2019

Effect of cannabidiol on endocannabinoid, glutamatergic and GABAergic signalling markers in male offspring of a maternal immune activation (poly I:C) model relevant to schizophrenia

Ashleigh L. Osborne
University of Wollongong, alo649@uowmail.edu.au

Nadia Solowij
University of Wollongong, *Australian Centre for Cannabinoid Clinical and Research Excellence*, nadia@uow.edu.au

Ilijana Babic
University of Wollongong, *Illawarra and Shoalhaven Health District*, ib171@uowmail.edu.au

Jeremy Lum
Schizophrenia Research Institute, University of Wollongong, jlum@uow.edu.au

Kelly A. Newell
University of Wollongong, knewell@uow.edu.au

See next page for additional authors

Publication Details

Osborne, A. L., Solowij, N., Babic, I., Lum, J. S., Newell, K. A., Huang, X. & Weston-Green, K. (2019). Effect of cannabidiol on endocannabinoid, glutamatergic and GABAergic signalling markers in male offspring of a maternal immune activation (poly I:C) model relevant to schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 95 109666-1-109666-10.

Effect of cannabidiol on endocannabinoid, glutamatergic and GABAergic signalling markers in male offspring of a maternal immune activation (poly I:C) model relevant to schizophrenia

Abstract

The mainstay treatment for schizophrenia is antipsychotic drugs (APDs), which are mostly effective against the positive symptoms (e.g. hallucinations), but provide minimal benefits for the negative symptoms (e.g. social withdrawal) and cognitive deficits. We have recently shown that treatment with the non-intoxicating phytocannabinoid, cannabidiol (CBD), can improve cognition and social interaction deficits in a maternal immune activation (MIA) model relevant to the aetiology of schizophrenia, however, the mechanisms underlying this effect are unknown. An imbalance in the main excitatory (glutamate) and inhibitory (GABA) neurotransmitter systems in the brain plays a role in the pathophysiology of schizophrenia. Therefore, the endocannabinoid system could represent a therapeutic target for schizophrenia as a regulator of glutamate and GABA release via the CB1 receptor (CB1R). This study investigated the effects of chronic CBD treatment on markers of glutamatergic, GABAergic and endocannabinoid signalling in brain regions implicated in social behaviour and cognitive function, including the prefrontal cortex (PFC) and hippocampus (HPC). Time-mated pregnant Sprague-Dawley rats (n = 16) were administered poly I:C (4 mg/kg, i.v.) or saline (control) on gestational day 15. Male offspring were injected with CBD (10 mg/kg, i.p.) or vehicle twice daily from postnatal day 56 for 3 weeks. The prefrontal cortex (PFC) and hippocampus (HPC) were collected for post-mortem receptor binding and Western blot analyses (n = 8 per group). CBD treatment attenuated poly I:C-induced deficits in cannabinoid CB1 receptor binding in the PFC and glutamate decarboxylase 67, the enzyme that converts glutamate to GABA, in the HPC. CBD treatment increased parvalbumin levels in the HPC, regardless of whether offspring were exposed to poly I:C in utero. Conversely, CBD did not affect N-methyl-d-aspartate receptor and gamma-aminobutyric acid (GABA) A receptor binding or protein levels of fatty acid amide hydrolase, the enzyme that degrades the endocannabinoid, anandamide. Overall, these findings show that CBD can restore cannabinoid/GABAergic signalling deficits in regions of the brain implicated in schizophrenia pathophysiology following maternal poly I:C exposure. These findings provide novel evidence for the potential mechanisms underlying the therapeutic effects of CBD treatment in the poly I:C model.

Disciplines

Medicine and Health Sciences

Publication Details

Osborne, A. L., Solowij, N., Babic, I., Lum, J. S., Newell, K. A., Huang, X. & Weston-Green, K. (2019). Effect of cannabidiol on endocannabinoid, glutamatergic and GABAergic signalling markers in male offspring of a maternal immune activation (poly I:C) model relevant to schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 95 109666-1-109666-10.

Authors

Ashleigh L. Osborne, Nadia Solowij, Ilijana Babic, Jeremy Lum, Kelly A. Newell, Xu-Feng Huang, and Katrina Weston-Green

Effect of cannabidiol on endocannabinoid, glutamatergic and GABAergic signalling markers in male offspring of a maternal immune activation (poly I:C) model relevant to schizophrenia.

