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Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures

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ABSTRACT

Cannabis sativa has been associated with contradictory effects upon seizure states despite its medicinal use by numerous people with epilepsy. We have recently shown that the phytocannabinoid cannabidiol (CBD) reduces seizure severity and lethality in the well-established *in vivo* model of pentylenetetrazole-induced generalised seizures, suggesting that earlier, small-scale clinical trials examining CBD effects in people with epilepsy warrant renewed attention. Here, we report the effects of pure CBD (1, 10 and 100 mg/kg) in two other established rodent seizure models, the acute pilocarpine model of temporal lobe seizure and the penicillin model of partial seizure. Seizure activity was video recorded and scored offline using model-specific seizure severity scales. In the pilocarpine model CBD (all doses) significantly reduced the percentage of animals experiencing the most severe seizures. In the penicillin model, CBD (≥10 mg/kg) significantly decreased the percentage mortality as a result of seizures; CBD (all doses) also decreased the percentage of animals experiencing the most severe tonic–clonic seizures. These results extend the anti-convulsant profile of CBD; when combined with a reported absence of psychoactive effects, this evidence strongly supports CBD as a therapeutic candidate for a diverse range of human epilepsies.
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1. Introduction

Dating back to 4000 BC, *Cannabis sativa* has a long history of medicinal use for the treatment of a variety of disorders such as rheumatism, chronic inflammation and pain management, in addition to control of convulsions.[1] More recently, *C. sativa* has been ascribed both pro-[2] and anti-convulsant effects[3] despite numerous people with epilepsy continuing to use *C. sativa* medicinally for seizure control.[4,5] Since Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent of *C. sativa* was isolated,[6] more than 60 other phytocannabinoids (cannabis-derived components) have also been identified, isolated and shown to possess varied pharmacological activity.[7] One such phytocannabinoid is cannabidiol (CBD), typically the second most prevalent phytocannabinoid in *C. sativa*, whose structure was first described by Mechoulam and Shvo.[8] CBD currently represents the most promising phytocannabinoid candidate for clinical utilisation due to its non-psychotropic properties, low toxicity and high tolerability in humans and other animal species.[9–11]

Early preclinical work demonstrated that CBD possesses anti-convulsant properties.[12–14] In rats, CBD was an effective and relatively potent anti-convulsant in the maximal electroshock (MES) and audiogenic seizure models; findings that compared favourably with the clinically used AEDs employed in the same study.[15] In mice, CBD pretreatment prevented tonic convulsions caused by either MES seizures, γ-aminobutyric acid (GABA) antagonists or inhibitors of GABA synthesis, in addition to reliably protecting against 3-mercaptoproprionic acid-induced lethality.[10] Overall, these pre-clinical seizure studies confirmed CBD's anti-convulsant profile and are consistent with an assertion of therapeutic benefits in human epilepsies.

Interestingly and despite these promising pre-clinical results, only one clinical trial has thus far explored the potential anti-convulsant effects of CBD in humans.[11] Fifteen people experiencing secondary generalised epilepsy with temporal lobe focus that was

Abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol; AED, anti-epileptic drug; CBD, cannabidiol; CCTVs, closed-circuit television cameras; CNS, central nervous system; GABA, γ-aminobutyric acid; mAChR, muscarinic acetylcholine receptor; MES, maximal electroshock; NMDA, *N*-methyl-D-aspartate; PTZ, pentylenetetrazole; TLE, temporal lobe epilepsy.

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unresponsive to prescribed AED treatments were recruited. Four of eight of those receiving CBD in conjunction with their existing AEDs remained virtually seizure-free during the supplementation period and the remainder of this patient group exhibited a marked improvement in seizure control.[11] Surprisingly however, no further clinical trials employing CBD have been published.

The therapeutic potential of the phytocannabinoids attracted renewed interest following the discovery and characterisation of the endocannabinoid signalling system that comprises the G protein-coupled cannabinoid CB_1 and CB_2 receptors, a family of endogenous cannabinoid receptor ligands and several enzymes involved in their metabolism and degradation.[16] Whilst a number of phytocannabinoid actions are mediated *via* CB_1 and/or CB_2 receptors,[7,17] including the now well-known CB1 receptor-mediated modulation of epileptiform and seizure activity,[18,19] CBD exhibits negligible affinity for either CB_1 and/or CB_2 receptors.[7,20,21] Consequently, it is likely that the anti-convulsant effects of CBD described above arise *via* cannabinoid receptor-independent mechanisms.[17,22–24]

Recently, we have shown that CBD inhibits epileptiform activity *in vitro* and reduces seizure severity and lethality in the pentylenetetrazole (PTZ) model of generalised seizures *in vivo*, strongly supporting reconsideration of the use of CBD in the treatment of human epilepsies.[22] However, in order to strengthen earlier findings and inform appropriate human study design, assessment of the anti-convulsant potential of CBD against untested seizure phenotypes *in vivo* is required.

In this present study, we have investigated whether CBD exerts anti-convulsant effects in the acute pilocarpine-induced model of temporal lobe seizure and the penicillin-induced model of partial seizure in rat. Furthermore, four behavioural tests were undertaken to assess the effects of CBD on rodent motor function, providing complementary evidence of CBD's lack of toxicity.

2. Materials and methods

2.1. Animals

Adult male Wistar Kyoto rats (Harlan, Bicester, UK) were used in both seizure models and the rotarod test described below (acute pilocarpine model of temporal lobe seizure: >P21, 70–110 g; penicillin model of partial seizure: >P40, 250–300 g; motor function tests: >P28, starting weight 110–140 g). Animals were housed at room temperature on a 12:12-h day/night cycle (lights on at 0800) and given *ad libitum* access to food and water. On days prior to seizure induction, animals were habituated to handling, experimental procedures and the test environment. All experiments were carried out in accordance with UK Home Office regulations (Animals (Scientific Procedures) Act, 1986).

2.2. CBD administration

CBD penetrates the blood–brain barrier such that 120 mg/kg delivered intraperitoneally in Wistar Kyoto rats provides C_{max} = 6.8 μg/g at T_{max} = 120 min and, at the same dosage, no major toxicity, genotoxicity, or mutagenicity has been observed (personal communication with GW Pharmaceuticals Ltd.; Study Report UNA-REP-02). Prior to seizure or motor function protocols, animals received (i.p.) 1, 10 or 100 mg/kg CBD in all seizure experiments or 50, 100 or 200 mg/kg CBD in motor function tests (GW Pharmaceuticals, Porton Down, Wiltshire, UK). The vehicle employed was a 1:1:18 solution of ethanol, Cremophor (Sigma–Aldrich, Poole, UK) and 0.9% (w/v) NaCl. In each experiment, a group of animals that received volume-matched doses of vehicle alone served as a negative control.

