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Accepted Version

Cannabidivarin is anticonvulsant in mouse and rat *in vitro* and *in seizure* models

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/j.1476-5381.2012.02207.x

Summary

Background and purpose: Phytocannabinoids in *Cannabis sativa* have diverse pharmacological targets extending beyond cannabinoid receptors and several exert notable anticonvulsant effects. For the first time, we investigated the anticonvulsant profile of the phytocannabinoid cannabidivarin (CBDV) *in vitro* and in *in vivo* seizure models.

Experimental approach: The effect of CBDV (1-100μM) on epileptiform local field potentials (LFPs) induced in rat hippocampal brain slices by 4-AP application or Mg^{2+}-free conditions was assessed by *in vitro* multi-electrode array recordings. Additionally, the anticonvulsant profile of CBDV (50-200 mg kg^{-1}) *in vivo* was investigated in four rodent seizure models: maximal electroshock (mES) and audiogenic seizures in mice, and pentylenetetrazole (PTZ) and pilocarpine-induced seizures in rat. CBDV effects in combination with commonly-used antiepileptic drugs were investigated in rat seizures. Finally, the motor side effect profile of CBDV was investigated using static beam and grip-strength assays.

Key results: CDBV significantly attenuated *status epilepticus*-like epileptiform LFPs induced by 4-AP and Mg^{2+}-free conditions. CBDV had significant anticonvulsant effects in mES (≥100 mg kg^{-1}), audiogenic (≥50 mg kg^{-1}) and PTZ-induced seizures (≥100 mg kg^{-1}). CBDV alone had no effect against pilocarpine-induced seizures, but significantly attenuated these seizures when administered with valproate or phenobarbital at 200 mg kg^{-1} CBDV. CBDV had no effect on motor function.

Conclusions and Implications: These results indicate that CBDV is an effective anticonvulsant across a broad range of seizure models, does not **significantly** affect normal motor function and therefore merits further investigation in chronic epilepsy models to justify human trials.

Keywords: Epilepsy, cannabinoid, cannabidivarin, seizure, side effect, hippocampus

Abbreviations:

AED: antiepileptic drugs; 4-AP: 4-aminopyridine; CBD: cannabidiol; CBDV: cannabidivarin; DG: dentate gyrus; LFP: local field potential; MEA: multi-electrode array; mES: maximal electroshock; PTZ: pentylenetetrazole; Δ^9-THC: Δ^9-tetrahydrocannabinol; TRP: transient receptor potential.

Introduction

Epilepsy is a central nervous system disorder affecting ~1% of the global population, and is symptomatically characterised by chronic, recurrent seizures. A range of treatments are available, although the need for more effective and better tolerated antiepileptic drugs (AEDs) remains, as dictated by the pharmacological intractability of ~30% of cases and the poor side effect profile of currently available AEDs (Kwan *et al.*, 2007). *Cannabis sativa* has a long history of use for control of human seizures (Mechoulam, 1986; O'Shaughnessy, 1840), and is legally used for this indication in some countries (Sirven *et al.*, 2004).

There are >100 phytocannabinoids present in *Cannabis sativa*, of which Δ^9-tetrahydrocannabinol (Δ^9-THC) is the most abundant (Elsohly *et al.*, 2005; Mehmedic *et al.*, 2010) and is responsible for the classical psychoactive effects of cannabis via partial agonism of the CB1 cannabinoid receptor (Pertwee, 2008). Although CB1 cannabinoid receptor agonism can exert anticonvulsant effects in *in vitro* and *in vivo* models (Chesher *et al.*, 1974; Deshpande *et al.*, 2007; Wallace *et al.*, 2003; Wallace *et al.*, 2001), the most promising non-psychoactive anticonvulsant phytocannabinoid investigated to date is cannabidiol (CBD) which exerts anticonvulsant actions via as yet unknown, non-CB1 cannabinoid receptor mechanism(s) *in vitro, in vivo* and in humans (Consroe *et al.*, 1982; Cunha *et al.*, 1980; Jones *et al.*, 2010; Wallace *et al.*, 2001); CBD's notable anticonvulsant properties led us to investigate the anticonvulsant potential of its propyl analogue, cannabidivarin (CBDV).

CBDV was first isolated in 1969 (Vollner *et al.*, 1969). Presently, little is known about the pharmacological properties of CBDV (Izzo *et al.*, 2009), although Scutt & Williamson reported that CBDV can act via CB2 cannabinoid receptor-dependent mechanisms (Scutt *et al.*, 2007). More recently, De Petrocellis and co-workers reported differential CBDV effects at transient receptor potential (TRP) channels *in vitro*, where it

acted as a human TRPA1, TRPV1 and TRPV2 agonist (EC_{50} values: 0.42, 3.6 and 7.3μM respectively) and a TRPM8 antagonist (IC_{50}: 0.90 μM) (De Petrocellis *et al.*, 2011a; De Petrocellis *et al.*, 2011b). Additionally, CBDV inhibited diacylglycerol lipase α (IC_{50} 16.6μM) *in vitro* (De Petrocellis *et al.*, 2011a), the primary synthetic enzyme of the endocannabinoid, 2-arachidonoylglycerol (Bisogno *et al.*, 2003). Whilst the pharmacological relevance of these effects remains unconfirmed *in vivo*, they further illustrate the diversity of non-Δ^9-THC phytocannabinoid pharmacology and underline the emergent role of multiple non-CBR targets (Hill *et al.*, 2012; Pertwee, 2010).

Here, we identify anticonvulsant effects of CBDV for the first time; thus, CBDV suppressed *in vitro* epileptiform activity in brain slices and acted as an anticonvulsant *in vivo*, normal motor function was **not significantly affected by CBDV**. Further investigation into the clinical development of CBDV as a novel AED is therefore warranted.

Methods

In vitro *electrophysiology*

Tissue preparation

All experiments were carried out in accordance with Home Office regulations (Animals (Scientific Procedures) Act, 1986). Transverse hippocampal slices (~450μm thick) for multielectrode array (MEA) recordings were prepared from female and male adult Wistar Kyoto rats (P>21; Harlan, UK) using a Vibroslice 725M (Campden Instruments Ltd., UK) as previously described (Jones *et al.*, 2010).

MEA recordings

MEA recordings and analyses were conducted as described in Hill *et al*. (2010). Once established (by addition of either 100μM 4-AP or omission of $MgSO_4.7H_2O$ without substitution), epileptiform activity was permitted to continue for 30 min (control bursting) before sequential addition of 1, 10 and 100μM CBDV (30 min each). **Epileptiform activity was characterised by spontaneous LFPs (local field potentials) recorded simultaneously from 59 electrodes covering the majority of the hippocampal slice preparation. The amplitude and duration of epileptiform LFPs was analysed for each electrode, where data from individual electrodes chosen based on their position in each hippocampal subregion were pooled to provide mean results for each subregion across n≥5 slices from n≥5 animals per model.** Matlab 6.5 and 7.0.4 (Mathworks, Natick, Massachusetts USA), Microsoft Excel (Microsoft, Redmond, USA), MC_DataTool and MC_Rack (Multi Channel Systems GmbH) were used to process and present data as described in Hill *et al*. (2010). Inherent changes in LFP amplitude and frequency were corrected for as described in (Hill *et al.*, 2010). For reference, the extent of amplitude rundown correction applied is illustrated in Figs. 1C and D. **LFP frequency was calculated per slice (n≥5 for each model)**

and represents the number of LFP bursts/unit time. Examples of single bursts from each model can be seen in Figs. 1A and B. Drug-induced changes in burst duration, amplitude and frequency were expressed as normalised proportions of control values ± S.E.M., corrected where necessary, and were analysed by Wilcoxon paired test with Holm's sequential Bonferroni correction.

In vivo seizure models

Animals

In all cases prior to seizure induction, animals were maintained on a 12:12 hour light/dark cycle with free access to food and water (with the exception of rats that received oral CBDV, see below). Audiogenic seizure experiments with DBA/2 mice (3-4 weeks old; Elevage Janvier, Le Genest-Saint-Isle, France) were performed at Porsolt Research Laboratory (Le Genest-Saint-Isle, France) in accordance with French legislation and under licence from the French Ministry for Agriculture and Fisheries. mES experiments with ICR (CD-1) mice (5 weeks old; SLC Japan Inc., Japan) were performed at Otsuka Pharmaceuticals Co, Ltd, Japan in accordance with the guidelines of the Physiological Society of Japan. Seizure studies in male Wistar Kyoto rats (Harlan, UK, 3-4 weeks old) were performed at the University Of Reading, UK; all experiments were carried out in accordance with UK Home Office regulations (Animals (Scientific Procedures) Act 1986).

