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Assessing the role of serotonergic receptors in cannabidiol's anticonvulsant efficacy

Madeline C. Pelz, Kathleen D. Schoolcraft, Chloe Larson, Mitchell G. Spring, Hassan H. López *

Skidmore College, 815 N. Broadway, Saratoga Springs, NY 12866, United States

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ABSTRACT

Cannabidiol (CBD) is a phytocannabinoid that has demonstrated anticonvulsant efficacy in several animal models of seizure. The current experiment validated CBD's anticonvulsant effect using the acute pentylenetetrazol (PTZ) model. Furthermore, it tested whether CBD reduces seizure activity by interacting with either the serotonergic 5HT1A or 5HT2A receptor. 120 male adolescent Wistar-Kyoto rats were randomly assigned to 8 treatment groups in two consecutive experiments. In both experiments, subjects received either CBD (100 mg/kg) or vehicle 60 min prior to seizure testing. In Experiment 1, subjects received either WAY-100635 (1 mg/kg), a 5HT1A antagonist, or saline vehicle injection 80 min prior to seizure testing. In Experiment 2, subjects received either MDL-100907 (0.3 mg/kg), a specific 5HT2A antagonist, or 40% DMSO vehicle 80 min prior to seizure testing. 85 mg/kg of PTZ was administered to induce seizure, and behavior was recorded for 30 min. Seizure behaviors were subsequently coded using a 5-point scale of severity. Across both experiments, subjects in the vehicle control groups exhibited high levels of seizure activity and mortality. In both experiments, CBD treatment significantly attenuated seizure activity. Pre-treatment with either WAY-100635 or MDL-100907 did not block CBD's anticonvulsant effect. WAY-100635 administration, by itself, also led to a significant attenuation of seizure activity. These results do not support the hypothesis that CBD attenuates seizure activity through activation of the 5HT1A or 5HT2A receptor. While this work further confirms the anticonvulsant efficacy of CBD and supports its application in the treatment of human seizure disorders, additional research on CBD's mechanism of action must be conducted.

1. Introduction

One-third of the 50 million people with epilepsy experience seizures that cannot be controlled with available treatments [1]. Current antiepileptic drugs (AED's) also cause many negative side effects, including fatigue, nausea, decreased appetite, dizziness, difficulty concentrating, memory problems, aggression, and hyperactivity. There is a substantial need for novel treatments for seizure disorders, and cannabinoids represent one highly promising option.

Cannabidiol (CBD) is the second-most abundant phytocannabinoid derived from the *Cannabis sativa* plant. CBD possesses antipsychotic, anxiolytic, and neuroprotective effects, and has also demonstrated anticonvulsant efficacy in several experimental studies [2,3]. Unlike Δ9-tetrahydrocannabinol (THC), the primary psychoactive cannabinoid, CBD does not induce psychoactive effects and is not reinforcing. As such, it is an especially attractive drug candidate for the treatment of seizures in children and adolescents. Recently, an open-label trial reported that CBD reduced seizure frequency in patients with severe treatment-resistant epilepsy [4]. CBD's anticonvulsant properties have also been tested in several different animal models of seizure and epilepsy [5,6]. The proconvulsant compound, pentylenetetrazol (PTZ), forms the basis of one of the most common animal models of generalized seizure and is often used to screen putative anticonvulsants [7]. Jones et al. (2010) reported that a 100 mg/kg dose of CBD was highly effective at reducing seizure severity in PTZ-treated rats [5]. One goal of the current experiments was to validate this anticonvulsant effect of CBD using the PTZ-model.

A second goal was to explore two possible mechanisms by which CBD may reduce seizure activity. CBD is a pharmacologically complex compound that targets many different molecules in the central nervous system and may affect seizure activity through multiple mechanisms [8]. Intriguingly, CBD has minimal affinity and shows low agonist activity at central cannabinoid receptors (CB1 and CB2). Among other targets, CBD binds to several serotonin receptors in the brain, including the 5HT1A and 5HT2A [9]. CBD's ability to influence serotonergic activity is relevant to seizure researchers for several reasons. First, serotonin and its receptors participate in the pathophysiology of epilepsy [10]. For

* Corresponding author at: Department of Psychology, Skidmore College, 815 N. Broadway, Saratoga Springs, NY 12866, United States.
E-mail addresses: mpelz@mit.edu (M.C. Pelz), kschoolc@skidmore.edu (K.D. Schoolcraft), clarson@skidmore.edu (C. Larson), hlopez@skidmore.edu (H.H. López).

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example, PET imaging studies have noted reduced expression of 5HT1A receptors in the hippocampus, entorhinal cortex, and parahippocampal gyrus of patients with temporal lobe epilepsy [11]. Numerous studies have documented that 5HT1A agonism attenuates seizure, although this literature is not entirely consistent [12]. Second, recent work has suggested that fenfluramine, a potent indirect 5HT agonist, can be an effective add-on treatment for Dravet's syndrome [13]. Fenfluramine works through multiple mechanisms to increase synaptic serotonin, but it is not known what post-synaptic effects are most relevant to its anticonvulsant efficacy. Third, there is growing awareness that 5HT2A receptor dysfunction may underlie comorbidity between major depressive disorder and epilepsy in human patients [14].

CBD's affinity for central 5HT1A receptors has been demonstrated through its ability to displace 8-OH-DPAT, and its efficacy has been observed through measurement of in vitro signal transduction [9]. Furthermore, antagonism of 5HT1A receptors blocks several of the physiological and behavioral effects of CBD administration. For instance, CBD's anti-depressant effects in mice were reversed following administration of the 5HT1A antagonist, WAY-100635 [15]. Similarly, CBD induced an anxiolytic effect in Wistar rats when injected directly into the bed nucleus of the stria terminalis (BNST) – an effect blocked by pre-treatment with WAY-100635 [16]. Perhaps most pertinent to its anticonvulsant potential, Ledgerwood et al. (2011) noted that CBD inhibits basal synaptic transmission in acute hippocampal slices, and this effect was terminated by administration of WAY-100135, a non-selective 5HT1A antagonist [17]. Russo et al. (2005) also demonstrated that CBD is active at the 5HT2A receptor, albeit less so than at the 5HT1A [9]. It is unknown whether any of CBD's physiological or behavioral effects are caused by activity at the 5HT2A receptor.