2.3. Acute pilocarpine in vivo seizure model

Pilocarpine is a muscarinic acetylcholine receptor agonist that, following systemic administration, causes localised seizure foci in the limbic system consistent with temporal lobe seizures[25] ($n \geq 14$ for each group). 15 min after CBD or vehicle administration, animals were injected with the muscarinic receptor antagonist methylscopolamine (Sigma–Aldrich, Poole, UK; 1 mg/kg; i.p.) to minimise peripheral pilocarpine-induced side-effects. 45 min later, pilocarpine (Sigma–Aldrich, Poole, UK; 380 mg/kg; i.p.) was administered to induce seizures and animal behaviour was monitored for a further 60 min. On completion of the experimental procedure animals were euthanised by CO_2 inhalation.

2.4. Penicillin in vivo seizure model

Penicillin selectively antagonises $GABA_A$-receptor mediated inhibitory postsynaptic potentials in the central nervous system (CNS).[26,27] Surgical implantations of cannulae were required to enable the focal administration of penicillin G potassium salt (penicillin; Sigma–Aldrich) directly into the cerebral ventricles to induce partial seizures.[28] Prior to surgery, animals were placed in an isoflurane anaesthetic induction chamber (Vet Tech Solutions Ltd., Cheshire, UK) which was attached to an isoflurane machine (Vet Tech Solutions Ltd.) with an isoflurane vaporiser, oxygen tank and active scavenging unit. The isoflurane (National Veterinary Services, Stoke on Trent, UK) concentration for induction was set to 5% and the oxygen flow rate was set to 2 L/min. The anaesthetised animals were then placed on a stereotaxic frame (David Kopf, Bilaney Consultants Ltd., Kent, UK) with an anaesthesia mask (David Kopf Model 906, Bilaney) attached to the patient breathing circuit of the isoflurane machine. Isoflurane concentration was initially 4% before reduction to 3.5% for maintenance of anaesthesia during the surgery. The oxygen flow rate was set to 1.5 L/min throughout the surgery. Fucithalmic Vet (Dechra Veterinary Products A/S, Uldum, Denmark) eye ointment was applied to the eyes during the surgery. After cranial midline incision, a 26-gauge guide cannula (Bilaney) was implanted using flat-skull stereotaxic technique into the right lateral cerebral ventricle. In all surgeries, bregma was used as a reference point and implantation co-ordinates were taken from the atlas of Paxinos and Watson[29] (lateromedial: +2.0 mm; anteroposterior: −0.6 mm; dorsoventral: −4.2 mm). After fixation to the skull with two stainless steel screws (1 mm diameter; Bilaney) and dental cement (Advanced Healthcare Limited, Kent, UK) each cannula was sealed with a stylet (Bilaney) to maintain patency. Post-operatively, buprenorphine hydrochloride (Reckitt Benckiser Healthcare (UK) Ltd., Hull, UK; 1 mg/kg; s.c.) and 0.9% (w/v) NaCl (1 ml; s.c.) were administered as required. Animals were housed individually and allowed at least one week to recover from surgery.

One hour after CBD administration, 525 IU penicillin was infused into the right lateral ventricle in 1.5 μl 0.9% (w/v) NaCl to induce partial seizures (n = 17–18 for each group). Intracerebroventricular infusions were made by attaching the implanted cannula to a 10 μl Hamilton syringe (Fisher Scientific, Loughborough, UK; infusion rate 1.5 μl/min) *via* a polyethylene tube (Bilaney). Animal behaviour was then monitored for 120 min after penicillin administration. On completion of the experimental procedure, animals were euthanised by CO_2 inhalation before being decapitated. Removed heads were placed in 4% (w/v) paraformaldehyde (Sigma), left to fix for one week at room temperature, then dissected and cannula placement confirmed as right lateral ventricle (conducted blind with respect to seizure scoring results). Results from any animals which exhibited an incorrect cannula position were omitted from the study.

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Table 1
Severity scoring scale for acute pilocarpine-induced temporal lobe seizures.[35]

Acute pilocarpine-induced temporal lobe seizures		
Seizure score	Behavioural expression	Righting reflex
0	No change in behaviour	Preserved
1	Mouth clonus	Preserved
2	Unilateral forelimb clonus	Preserved
3	Bilateral forelimb clonus	Preserved
4	Bilateral forelimb clonus with rearing and falling	Preserved
5	Tonic–clonic seizure	Lost

2.5. Seizure analysis

An observational system utilising closed-circuit television cameras (CCTVs)[30] was used to monitor the behaviour of up to ten animals simultaneously and was started prior to CBD administration. Input from CCTVs was managed on a PC and recorded by Zoneminder (v1.2.3; Triornis Ltd., Bristol, UK) software before post-processing to yield complete videos for each animal. Videos of seizure behaviour were scored offline according to modified seizure severity scales appropriate for the acute pilocarpine (Table 1; Ref. 31) and penicillin models (Table 2; adapted from Bostanci and Bagirici[28]) using Observer Video-Pro software (Noldus, Wageningen, the Netherlands). Specific markers of seizure behaviour and development were assessed and compared between vehicle control and CBD groups. The percentage of animals that developed the two most severe seizure states was noted for each seizure model (see Tables 1 and 2). In addition, the mean number of incidences of each state that occurred within the total recording period was calculated. Finally, the median severity, the percentage of animals that remained seizure-free (severity score = 0), and the percentage mortality in each group was determined for each seizure model.