CBDV administration

CBDV (50, 100 or 200 mg kg^{-1}; GW Pharmaceuticals Ltd., Salisbury, UK) was administered by intra-peritoneal (I.P.) injection one hour before seizure induction in all models except mES (30 min) in an ethanol:Cremophor:0.9%$^{w}/_{v}$ NaCl vehicle (2:1:17; all Sigma, Poole, UK). All experiments included a control group that received volume-matched vehicle, against

which other groups were assessed. In mice experiments, n=10/group; in rat, n=15/group. In experiments where CBDV was administered orally (gavage), 400 mg kg^{-1} CBDV or volume-matched vehicle (20% solutol (Sigma) in 0.9%$^{w}/_{v}$ NaCl) was administered after 13.5 hours of fasting and 3.5 hours before I.P. administration of PTZ, n=15 for both groups (see supplementary methods for details on oral dose levels).

Seizure induction

mES seizures were induced in mice by a stimulator (Ugo Basile ECT, Italy) via earlap clamps at a current of 30 mA delivered at 100 Hz for 200 ms. DBA/2 mice were placed in a Plexiglas jar one hour after CBDV/vehicle administration. A mounted bell (110-120 dB) was activated until occurrence of a tonic audiogenic seizure or for a maximum of 60s. 85 mg kg^{-1} PTZ was injected I.P. to induce generalised seizures in rats. ***Status epilepticus*** **with a temporal lobe focus was induced in rats** by 380 mg kg^{-1} I.P. pilocarpine hydrochloride (Sigma; in 0.9%$^{w}/_{v}$ NaCl), 45 min prior to pilocarpine, 1 mg kg^{-1} methyl scopolamine (in 0.9% $^{w}/_{v}$ NaCl; Sigma) was administered I.P. to block peripheral effects of pilocarpine.

Seizure analysis

In mES experiments, mice were observed for 10 s during electroshock, tonic hindlimb extension occurrence was noted and expressed as a percentage of the total number of animals for each group. Audiogenic seizure behaviour was observed visually, whilst rat seizures were video recorded (Farrimond *et al.*, 2009). For audiogenic seizures the incidence (as a percentage) of the most severe (tonic-clonic) seizures, mortality and seizure-free animals were calculated for each group. These parameters, as well as seizure duration and severity, were also determined for rat seizures. Rat behaviour was coded blind offline using The

Observer Pro software (Noldus, Wageningen, The Netherlands) and seizure severity scales appropriate to each seizure type (Table 1). Values are expressed as mean ± S.E.M throughout.

Co-administration experiments

The effect of co-administration of clinically-used AEDs with 200 mg kg^{-1} CBDV on PTZ- and pilocarpine-induced seizures was investigated. For details see supplementary methods. Briefly, in each experiment an AED was administered IP at either ~20, ~40 or ~70% maximal effective dose in the absence or presence of 200 mg kg^{-1} CBDV (n=15/group, 120/experiment), convulsant (PTZ or pilocarpine) was administered one hour after CBDV or its vehicle. Experimental design is illustrated and summarised in Table 2. **In the PTZ model, CBDV was co-administered with valproate or ethosuximide prior to PTZ, and with valproate or phenobarbital prior to pilocarpine. These AEDs were chosen based on their clinical profile and their reported efficacy in the models used here, with valproate suppressing both seizure types and ethosuximide and phenobarbital suppressing PTZ and pilocarpine respectively (Lindekens *et al.*, 2000; Loscher, 2011; Loscher *et al.*, 1991; Shantilal *et al.*, 1999; Sofia *et al.*, 1993).** In co-administration experiments, seven (2.9%) rats exhibited a fatal reaction to CBDV administration. Behaviourally this manifested as rapid development (within 300 s) of lethargic convulsive movements followed by death. Overall this effect was seen in 2.6% of all rats that received 200 mg kg^{-1} CBDV across all PTZ and pilocarpine experiments, and not at all in side effect tests. No adverse effects of other CBDV doses were observed in rats, and none at any dose in mice. These animals were omitted from all analyses.

Statistics

In experiments where I.P. CBDV alone was administered, the effects of CBDV on seizure severity, onset latency and seizure duration were assessed by one-way (ANOVA) with *post hoc* Tukey tests as appropriate. Chi squared tests followed by *post hoc* Fisher's exact tests were used where appropriate to assess differences in incidence parameters. Where CBDV was co-administered with an AED, two-way ANOVA or log-linear modelling was used to analyse the effects of CBDV and AEDs. **Log-linear modelling was used to model the interactions between drug co-administration and incidence parameters (e.g. mortality, % seizure-free). If the model indicated a significant effect of drug treatment, further analysis to determine the contribution of CBDV, the relevant AED and any drug x drug interaction was performed; these analyses are given in the text and in Supplementary Tables 1 and 2.**

Motor function assays

The effects of CBDV (50, 100 and 200 mg kg^{-1}) and valproate (125, 250 and 350 mg kg^{-1}) on normal rat motor function were assessed on a 1 m raised static beam and by a grip strength test (see Supplementary Methods for details).

All receptor an ion channel nomenclature conforms to Alexander *et al*., 2011.

Results

Effects of pure CBDV in the Mg^{2+}-free and 4-AP models of in vitro *epileptiform activity*

The effects of CBDV (1-100 μM) on epileptiform activity in rat acute hippocampal slices induced by Mg^{2+}-free aCSF (Fig. 1A) or 100 μM 4-AP (Fig. 1B) were examined. CBDV significantly decreased the amplitude and duration of epileptiform local field potentials (LFPs) induced by Mg^{2+}-free aCSF (Fig. 1C&D; $p \leq 0.05$); significant effects were seen at ≥10 μM, and CA3 was more resistant to the effects of CBDV than DG or CA1 (Fig. 1C&D). Conversely, CBDV significantly increased Mg^{2+}-free-induced LFP frequency (≥10 μM; Fig. 1E; $p \leq 0.05$).

An anti-epileptiform effect of 100 μM CBDV **on the amplitude of 4-AP-induced epileptiform LFPs** was observed in the CA1 region alone (Fig. 1F; $p \leq 0.05$), whereas LFP duration was significantly lowered in all hippocampal regions by ≥10 μM CBDV (Fig. 1G) and, by contrast to the Mg^{2+}-free model, 4-AP-induced LFP frequency was significantly decreased by all CBDV concentrations tested (Fig. 1H; $p \leq 0.05$). Thus, CBDV attenuated the duration of amplitude of LFPs in both models, and had differential effects on frequency.

Effects of CBDV on maximal electroshock (mES) and audiogenic seizures in mice

The effects of CBDV (50-200 mg kg^{-1}) on mES convulsions and audiogenic seizures in mice were investigated. CBDV had a significant anticonvulsant effect on animals displaying tonic hind-limb extension after mES ($X^2(3)=15.000$; $p \leq 0.001$; Fig. 2A); significantly fewer animals that received 100 or 200 mg kg^{-1} CBDV exhibited hind-limb extension (both groups 30%) than those that received vehicle (90%, Fig. 2A; $p \leq 0.001$ vs. vehicle treated group). Audiogenic seizures were also significantly attenuated by CBDV (Fig. 2B-D). **The incidence of tonic convulsions** was significantly lower after CBDV

administration ($X^2(3)=19.436$, $p\leq0.001$; Fig. 2B); 80% of vehicle-treated animals developed tonic convulsions compared to only 20% (50 mg kg^{-1} CBDV), 10% (100 mg kg^{-1} CBDV) and 0% (200 mg kg^{-1} CBDV) after drug treatment (each $p\leq0.001$ *vs* vehicle). The proportion of animals that remained seizure-free was significantly higher after administration of 200 mg kg^{-1} CBDV (90%) than vehicle (0%; $X^2(3)=27.461$, $p\leq0.001$; Fig. 2C). Finally, a **statistical trend** was observed for the mortality rate ($X^2(3)=6.667$, $p\leq0.1$), with lower mortality after 100 and 200 mg kg^{-1} CBDV treatment than vehicle (0% vs 30% respectively; Fig. 2D). Thus, CBDV exhibits strong and significant anticonvulsant effects in two broad-screen mouse seizure models. We therefore next investigated the anticonvulsant potential of CBDV in two further models of seizure in rat that emulate more specific seizure types.