The current studies were designed to further assess CBD's anticonvulsant efficacy and to specifically determine whether CBD is working through the serotonergic 5HT1A or 5HT2A receptor. Our study is the first attempt to explore these hypotheses and serves as a critical step in isolating CBD's mechanism of action. In two separate experiments, adolescent Wistar Kyoto rats were given PTZ to induce severe seizure. Half were given 100 mg/kg CBD, which has previously been shown to significantly reduce seizure severity [5]. In Experiment 1, we tested whether administration of the specific 5HT1A antagonist, WAY-100635, attenuates CBD's anticonvulsant effect. In Experiment 2, we conducted a similar experiment using MDL-100907, a specific 5HT2A antagonist. If CBD reduces seizure activity by serving as an agonist at either of these receptors, then pre-treatment with a suitable antagonist should block its anticonvulsant properties.

2. Material & methods

2.1. Subjects

120 male, Wistar Kyoto rats (Charles River Laboratories, Wilmington, MA) were used in two successive experiments. They were tested in two separate cohorts: 60 subjects in Experiment 1 and 60 in Experiment 2. Subjects arrived at 21 days of age and were pair-housed in plastic cages with corncob bedding within a temperature-controlled (23 ± 2 °C) vivarium. The vivarium was programmed with a 12:12 light–dark schedule (lights on 6:00–18:00 h). Food and water were available ad libitum, and all subjects' cages were environmentally enriched with a Nylabone® and a short length of PVC pipe. All experimental protocols were approved by the campus Institutional Animal Care and Use Committee in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Procedure

Subjects were handled for several days prior to any experimental procedures. For four days leading up to the seizure tests, subjects were habituated to the seizure testing room and apparatus; each subject was placed in a seizure observation chamber for 15 min per day.

In Experiment 1, we tested whether the 5HT1A receptor is implicated in CBD's anticonvulsant activity. 60 subjects were randomly assigned to one of the four treatment conditions (n = 15 each), using a 2 (CBD vs. vehicle) × 2 (WAY-100635 vs. vehicle) design. The four groups were: 1) vehicle + vehicle, 2) CBD + vehicle, 3) CBD + WAY-100635, and 4) vehicle + WAY-100635. CBD was administered via intraperitoneal (IP) injection at a dose of 100 mg/kg, 60 min prior to seizure induction [18]. CBD was dissolved in a vehicle of 2:1:17 ethanol: Kolliphor: physiological saline. CBD was generously donated from GW Pharmaceuticals (Cambridge, UK). WAY-100635 (Sigma-Aldrich, St. Louis, MO) was administered via subcutaneous (SC) injection at a dose of 1 mg/kg, 20 min prior to CBD administration [19]. Vehicle was physiological saline. WAY-100635 was chosen because it is one of the most commonly used, selective 5HT1A antagonists used in behavioral pharmacology [20] and has been used previously to test the mechanism of action for other behavioral effects of CBD [15,16]. Dosage was based on previous research, including Lopez-Meraz et al. (2005), who used 1 mg/kg in male Wistar rats to assess the effects of various 5HT1A agonists on epileptic seizures in several different models [21]. Notably, 1 mg/kg of WAY-100635 did not significantly affect seizure incidence or intensity on its own.

In Experiment 2, we tested whether the 5HT2A receptor is implicated in CBD's anticonvulsant effects. 60 subjects were randomly assigned to one of four treatment conditions (n = 15 each), using a 2 (CBD vs. vehicle) × 2 (MDL-100907 vs. vehicle) design. These four groups were: 1) vehicle + vehicle, 2) vehicle + CBD, 3) MDL-100907 + vehicle, and 4) MDL-100907 + CBD. MDL-100907 (Sigma-Aldrich, St. Louis, MO) was administered via subcutaneous (SC) injection at a dose of 0.3 mg/kg, 80 min prior to seizure induction [22]. MDL-100907 was chosen because it is considered to be one of the most selective 5HT2A receptor antagonists available for preclinical studies [23–25]. With regard to dose, 0.3 mg/kg falls within the range used by previous researchers to successfully examine the functionality of the 5HT2A receptor in a variety of behavioral domains [25–27]. MDL-100907 was dissolved in a vehicle of 40% DMSO in physiological saline. CBD, as before, was administered via intraperitoneal (IP) injection at a dose of 100 mg/kg, 60 min prior to seizure induction.

The following procedures were identical for both Experiment 1 and Experiment 2. Between post-natal day (PND) 29–33, subjects were tested for seizure susceptibility. Seizure testing took place in a separate room under normal light conditions, beginning around 10:00 h. To induce seizure, subjects were administered a single intraperitoneal (IP) injection of 85 mg/kg of pentylenetetrazol (PTZ). Immediately after PTZ administration, each subject was transferred to a transparent plastic cage (27 × 16.5 × 12 cm) for observation. Four subjects — one from each treatment group — were tested simultaneously. A wooden divider was placed between cages so that subjects being tested simultaneously were not visible to each other. The seizure test lasted for 30 min. Tests were video-recorded using a tripod-mounted digital camera (NTSE SONY XC-EI50). Video was digitized at 60 Hz in 640 × 840 resolution onto a Macintosh computer. Video was processed using unpublished software (RatCam) using a frame-by-frame accurate timecode.

Seizure videos were subsequently coded by experimenters blind to the treatment condition of each subject. Individual seizures were scored on a 5-point severity scale appropriate for generalized seizures with forebrain origin [28,29]: 1, isolated myoclonic jerks; 2, atypical clonic seizure; 3, fully developed bilateral forelimb clonus; 3.5, forelimb clonus with tonic component and body twist; 4, tonic–clonic seizure with suppressed tonic phase, loss of righting; 5, fully developed tonic–clonic seizure with loss of righting. For each subject, latency to first sign of seizure and maximum seizure severity achieved were coded. The following group variables were then calculated: 1) mean latency to first seizure event, 2) median maximum seizure severity, 3) percentage of subjects that displayed any seizure activity (1 or greater), 4) percentage that

achieved tonic–clonic seizure (4 or 5), and 5) percentage mortality. Mortality was defined by the subject ceasing all respiratory and cardiac function at some point during the 30-min test. The group variables were based upon those described in Jones et al. [5].

2.3. Statistical analyses

Latencies were analyzed with a 2 × 2 analysis of variance (ANOVA). Median maximum seizure severities were analyzed with both a Kruskal–Wallis test and a median test, following by post-hoc Mann–Whitney tests. Group percentages were analyzed using multiple non-parametric binomial tests (as in Jones et al.), using the Holm–Bonferroni method to adjust alpha for multiple comparisons [5].

3. Results

3.1. Experiment 1

Table 1 summarizes the results of Experiment 1.

Fig. 1 displays the mean (±SEM) latency to first seizure event (for those subjects that experienced a seizure) for the four treatment groups. A 2 (CBD treatment) × 2 (WAY-100635 treatment) ANOVA on these data revealed no significant main effect of CBD ($F(1,36) = 3.27$, $p = 0.08$), no main effect of WAY-100635 ($F(1,36) = 0.58$, $p = 0.45$), and no interaction ($F(1,36) = 0.004$, $p = 0.95$).

