L. (2003). Behaviorally active doses of the CB_1 receptor antagonist SR 141716A increase brain serotonin and dopamine levels and turnover. *Pharmacology Biochemistry and Behavior*, *75*(4), 777–787. https://doi.org/10.1016/s0091-3057(03)00150-3

De Petrocellis, L., Ligresti, A., Moriello, A. S., Allarà, M., Bisogno, T., Petrosino, S., … Di Marzo, V. (2011). Effects of cannabinoids and cannabinoid-enriched *Cannabis* extracts on TRP channels and endocannabinoid metabolic enzymes. *British Journal of Pharmacology*, *163*(7), 1479–1494. https://doi.org/10.1111/j.1476-5381.2010.01166.x

Dursun, S. M., & Handley, S. L. (1996). Similarities in the pharmacology of spontaneous and DOI-induced head-shakes suggest 5HT2A receptors are active under physiological conditions. *Psychopharmacology*, *128*(2), 198–205. https://doi.org/10.1007/s002130050125

Egashira, N., Koushi, E., Mishima, K., Iwasaki, K., Oishi, R., & Fujiwara, M. (2007). 2,5-Dimethoxy-4-iodoamphetamine (DOI) inhibits Δ^9-tetrahydrocannabinol-induced catalepsy-like immobilization in mice. *Journal of Pharmacological Sciences*, *105*(4), 361–366. https://doi.org/10.1254/jphs.fp0071247

Egashira, N., Okuno, R., Shirakawa, A., Nagao, M., Mishima, K., Iwasaki, K., … Fujiwara, M. (2012). Role of 5-hydroxytryptamine$_{2C}$ receptors in marble-burying behavior in mice. *Biological & Pharmaceutical Bulletin*, *35*(3), 376–379. https://doi.org/10.1248/bpb.35.376

Fernandez-Ruiz, J., Sagredo, O., Pazos, M. R., Garcia, C., Pertwee, R., Mechoulam, R., & Martinez-Orgado, J. (2013). Cannabidiol for neurodegenerative disorders: Important new clinical applications for this phytocannabinoid? *British Journal of Clinical Pharmacology*, *75*(2), 323–333. https://doi.org/10.1111/j.1365-2125.2012.04341.x

Greydanus, D. E., & Tullio, J. (2020). Tourette's disorder in children and adolescents. *Translational Pediatrics*, *9*(Suppl 1), S94–s103. https://doi.org/10.21037/tp.2019.09.11

Hasan, A., Rothenberger, A., Munchau, A., Wobrock, T., Falkai, P., & Roessner, V. (2010). Oral Δ9-tetrahydrocannabinol improved refractory Gilles de la Tourette syndrome in an adolescent by increasing intracortical inhibition: A case report. *Journal of Clinical Psychopharmacology*, *30*(2), 190–192. https://doi.org/10.1097/JCP.0b013e3181d236ec

Hawkins, M. F., Uzelac, S. M., Baumeister, A. A., Hearn, J. K., Broussard, J. I., & Guillot, T. S. (2002). Behavioral responses to stress following central and peripheral injection of the 5-HT_2 agonist DOI. *Pharmacology, Biochemistry, and Behavior*, *73*(3), 537–544. https://doi.org/10.1016/S0091-3057(02)00822-5

Hines, L. A., Freeman, T. P., Gage, S. H., Zammit, S., Hickman, M., Cannon, M., … Heron, J. (2020). Association of high-potency cannabis use with mental health and substance use in adolescence. *JAMA Psychiatry*, *77*, 1044–1051. https://doi.org/10.1001/jamapsychiatry.2020.1035

Jakubovski, E., & Muller-Vahl, K. (2017). Speechlessness in Gilles de la Tourette syndrome: Cannabis-based medicines improve severe vocal blocking tics in two patients. *International Journal of Molecular Sciences*, *18*(8). https://doi.org/10.3390/ijms18081739

Janoyan, J. J., Crim, J. L., & Darmani, N. A. (2002). Reversal of SR 141716A-induced head-twitch and ear-scratch responses in mice by Δ^9-THC and other cannabinoids. *Pharmacology, Biochemistry, and Behavior*, *71*(1–2), 155–162. https://doi.org/10.1016/S0091-3057(01)00647-5

Kanaan, A. S., Jakubovski, E., & Muller-Vahl, K. (2017). Significant tic reduction in an otherwise treatment-resistant patient with Gilles de la Tourette syndrome following treatment with nabiximols. *Brain Sciences*, *7*(5), 47. https://doi.org/10.3390/brainsci7050047

Klimkeit, E., Rinehart, N., May, T., & Bradshaw, J. (2017). Neurodevelopmental disorders. In S. R. Quah (Ed.), *International encyclopedia of public health (second edition)* (pp. 223–230). Oxford: Academic Press.