Effects of CBDV on PTZ- and pilocarpine-induced seizures in rat

We investigated the effect of CBDV (50-200 mg kg^{-1}) on PTZ-induced seizures. CBDV significantly decreased PTZ seizure severity ($F_{3,58}=4.423$, $p\leq0.05$; Fig. 3A); the median seizure severity after vehicle administration was tonic-clonic convulsion (score [5]), but after 200 mg kg^{-1} CBDV administration seizure severity was significantly lowered to a median severity of bilateral clonic convulsion (score [3]; $p\leq0.05$). CBDV also significantly reduced mortality ($X^2(3)=10.356$, $p\leq0.05$; Fig. 3B) at 100 and 200 mg kg^{-1} CBDV ($p\leq0.01$). The proportion of animals remaining seizure-free was significantly increased by CBDV administration ($X^2(3)=7.809$, $p\leq0.05$; Fig. 3C); 33.3% of animals that received 200 mg kg^{-1} CBDV exhibited no signs of seizure, compared to only 6.7% of animals that received vehicle ($p\leq0.01$). Furthermore, seizure onset was significantly delayed by CBDV treatment ($F_{3,50}=2.971$, $p\leq0.05$; Fig. 3D); mean onset latency was significantly **longer** after administration of 200 mg kg^{-1} CBDV than vehicle (65±11s and 40±4s respectively; $p\leq0.05$). Thus, CBDV administered alone exhibited strong and significant anticonvulsant effects on

PTZ seizures at 200 mg kg^{-1} (Fig. 3A-D) with more limited, but significant, effects at 100 mg kg^{-1} (Fig. 3B).

We extended our studies to investigate the effects of CBDV (50-200 mg kg^{-1}) on the convulsions associated with pilocarpine-induced *status epilepticus* (380 mg kg^{-1}). CBDV (50-200 mg kg^{-1}) had no significant effect on the severity ($F_{3,59}$=0.049, p>0.1; Fig. 3E) or resultant mortality of pilocarpine convulsions ($X^2(3)$=1.779, p>0.1; Fig. 3F). Similarly, CBDV did not significantly affect the proportion of animals that remained seizure-free ($X^2(3)$=0.110, p>0.1; Fig. 3G) or the latency to the onset of convulsions ($F_{3,53}$=0.404, p>0.1; Fig. 3H).

Effect of co-administration of CBDV and AEDs on PTZ- and pilocarpine-induced seizures in rat

We investigated the properties of CBDV when co-administered with AEDs prior to PTZ or pilocarpine treatment. The effects of combined drug treatment (CBDV + AED) on seizure parameters are described here and illustrated in Figures 4 and 5, as is the contribution of CBDV to these effects. The contribution of AEDs is illustrated in Figures 4 and 5 while statistical analyses of AED effects and any interaction between CBDV and AEDs are shown in Supplementary Table 1.

200 mg kg^{-1} CBDV was co-administered with valproate (50-250 mg kg^{-1}) or ethosuximide (60-175 mg kg^{-1}). In CBDV + valproate experiments, drug co-administration had significant anticonvulsant effects on all seizure parameters except the proportion of animals remaining seizure-free. CBDV and valproate co-administration significantly decreased seizure severity ($F_{7,112}$=10.449, p≤0.001; Fig. 4A).

When modelled by log linear analyses, our data indicated that drug co-administration decreased mortality (Fig. 4B) and the incidence of the most severe (tonic-clonic) seizures (Fig. 4C). Seizure onset was significantly delayed by drug co-administration ($F_{7,109}$=13.285, p≤0.001; Fig. 4D) and the mean duration of seizures was increased ($F_{7,103}$=5.250, p≤0.001). Valproate contributed significantly to all these effects (Fig. 4A-D, Supplementary Table 1). CBDV significantly contributed to the overall decrease in severity ($F_{1,112}$=5.748, p≤0.05; Fig. 4A) and mortality (X^2(1)=6.639, p≤0.01; Fig. 4B) and the increase in onset latency ($F_{1,109}$=7.393, p≤0.01; Fig. 4C). CBDV did not significantly affect tonic-clonic seizure incidence (Fig. 4D) or seizure duration (p>0.1). No effect of drug treatment on the number of seizure-free animals was observed (X^2(14)=8.930, p>0.1) and no significant positive or negative interactions between the effects of 200 mg kg^{-1} CBDV and valproate were observed (Supplementary Table 1; p>0.1).

Co-administration of 200 mg kg^{-1} CBDV and ethosuximide (60-175 mg kg^{-1}) had significant anticonvulsant effects on all parameters of PTZ-induced seizures: CBDV and ethosuximide co-administration significantly decreased seizure severity ($F_{7,110}$=12.556, p≤0.001; Fig. 4E), when modelled with log linear analysis, our data indicated that co-administration also decreased mortality (Fig. 4F) and the incidence of the most severe seizures (Fig. 4G). Seizure onset latency was significantly increased ($F_{7,76}$=7.885, p≤0.001; Fig. 4H), as was the proportion of animals that remained seizure-free (log linear model; Fig 4I); seizure duration was significantly decreased ($F_{7,102}$=6.934, p≤0.001). Ethosuximide significantly contributed to all anticonvulsant effects (Fig. 4E-I; Supplementary Table 1). CBDV contributed significantly to the overall decreases in seizure severity ($F_{1,112}$=7.474, p≤0.01; Fig. 4E) and mortality (X^2(1)=5.174, p≤0.05; Fig. 4F); the contribution of CBDV to the increase in onset latency showed a statistical trend ($F_{1,76}$=2.791, p≤0.1; Fig. 4H). CBDV did not significantly

contribute to the effects on seizure duration, the proportion of animals that remained seizure-free (both p>0.1) or the incidence of the most severe seizures (p>0.1; Fig. 4G). No significant positive or negative interactions between the effects of 200 mg kg^{-1} CBDV and ethosuximide were observed (Supplementary Table 1; p>0.1).

We next investigated whether 200 mg kg^{-1} CBDV affected the anticonvulsant actions of valproate or phenobarbital on pilocarpine-induced convulsions. Interestingly, these co-administration experiments highlighted significant anticonvulsant effects of 200 mg kg^{-1} CBDV not previously observed when CBDV was administered alone. Co-administration of valproate (50-250 mg kg^{-1}) with 200 mg kg^{-1} CBDV had significant anticonvulsant effects on all parameters except the proportion of animals that remained convulsion-free: CBDV and valproate co-administration significantly decreased severity ($F_{7,100}$=16.477, p≤0.001; Fig. 5A); when modelled by log linear analysis, our data indicated that mortality (Fig. 5B) and the incidence of the most severe (tonic-clonic) convulsions (Fig. 5C) were also decreased by drug co-administration; onset latency was significantly increased ($F_{7,105}$=8.649, p≤0.001; Fig. 5D). Valproate contributed significantly to all anticonvulsant effects (Fig. 5A-D, Supplementary Table 2) with the interesting exception of mortality. Although not significantly so, mortality was higher after 62.5 and 125 mg kg^{-1} valproate were co-administered with vehicle (Fig. 5B), however CBDV had an anticonvulsant effect, significantly decreasing mortality compared to administration of its vehicle (X^2(1)=4.010, p≤0.05; Fig. 5D). CBDV also significantly contributed to the overall anticonvulsant effects of treatment on severity ($F_{1,110}$=22.711, p≤0.001; Fig. 5A) and the incidence of tonic-clonic convulsions (X^2(1)=4.010, p≤0.01; Fig. 5C) although had no significant effect on onset latency (p>0.1; Fig. 5D). The proportion of animals that remained convulsion-free (X^2(6)=1.564, p>0.1)

was unaffected by treatment. No significant interactions between CBDV and valproate effects were observed (Supplementary Table 2; p>0.1).

Co-administration of 200 mg kg^{-1}CBDV and phenobarbital (10-40 mg kg^{-1}) had significant anticonvulsant effects on the severity of pilocarpine-induced convulsions ($F_{7,108}$=19.352, p≤0.001; Fig. 5E). When modelled with log linear analyses, our data indicated that there was no effect of treatment on mortality was observed (Fig. 5F), whereas the proportion of animals that developed tonic-clonic convulsions was significantly decreased (Fig. 5G). No effect of drug treatment was observed on seizure onset latency (p>0.1; Fig. 5H), however, when modelled with log linear analysis, our data indicated that the proportion of animals that remained convulsion-free was significantly increased (Fig. 5I). Phenobarbital significantly contributed to all anticonvulsant effects (Fig. 5E-I; Supplementary Table 2). CBDV significantly contributed to the overall decrease seen in severity ($F_{1,108}$=4.480, p≤0.05), and the effects of CBDV and phenobarbital interacted significantly due to a convergence of the severity observed in the absence and presence of CBDV (Fig. 5F Supplementary Table 2; $F_{3,108}$=3.105, p≤0.05), no further significant interactions between the effects of CBDV and phenobarbital were observed (p>0.1; Supplementary Table 2).