Fig. 2 shows the percentage of subjects within each group that achieved each Racine stage (1–5). Fig. 3 displays the median maximum seizure severity for the four treatment groups. A non-parametric Kruskal–Wallis test revealed a significant difference in medians among all 4 groups (Chi-Square (3, N = 60) = 8.72, $p = 0.033$). A median test also revealed a significant difference between groups (Chi-Square (3, N = 60) = 8.80, $p = .032$). Post-hoc analysis consisted of three one-tailed Mann–Whitney tests, in which each experimental group was compared to the Veh + Veh control. An additional two-tailed Mann–Whitney test compared the Veh + CBD group to the WAY + CBD group to specifically assess whether WAY-100635 administration impacted the effects of CBD. The Holm–Bonferroni method was used to adjust alpha for 4 multiple comparisons (using an omnibus alpha of 0.05). The median maximum seizure in the Veh + Veh control group (median = 5) was significantly higher than the median value of each other experimental group. There was no significant difference between the Veh + CBD group and the WAY + CBD group.

Fig. 4 displays the percentage of subjects within each treatment group that a) showed any seizure activity, b) attained tonic–clonic seizure, and c) died during seizure testing. A non-parametric binomial test was utilized for each dependent variable, comparing the control (vehicle–vehicle) proportion to each other group's proportion. In addition, the Veh + CBD group was compared to the WAY + CBD group to specifically assess whether WAY-100635 administration impacted the effects of CBD. The Holm–Bonferroni method was again used to adjust alpha for 4 multiple comparisons (using an omnibus alpha of 0.05). For percentage showing any seizure activity (Fig. 4A), the Veh + CBD group (53.3%) and WAY + Veh group (60.0%) expressed significantly lower proportions than the control (86.7%). For the percentage showing tonic–clonic seizure (Fig. 4B), the control group percentage (53.3%) was significantly higher than that of the Veh + CBD group (20.0%), the WAY + Veh group (13.3%), and the WAY + CBD group (13.3%). For percentage mortality (Fig. 4C), the control group percentage (26.7%) was significantly higher than that of the WAY + Veh group (0.0%) and the WAY + CBD group (0.0%).

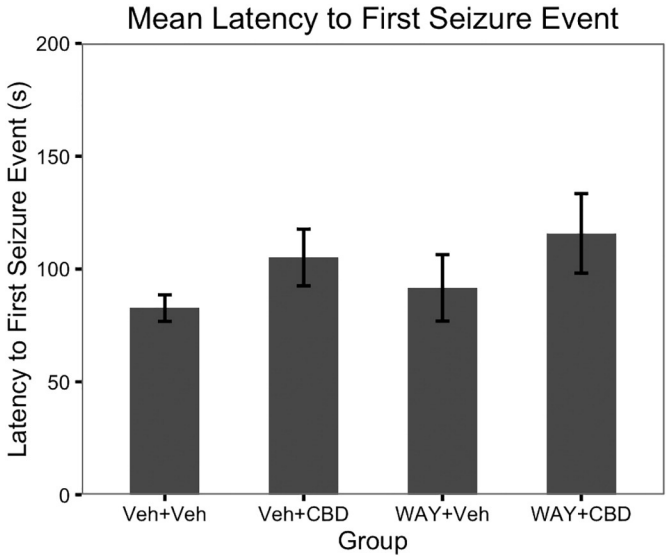

Fig. 1. The mean (±SEM) latency to the first seizure event for the four treatment groups in Experiment 1. Veh = Vehicle; CBD = cannabidiol; WAY = WAY-100635.

3.2. Experiment 2

Data from three subjects were lost due to corrupted video files. Table 2 summarizes the results of the MDL-100907 experiment.

Fig. 5 displays the mean (±SEM) latency to first seizure event (for those subjects that experienced a seizure) for the four treatment groups. A 2 (MDL treatment) × 2 (CBD treatment) ANOVA on these data revealed a significant main effect of CBD treatment ($F(1,43) = 5.84$, $p = 0.02$). The estimated marginal mean for vehicle-treated subjects was 79.7 s, while that for CBD-treated subjects was 99.3 s. There was no significant interaction between MDL and CBD treatment ($F(1,43) = 1.15$, $p = 0.29$).

Fig. 6 shows the percentage of subjects within each group that achieved each Racine stage (1–5). Fig. 7 shows the median maximum seizure severity for the four treatment groups. A non-parametric Kruskal–Wallis test revealed a significant difference in medians among all 4 groups (Chi-Square (3, N = 57) = 8.89, $p = 0.03$). A median test also revealed a significant difference between groups (Chi-Square (3, N = 57) = 11.84, $p = 0.008$). Post-hoc analysis consisted of three one-tailed Mann–Whitney tests, in which each experimental group was compared to the Veh + Veh control. In addition, a two-tailed test compared the Veh + CBD group to the MDL + CBD group to specifically assess whether MDL administration impacted the effects of CBD. The Holm–Bonferroni method was used to adjust alpha for 4 multiple comparisons (using an omnibus alpha of 0.05). These tests revealed no significant differences between individual groups.

Table 1
The Effects of WAY-100635 on CBD's anticonvulsant efficacy.

	Veh + Veh (n = 15)	Veh + CBD (n = 15)	WAY + Veh (n = 15)	WAY + CBD (n = 15)
Mean (±SEM) latency to 1st seizure (sec)	82.7 (±5.9)	105.3 (±12.5)	91.7 (±14.7)	115.8 (±17.6)
Median (±IQR) max seizure severity	5.0 (±1.5)	3.0 (±3.5)	3.5 (±3.5)	1.0 (±3.5)
% showing any seizure	86.7	53.3	60.0	66.7
% showing tonic–clonic seizure	53.3	20.0	13.3	13.3
% mortality	26.7	6.7	0.0	0.0

Veh = Vehicle; CBD = cannabidiol; WAY = WAY-100635.

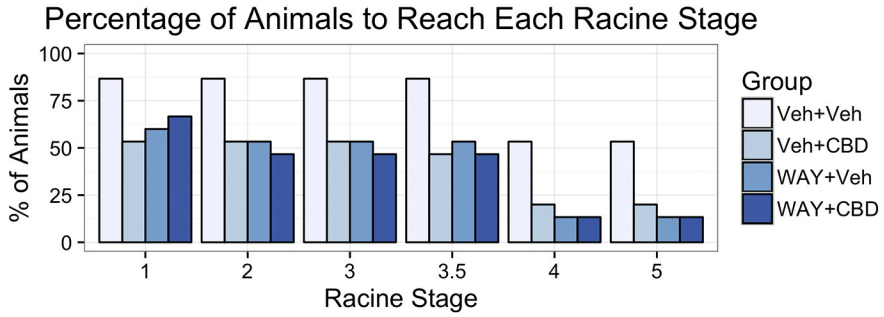

Fig. 2. The percentage of subjects within each treatment group in Experiment 1 that achieved each stage of the Racine seizure severity scale. Veh = Vehicle; CBD = cannabidiol; WAY = WAY-100635.

