Supporting Information

Supporting Information Table 1

Rank	Low dose (1.08/1.00 THC/CBD mg kg^{-1})				High dose (40.5/37.5 THC/CBD mg kg^{-1})			
	Acute	**Median % (IQR)**	**Persistent**	**Median % (IQR)**	**Acute**	**Median % (IQR)**	**Persistent**	**Median % (IQR)**
7							Mouth rubbing	0 (0-0)
8			Hunched posture	0 (0-0)			**Chewing**	0 (0-0)
9			Exopthalmos	0 (0-0)			**Twitch**	0 (0-0)
10			Fasciculations	0 (0-0)			**Tremor**	0 (0-0)
11	Piloerection	0 (0-0)	Piloerection	0 (0-0)			Behavioural arrest	0 (0-0)
12	Digit biting	0 (0-0)	Digit biting	0 (0-0)			**Forelimb flickering**	0 (0-0)
13	**Twitch**	0 (0-0)	Mouth rubbing	0 (0-0)	Digit biting	0 (0-0)	**Forelimb clonus**	0 (0-0)
14	**Tremor**	0 (0-0)	**Chewing**	0 (0-0)	**Tremor**	0 (0-0)	**Popping**	0 (0-0)
15	Behavioural arrest	0 (0-0)	**Twitch**	0 (0-0)	**Forelimb clonus**	0 (0-0)	Fasciculations	0 (0-0)
16	**Forelimb clonus**	0 (0-0)	**Tremor**	0 (0-0)	**Popping**	0 (0-0)	Writhing	0 (0-0)
17	**Popping**	0 (0-0)	Behavioural arrest	0 (0-0)	Fasciculations	0 (0-0)	Licking	0 (0-0)
18	Fasciculations	0 (0-0)	**Forelimb clonus**	0 (0-0)	Writhing	0 (0-0)	Salivation	0 (0-0)
19	Hunched posture	0 (0-0)	**Popping**	0 (0-0)	Licking	0 (0-0)	Hind limb extension	0 (0-0)
20	Licking	0 (0-0)	Licking	0 (0-0)	Hind limb extension	0 (0-0)	Head searching	0 (0-0)
21	Exopthalmos	0 (0-0)	Salivation	0 (0-0)	Head searching	0 (0-0)	**Myoclonic jerk**	0 (0-0)
22	Hind limb extension	0 (0-0)	Hind limb extension	0 (0-0)	**Myoclonic jerk**	0 (0-0)	**Convulsion**	0 (0-0)
23	Head searching	0 (0-0)	Head searching	0 (0-0)	**Convulsion**	0 (0-0)	Hunched posture	0 (0-0)
24	**Myoclonic jerk**	0 (0-0)	**Myoclonic jerk**	0 (0-0)	Hunched posture	0 (0-0)	Exopthalmos	0 (0-0)
25	**Convulsion**	0 (0-0)	**Convulsion**	0 (0-0)	Exopthalmos	0 (0-0)	Digit biting	0 (0-0)

Supporting Information Table 2

Behaviour	All behaviours			
	F1		F2	
	Squared cosine	Contribution (%)	Squared cosine	Contribution (%)
Piloerection	**0.704**	3.205	0.282	13.661
Ptosis	**0.731**	8.931	0.250	32.564
Digit biting	**0.324**	0.057	0.031	0.059
Increased grooming	**0.704**	2.813	0.091	3.861
Increased scratching	**0.510**	1.229	0.449	11.532
Wet dog shakes	**0.846**	3.210	0.152	6.148
Mouth rubbing	**0.957**	38.458	0.043	18.400
Chewing	**0.990**	3.395	0.001	0.030
Twitch	0.375	0.040	**0.623**	0.703
Tremor	**0.200**	0.004	0.161	0.037
Behavioural arrest	**0.680**	0.137	0.279	0.598
Forelimb flickering	**0.878**	3.703	0.118	5.318
Forelimb paddling	**0.999**	32.490	0.000	0.000
Forelimb clonus	**0.674**	0.051	0.215	0.173
Popping	**0.569**	0.002	0.282	0.008
Fasciculations	**0.391**	0.000	0.107	0.000
Writhing	0.222	0.036	**0.653**	1.140
Licking	**0.676**	0.000	0.058	0.000
Salivation	**0.791**	2.236	0.190	5.702
Hind limb extension	**0.627**	0.002	0.371	0.012
Head searching	0.032	0.000	**0.456**	0.002
Myoclonic jerk	0.082	0.000	**0.748**	0.001
Convulsion	0.040	0.000	**0.725**	0.049
Hunched posture	**0.535**	0.000	0.428	0.000
Exopthalmos	**0.535**	0.000	0.428	0.000
Piloerection	**0.704**	3.205	0.282	13.661