Data from the co-administration experiments demonstrate that the AEDs strongly suppress PTZ-induced seizures and pilocarpine-induced convulsions in a dose-dependent manner as expected (Figures 4 and 5). 200 mg kg^{-1} CBDV significantly contributed to the anticonvulsant effects observed in these experiments as assessed by several, but not all, parameters examined. To more precisely assess the effect of CBDV on AED actions in these studies, we performed pairwise comparisons at each dose of AED between groups which received CBDV vehicle and groups that received 200 mg

kg^{-1} CBDV; these analyses were only performed if two-way ANOVA or log linear analysis results indicated an overall effect of CBDV upon a given parameter. Based on these analyses and Figs. 5F-I, the effect of CBDV on the actions of phenobarbital in the pilocarpine model appears limited and is not significant. Similarly, the effect of CBDV on the actions of valproate in the PTZ model was limited (Figs. 4A-D); the primary effect of CBDV is on delaying seizure onset, as 200 mg kg^{-1} CBDV significantly improved the effect of 50 mg kg^{-1} valproate ($p \leq 0.05$; Fig. 4D) and showed a statistical trend towards the same effect at 100 mg kg^{-1} valproate ($p < 0.1$). More notably, CBDV significantly improved the effect of 60 mg kg^{-1} ethosuximide on PTZ-induced seizure severity and onset latency ($p \leq 0.05$; Figs. 4E and H), also showing a statistical trend to improvement of the 120 mg kg^{-1} ethosuximide effect for both these measures ($p < 0.1$). Furthermore, when 200 mg kg^{-1} CBDV was administered together with valproate prior to pilocarpine administration, it significantly improved the effects of valproate on severity (62.5 and 250 mg kg^{-1}; $p \leq 0.05$), mortality (62.5 and 125 mg kg^{-1}; $p \leq 0.05$) and the proportion of animals that experienced the most severe seizures (all doses, $p \leq 0.01$; Figs. 5A-C).

Thus, CBDV is well-tolerated when co-administered with AEDs and does not interact antagonistically with any of the AEDs studied in either seizure model. Furthermore, CBDV has significant anticonvulsant properties when co-administered with ethosuximide in the PTZ model and even greater effects when co-administered with valproate in the pilocarpine model where beneficial effects generally were observed at low and medium AED doses. CBDV had no impact on the effects of phenobarbital in the pilocarpine model and only very limited effects on the onset of seizures when co-administered with valproate prior to PTZ treatment.

CBDV motor side effect profile and anticonvulsant efficacy via oral administration

To further determine the suitability of CBDV as a clinical candidate, we assessed both its motor side effect profile and whether it could suppress seizures when administered orally prior to PTZ treatment. Many currently-used AEDs cause significant side effects at clinically effective doses, particularly on motor function (Schachter, 2007), whilst orally-delivered seizure suppression is a prerequisite for human epilepsy treatment.

We used two motor tasks to investigate the side effect profile of CBDV (50-200 mg kg^{-1}): a static beam test to assess motor coordination (Roberts *et al.*, 2006; Stanley *et al.*, 2005) and a grip-strength test to assess drug-induced muscle relaxation and functional neurotoxicity (Crofton *et al.*, 1996; Nevins *et al.*, 1993). CBDV had no **significant** effect on motor performance at any dose compared to vehicle treatment (Fig. 6A-D). In the static beam assay, the pass rate ($X^2(3)=4.053$; $p>0.1$; Fig. 6A) and mean distance travelled ($F_{3,79}=1.335$; $p>0.1$; data not shown) were both unaffected by CBDV. CBDV had no significant overall effect on the mean number of foot slips ($F_{3,79}=0.858$; $p>0.1$; Fig. 6B), **although we did note a non-significant increase in foot slips in animals treated with 200 mg kg^{-1} CBDV (0.70±0.25 slips, compared to 0.30±0.11 slips after vehicle treatment).** CBDV had no effect on grip strength ($F_{3,79}=0.465$; $p>0.1$, Fig. 6C). To validate the tests' ability to detect AED-induced motor deficits, a second group of animals received valproate (125-350 mg kg^{-1}) or saline vehicle. Valproate significantly affected the proportion of animals that successfully completed the static beam test ($X^2(3)=35.084$; $p\leq0.001$; Fig. 6A), with doses ≥250 mg kg^{-1} significantly decreasing the pass rate ($p\leq0.01$). Similarly, both the number of foot slips ($F_{3,78}=9.140$; $p\leq0.001$; Fig. 6B) and the mean distance travelled ($F_{3,78}=15.561$; $p\leq0.001$; data not shown) were significantly, negatively and dose-dependently affected by treatment with

≥250 mg kg^{-1} valproate ($p \leq 0.01$). Valproate also significantly affected the grip strength of animals ($F_{3,79}=3.175$; $p \leq 0.05$; Fig. 6C), with a small but significant decrease in mean strength induced by 350 mg kg^{-1} valproate ($p \leq 0.05$).

Finally, we investigated the ability of orally administered 400 mg kg^{-1} CBDV (see supplementary methods for dose details) to suppress PTZ seizures (90 mg kg^{-1}). 400 mg kg^{-1} CBDV significantly lowered the severity of PTZ-induced seizures (Fig. 6D, $p \leq 0.05$) from [5] to [3.5]. There were no significant effects of CBDV on seizure onset latency (vehicle 58.6±3.7 s; CBDV 61.8±5.2 s; $p>0.1$), percentage mortality (vehicle 26.7%; CBDV 20%; $p>0.1$) or development of tonic-clonic seizures (vehicle 53.3; CBDV 33.3; $p>0.1$). Overall, we demonstrate that the anticonvulsant effects of CBDV in rat are due to genuine anticonvulsant properties and not motor suppression, and that CBDV is anticonvulsant when administered orally as well as I.P in the PTZ model.

Discussion

This study demonstrates the anticonvulsant properties of CBDV for the first time and is the only to-date that has investigated CBDV effects in whole animals. Our main finding is that CBDV suppresses seizures in four *in vivo* seizure models at doses ≥50 mg kg^{-1}. CBDV also does not affect normal motor function and is well-tolerated when co-administered with AEDs. Moreover, CBDV suppresses epileptiform activity *in vitro*.

In vitro *effects of CBDV*

In both *in vitro* models of epileptiform activity, LFP duration and amplitude were significantly decreased by CBDV, with efficacy varying between hippocampal subregions and models. CA3 was most resistant to CBDV effects, potentially due to its role as the epileptiform focus (Hill *et al.*, 2010; Perreault *et al.*, 1992). It is also reported that a smaller proportion of neurons in CA1 contribute to burst activity than CA3 (Perreault *et al.*, 1992), potentially rendering CA1 more sensitive to anti-epileptiform drug effects. CBDV effects on LFP frequency in the two models were opposite; CBDV increased Mg^{2+}-free-induced LFP frequency, but decreased 4-AP-induced LFP frequency. **This may be due to a genuine, model-dependent CBDV effect on LFP frequency; however the response of frequency in the Mg^{2+}-free model is in direct contrast to all other findings across both models, where varying degrees of anti-epileptiform effects were observed. In addition, we have observed that LFPs in the Mg^{2+}-free model exhibit greater variation in frequency than the 4-AP model, occurring in sporadic bursts of LFPs with periods of relative quiescence between (see Hill *et al.*, 2010), thus, whilst the frequency of LFPs in this model was corrected to allow for inherent increases, it may be that the unpredictability of LFP incidence limits the accuracy of this process. Overall, the magnitude of CBDV**

effects on LFP amplitude and duration are comparable with both CBD and clinically-used AEDs (Hill *et al.*, 2010; Jones *et al.*, 2010; Sagratella, 1998).

In vivo *effects of CBDV and clinical implications*

CBDV possessed significant anticonvulsant properties in four seizure models with different bases across two species. CBDV was effective in three models of generalised seizure – mES and audiogenic in mice and PTZ in rat. **In particular, CBDV (200 mg kg^{-1} completely prevented tonic-clonic convulsions in the audiogenic seizure model and had robust effects in the mES model in line with the reported efficacy of valproate and other AEDs in these models (Gareri *et al.*, 2004; Luszczki *et al.*, 2011; Luszczki *et al.*, 2012). Moreover,** positive findings in the mES model - a primary screen for putative anticonvulsants (Loscher, 2011) - are predictive of clinical efficacy against generalised human seizures (Loscher, 2011), whilst audiogenic seizures, although providing limited predictive differentiation of future efficacy against human seizure types (Loscher, 2011), are also a useful model of generalised seizure (Pitkanen et al., 2006). As well as predicting effective suppression of generalised seizures in humans, attenuation of PTZ-induced seizures can be predictive of efficacy against absence seizures (Veliskova, 2006), thus CBDV should also be investigated in non-convulsive seizure models (e.g. WAG/Rjj rats (Coenen *et al.*, 2003)). Importantly, P.O. CBDV (400 mg kg^{-1}) also suppressed PTZ-induced seizures, showing that CBDV can exert anticonvulsant effects via oral administration routes.

Systemic administration of pilocarpine induces status epilepticus with a temporal lobe focus that subsequently generalises **and is associated with motor convulsions** (Curia *et al.*, 2008). Interestingly, the anticonvulsant effects of CBDV only became apparent when 200 mg kg^{-1} CBDV and AEDs were co-administered. Thus, effects were observed only in higher-power experiments in which 60, as opposed to 15, animals received 200 mg kg^{-1} CBDV.