Fig. 8 displays the percentage of subjects within each treatment group that a) showed any seizure activity, b) attained tonic–clonic seizure, and c) died during seizure testing. A non-parametric binomial test was utilized for each of these dependent variables, comparing the control (Veh–Veh) proportion to each other group's proportion. In addition, the Veh + CBD group was compared to the MDL + CBD group to specifically assess whether MDL administration impacted the effects of CBD. The Holm–Bonferroni method was used to adjust alpha for 4 multiple comparisons (using an omnibus alpha of 0.05). For percentage showing any seizure activity (Fig. 8A), there were no significant group differences. For percentage showing tonic–clonic seizure (Fig. 8B), the Veh + CBD group (40%) and MDL + CBD group (7.1%) had significantly lower percentages than the Veh + Veh control (71.4%). In addition, the MDL + CBD group expressed a significantly lower percentage than the Veh + CBD group. For percentage mortality (Fig. 8C), the Veh + CBD group (6.7%) and MDL + CBD group (0.0%) had significantly lower percentages than the Veh + Veh control (57.1%).

4. Discussion

We had two goals in conducting this research: 1) to independently replicate and validate the anticonvulsant effects of CBD in a common animal model of generalized seizure, and 2) explore CBD's anticonvulsant mechanism of action. With regard to goal 1, both experiments demonstrated that 100 mg/kg CBD significantly attenuated PTZ-induced seizure in adolescent Wistar-Kyoto rats. In Experiment 1, CBD significantly reduced the 1) median maximum seizure severity, 2) the percentage of subjects experiencing any seizure activity, and 3) the percentage of subjects achieving tonic–clonic seizure. There was a strong, but non-significant, trend for CBD to also reduce mortality rate. In Experiment 2, CBD treatment significantly 1) increased the latency to first seizure event in those subjects that displayed seizure activity, 2) reduced the percentage of subjects showing tonic–clonic seizure, and 3) reduced mortality. There was a strong, but non-significant, trend for CBD to reduce maximum seizure severity.

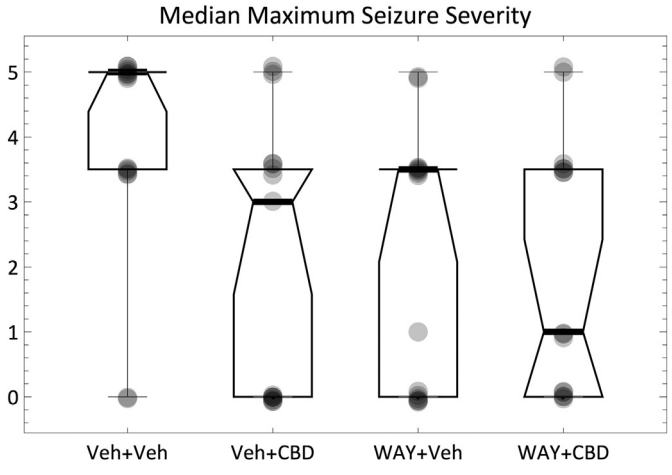

Fig. 3. Box & whisker plots for the median maximum seizure severities of the four treatment groups in Experiment 1. Medians are thick black lines, with boxes showing 25th and 75th percentiles; whiskers denote 5th and 95th percentiles. Individual (semi-transparent) data points are overlaid with slight jitter. Veh = Vehicle; CBD = cannabidiol; WAY = WAY-100635. A non-parametric Kruskal–Wallis test revealed an overall significant difference in medians. Post-hoc Mann–Whitney tests (with alpha adjusted via the Holm-Bonferroni method) indicated that the median maximum seizure of the Veh + Veh control group was significantly higher than the median value of each other experimental group.

The slight variation in CBD results observed between the experiments may have been caused by using different cohorts of animals or different lot numbers of PTZ. For example, in Experiment 2, we observed more intense seizure activity overall in our Veh + Veh controls compared to the Veh + Veh controls in Experiment 1. This included a higher rate of tonic–clonic seizure (71.4% in Experiment 2 vs. 53.3% in Experiment 1) and mortality (57.1% in Experiment 2 vs. 26.7% in Experiment 1). Importantly, however, in both experiments the mortality rate of subjects given CBD was strikingly low and consistent (6.7% in each case). Furthermore, while there was some variation in which dependent variables were impacted most strongly by CBD across experiments, the overall pattern of results between them was quite similar.

Taken as a whole, these results provide compelling evidence that CBD is capable of calming neurological seizure activity and preventing the most severe consequences of seizure (e.g., progression to full-body, tonic–clonic activity and death). Recent years have witnessed burgeoning interest among scientists, clinicians, and patients in the potential therapeutic application of CBD for treatment of severe epilepsy disorders, including Dravet's syndrome [30]. Animal work has largely been consistent in showing an anticonvulsant effect of CBD via many different seizure models, including maximal electroshock, cobalt, pilocarpine, penicillin, and numerous GABA antagonists [2,3]. Clinical trials testing CBD's effectiveness are currently ongoing; one recent report indicated that CBD has an adequate safety profile in children and young adults with severe epilepsy and may significantly reduce seizure [4]. However, large randomized controlled trials are needed to confirm these hypotheses.

The second goal of the current experiment was to test whether CBD's anticonvulsant effects are mediated through either the serotonergic 5HT1A or 5HT2A receptor. As such, we hypothesized that pretreatment with appropriate antagonists (WAY-100635 or MDL-100907, respectively) 20 min prior to CBD administration would significantly attenuate CBD's anticonvulsant effects. Our results did not support these hypotheses. Within Experiment 1, there were no significant differences between the Veh + CBD group and the WAY + CBD group on any seizure variable, including: median seizure severity, percentage experiencing any seizure (53.3% vs. 66.7%), percentage displaying tonic–clonic seizure (20.0% vs. 13.3%), and percentage mortality (6.7% vs. 0.0%).