2.6. Motor function tests

Four behavioural tasks were used to assess motor function after CBD (50, 100 or 200 mg/kg; i.p.) or vehicle administration. The motor function of one group of rats ($n = 12$) was tested on an accelerating rotarod, whilst a second group ($n = 10$) was tested on a static beam task, grip strength task and an inclined screen test. Each animal received either CBD (50, 100 or 200 mg/kg) or vehicle on a given experimental day with a rest period of two or more days between successive treatments. In each case, the order of drug administration was randomised. The rotarod test commenced one hour after CBD or vehicle administration. The first group of animals were placed on the rotarod (Panlab/Harvard Apparatus, Holliston, USA) that linearly increased rotation speed from 4 to 40 rpm during a 300 s period. An accelerating protocol was employed to eliminate the need for habituation to the rotarod (based upon Baytan et al.[32]). Latency to fall from the rotarod in seconds was compared between vehicle control and CBD groups to assess motor function. Each animal undertook three accelerating rotarod runs per experimental day and was permitted a 5 min recovery between each run to avoid any fatigue-induced decline in motor performance. One hour after CBD or vehicle administration, animals from the second group were placed at the illuminated, open end of a 100 cm cylindrical static wooden beam (diameter: 32 mm) after being habituated for five days to run to the enclosure (40 cm × 40 cm × 20 cm) at the other end of the beam. On two successive occasions separated by a short rest period, animals were permitted 5 min to traverse the beam. Following a 5 min rest period, animals then undertook a grip strength test such that animals gripped a horizontal trapeze bar attached to a digital force gauge (Sauter FH 50; Scalesmart, Leicester, UK) and were pulled in a uniform manner until their grip released at which point peak force (kgf) was recorded. A second trial was performed after a 30 s rest period. Finally, animals were allowed a 15 min rest period prior to placement on an inclined screen (6 mm × 6 mm mesh; 60° from horizontal; test area 10 cm × 43 cm; 100 cm above the floor). The animals were placed facing upwards (~5 cm from the top edge of the apparatus). The latency to fall from the screen was recorded, with a maximum allowed time of 30 min (at which point the duration of maintenance of grip on the screen was recorded as 1800 s). In all cases, behavioural testing was completed within 1 h of starting, and therefore within 2 h of CBD administration.

Table 2
Modified severity scoring scale for penicillin-induced partial seizures adapted from Bostanci and Bagirici.[32]

Penicillin-induced partial seizures		
Seizure score	Behavioural expression	Righting reflex
0	No change in behaviour	Preserved
1	Wild running/leaping	Preserved
2	Myoclonic phase	Preserved
3	Unilateral forelimb clonus	Preserved
4	Bilateral forelimb clonus	Preserved
4.5	Tonic–clonic seizure with postural control retained	Preserved
5	Tonic–clonic seizure without postural control	Lost

2.7. Statistical analysis

All statistical procedures were performed using SPSS 15.0.0 software (SPSS Inc., Chicago, IL, USA). Differences between groups for the mean number of occurrences of each seizure state and median seizure severity values were assessed using one-way analysis of variance (ANOVA) with a *post hoc* Tukey test. Differences between groups in the percentage of animals that remained seizure-free, percentage of animals that developed the most severe seizure states and percentage mortality were assessed using a nonparametric binomial test. Differences between groups for rotarod performance were assessed using a two-way ANOVA with 'latency' and 'run' as factors. Differences between groups in the proportions of animals that passed the static beam were assessed using a nonparametric binomial test; differences in mean numbers of footslips, distance travelled, time taken, grip strength, and time on inclined screen were assessed by one-way ANOVA with *post hoc* Tukey test. In all cases, $P \leq 0.05$ was considered significant.

3. Results

3.1. Acute pilocarpine model of temporal lobe seizure

In the acute pilocarpine model, all doses of CBD (1, 10 and 100 mg/kg) significantly reduced the percentage of animals manifesting with bilateral forelimb clonus with rearing and falling (seizure score of 4; Table 1); a reduction from 71% (vehicle-dosed animals) to 43% following 1 mg/kg CBD ($n = 14$, $P \leq 0.05$), 47% following 10 mg/kg CBD ($n = 15$, $P \leq 0.05$), and 29% following 100 mg/kg CBD ($n = 14$, $P \leq 0.01$; Fig. 1A). However, despite the significant reduction in the number of animals exhibiting this seizure state, no significant CBD effect upon the mean number of occurrences for each animal of this state was seen at any dose ($F_{3,56} = 0.575$, $P = 0.634$; Fig. 1B). Thus, although CBD significantly decreased the percentage of animals exhibiting bilateral forelimb clonus with rearing and falling, it did not significantly decrease the number of occurrences.

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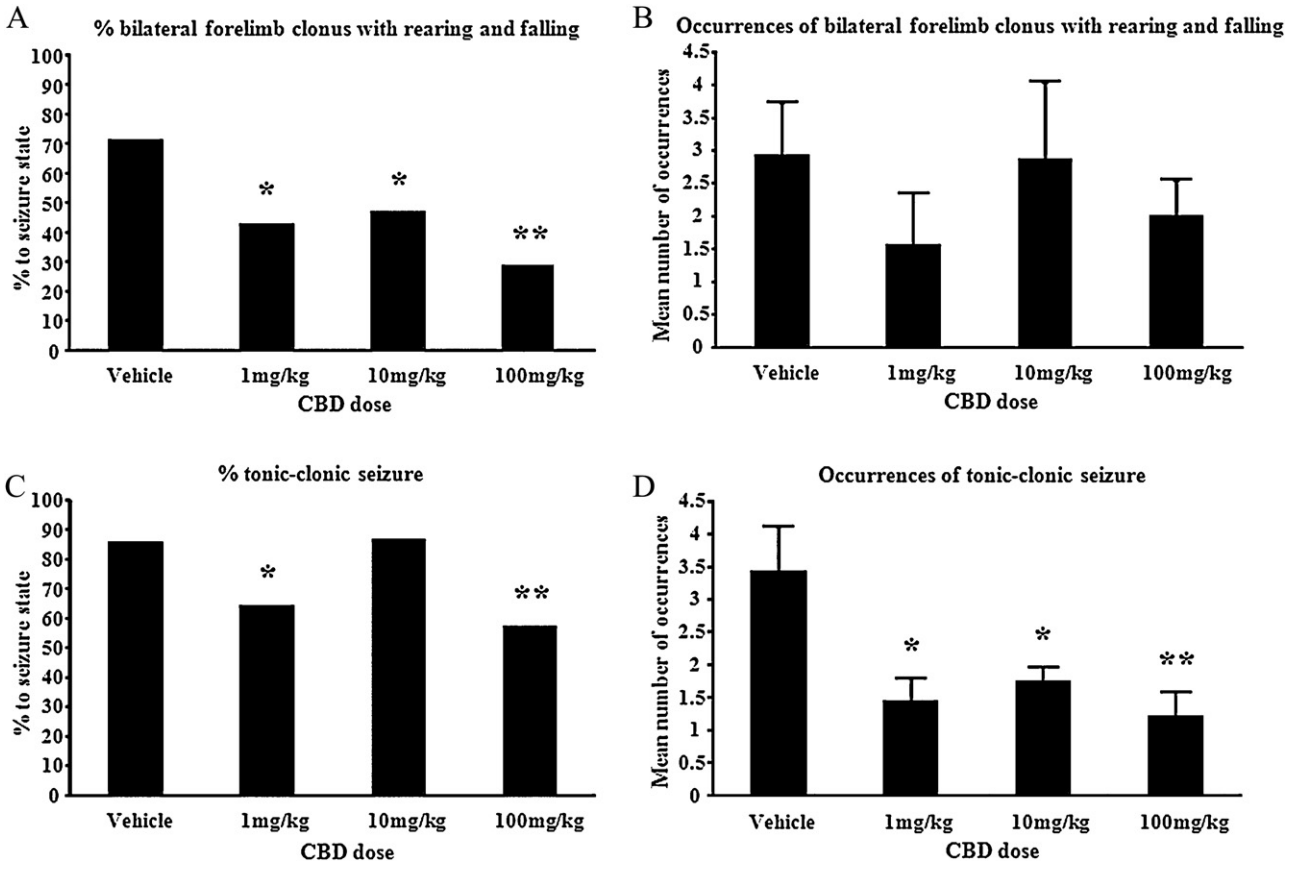