Laprairie, R. B., Bagher, A. M., Kelly, M. E. M., & Denovan-Wright, E. M. (2015). Cannabidiol is a negative allosteric modulator of the cannabinoid CB_1 receptor. *British Journal of Pharmacology*, *172*(20), 4790–4805. https://doi.org/10.1111/bph.13250

Lilley, E., Stanford, S. C., Kendall, D. E., Alexander, S. P., Cirino, G., Docherty, J. R., … Ahluwalia, A. (2020). ARRIVE 2.0 and the *British Journal of Pharmacology*: Updated guidance for 2020. *British Journal of Pharmacology*, 3611–3616. https://doi.org/10.1111/bph.15178

McGrath, J. C., & Lilley, E. (2015). Implementing guidelines on reporting research using animals (ARRIVE etc.): New requirements for publication in *BJP*. *British Journal of Pharmacology.*, *172*, 3189–3193.

McNaught, K. S. P., & Mink, J. W. (2011). Advances in understanding and treatment of Tourette syndrome. *Nature Reviews Neurology*, *7*(12), 667–676. https://doi.org/10.1038/nrneurol.2011.167

Milosev, L. M., Psathakis, N., Szejko, N., Jakubovski, E., & Muller-Vahl, K. R. (2019). Treatment of Gilles de la Tourette syndrome with cannabis-based medicine: Results from a retrospective analysis and online survey. *Cannabis and Cannabinoid Research*, *4*(4), 265–274. https://doi.org/10.1089/can.2018.0050

Muller-Vahl, K. R., Bindila, L., Lutz, B., Musshoff, F., Skripuletz, T., Baumgaertel, C., & Suhs, K. W. (2020). Cerebrospinal fluid endocannabinoid levels in Gilles de la Tourette syndrome. *Neuropsychopharmacology*, *45*, 1323–1329. https://doi.org/10.1038/s41386-020-0671-6

Muller-Vahl, K. R., Schneider, U., Prevedel, H., Theloe, K., Kolbe, H., Daldrup, T., & Emrich, H. M. (2003). Δ9-tetrahydrocannabinol (THC) is effective in the treatment of tics in Tourette syndrome: A 6-week randomized trial. *The Journal of Clinical Psychiatry*, *64*(4), 459–465. Retrieved from. http://www.ncbi.nlm.nih.gov/pubmed/12716250

Navarro, M., Fernandez-Ruiz, J. J., De Miguel, R., Hernandez, M. L., Cebeira, M., & Ramos, J. A. (1993). Motor disturbances induced by an acute dose of Δ^9-tetrahydrocannabinol: Possible involvement of nigrostriatal dopaminergic alterations. *Pharmacology, Biochemistry, and Behavior*, *45*(2), 291–298. https://doi.org/10.1016/0091-3057(93)90241-k

Nespoli, E., Rizzo, F., Boeckers, T. M., Hengerer, B., & Ludolph, A. G. (2016). Addressing the complexity of Tourette's syndrome through the use of animal models. *Frontiers in Neuroscience*, *10*, 133. https://doi.org/10.3389/fnins.2016.00133

Paramlall, M., & Tyagi, H. (2020). 34 case report of sertraline exacerbation of tics in Tourette's with OCD. *Journal of Neurology, Neurosurgery, and Psychiatry*, *91*(8), e22. https://doi.org/10.1136/jnnp-2020-BNPA.51

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., … Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biology*, *18*(7), e3000410. https://doi.org/10.1371/journal.pbio.3000410

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P., Di Marzo, V., Elphick, M. R., … Ross, R. A. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: Beyond CB_1 and CB_2. *Pharmacological Reviews*, *62*(4), 588–631. https://doi.org/10.1124/pr.110.003004 62/4/588 [pii]

Pichler, E. M., Kawohl, W., Seifritz, E., & Roser, P. (2019). Pure delta-9-tetrahydrocannabinol and its combination with cannabidiol in treatment-resistant Tourette syndrome: A case report. *International Journal of Psychiatry in Medicine*, *54*(2), 150–156. https://doi.org/10.1177/0091217418791455

Rock, E. M., Bolognini, D., Limebeer, C. L., Cascio, M. G., Anavi-Goffer, S., Fletcher, P. J., … Parker, L. A. (2012). Cannabidiol, a non-psychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT_{1A} somatodendritic autoreceptors in the dorsal raphe nucleus. *British Journal of Pharmacology*, *165*(8), 2620–2634. https://doi.org/10.1111/j.1476-5381.2011.01621.x