Contrary to expectation, subjects given just WAY-100635 (without CBD) displayed a significant reduction in seizure activity compared to vehicle controls. Furthermore, there was little identifiable difference in

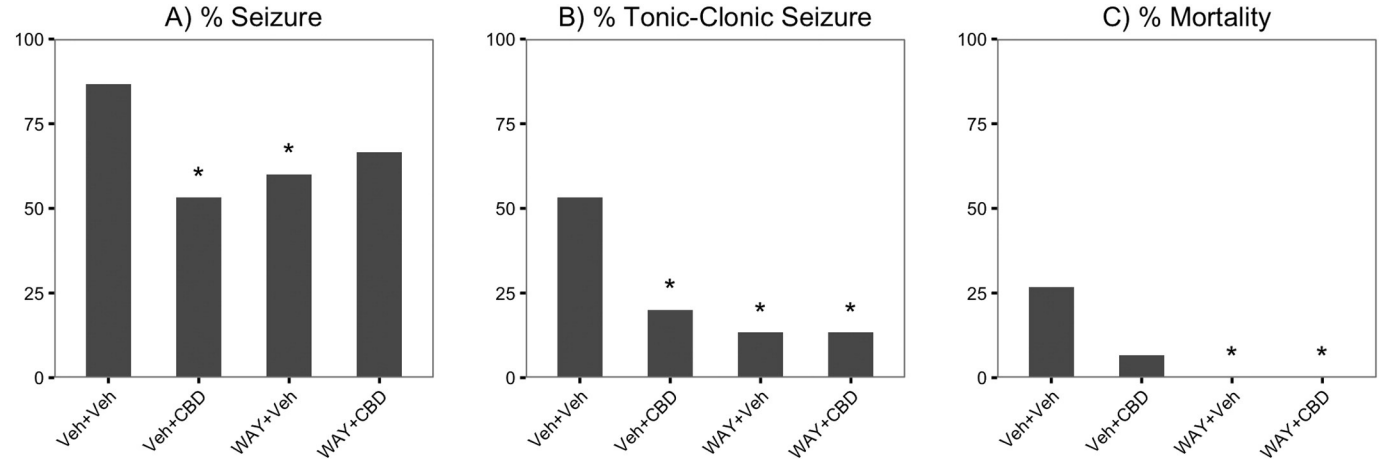

Fig. 4. The percentage of subjects within each group of Experiment 1 that A) displayed any seizure activity, B) displayed tonic–clonic seizure, and C) expired during testing. Veh = Vehicle; CBD = cannabidiol; WAY = WAY-100635. * indicates a significant difference from the Veh + Veh control, as assessed by a non-parametric binomial test (adjusted via the Holm–Bonferroni method for multiple comparisons).

the behavior of these subjects compared to either the CBD-treated group or the CBD + WAY group. This was unexpected, given previous evidence that 5HT1A receptor agonists are often anticonvulsant [12]. However, the literature in this area has been inconsistent. For example, Lopez-Meraz et al. (2005) tested the effects of two 5HT1A agonists (8-OH-DPAT and Indorenate) and one antagonist (WAY-100635) on PTZ-induced (60 mg/kg) seizures in male Wistar rats [21]. 8-OH-DPAT treatment dose-dependently reduced the percentage of rats with tonic extension and the mortality rate. Conversely, 1 mg/kg 8-OH-DPAT significantly increased the number of clonic seizures (an effect which was blocked by WAY-100635). Indorenate dose-dependently increased seizure latencies and reduced the percentage of subjects presenting clonic seizure, tonic extension, and death. WAY-100635 (1 mg/kg, SC) by itself had no significant effect on seizure activity.

There are additional findings that other 5HT1A antagonists may possess anticonvulsant efficacy under certain experimental conditions. For example, Moreau at el. reported that (S)-UH-301, a selective 5HT1A antagonist, exhibited dose-dependent anticonvulsant activity in two different mouse models of acute seizure (audiogenic seizures and ICV administration of NMDA in DBA/2J mice) [31]. Graf et al. (2004) noted an anticonvulsant effect of WAY-100635 in WAG/Rij rats, which exhibit spontaneously occurring spike–wave discharges (SWDs) and behavioral symptoms similar to those seen in human absence epilepsy [32]. Subjects were given 0.2 mg/kg of WAY-100635 (IP) prior to 5 h of EEG and EMG recordings. Interestingly, WAY-100635 caused an initial increase in SWDs and paroxysms (30–60 min after administration), but then significantly reduced seizure activity in the following 4 h. These results suggest that there may be biphasic effects of 5HT1A antagonism on seizure activity.

Brain 5HT1A receptors can be either pre-synaptic, inhibitory autoreceptors (within the raphe nuclei, for instance), or post-synaptic (within forebrain/limbic terminal regions). Dose-dependent and biphasic effects of 5HT1A antagonists may, therefore, result from differential activation of pre- vs. post-synaptic receptors and potentially distinctive roles of these receptor categories in regulating seizure activity. For example, if a particular dose of WAY-100635 preferentially blocks autoreceptors within the raphe nuclei, terminal regions like the hippocampus may experience a boost of 5HT transmission that has an anti-seizure effect. Complex 5HT1A-mediated effects like these have been noted in the preclinical anxiety literature; 5HT1A antagonists can both block the anxiolytic effects of other drugs, and induce anxiolytic effects on their own following systemic administration [16,33].

One final factor to consider is that WAY-100635 may not be as selective an antagonist as many pharmacologists have previously assumed; specifically, it appears to act as a potent agonist at the dopaminergic D4 receptor [34]. While there is no conclusive evidence that D4 agonists possess anticonvulsant efficacy, D4 receptors (like other members of the D2 family) may help inhibit neuronal hyperexcitability in limbic regions [35].

In Experiment 2, we found no evidence that antagonism of 5HT2A receptors attenuated CBD's anticonvulsant effects. The Veh + CBD group and MDL + CBD group did not differ on: 1) median seizure severity, percentage experiencing any seizure (93.3% vs. 92.9%), or percentage mortality (6.7% vs. 0.0%). However, our analysis of tonic–clonic seizure revealed that the Veh + CBD group expressed a *higher* percentage compared to the MDL + CBD group (40.0% vs. 7.1%). The direction of this effect suggests that MDL pre-treatment may have boosted CBD's ability to prevent tonic–clonic seizure. However, given that MDL did not have a similar effect on any other seizure variable, we must be cautious in our interpretation. Notably, MDL treatment by itself did not affect seizure severity; there were no significant differences between the Veh + Veh control and the MDL + Veh group on any dependent variable.

The most likely interpretation of the data from both Experiment 1 and Experiment 2 is that CBD is not working through the 5HT1A or 5HT2A receptor to block or reduce seizure activity. While serotonergic signaling is implicated in seizure susceptibility [12] and CBD shows binding affinity for both 5HT1A and 5HT2A [9], these signaling systems may not mediate CBD's anticonvulsant effects. However, a limitation of the current experiments was utilization of only a single dose of both

Table 2
The Effects of MDL-100907 on CBD's anticonvulsant efficacy.