Factor scores / **Observations**	F1	F2
Low dose/persistent	-57.6	-2.4
Low dose/acute	38.7	-26.6
High dose/persistent	-52.2	9.5
High dose/acute	71.1	19.5

Supporting Information Table 3

Animal ID	Dose group	Treatment day	During treatment or observation period	Motor convulsion	EEG record obtained	Epileptiform event present in EEG	Epileptiform event duration (s)
6	Low	64	No	No	Yes	Yes	28
13	High	46*	Yes	Yes	Yes	Yes	95
	High	46*	Yes	Yes	Yes	Yes	27
	High	46*	Yes	Yes	Yes	Yes	22
	High	58	Yes	Yes	Yes	Yes	50
	High	61	Yes	Yes	No	N/A	N/A
	High	61	Yes	Yes	No	N/A	N/A
	High	61	Yes	Yes	No	N/A	N/A
15	High	36	No	No	Yes	Yes	16
	High	55	No	Yes	No	N/A	N/A
	High	62*	No	Yes	Yes	Yes	65
	High	62*	No	Yes	Yes	Yes	44
16	High	21	Yes	Yes	Yes	Yes	74
	High	36	No	No	Yes	Yes	23
	High	44	Yes	Yes	Yes	Yes	115
17	High	39*	Yes	Yes	Yes	Yes	104
	High	39*	Yes	Yes	Yes	Yes	16
	High	39*	Yes	Yes	Yes	Yes	37
	High	69	No	Yes	Yes	Yes	57
	High	71	No	Yes	Yes	Yes	114
	High	76	No	Yes	No	N/A	N/A
19	High	47	No	Yes	No	N/A	N/A
	High	50	Yes	Yes	No	N/A	N/A
	High	58	No	Yes	No	N/A	N/A
	High	69	Yes	Yes	Yes	Yes	87
20	High	63	No	No	Yes	Yes	N/A**
23	High	61	Yes	No	No	N/A	N/A
	High	90	Yes	Yes	Yes	Yes	N/A**
24	High	91	Yes	Yes	No	N/A	N/A

Supporting Information Table Legends

Supporting Information Table 1

Incidence of behaviours in rats (n=10 per group) observed immediately after (10 min; 'acute') or ~23 hours after ('persistent') daily oral administration of low dose (1.08 mg kg^{-1} Δ^9-THC + 1 mg kg^{-1} CBD) or high dose (40.5 mg kg^{-1} Δ^9-THC + 37.5 mg kg^{-1} CBD) cannabis extract for 13 weeks that exhibited a median and IQR of zero. Behavioural events conventionally associated with generalised seizures in rodents are highlighted in bold.

Supporting Information Table 2

Table showing squared cosine and percentage contribution of each measured behaviour following variance-covariance principal component analysis applied to all behaviours recorded in low dose (1.08 mg kg^{-1} Δ^9-THC + 1 mg kg^{-1} CBD) and high dose (40.5 mg kg^{-1} Δ^9-THC + 37.5 mg kg^{-1} CBD) cannabis extract treated animals. Behaviours highlighted in bold show those conventionally associated with primary generalised seizures in rodents (see also: **Figures 1 & 2**). Squared cosine values shown in bold highlight the principal component in which the value exhibited its highest value. Table also shows factor values for each observation for the first two principal components (**F1** and **F2**).