These effects were limited (see below), suggesting that CBDV acts less efficaciously in this model than in the others studied here, however our statistical analyses assert that CBDV effects in these experiments were independent of, and separate to, AED actions. In the future, **it would be of interest to continue characterising CBDV effects on pilocarpine-induced *status epilepticus* using direct recordings of brain activity, for example via electroencephalographic or electrocorticographic recordings in this model as *status epilepticus* activity can persist in the absence of motor activity.**

Many AEDs exert significant motor side effects (Schachter, 2007) which can limit patient quality of life. To address this and confirm that CBDV's anticonvulsant actions were due to direct actions on seizures and not motor suppression, we investigated the performance of rats treated with CBDV in static beam and grip strength tasks. These tests assess balance, coordination, muscle relaxation and drug-induced functional neurotoxicity (Crofton *et al.*, 1996; Muller *et al.*, 2008; Nevins *et al.*, 1993; Stanley *et al.*, 2005). CBDV did not affect grip strength, **and although the number of foot slips did increase after 200 mg kg^{-1} CBDV treatment, this effect was not significant.** Our tests were validated by the finding that valproate negatively affected all motor parameters, consistent with previous studies (Roks *et al.*, 1999).

The above *in vivo* results indicate that CBDV has comparatively strong anticonvulsant effects in a range of seizure models, has significant potential for the treatment of generalised, human seizures and should be further investigated against temporal lobe seizures. Furthermore, data from motor function assays indicate that CBDV does not cause significant adverse motor effects at anticonvulsant doses. Our tests were validated by the finding that valproate negatively affected all motor parameters, consistent with previous studies (Roks *et al.*, 1999). **In future, it will be of great interest to investigate CBDV's properties in**

models of chronic epilepsy and hyperexcitability. The impact of chronic CBDV treatment on behaviour in healthy and epileptic animals is also worthy of investigation.

Co-administration studies

Clinical investigation of new anticonvulsants is typically performed using the candidate AED as an adjunctive treatment to the patient's current treatment regimen (French, 2001) (French et al., 2001). Therefore, we investigated the effects of CBDV (200 mg kg^{-1}) when co-administered with clinically used anticonvulsants. The three anticonvulsants used were chosen based on their use as prescribed AEDs and, more pragmatically, reported efficacy in the seizure models used (Lindekens *et al.*, 2000; Loscher, 2011; Loscher *et al.*, 1991; Shantilal *et al.*, 1999; Sofia *et al.*, 1993). **No negative interactions between CBDV and the AEDs were observed, indicating that CBDV is well-tolerated when co-administered with the three clinically used AEDs employed in these studies. The anticonvulsant effect of CBDV beyond that of these AEDs was variable, in our study. When administered with ethosuximide prior to PTZ or valproate prior to pilocarpine, CBDV contributed significantly to the effects seen on severity (both cases), mortality (valproate in pilocarpine only), latency (ethosuximide only) and the incidence of tonic-clonic convulsions (valproate in pilocarpine only). The majority of CBDV's significant facilitatory effects were seen at the lower two doses, this is could due to the greater potential for anticonvulsant actions when the AED is not producing a maximal effect itself. However, 200 mg kg^{-1} CBDV appeared to have little effect on pilocarpine-induced convulsions when administered with phenobarbital at any dose, although it should be noted that all doses of phenobarbital strongly suppressed seizure activity, likely to limit CBDV's impact. CBDV had limited effects on PTZ-induced seizures when co-administered with valproate. Thus, CBDV had AED-dependent effects in these**

experiments, producing notable improvements over AED treatment alone in two of four experiments. Based on these data, we postulate that CBDV is well-tolerated when co-administered with three AEDs used in the clinic for a variety of epileptic syndromes, but that further investigation of its anticonvulsant properties in combination with other drugs is required, for example, using isobolographic experimental design and analysis (e.g. Luszczki *et al.*, 2010).

Anticonvulsant mechanisms of CBDV

This is the first investigation of CBDV effects in any *in vivo* model or system; *in vitro* information on CBDV pharmacological properties, whilst growing, is limited (De Petrocellis *et al.*, 2011a; De Petrocellis *et al.*, 2011b; Scutt *et al.*, 2007) and remains of unknown *in vivo* or clinical relevance. For example, reported effects of CBDV at recombinant TRP channels are as yet unconfirmed in native tissue and it is unknown how such TRP-based mechanisms of action could affect excitability in epileptogenic areas. Whilst TRPV1 expression in brain areas including the hippocampus remain controversial (Cavanaugh *et al.*, 2011; Mezey *et al.*, 2000), the functional expression of other TRP subtypes in relevant parts of the brain has yet to be confirmed (Crawford *et al.*, 2009; Hirata *et al.*, 2010). CBDV has also been reported to inhibit DAGLα (De Petrocellis *et al.*, 2011a), the enzyme responsible for production of the endocannabinoid 2-arachidonoylglycerol **(2-AG; Stella *et al.*, 1997). The effect of inhibiting 2-AG production is likely to be complex. The initial effect would be to decrease 2-AG levels and subsequent CB1 cannabinoid receptor activation. However, the overall effect of this on seizure activity would depend on proportional CB1 cannabinoid receptor expression and localisation on different presynapses (i.e. excitatory or inhibitory) and the contribution of inhibitory GABAergic circuits in brain areas crucial to epileptogenesis, as a decrease in 2-AG would result in less suppression**

of both excitatory and inhibitory synapses. Furthermore, over longer time courses, it has been reported that CB1 cannabinoid receptor levels can be affected by changes in agonist levels i.e. higher levels of CB1 cannabinoid receptor agonist can increase internalisation of the receptor (Coutts *et al.*, 2001). Thus, reduced 2-AG levels could cause increased CB1 cannabinoid receptor at the membrane. Additionally, whilst this study investigated CBDV effects only upon acute seizures, CB1 cannabinoid receptor expression changes during both animal models (e.g. pilocarpine-induced SRS as a model of temporal lobe epilepsy) of chronic epilepsy and in human epilepsy (Karlocai *et al.*, 2011; Magloczky *et al.*, 2010), which could affect the consequences of changes in endocannabinoid levels upon seizure activity. Direct anticonvulsant action via CB1 cannabinoid receptor agonism has been reported for Δ^9-THC (Wallace *et al.*, 2001), however CBDV effects at the CB1 cannabinoid receptor remain to be characterised and, furthermore, 200 mg kg^{-1} CBDV caused no **significant** effects in the motor function assays used here, whilst CB1 cannabinoid receptor agonists produce significant motor deficits (Carlini *et al.*, 1974), suggesting that CBDV does not act via CB1 cannabinoid receptor agonism.

CBDV is the propyl analogue of CBD. **A solely qualitative comparison of the effects of CBD and CBDV on PTZ-induced seizures shows that both compounds improve mortality and severity; CBD showing these effects at 100 mg kg^{-1}, a dose at which CBDV did not affect severity. CBD showed no indication of affecting onset latency (≤100 mg kg^{-1}), CBDV delayed seizure onset in a dose-dependent manner that reached significance at 200 mg kg^{-1}. The comparison between CBD and CBDV in the pilocarpine model is less simple as CBDV at 200 mg kg^{-1} had wider-ranging anticonvulsant effects in our co-administration experiments (on severity, mortality and latency as well as the proportion of animals that developed tonic-clonic convulsions) but was not effective in initial experiments at any dose, whereas low-dose CBD affected**

tonic-clonic convulsions but no other measures. A direct experimental comparison both of efficacy and how similarly CBD and CBDV affect seizures may be of interest. Although assumptions of pharmacological similarity on the basis of structural homology should be made with caution (e.g. the opposing effects of Δ^9-THC and Δ^9-THCV on CB1 cannabinoid receptors), CBD is anticonvulsant in animals and humans and more is known about CBD's pharmacological properties, if not its specific anticonvulsant mechanism(s) of action. CBD is unlikely to act via CB1 cannabinoid receptor, but has a wide range of known pharmacological targets that could underlie its anticonvulsant effects (Hill *et al.*, 2012). These include inhibition of T-type Ca^{2+} channels (Ross *et al.*, 2008), inhibition of GPR55 in some tissues/preparations (Ryberg *et al.*, 2007), modulation of mitochondrial calcium handling in neurons (Ryan *et al.*, 2009) and increased activity of inhibitory non-cannabinoid G protein-coupled receptors including 5-HT1A (direct agonism; (Russo *et al.*, 2005)) and adenosine A1 (via effects on adenosine uptake;(Carrier *et al.*, 2006)). Thus, if CBDV shares some or all of CBD's pharmacological targets, it is possible that CBDV also acts via multiple mechanisms to produce its overall anticonvulsant effect, as opposed to exerting a high-efficacy action at a single target. However, there is no *a priori* reason to assume a common target and there is clearly some divergence between CBD and CBDV properties, for example CBD, but not CBDV, inhibits FAAH (De Petrocellis *et al.*, 2011a).