	Veh + Veh (n = 14)	Veh + CBD (n = 15)	MDL + Veh (n = 14)	MDL + CBD (n = 14)
Mean (±SEM) latency to 1st seizure (sec)	82.3 (±11.7)	93.2 (±6.2)	76.6 (±4.6)	104.8 (±10.1)
Median (±IQR) max seizure severity	5.0 (±3.0)	3.5 (±1.5)	5.0 (±1.5)	3.25 (±2.0)
% showing any seizure	78.6	93.3	100.0	92.9
% showing tonic–clonic seizure	71.4	40.0	57.1	7.1
% mortality	57.1	6.7	35.7	0.0

Veh = Vehicle; CBD = cannabidiol; MDL = MDL-100907.

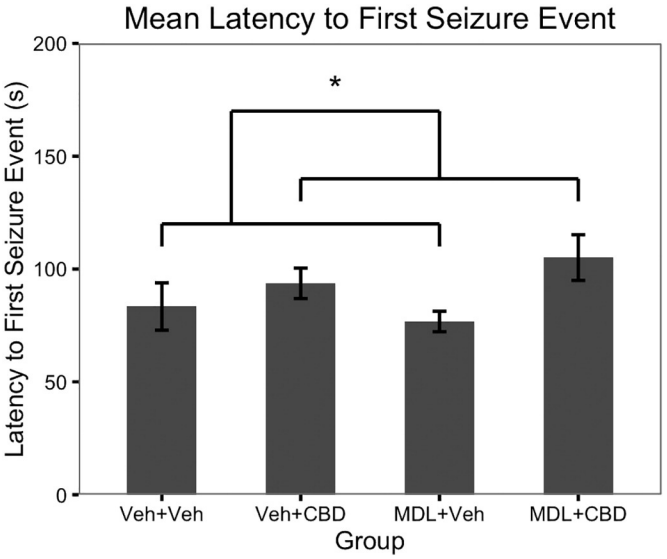

Fig. 5. The mean (±SEM) latency to the first seizure event for the four treatment groups in Experiment 2. Veh = Vehicle; CBD = cannabidiol; MDL = MDL-100907. There was a significant main effect of CBD treatment ($F(1,43) = 5.84$, $p = 0.02$), such that the CBD-treated subjects expressed a higher mean latency to first seizure (estimated marginal mean = 99.3 s) compared to vehicle-treated subjects (estimated marginal mean = 79.7 s).

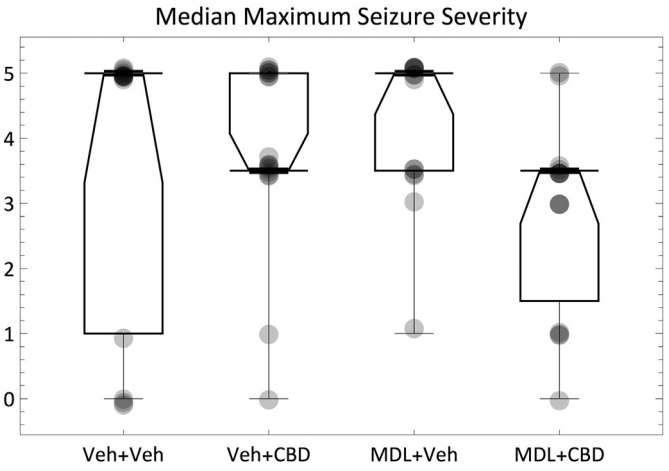

Fig. 7. Box & whisker plots for the median maximum seizure severities of the four treatment groups in Experiment 2. Medians are thick black lines, with boxes showing 25th and 75th percentiles; whiskers denote 5th and 95th percentiles. Individual (semi-transparent) data points are overlaid with slight jitter. Veh = Vehicle; CBD = cannabidiol; MDL = MDL-100907. A non-parametric Kruskal–Wallis test revealed an overall significant difference in medians between the 4 groups, although post-hoc comparisons revealed no significant group by group differences.

CBD and the selected 5HT antagonist. One implication of this is that we cannot be absolutely certain that a 100 mg/kg dose of CBD produces a brain concentration that allows for activation of 5HT1A/5HT2A receptors. In vitro evidence of CBD's activity at 5HT1A receptors, for instance, is based upon relatively high concentrations: 16 and 32 μM in Russo et al. and 10 μM in Ledgerwood et al. [9,17]. A pharmacokinetic profile of CBD revealed that an IP dose of 120 mg/kg in rats led to substantial brain concentrations following rapid absorption (brain $C_{max} = 6.8$ μg/ml; $T_{max} = 120$ min), but no laboratory has measured specific concentrations of CBD within regions of interest, such as the hippocampus, following systemic administration [18]. However, we note that Resstel et al. [36] found that a 0.1 mg/kg systemic dose of WAY-100635 blocked the anxiolytic effect of 10 mg/kg CBD (administered intraperitoneally to male Wistar rats). Thus, there is behavioral evidence that systemic doses of CBD lower than 100 mg/kg act upon 5HT1A receptors involved in emotion and behavior [36].

As such, future studies should continue to explore the role of serotonergic receptors in mediating CBD's anticonvulsant activity using other pharmacological and non-pharmacological tools. In particular, it would be extraordinarily valuable for researchers to assess CBD's antiseizure effects in genetic models characterized by reduced 5HT1A/5HT2A functionality: e.g., in vitro effects in tissue with 5HT receptor targeted knockdown or in vivo effects in 5HT receptor KO mice. Lack of CBD's efficacy in such models would give us increased confidence in rejecting 5HT1A and 5HT2A as putative molecular targets.

While the current data do not support the hypothesis that 5HT1A receptors are implicated in CBD's anticonvulsant mechanism of action, it is still possible that CBD acts through this receptor for other physiological and behavioral effects. The strongest evidence for this may lie within the domain of anxiety. Systemic CBD administration has demonstrated anxiolytic effects in a number of animal models [e.g., [36–38]], and 5HT1A receptor antagonism reliably attenuates these anxiolytic effects [16,36,39,40].