Fig. 1. Cannabidiol (CBD) attenuates acute pilocarpine-induced temporal lobe seizures. Percentage reaching: (A) bilateral forelimb clonus with rearing and falling seizures, (C) tonic–clonic seizures. Mean number of occurrences: (B) bilateral forelimb clonus with rearing and falling seizures, (D) tonic–clonic seizures. Each data set $n \geq 14$ animals. Statistical testing was performed using either a binomial test (panels A and C) or one-way ANOVA with *post hoc* Tukey test (panels B and D). $^{*}P < 0.05$, $^{**}P < 0.01$. $n = 15$ for all groups.

Analysis of tonic–clonic seizure events revealed a significant decrease in the percentage of animals that developed this most severe state (seizure score 5; Table 1), which was reduced from 86% in vehicle-dosed animals to 64% following 1 mg/kg CBD treatment ($n = 14$, $P \leq 0.05$) and 57% following 100 mg/kg CBD treatment ($n = 14$, $P \leq 0.01$). However, no reduction in the percentage of animals that developed tonic–clonic seizures was seen following 10 mg/kg CBD treatment (87%; $P > 0.1$; Fig. 1C). CBD significantly reduced the occurrence of tonic–clonic seizure state seizures at all doses ($F_{3,56} = 5.306$, $P = 0.003$). For individual CBD doses, occurrence was decreased from 3.4 ± 0.7 in vehicle-dosed animals to 1.4 ± 0.4 following 1 mg/kg CBD treatment ($n = 14$, $P \leq 0.05$), 1.7 ± 0.2 following 10 mg/kg CBD treatment ($n = 15$, $P \leq 0.05$) and 1.2 ± 0.4 following 100 mg/kg CBD treatment ($n = 14$, $P \leq 0.01$; Fig. 1D). Thus, administration of 1 mg/kg and 100 mg/kg CBD significantly reduced the percentage of animals exhibiting tonic–clonic seizures, whilst CBD administration at all doses significantly reduced the mean number of occurrences of those animals reaching this state.

In contrast, 1 and 100 mg/kg CBD had no effect on percentage mortality when compared to vehicle ($P > 0.1$), although 10 mg/kg CBD significantly increased percentage mortality ($n = 15, P \leq 0.05$). However, when the severity of pilocarpine-induced seizures is considered, CBD had neither a pro- nor anti-convulsant effect as all animal groups reached a median severity score of 5 ($F_{3,56} = 1.902$, $P = 0.140$). Furthermore, no CBD doses had an effect on the percentage of animals that remained seizure-free, with all animals experiencing a pilocarpine-induced seizure event during the experiment.

3.2. Penicillin model of partial seizure

An analysis of seizure severity in penicillin-treated animals revealed that vehicle-dosed animals reached a median seizure severity score of 5 (tonic–clonic seizure without postural control; the most severe on the scoring scale; Table 2) (Fig. 2A), whilst in groups that received 10 or 100 mg/kg CBD the median decreased to 4.5 (tonic–clonic seizure maintaining postural control; Table 2) due to the greater number of animals with more moderate seizures compared to the vehicle group, although this was not significant (Fig. 2A; $P > 0.1$). Similarly, no effect of CBD treatment on the proportion of animals that remained seizure-free was observed ($P > 0.1$; Fig. 2B). However, CBD treatment ($\geq$10 mg/kg) did cause significant reductions in the percentage mortality of animals exhibiting penicillin-induced partial seizures ($P \leq 0.001$ for both doses; Fig. 2C). Whilst no overall effect on severity was seen, the percentage of animals that developed tonic–clonic seizures whilst retaining postural control (seizure score 4.5; Table 2) was significantly decreased by all CBD doses; 94% of vehicle treated animals exhibited this seizure behaviour; this was reduced to 76% ($P \leq 0.05$), 61% ($P \leq 0.001$) and 59% ($P \leq 0.001$) by treatment with 1, 10 and 100 mg/kg CBD respectively (Fig. 3A). Despite the reduction in the number of animals exhibiting tonic–clonic seizures with retained postural control, CBD did not affect the mean number of occurrences of this state ($F_{3,68} = 0.830$, $P > 0.1$) (Fig. 3B). Therefore, despite the percentage of animals exhibiting this state decreasing, those that reached this state after CBD treatment exhibited the same number of occurrences as vehicle-dosed animals.