In conclusion, **our most important finding is that CBDV possesses strong anticonvulsant properties in a range of *in vivo* seizure models** that parallel a variety of human seizure types and pathologies; anticonvulsant effects were also seen after oral, as well as I.P., administration. As with many clinically-used AEDs, further work is required to determine the anticonvulsant mechanism of CBDV**, but the significant anticonvulsant effects and favourable motor side effect profile demonstrated in this study identify**

CBDV as a potential standalone AED or as a clinically useful adjunctive treatment alongside other AEDs.

Acknowledgments

UoR authors thank GW Pharmaceuticals and Otsuka Pharmaceuticals for research sponsorship and the provision of CBDV and thank Simon Marshall for technical assistance.

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Figure 1. Effects of CBDV on hippocampal epileptiform activity

A & B: Example traces showing effects of 100 μM CBDV on epileptiform LFPs induced by Mg^{2+}-free conditions **(A)** or 100 μM 4-AP **(B)** in the CA1 region. The black bar represents amplitude as corrected for inherent rundown (see Methods); the dotted line below represents control burst duration. Scale in A: 100μV/200 ms; B: 150μV/200 ms. **C-H:** Effects of CBDV on amplitude **(C&F)**, duration **(D&G)** and frequency **(E&H)** of epileptiform LFPs induced by Mg^{2+}-free conditions **(C-E)** or 100 μM 4-AP **(F-H)**. Data is presented as mean ± S.E.M. normalised to control (pre-drug) conditions and corrected for background changes where appropriate (see Methods). LFP amplitude and duration values are expressed for each hippocampal region as in the key. n=9-12. *= $p \leq 0.05$, **= $p \leq 0.01$ and ***= $p \leq 0.001$.

Figure 2. Effects of CBDV on mES and audiogenic seizures in mice

A: The effect of CBDV on the percentage of animals that exhibited tonic hindlimb extension in response to mES. **B-D**: The effect of CBDV (50-200 mg kg^{-1}) on the percentage of animals that displayed tonic convulsions (**B**), remained seizure-free (**C**) or suffered mortality (**D**) as a result of audiogenic seizure induction. n=10 in all cases, *** = $p \leq 0.001$.

Figure 3. Effects of CBDV on PTZ- and pilocarpine-induced seizures in rat

A-D: The effect of CBDV on PTZ-induced seizures: seizure severity (**A**), mortality (**B**), the proportion of animals remaining seizure-free (**C**) and the onset latency (**D**). **E-H:** The effect of CBDV on pilocarpine-induced convulsions: severity (**E**), mortality (**F**), the proportion of animals remaining seizure-free (**G**) and the onset latency (**H**). In **D** & **H**, onset latency is presented ± S.E.M. In **A** & **E** median severity is represented by a thick horizontal line, the

25th & 75th percentiles by the box and maxima and minima are represented by "whiskers". n=15 in all cases. *= p≤0.05, **= p≤0.01 and ***= p≤0.001.

Figure 4. Effects of co-administration of CBDV and AEDs on PTZ-induced seizures in rat

The effects of CBDV co-administration with valproate (**A-D**) or ethosuximide (**E-I**) on PTZ induced seizures: severity (**A** & **E**), mortality (**B** & **F**), the incidence of tonic-clonic seizures (**C** & **G**), onset latency (**D** & **H**) and (for CBDV+ethosuximide only) the proportion of animals that remained seizure-free. In **D** & **H**, onset latency is presented ± S.E.M. In **A** & **E**, median severity is represented by a thick horizontal line, the 25th & 75th percentiles by the box and maxima and minima are represented by "whiskers". Co-administration of 200 mg kg^{-1} CBDV is represented by grey plots, CBDV vehicle by black. Significance of CBDV treatment is given in text. n=15 in all cases. *= p≤0.05, **= p≤0.01 and ***= p≤0.001 for AED effects.

Figure 5. Effects of co-administration of CBDV and AEDs on pilocarpine-induced convulsions in rat

The effects of CBDV co-administration with valproate (**A-D**) or phenobarbital (**E-I**) on pilocarpine induced convulsions: severity (**A** & **E**), mortality (**B** & **F**), the incidence of tonic-clonic convulsions (**C** & **G**), onset latency (**D** & **H**) and (for CBDV+phenobarbital only) the proportion of animals that remained seizure-free. In **D** & **H**, onset latency is presented ± S.E.M. In **A** & **E**, median severity is represented by a thick horizontal line, the 25th & 75th percentiles by the box and maxima and minima are represented by "whiskers". Co-

administration of 200 mg kg^{-1} CBDV is represented by grey plots, CBDV vehicle by black. Significance of CBDV treatment is given in text. n=15 in all cases. *= $p \leq 0.05$, **= $p \leq 0.01$ and ***= $p \leq 0.001$ for AED effects.

Figure 6. Effects of CBDV on performance in the static beam and forelimb grip strength assays in rat and as an orally-administered anticonvulsant

A&B: Static beam performance; including the pass rate **(A)** and foot slips **(B)**. **C:** Performance in the grip strength assay. **A,** pass rate is represented as percentage; **B&C,** represented as mean ± S.E.M. n=20 for static beam data, 10 for grip strength. **D** Effect of orally administered 400 mg kg^{-1} CBDV on the severity of PTZ-induced seizures. A-C: n=20, D: n=15. *= $p \leq 0.05$, **= $p \leq 0.01$ and ***= $p \leq 0.001$ respectively. **A-C:** "V": CBDV vehicle, "S": valproate vehicle (saline).

TABLE 1

Score	PTZ-induced seizures	Pilocarpine-induced seizures
0	Normal behaviour	Normal behavior
1	Isolated myoclonic jerks	Mouth clonus
2	Atypical clonic seizure	Unilateral forelimb clonus
3	Fully developed bilateral forelimb clonus	Bilateral forelimb clonus
3.5	Forelimb clonus with tonic component and body twist	N/A
4	Tonic–clonic seizure with suppressed tonic phase*	Bilateral forelimb clonus with rearing and falling
4.5	N/A	Tonic-clonic seizure with postural control retained
5	Fully developed tonic–clonic seizure*	Tonic-clonic seizure*

Table 1. Seizure behaviour scoring scales for PTZ and pilocarpine-induced seizures.

Seizure severity scoring scales are shown for each model although no equivalency of severity should be assumed between scales for different models. * indicates a loss of righting reflex.

TABLE 2

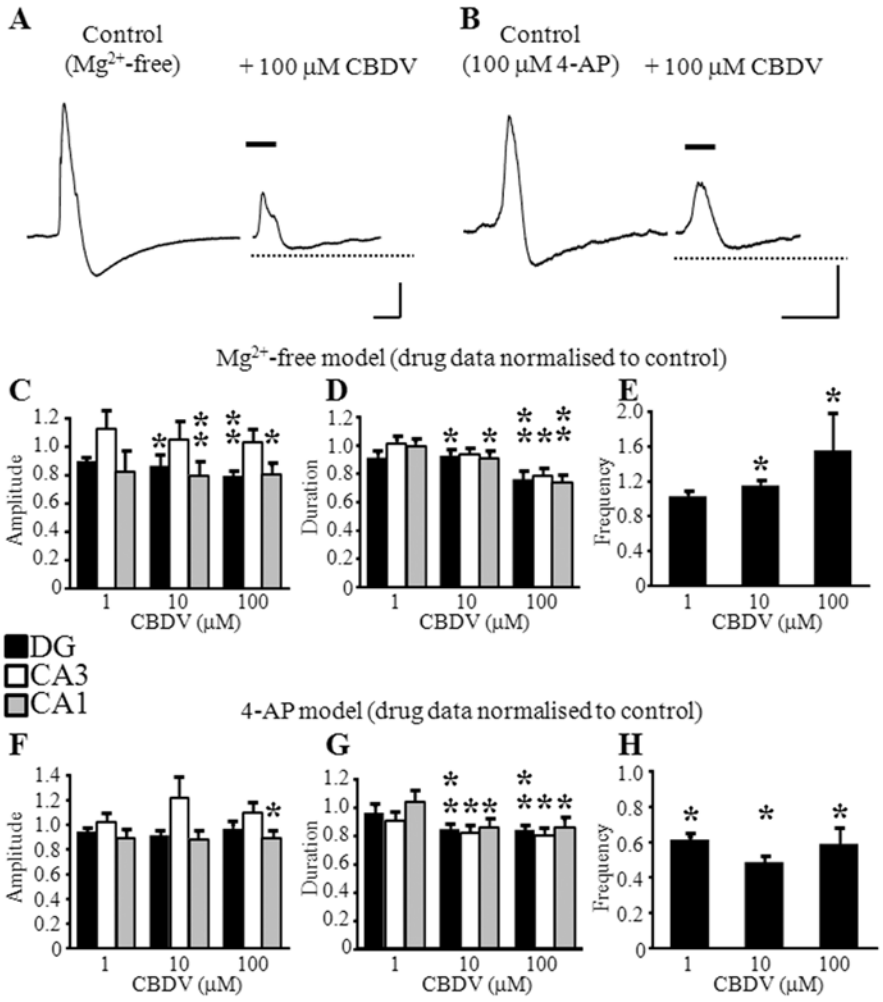
		60 min			
	CBDV/vehicle treatment (I.P.)	**Time A**	**AED treatment (I.P.)**	**Time B**	**Seizure induction and recording**
PTZ experiments	200 mg kg^{-1} CBDV (n=60)	**30 min**	Valproate (VPA) vehicle, 50, 100, 250 mg kg^{-1} VPA (n=15 each)	**30 min**	85mg kg^{-1} PTZ 30 min recording
	CBDV vehicle (n=60)		VPA vehicle, 50, 100, 250 mg kg^{-1} VPA (n=15 each)		
	200 mg kg^{-1} CBDV (n=60)	**30 min**	Ethosuximide (ESM) vehicle, 60, 120, 175mg kg^{-1} ESM (n=15 each)	**30 min**	
	CBDV vehicle (n=60)		ESM vehicle, 60, 120, 175mg kg^{-1} ESM (n=15 each)		
Pilocarpine experiments	200 mg kg^{-1} CBDV (n=60)	**15 min**	VPA vehicle, 62.5, 125, 250 mg kg^{-1} VPA (n=15 each)	**45 min**	380 mg kg^{-1} pilocarpine 60 min recording
	CBDV vehicle (n=60)		VPA vehicle, 62.5, 125, 250 mg kg^{-1} VPA (n=15 each)		
	200 mg kg^{-1} CBDV (n=60)	**15 min**	Phenobarbital (PB) vehicle, 10, 20, 40 mg kg^{-1} PB (n=15 each)	**45 min**	
	CBDV vehicle (n=60)		PB vehicle, 10, 20, 40 mg kg^{-1} PB (n=15 each)		