As noted in the Introduction section, CBD is a pharmacologically complex molecule that may rely upon several biochemical mechanisms to attenuate seizure activity [2,3,8]. There is evidence, largely based on in vitro experimentation, that CBD can affect dozens of different receptors, enzymes, ion channels, and transporters. However, a primary focus of current researchers is to identify those molecular targets which are most plausibly linked to CBD's therapeutic effects — a valid concern, given CBD's limited bioavailability and the utilization of supraphysiological concentrations in many in vitro experiments [8]. Two of the more likely mechanisms are blockade of voltage-gated calcium channels and blockade of the orphan receptor, GPR55; both involve regulation of intracellular calcium. In addition, there is significant interest in CBD's ability to enhance adenosine activity via uptake inhibition. This may be linked to neuronal hyperpolarization and reduced

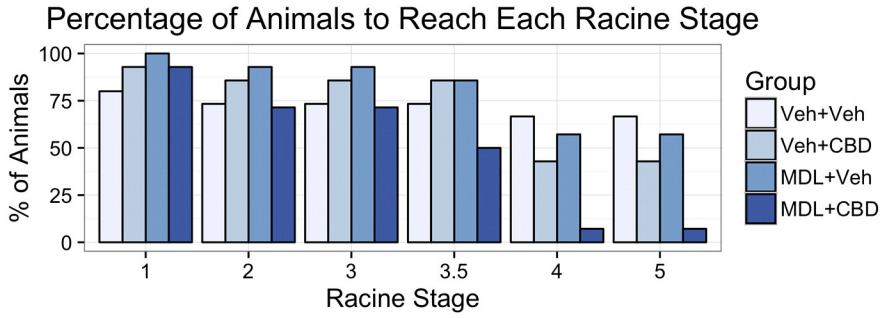

Fig. 6. The percentage of subjects within each treatment group in Experiment 2 that achieved each stage of the Racine seizure severity scale. Veh = Vehicle; CBD = cannabidiol; MDL = MDL-100907.

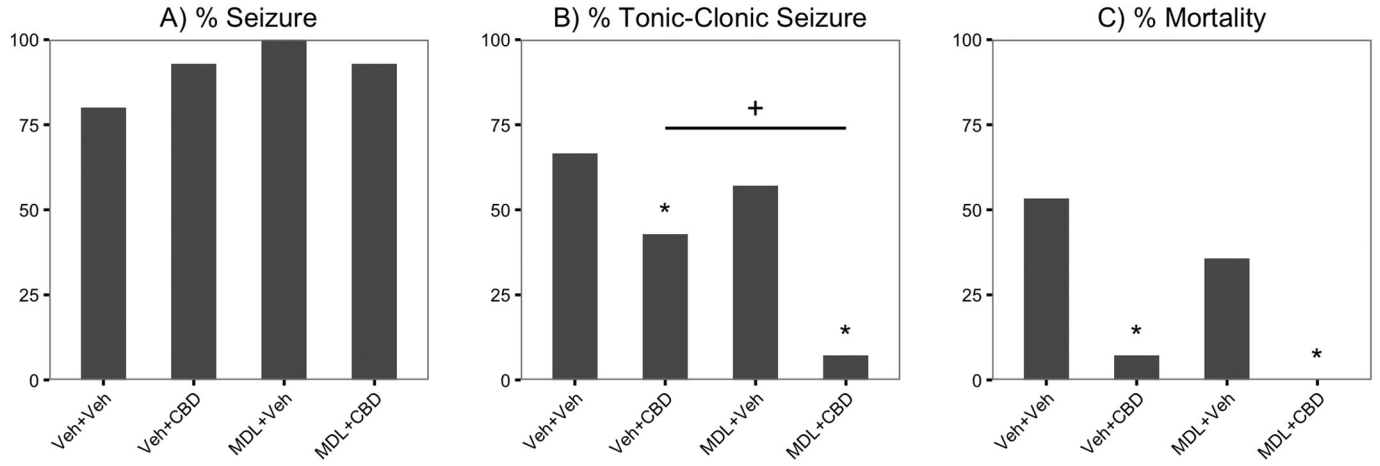

Fig. 8. The percentage of subjects within each group of Experiment 2 that A) displayed any seizure activity, B) displayed tonic–clonic seizure, and C) expired during testing. Veh = Vehicle; CBD = cannabidiol; MDL = MDL-100907. * indicates a significant difference from the Veh + Veh control, as assessed by a non-parametric binomial test (adjusted via the Holm–Bonferroni method for multiple comparisons). + indicates a significant difference between the Veh + CBD group and the MDL + CBD group.

exocytosis, as well as neuroprotection against damage occurring during seizure [8,41]. We must not be thwarted by the complexity of CBD, but continue exploring the various mechanisms by which it exerts its clinical effects, if CBD and other cannabinoids are to gain traction within the biomedical community as viable treatment options for neurological disorders.

5. Conclusion

Further validating previous preclinical studies [5,6], we found that 100 mg/kg of CBD significantly attenuated the most severe aspects of acute seizure induced by PTZ in male Wistar Kyoto rats. Many researchers have speculated that CBD's anticonvulsant properties may, in part, be linked to agonism at either the serotonergic 5HT1A or 5HT2A receptor [2,8]. Our study is the first to explore these specific hypotheses regarding CBD's mechanism of action. We found no evidence that CBD worked through 5HT1A or 5HT2A to reduce seizure activity in this model.

Conflicts of interest

None of the authors has any conflict of interest to disclose.