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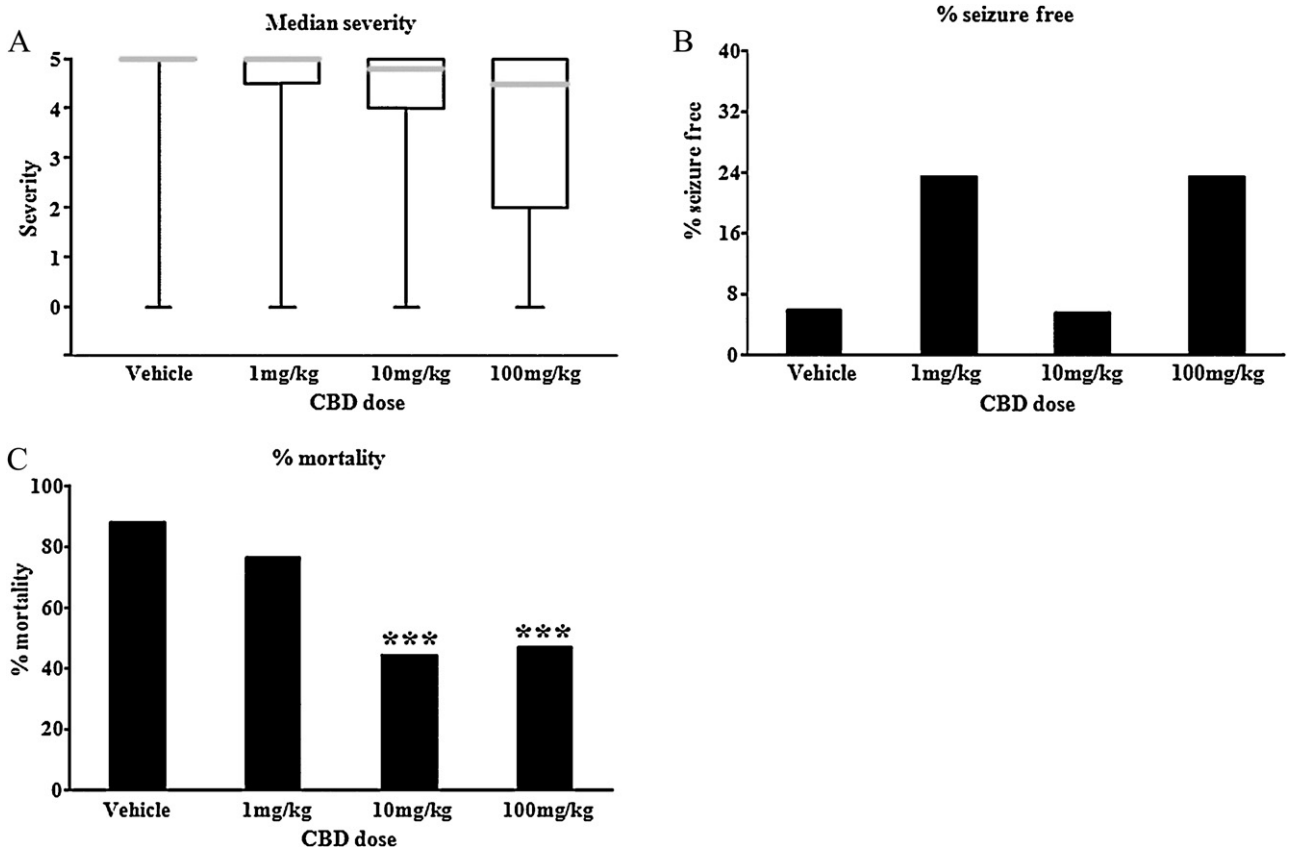

Fig. 2. Cannabidiol (CBD) attenuates penicillin-induced partial seizures. (A) Median seizure severity. Grey lines show median severity, black boxes show 25th and 75th percentiles and error bars indicate 0th and 100th percentiles. (B) Percentage of seizure-free animals. (C) Percentage mortality. Statistical testing was performed using either a binomial test (panels B and C) or one-way ANOVA with *post hoc* Tukey test (panel A). $^{*}P < 0.05$, $^{***}P < 0.001$. $n = 17–18$ per group.

Investigation of the most severe seizure state, tonic–clonic seizures without postural control (seizure score 5; Table 2), revealed a marked decrease in the percentage of animals that developed this most severe state which was reduced from 82% in vehicle-dosed animals to 50% after 10 mg/kg CBD ($P \leq 0.001$) and 47% following 100 mg/kg CBD ($P \leq 0.001$; Fig. 3C). No effect of CBD was observed on the mean number of occurrences of tonic–clonic seizures without postural control ($F_{3,68} = 2.162$, $P > 0.1$; Fig. 3D). Thus CBD decreases both the lethality of penicillin-induced seizures and the proportion of animals that developed the most severe seizures.

3.3. Motor function tests

In the accelerating rotarod test, CBD (50, 100 or 200 mg/kg) had no effect on the latency to fall when compared to vehicle-dosed animals ($F_{3,99} = 0.568$, $P = 0.637$; Fig. 4A). Furthermore, no significant differences between runs were found ($F_{2,33} = 1.378$, $P = 0.266$) nor was any significant interaction between drug and run found ($F_{6,99} = 0.284$, $P = 0.943$). The percentage of animals that successfully traversed the static beam was not significantly affected by CBD treatment ($P > 0.1$; Fig. 4B). CBD did however have a significant effect on the mean number of footslip errors that rats made ($F_{3,79} = 2.915$, $P \leq 0.05$; Fig. 4C), which was apparent only at 100 mg/kg CBD ($P \leq 0.05$). The effect of CBD on footslips however had no significant impact on the average time taken for animals to traverse the static beam (vehicle: 11.8 ± 2.6 s; 50 mg/kg: 11.2 ± 2.9 s; 100 mg/kg: 19.1 ± 5.2 s; 200 mg/kg: 9.8 ± 2.8 s; $F_{3,74} = 1.405$, $P > 0.1$). Similarly, CBD treatment had no effect on the average distance completed (vehicle: 98.5 ± 1.5 cm; 50 mg/kg: 97.5 ± 2.5 cm; 100 mg/kg: 90.5 ± 5.6 cm; 200 mg/kg: 100 cm; $F_{3,79} = 1.758$, $P > 0.1$). The grip strength test was used to assess effects of CBD treatment on muscle strength; it is also a putative test for functional neurotoxicity.[33,34] CBD treatment had no effect on the mean grip strength of rats (Fig. 4D; $F_{3,79} = 0.115$, $P > 0.1$). Muscle tone, as assessed by the inclined screen test, was also unaffected by CBD treatment, with no significant difference in the duration animals remained on the screen being observed between any group (vehicle, 100 mg/kg and 200 mg/kg: 180 0s; 50 mg/kg: 1748.1 ± 42.1 s; $F_{3,39} = 1.522$, $P > 0.1$). These data indicate that, overall, CBD had very little effect on motor function that may compromise data indicating that CBD is an anti-convulsant collected in this study.