Table 2. Experimental design and timecourse of co-administration experiments

"Time A" column: time between CBDV/CBDV vehicle and AED administration. "Time B" column: time between AED/vehicle and convulsant. The duration of seizure recording is indicated in the final column.

CONFLICT OF INTEREST STATEMENT:

The work reported was funded by grants to BJW, CMW & GJS from GW Pharmaceuticals and Otsuka Pharmaceuticals. BJW, AJH, NAJ, CMW & GJS were responsible for experimental design. YY and TF are employees of Otsuka Pharmaceuticals and hold stocks in this company. MD and CGS are GW Pharmaceuticals employees, and CGS is a stockholder.



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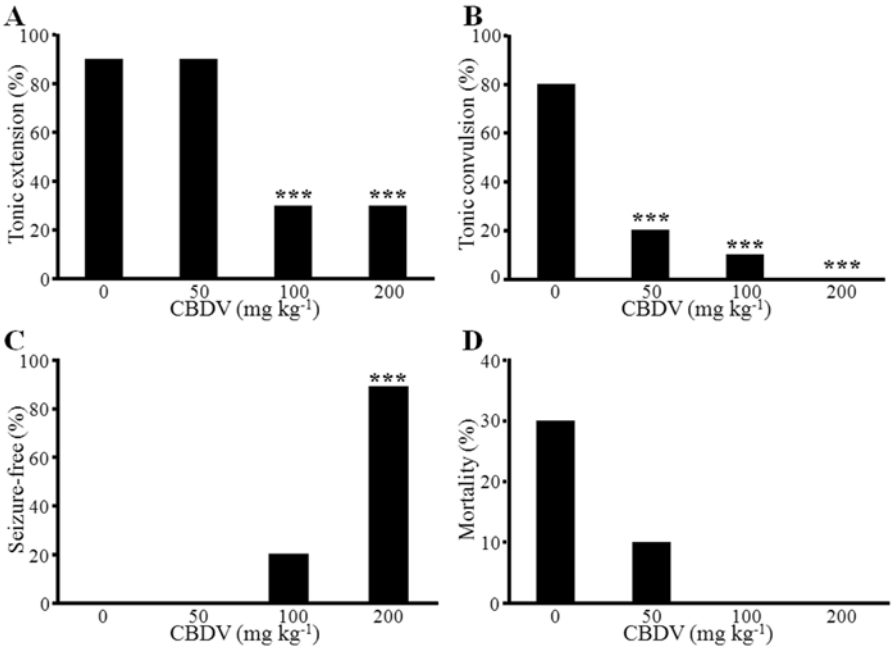

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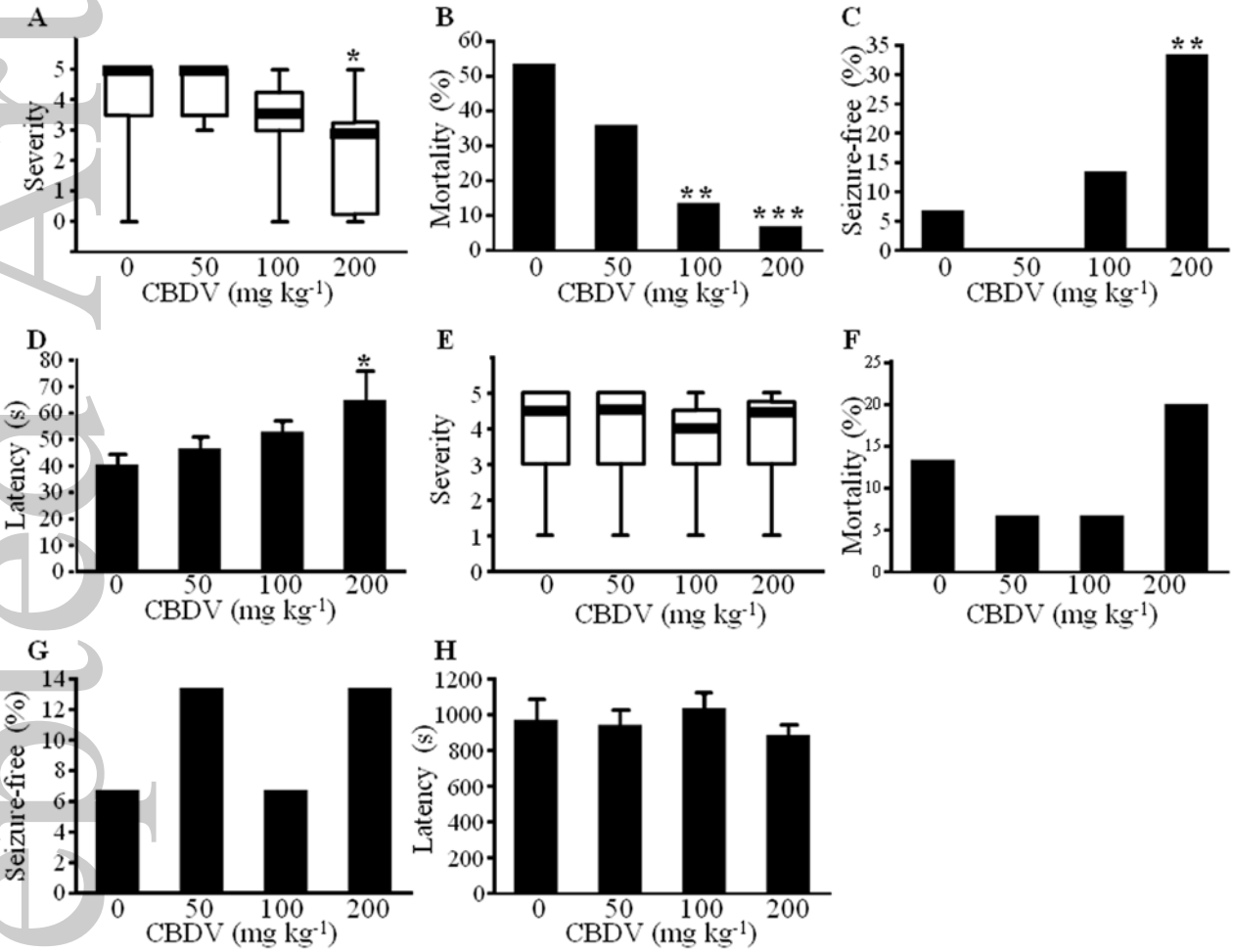