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References

[1] Kwan P, Brodie MJ. Emerging drugs for epilepsy. Expert Opin Emerg Drugs 2007;12(3):407–22.
[2] Devinsky O, Cilio MR, Cross H, Fernandez-Ruiz J, French J, Hill C, et al. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. Epilepsia 2014;55(6):791–802.
[3] Leo A, Russo E, Elia M. Cannabidiol and epilepsy: rationale and therapeutic potential. Pharmacol Res 2016;107:85–92.
[4] Devinsky O, Marsh E, Friedman D, Thiele E, Laux L, Sullivan J, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. Lancet Neurol 2016;15(3):270–8.
[5] Jones NA, Hill AJ, Smith I, Bevan SA, Williams CM, Whalley BJ, et al. Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. J Pharmacol Exp Ther 2010;332(2):569–77.
[6] Jones NA, Glyn SE, Akiyama S, Hill TD, Hill AJ, Weston SE, et al. Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. Seizure 2012;21(5):344–52.
[7] Kliqueva IA, van Luijtelaar EL, Chepurnova NE, Chepurnov SA. PTZ-induced seizures in rats: effects of age and strain. Physiol Behav 2001;72(3):421–6.
[8] Bih CI, Chen T, Nunn AV, Bazelot M, Dallas M, Whalley BJ. Molecular targets of cannabidiol in neurological disorders. Neurotherapeutics 2015;12(4):699–730.
[9] Russo EB, Burnett A, Hall B, Parker KK. Agonistic properties of cannabidiol at 5-HT1a receptors. Neurochem Res 2005;30(8):1037–43.
[10] Theodore WH. Does serotonin play a role in epilepsy? Epilepsy Curr 2003;3(5):173–7.
[11] Toczek MT, Carson RE, Lang L, Ma Y, Spanaki MV, Der MG, et al. PET imaging of 5-HT1A receptor binding in patients with temporal lobe epilepsy. Neurology 2003;60(5):749–56.
[12] Gharedaghi MH, Seyedabadi M, Ghia JE, Dehpour AR, Rahimian R. The role of different serotonin receptor subtypes in seizure susceptibility. Exp Brain Res 2014;232(2):347–67.
[13] Ceulemans B, Boel M, Leyssens K, Van Rossem C, Neels P, Jorens PG, et al. Successful use of fenfluramine as an add-on treatment for Dravet syndrome. Epilepsia 2012;53(7):1131–9.
[14] Guiard BP, Di Giovanni G. Central. Serotonin-2A (5-HT2A) receptor dysfunction in depression and epilepsy: the missing link? Front Pharmacol 2015;6:46.
[15] Zanelati TV, Biojone C, Moreira FA, Guimaraes FS, Joca SR. Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. Br J Pharmacol 2010;159(1):122–8.
[16] Gomes FV, Resstel LB, Guimarães FS. The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT1A receptors. Psychopharmacology (Berl) 2011;213(2–3):465–73.
[17] Ledgerwood CJ, Greenwood SM, Brett RR, Pratt JA, Bushell TJ. Cannabidiol inhibits synaptic transmission in rat hippocampal cultures and slices via multiple receptor pathways. Br J Pharmacol 2011;162(1):286–94.
[18] Deiana S, Watanabe A, Yamasaki Y, Amada N, Arthur M, Fleming S, et al. Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive–compulsive behaviour. Psychopharmacology (Berl) 2012;219(3):859–73.
[19] Fletcher A, Forster EA, Bill DJ, Brown G, Cliffe IA, Hartley JE, et al. Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT 1A receptor antagonist. Behav Brain Res 1995;73(1):337–53.
[20] Forster EA, Cliffe IA, Bill DJ, Dover GM, Jones D, Reilly Y, et al. A pharmacological profile of the selective silent 5-HT1A receptor antagonist, WAY-100635. Eur J Pharmacol 1995;281(1):81–8.
[21] López-Meraz ML, González-Trujano ME, Neri-Bazán L, Hong E, Rocha LL. 5-HT 1A receptor agonists modify epileptic seizures in three experimental models in rats. Neuropharmacology 2005;49(3):367–75.
[22] Knauer CS, Campbell JE, Galvan B, Bowman C, Osgood S, Buist S, et al. Validation of a rat in vivo [3 H] M100907 binding assay to determine a translatable measure of 5-HT 2A receptor occupancy. Eur J Pharmacol 2008;591(1):136–41.
[23] Herth MM, Kramer V, Piel M, Palner M, Riss PJ, Knudsen GM, et al. Synthesis and in vitro affinities of various MDL 100907 derivatives as potential 18 F-radioligands for 5-HT 2A receptor imaging with PET. Bioorg Med Chem 2009;17(8):2989–3002.
[24] Kehne JH, Baron BM, Carr AA, Chaney SF, Elands J, Feldman DJ, et al. Preclinical characterization of the potential of the putative atypical antipsychotic MDL 100,907 as a potent 5-HT2A antagonist with a favorable CNS safety profile. J Pharmacol Exp Ther 1996;277(2):968–81.
[25] Nic Dhonnchadha BA, Fox RG, Stutz SJ, Rice KC, Cunningham KA. Blockade of the serotonin 5-HT2A receptor suppresses cue-evoked reinstatement of cocaine-seeking behavior in a rat self-administration model. Behav Neurosci 2009;123(2):382.

[26] Boothman LJ, Allers KA, Rasmussen K, Sharp T. Evidence that central 5-HT2A and 5-HT2B/C receptors regulate 5-HT cell firing in the dorsal raphe nucleus of the anaesthetised rat. Br J Pharmacol 2003;139(5):998–1004.
[27] Hitchcock JM, Lister S, Fischer TR, Wettstein JG. Disruption of latent inhibition in the rat by the 5-HT 2 agonist DOI: effects of MDL 100,907, clozapine, risperidone and haloperidol. Behav Brain Res 1997;88(1):43–9.
[28] Pohl M, Mares P. Effects of flunarizine on metrazol-induced seizures in developing rats. Epilepsy Res 1987;1(5):302–5.
[29] Velíšková JA. Behavioral characterization of seizures in rats. Models of seizures and epilepsy. Amsterdam: Elsevier Academic Press; 2006. p. 601–11.
[30] Cilio MR, Thiele EA, Devinsky O. The case for assessing cannabidiol in epilepsy. Epilepsia 2014;55(6):787–90.
[31] Moreau JL, Griebel G, Jenck F, Martin JR, Widmer U, Haefely WE. Behavioral profile of the 5HT 1A receptor antagonist (S)-UH-301 in rodents and monkeys. Brain Res Bull 1992;29(6):901–4.
[32] Graf M, Jakus R, Kantor S, Levay G, Bagdy G. Selective 5-HT 1A and 5-HT 7 antagonists decrease epileptic activity in the WAG/Rij rat model of absence epilepsy. Neurosci Lett 2004;359(1):45–8.
[33] Griebel G, Rodgers RJ, Perrault G, Sanger DJ. The effects of compounds varying in selectivity as 5-HT(1A) receptor antagonists in three rat models of anxiety. Neuropharmacology 2000;39(10):1848–57.
[34] Chemel BR, Roth BL, Armbruster B, Watts VJ, Nichols DE. WAY-100635 is a potent dopamine D4 receptor agonist. Psychopharmacology (Berl) 2006;188(2):244–51.
[35] Bozzi Y, Borrelli E. The role of dopamine signaling in epileptogenesis. Front Cell Neurosci 2013;7:157.
[36] Resstel L, Tavares RF, Lisboa SF, et al. 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. Br J Pharmacol 2009;156(1):181–8.
[37] Guimarães FS, Chiaretti TM, Graeff FG, Zuardi AW. Antianxiety effect of cannabidiol in the elevated plus-maze. Psychopharmacology (Berl) 1990;100(4):558–9.
[38] Resstel L, Tavares RF, Lisboa SF, Joca SR, Correa F, Guimarães FS. Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. Prog Neuropsychopharmacol Biol Psychiatry 2006;30(8):1466–71.
[39] Campos AC, Guimarães FS. Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. Psychopharmacology (Berl) 2008;199(2):223–30.
[40] de Paula Soares V, Campos AC, de Bortoli VC, Zangrossi H, Guimarães FS, Zuardi AW. Intra-dorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors. Behav Brain Res 2010;213(2):225–9.
[41] Świąder MJ, Kotowski J, Łuszczki JJ. Modulation of adenosinergic system and its application for the treatment of epilepsy. Pharmacol Rep 2014;66(3):335–42.