4. Discussion

In the present study, we examined the anti-convulsant potential of CBD, the most prevalent non-psychoactive phytocannabinoid found in *C. sativa*, in models of temporal lobe and partial seizures. In the acute pilocarpine model, CBD showed modest anti-convulsant effects, significantly lowering the incidence of the most severe seizures. However, these findings were not reflected in effects on mortality and severity. By contrast, strong anti-convulsant CBD effects were seen in the penicillin model of partial seizure. CBD significantly reduced mortality and significantly lowered the percentage of animals experiencing tonic–clonic seizures both with and without maintained postural control. Furthermore, four separate tests of motor function and muscle strength and tone indicated that CBD exerted only limited effects on motor function. Thus, here we demonstrate CBD's potential as a novel AED in temporal lobe and partial seizures, complementing previous research in other *in vivo* animal models.[15,20,27]

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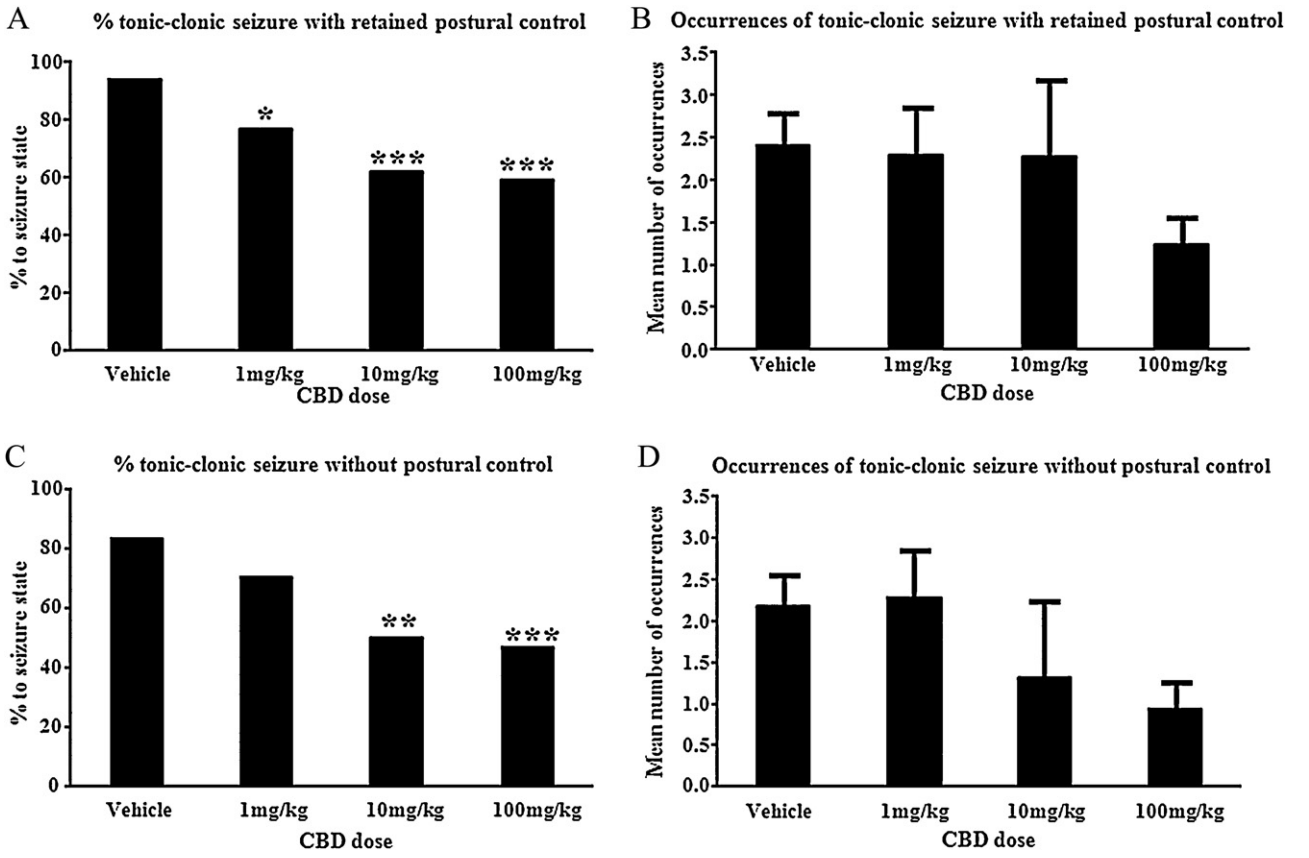

Fig. 3. Effects of cannabidiol (CBD) on penicillin-induced partial tonic–clonic seizures. Percentage of animals reaching: (A) tonic–clonic seizures with retained postural control, (C) tonic–clonic seizures without postural control. Mean (±S.E.M.) number of occurrences: (B) tonic–clonic seizures with retained postural control, (D) tonic–clonic seizures without postural control. $n = 17–18$ animals per group. Statistical testing was performed using either a binomial test (panels A and C) or one-way ANOVA with *post hoc* Tukey test (panels B and D). $^{*}P < 0.05$, $^{***}P < 0.001$. $n = 17–18$ per group.

4.1. Acute pilocarpine model of temporal lobe seizure

In the temporal lobe model, acute pilocarpine-induced seizure development is thought to be dependent on the activation of the muscarinic acetylcholine receptor (mAChR) M1 subtype since $M1R^{-/-}$ mice do not develop seizures in response to pilocarpine.[35] However, once initiated, seizure maintenance is dependent on *N*-methyl-D-aspartate (NMDA) receptor activation, and centrally acting muscarinic antagonists (*e.g.* atropine) fail to abolish pilocarpine-induced seizures.[36] Although CBD did not reduce mortality in the acute pilocarpine model, this could be a consequence of the well-reported high mortality rate associated with this model[37–40] which is attributed to an increased likelihood of respiratory failure associated with severe tonic–clonic seizure states compared to other models. Moreover, any respiratory depression could obscure or confound potentially mitigating drug effects upon mortality. Conversely, the lack of CBD effects upon other parameters could also have occurred as a result of CBD lacking activity against the initiation of seizures induced by pilocarpine, which is *via* M1 mAChR activation. Since CBD significantly reduced the number of occurrences of tonic–clonic seizures but did not affect seizure initiation, it is possible that CBD's anti-convulsant effects mitigate against NMDAR-mediated maintenance, but not mAChR-mediated initiation of acute pilocarpine-induced seizures.