Supporting Information Table 3

Incidence of all convulsive motor events and/or epileptiform events exhibited in rats treated with low dose (1.08 mg kg^{-1} Δ^9-THC plus 1 mg kg^{-1} CBD) or high dose (40.5 mg kg^{-1} Δ^9-THC plus 37.5 mg kg^{-1} CBD) cannabis extract for 13 weeks. Note that in some case, accompanying EEG recordings (see Figure 4) showed (*) multiple, discrete, epileptiform events during a single motor convulsion and (**) animal handling or severity of motor convulsion that prevented acquisition of valid EEG data.

Supporting Information Methods

Acquisition and processing of EEG data

EEG data were acquired via the implants and wirelessly transmitted to PhysioTel receivers (DSI) connected via a Matrix MX2 interface (DSI) to a PC for storage until offline analysis. Data were sampled at 500 Hz. Epileptiform activity in EEG was detected using an automated seizure detection algorithm implemented in Neuroscore (DSI) where perturbations of >5x standard deviations of signal amplitude from pre-treatment baseline per animal were manually inspected and assessed. Mean epileptiform event duration was calculated from recorded EEG events that were not contaminated by handling-related artefacts. Prior to spectrographic analysis, EEG data which included all detected epileptiform events were exported from Neuroscore as per animal European Data Format (EDF) files, high pass filtered (1 Hz cutoff) using EDF Browser (http://www.teuniz.net/edfbrowser/) before import into Matlab (The Mathworks, Natick, MA, USA), where each recording was converted to a format suitable for import to Neuroexplorer 4.0 (Nex Technologies, Madison, AL, USA) and OriginPro 8.6 (OriginLab, Northampton, MA, USA). Spectrograms were constructed (1-20 Hz; 2048 values; 320 temporal shifts of 0.5 s) and normalised to the sum of spectrum values as the mean squared value of the whole signal within the frequency range specified. Power spectral density (PSD; 1-50 Hz; 256 values) was normalised to percentage of total PSD of the signal. Spectrograms and PSD plots were smoothed using a 3-point boxcar filter. Representative traces of epileptiform events were constructed in OriginPro from high pass filtered, continuous data.

Preparation and analysis of bioanalytes

Samples, negative controls and calibration standards (provided by GW Research Ltd) were prepared for UPLC-MS/MS by mixing 100 µl sample, standard or control with 100 µl 1% Tween80 in dH_2O in a 96-well plate before vortex mixing (60 s; 1000 rpm) and subsequent addition of 20 µl dehydrated acetonitrile to all blank samples and 20 µl internal standard working solution to all other wells. Resulting solutions were vortex mixed (30 s; 1200 rpm) before 200 µl ASTED conditioning solution (30 g trichloroacetic acid, 24.5 g Trizma ® Base tris and 0.05 g sodium azide in 200 ml UPLC water) was added before vortex mixing again (30 s; 1200 rpm), followed by addition of 200 µl dH_2O and vortex mixing (30 s; 1200 rpm). Samples were then loaded onto a solid phase extraction plate (Waters Oasis HLB) before being washed twice with 1 ml

dH_2O and then washed twice with 1 ml methanol:water (20:80$^{v}/_{v}$). Samples were then dried under full flow for 1 min before being washed twice with 1 ml hexane and subsequent re-drying under full flow for 30 s. Samples were eluted into a 1 ml collection plate using two volumes (400 µl) methanol and evaporated at 30ºC under N_2. Samples were then reconstituted in 100 µl 1% Tween80 in dH_2O:methanol (60:40$^{v}/_{v}$), vortex mixed (30 s; 1200 rpm), centrifuged (5 min; 2600 g) and inject onto an Acquity Binary Solvent Manager UPLC-MS/MS system for analysis. A UPLC flow rate of 0.6 ml/min with a run time of 6.8 min at 65ºC that used mobile phases comprising 0.1% ammonia in methanol and 5 mM aqueous ammonium formate (pH 9) was used and was connected to an Applied Biosystems API5000 mass spectrometer with a heated nebuliser at 500ºC (5 amps) and the following parameters used: gas pressure: 40 psi; curtain gas pressure: 35 psi; collision gas pressure: 7 psi; probe position; 5 mm/5 mm. Cannabinoid and metabolite concentrations in samples were determined by comparison to standard curves.