Rojas-Corrales, M. O., Gibert-Rahola, J., & Mico, J. A. (2007). Role of atypical opiates in OCD. Experimental approach through the study of $5\text{-HT}_{2A/C}$ receptor-mediated behavior. *Psychopharmacology*, *190*(2), 221–231. https://doi.org/10.1007/s00213-006-0619-5

Rua, A., & Damásio, J. (2014). Tics induced by sertraline: Case report and literature review. *Movement Disorders Clinical Practice*, *1*(3), 243–244. https://doi.org/10.1002/mdc3.12044

Russo, E. B., Burnett, A., Hall, B., & Parker, K. K. (2005). Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochemical Research*, *30*(8), 1037–1043. https://doi.org/10.1007/s11064-005-6978-1

Sano, K., Mishima, K., Koushi, E., Orito, K., Egashira, N., Irie, K., … Fujiwara, M. (2008). Δ^9-Tetrahydrocannabinol-induced catalepsy-like immobilization is mediated by decreased 5-HT neurotransmission in the nucleus accumbens due to the action of glutamate-containing neurons. *Neuroscience*, *151*(2), 320–328. https://doi.org/10.1016/j.neuroscience.2007.10.026

Taylor, J. L., Rajbhandari, A. K., Berridge, K. C., & Aldridge, J. W. (2010). Dopamine receptor modulation of repetitive grooming actions in the rat: Potential relevance for Tourette syndrome. *Brain Research*, *1322*, 92–101. https://doi.org/10.1016/j.brainres.2010.01.052

Thomas, A., Baillie, G. L., Phillips, A. M., Razdan, R. K., Ross, R. A., & Pertwee, R. G. (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB_1 and CB_2 receptor agonists *in vitro*. *British Journal of Pharmacology*, *150*(5), 613–623. https://doi.org/10.1038/sj.bjp.0707133

Tikhonova, M. A., Kulikov, V. A., & Kulikov, A. V. (2011). Effects of LPS and serotonergic drugs on hygienic behavior in mice. *Pharmacology, Biochemistry, and Behavior*, *98*(3), 392–397. https://doi.org/10.1016/j.pbb.2011.02.005

Trainor, D., Evans, L., & Bird, R. (2016). Severe motor and vocal tics controlled with Sativex®. *Australasian Psychiatry*, *24*(6), 541–544. https://doi.org/10.1177/1039856216663737

Varvel, S. A., Bridgen, D. T., Tao, Q., Thomas, B. F., Martin, B. R., & Lichtman, A. H. (2005). Δ^9-Tetrahydrocannbinol accounts for the antinociceptive, hypothermic, and cataleptic effects of marijuana in mice. *The Journal of Pharmacology and Experimental Therapeutics*, *314*(1), 329–337. https://doi.org/10.1124/jpet.104.080739

Wettstein, J. G., Host, M., & Hitchcock, J. M. (1999). Selectivity of action of typical and atypical anti-psychotic drugs as antagonists of the behavioral effects of 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI). *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *23*(3), 533–544. https://doi.org/10.1016/s0278-5846(99)00014-7

Winslow, J. T., & Insel, T. R. (1991). Serotonergic modulation of the rat pup ultrasonic isolation call: Studies with $5HT_1$ and $5HT_2$ subtype-selective agonists and antagonists. *Psychopharmacology*, *105*(4), 513–520. https://doi.org/10.1007/BF02244372

Wong, D. F., Brasic, J. R., Singer, H. S., Schretlen, D. J., Kuwabara, H., Zhou, Y., … Grace, A. A. (2008). Mechanisms of dopaminergic and serotonergic neurotransmission in Tourette syndrome: Clues from an in vivo neurochemistry study with PET. *Neuropsychopharmacology*, *33*(6), 1239–1251. https://doi.org/10.1038/sj.npp.1301528

Wu, W.-H., & Cooper, R. L. (2012). Serotonin and synaptic transmission at invertebrate neuromuscular junctions. *Experimental Neurobiology*, *21*(3), 101–112. https://doi.org/10.5607/en.2012.21.3.101

Yang, K. H., Galadari, S., Isaev, D., Petroianu, G., Shippenberg, T. S., & Oz, M. (2010). The nonpsychoactive cannabinoid cannabidiol inhibits 5-hydroxytryptamine$_{3A}$ receptor-mediated currents in *Xenopus laevis* oocytes. *The Journal of Pharmacology and Experimental Therapeutics*, *333*(2), 547–554. https://doi.org/10.1124/jpet.109.162594

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Gorberg V, McCaffery P, Anavi-Goffer S. Different responses of repetitive behaviours in juvenile and young adult mice to Δ^9-tetrahydrocannabinol and cannabidiol may affect decision making for Tourette syndrome. *Br J Pharmacol*. 2021;178:614–625. https://doi.org/10.1111/bph.15302