In models of temporal lobe epilepsy (TLE), manifestation of the more severe seizure states (*e.g.* tonic–clonic seizures) is correlated with the eventual development of spontaneous recurrent excitation.[41] Consequently, the CBD-induced reduction in the percentage of animals developing such severe seizure states suggests that further investigation of CBD effects in the chronic pilocarpine model of spontaneously recurrent seizures is warranted. This assessment of CBD against spontaneous recurrent and secondarily generalised seizures modelled on TLE may prove worthwhile as Cunha and colleagues[11] reported beneficial CBD effects in people with secondary generalised epilepsy with a temporal lobe focus. A significant co-morbidity associated with epilepsy is depression. Serotonin dysfunction has been implicated in depression in people without epilepsy,[42] and has also been described as a possible causative factor in depression associated with epilepsy (for reviews see Refs. 43,44). CBD could therefore also hold additional benefits for people with TLE that exhibit a high co-morbid association with depression as a result of decreased serotonergic function,[45,46] as CBD as shown agonistic properties at 5-HT_{1A} receptors *in vitro* resulting in increased serotonergic function, albeit at concentrations of $>10\ \mu M$.[47] Additionally, CBD has exhibited significant anti-depressant-like effects in the forced swim and tail suspension tests, without changing the exploratory behaviour of Swiss mice *in vivo*.[48,49] In future, CBD should be tested for anti-depressant-like effects on behaviour in epileptic animals (as in *e.g.* Ref. 50) to investigate whether this potential benefit translates in an epileptic phenotype.

4.2. Penicillin model of partial seizure

In the penicillin model of partial seizure, penicillin administration into a cerebral ventricle leads to the local suppression of GABA-mediated inhibitory neurotransmission (overall causing disinhibition of the local circuitry) and, consequentially, partial seizures. In the present study, we have shown that CBD (≥10 mg/

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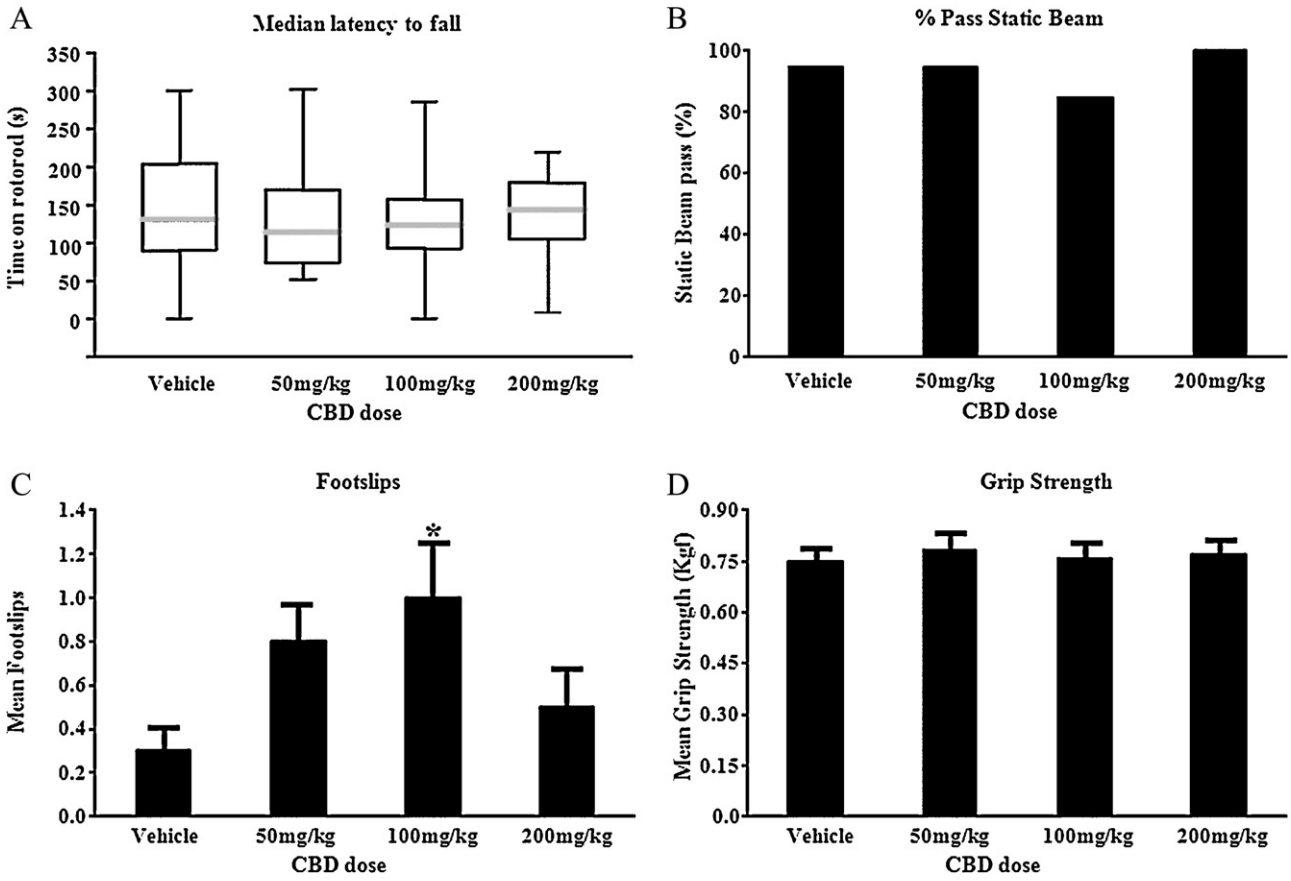