Radioligand Binding

Membrane preparation

Cerebellar tissue was pooled according to dose group and treatment time. After sacrifice, cerebellae from male mice, rats and chickens were rapidly removed and flash frozen in liquid nitrogen and stored at -80ºC until use. Male beagle and human cerebellae were supplied, already flash frozen. Irrespective of tissue type, membranes were prepared and used as described hereafter. Tissue was suspended in a membrane buffer containing Tris-HCl 50 mM, $MgCl_2$ 5 mM, EDTA 2mM and 0.5 mg ml^{-1} fatty acid-free BSA and complete protease inhibitor (pH 7.4) and subsequently homogenised using an Ultra-Turrax blender. Homogenates were centrifuged at 1200 g for 10 min and supernatants decanted. The resulting pellets were homogenised and centrifugation repeated. Pooled supernatants were then centrifuged at 39,000 g for 60 min in a high-speed centrifuge and supernatants discarded. Remaining pellets were re-suspended in membrane buffer and protein content determined by Lowry assay (Lowry, 1951).

Saturation binding

All drug stocks and membrane preparations were diluted in assay buffer (20 mM HEPES, 1 mM EDTA, 1 mM EGTA, 0.5%$^{w}/_{v}$ fatty acid-free BSA, pH 7.4) and stored on ice prior to use. Assay tubes contained a

final volume of 1ml with [^{3}H]SR141716A at final concentrations of (nM): 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 or cold SR141716A at 100 µM to determine non-specific binding. Assays were initiated by addition of 30 µg membrane protein before incubation for 3 h at 25°C and terminated by rapid filtration through Whatman GF/C filters using a Brandell cell harvester. Unbound radioactivity was removed by four washes with 3 ml ice-cold PBS (0.14 M NaCl, 3 mM KCl, 1.5 mM KH_2PO_4, 5 mM Na_2HPO_4; pH 7.4). Filters were soaked in 2 ml scintillation fluid overnight and radioactivity quantified by liquid scintillation spectrometry (Wallace 1414).

[^{35}S]-GTPγS binding

Assays were carried out in assay buffer containing (in mM) HEPES 20, $MgCl_2$ 3, NaCl 60, EGTA 1 and 0.5 mg ml^{-1} fatty acid free BSA; pH 7.4. Stock solutions of drugs and membrane preparations were diluted in assay buffer immediately before use and stored on ice prior to incubation. Assay tubes contained a final volume of 1 ml and guanosine 5'-diphosphate (GDP) at a final concentration of 10 µM, together with drugs at the desired final concentration, vehicle at an equivalent concentration, or additional assay buffer (when determining basal binding). Assays were initiated by addition of 10 µg membrane protein, pre-incubated for 30 min at 30°C prior to addition of [^{35}S]-GTPγS to a final concentration of 0.1 nM and terminated after a further 30 min incubation at 30°C by rapid filtration (Whatman GF/C filters) using a Brandell cell harvester and three washes with ice-cold phosphate buffered saline to remove unbound radioactivity. Filters were incubated overnight in 2 ml scintillation fluid, and radioactivity quantified by liquid scintillation spectrometry. [^{35}S]-GTPγS binding was expressed as percentage increase in radioactivity (measured as dpm) in the presence of drugs relative to basal levels of binding. Basal binding was defined as radioactivity measured in conditions of no agonist stimulation in the presence of 10 µM GDP and determined via a 10 pM-100 µM GDP dependency curve in triplicate on four separate occasions for each membrane preparation using 0.1 nM [^{35}S]-GTPγS.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein Measurement with the folin phenol reagent. J. Biol. Chem 193:265-275.