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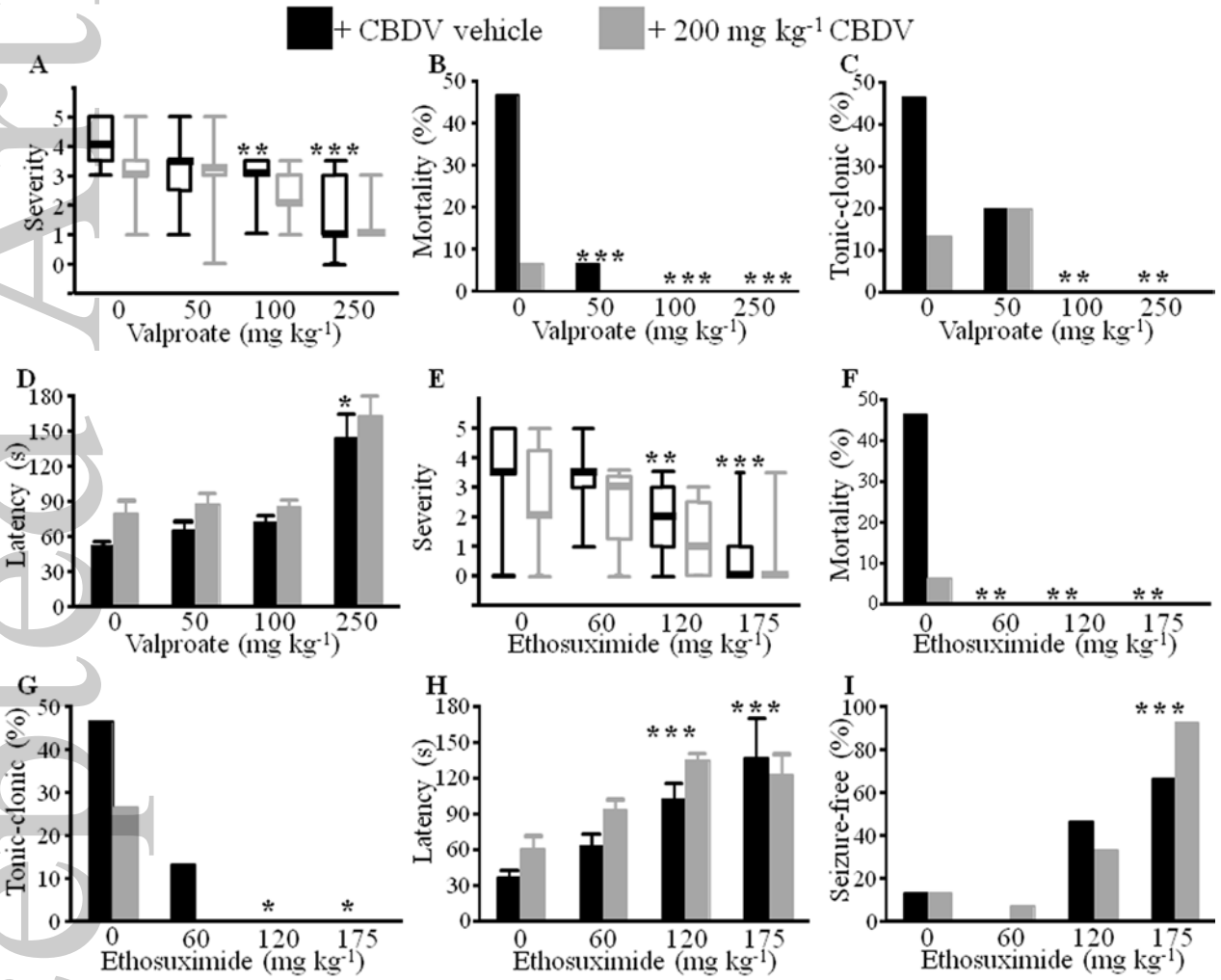

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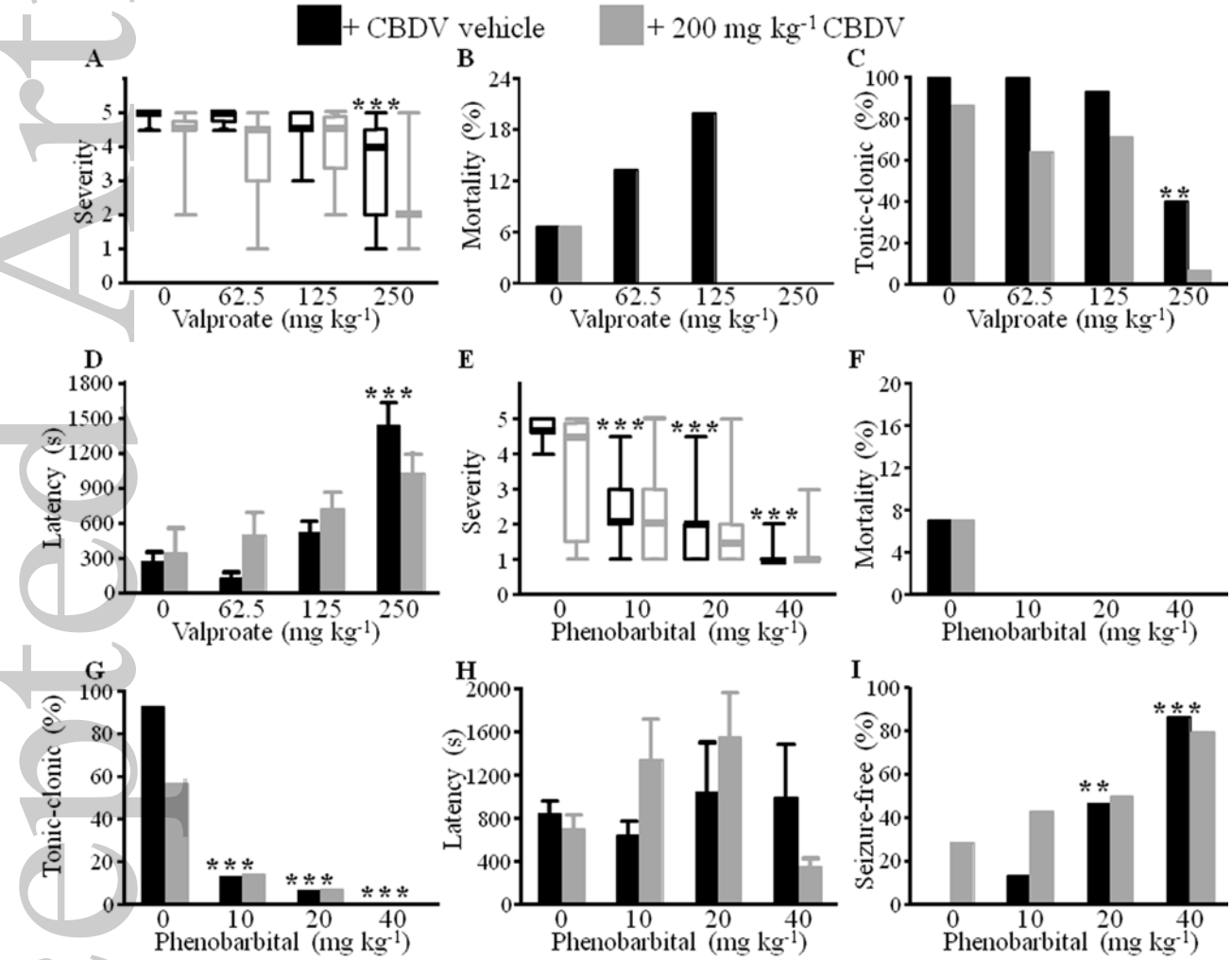

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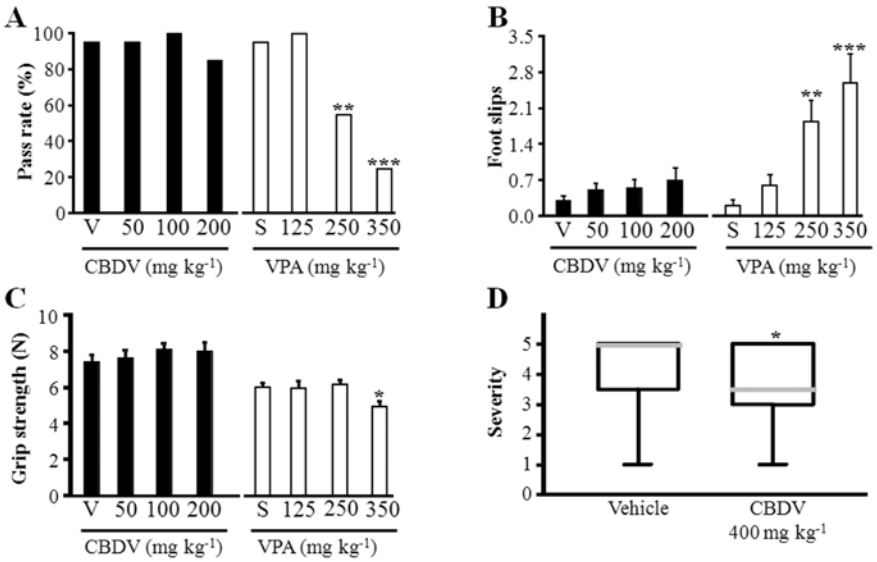

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