Fig. 4. Effects of CBD treatment on motor performance. (A) Box plot showing the latency to fall for vehicle and CBD (50, 100 and 200 mg/kg) treated animals on the accelerating rotarod test for motor function. Grey lines show median latency to fall from the rotarod, black boxes show 25th and 75th percentiles and error bars indicate 10th and 90th percentiles. $n = 12$ animals. Statistical testing was performed using a two-way ANOVA, revealing no significant differences in motor function. (B) Percentage passing static beam test. (C) Mean number of footslips per animal observed on static beam test (±S.E.M.). (D) Mean grip strength of rats (±S.E.M.; kilograms of force, kgf). (B and C) $n = 10$ animals, two values from each animal per group. (D) $n = 10$ animals. Statistical testing in (A) was performed using a two-way ANOVA, revealing no significant differences in motor function; in (B) by binomial test and in (C) and (D) by one-way ANOVA with *post hoc* Tukey test. $^*P < 0.05$.

kg) exerts clear anti-convulsant effects with significant reductions in the lethality of seizures and the proportions of animals developing the most severe (tonic–clonic) seizure types. The anti-convulsant effects in this model are comparable to data previously reported, showing CBD (100 mg/kg) to be effective in the PTZ model of generalised seizure.[22] Previously, Consroe and colleagues using several other seizure models found CBD to be exerting an anti-convulsant effect *via* the disinhibition of GABA, resulting in a proposed GABA-related mechanism of action.[10] Therefore, CBD could also be exerting its beneficial effects here in the penicillin model through the same GABA related mechanism of action. However, this provides only an indirect assessment, so further investigation is therefore required before definitive mechanistic conclusions regarding CBD's anti-convulsant effects can be drawn.

4.3. Motor function tests

Although CBD has previously been reported to be devoid of motor side-effects,[9–11] we investigated CBD effects upon performance in a variety of motor tasks. With the exception of an effect of 100 mg/kg CBD on the number of footslips as animals traversed a static beam, no effect of CBD treatment on motor function was observed in this study. Footslips on the static beam apparatus have been reported to correlate with deficits in sensorimotor coordination,[51] however the effect of 100 mg/kg CBD on footslips did not affect the ability of animals to complete the test, or their speed on the beam, and was not reflected in a poor performance on the rotarod. CBD also had no effect on grip strength, which represents both muscle strength and can be used as a measure of functional neurotoxicity,[33,34] or muscle tone as assessed by the inclined screen. Thus, we have demonstrated that CBD had minimal effects on motor function at doses up to 200 mg/kg. In contrast, all AEDs licensed for clinical use in the UK cause significant and strong motor side-effects emphasising the advantage of CBD as a potential clinical anti-convulsant.[52]

4.4. Mechanisms

The specific cellular mechanisms underlying lethality in both the pilocarpine and penicillin models are unknown, so we are presently unable to rationalise the observed CBD-induced reduction in mortality in the penicillin model of seizure only. However, CBD has a significant anti-convulsant effect by reducing the percentage of animals developing the most severe tonic–clonic seizure states in both models employed. Therefore, in these models, CBD may act preferentially to reduce seizure spread irrespective of its focal origin in the brain.[10] Moreover, if CBD is indeed preventing seizure spread, this is unlikely to also affect normal biophysical signal propagation, as demonstrated by our previous findings in *in vitro* hippocampal brain slice models of "epileptiform" activity,[22] where CBD did not affect the speed of signal propagation but did attenuate chemically induced seizure-like activity. This further supports the positive side-effect profile of

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CBD,[9–11] which is not shared by most clinically used AEDs. Consistent with our previous findings,[22] CBD appears to hold the greatest potential for the treatment of partial and generalised seizures, rather than temporal lobe seizures. It is already well known that CBD has only very low affinity for both endogenous CB_1 and CB_2 receptors[7,20,21] and is therefore likely to be exerting its anti-convulsant activity *via* cannabinoid receptor-independent mechanisms.[17,22,23]

Thus far, CBD has shown a poly-pharmacological profile, potentially modulating neuronal hyperexcitability *via* a number of different mechanisms (see also Jones et al.[22]). In this regard, proposed mechanisms include: (1) the bidirectional regulation of Ca^{2+} homeostasis *via* the mitochondrial Na^+/Ca^{2+}-exchanger to either elevate or decrease cytosolic Ca^{2+} levels, dependent on whether the neuron is under normal physiological or a highly excitable state[53]; (2) agonistic properties at 5-HT_{1A} receptors,[47,54–56] with receptor activation eliciting membrane hyperpolarising responses, consistent with an inhibitory role in seizure generation[46,57]; (3) enhancing endogenous adenosine levels in the CNS by reducing adenosine reuptake,[58,59] thereby increasing inhibitory adenosinergic tone to aid seizure suppression. Moreover, numerous additional cellular and molecular CBD effects and mechanisms of action have also been proposed, but are less likely to be related to CBD's anti-convulsant profile but, for example, have pharmacological relevance in CNS disorders, pain, inflammation and cancer (reviewed in Hill et al.[24] and Izzo et al.[60]). In summary, CBD's anti-convulsant effects may not be due to be one specific mechanism of action but the result of numerous cannabinoid receptor-independent mechanisms. The understanding of these mechanisms of action will be critical to improve CBD's efficacy, safety profile and to enhance drug combination strategies for this potential anti-convulsant in the future.

4.5. Therapeutic potential

We propose that CBD exerts a cumulative anti-convulsant effect; this may be achieved by a poly-pharmacological profile, with CBD simultaneously modulating a number of endogenous systems to attenuate and/or prevent epileptic neuronal hyperexcitability. Importantly, despite numerous potential targets, CBD has an excellent side-effect profile, as revealed in this investigation and others.[9–11] Moreover, CBD may have attractive synergistic or additive effects when co-administered with currently prescribed AEDs, noting that it is compulsory for new therapeutic agents to firstly be co-administered with currently available AEDs. Therefore, adjunctive CBD treatment may potentially have beneficial effects as an anti-convulsant, as well as decreasing the necessary dose, and therefore the undesirable side-effects of current AED treatments.

5. Conclusions

Overall, we demonstrate the anti-convulsant actions of CBD for the first time in the acute pilocarpine and penicillin models of temporal lobe and partial seizures respectively. These results clearly extend previously published data from other *in vivo* models which point to CBD being of potential therapeutic use (alone or as an adjunct) in the treatment of epilepsies. Future research is now required to investigate the potential synergistic or additive effects of CBD on AEDs and to establish in greater detail this important phytocannabinoid's anti-convulsant mechanism(s) of action.

Conflict of interest statement

YY and SA are employees of Otsuka Pharmaceuticals. The experimental design was by NAJ, CMW, BJW and AJH.

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