Authors: Ashleigh L. Osborne (BMHlthSc Hons) [a, b, c], Nadia Solowij (PhD) [d, e], Ilijana Babic (BMHlthSc Hons) [a, b, c, f], Jeremy S. Lum (BMSc Hons) [a, b, c], Kelly A. Newell (PhD) [a, b, c], Xu-Feng Huang (DSc, PhD, MBBS) [b, c, e], Katrina Weston-Green (PhD) [a, b, c, e*]

Affiliations:

[a] Neuropharmacology and Molecular Psychiatry Laboratory, School of Medicine, University of Wollongong, Wollongong, NSW, 2522, Australia

[b] Centre for Translational Neuroscience, Illawarra Health and Medical Research Institute, Wollongong, NSW, 2522, Australia

[c] Molecular Horizons, University of Wollongong, Wollongong, NSW, 2522, Australia

[d] School of Psychology, University of Wollongong, and Illawarra Health and Medical Research Institute, Wollongong, NSW, 2522, Australia

[e] Australian Centre for Cannabinoid Clinical and Research Excellence, New Lambton Heights, NSW, 2305 Australia

[f] Illawarra and Shoalhaven Health District, Wollongong, NSW, 2500, Australia

*Corresponding Author:

Dr Katrina Weston-Green

School of Medicine

Faculty of Science, Medicine and Health

University of Wollongong

Northfields Avenue, Wollongong, NSW 2522 Australia

Tel: +61 2 4252 8506

Email: katrina_green@uow.edu.au

Keywords: cannabidiol, maternal immune activation, schizophrenia, cannabinoid, glutamate, GABA, cognition, brain

Word count: 6091

Abstract

The mainstay treatment for schizophrenia is antipsychotic drugs (APDs), which are mostly effective against the positive symptoms (e.g. hallucinations), but provide minimal benefits for the negative symptoms (e.g. social withdrawal) and cognitive deficits. We have recently shown that treatment with the non-intoxicating phytocannabinoid, cannabidiol (CBD), can improve cognition and social interaction deficits in a maternal immune activation (MIA) model relevant to the aetiology of schizophrenia, however, the mechanisms underlying this effect are unknown. An imbalance in the main excitatory (glutamate) and inhibitory (GABA) neurotransmitter systems in the brain plays a role in the pathophysiology of schizophrenia. Therefore, the endocannabinoid system could represent a therapeutic target for schizophrenia as a regulator of glutamate and GABA release via the CB1 receptor (CB1R). This study investigated the effects of chronic CBD treatment on markers of glutamatergic, GABAergic and endocannabinoid signalling in brain regions implicated in social behaviour and cognitive function, including the prefrontal cortex (PFC) and hippocampus (HPC). Time-mated pregnant Sprague-Dawley rats (n = 16) were administered poly I:C (4 mg/kg, i.v.) or saline (control) on gestational day 15. Male offspring were injected with CBD (10 mg/kg, i.p.) or vehicle twice daily from postnatal day 56 for 3 weeks. The prefrontal cortex (PFC) and hippocampus (HPC) were collected for post-mortem receptor binding and Western blot analyses (n = 8 per group). CBD treatment attenuated poly I:C-induced deficits in cannabinoid CB1 receptor binding in the PFC and glutamate decarboxylase 67, the enzyme that converts glutamate to GABA, in the HPC. CBD treatment increased parvalbumin levels in the HPC, regardless of whether offspring were exposed to poly I:C in utero. Conversely, CBD did not affect *N*-methyl-D-aspartate receptor and gamma-aminobutyric acid (GABA) A receptor binding or protein levels of fatty acid amide hydrolase, the enzyme that degrades the endocannabinoid, anandamide. Overall, these findings show that CBD can restore

cannabinoid/GABAergic signalling deficits in regions of the brain implicated in schizophrenia pathophysiology following maternal poly I:C exposure. These findings provide novel evidence for the potential mechanisms underlying the therapeutic effects of CBD treatment in the poly I:C model.

1. Introduction

The prefrontal cortex (PFC) and hippocampus (HPC) are anatomically connected and synchrony between the two regions in important for normal brain function (Li et al., 2015). However, people with schizophrenia exhibit structural abnormalities in these brain regions, including reduced hippocampal volume and cortical thinning in the PFC (Dietsche et al., 2017; van Erp et al., 2016). In addition, patients show abnormal activity in the default mode network (i.e. the network of brain regions that are active at rest) and during memory tasks (e.g. evidenced by reduced gamma oscillations), suggesting that dysfunction in these regions may underlie the symptomatology of the disorder, particularly the negative and cognitive symptom domains (Gonzalez-Burgos and Lewis, 2012; Guo et al., 2017). Unfortunately, the negative and cognitive symptoms tend to precede the onset of psychosis, are persistent over the course of the disorder, and are associated with poor functional outcomes in patients (Lindenmayer et al., 2013; Barch and Ceaser, 2012). Although antipsychotic drugs (APDs) are generally effective at controlling the positive symptoms of schizophrenia (e.g. hallucinations and delusions), the drugs have poor efficacy against the negative symptoms (e.g. social withdrawal) (Lindenmayer et al., 2013) and cognitive deficits of schizophrenia (Goff et al., 2011), and in some cases can worsen cognition (Hill et al., 2010). Despite the introduction of newer 'second generation' APDs to the market, this drug class does not exhibit superior efficacy to 'first generation' APDs (reviewed in MacKenzie et al., 2018). Therefore, new therapeutic options that can address the negative and cognitive symptom domains of schizophrenia are required.

Cannabidiol (CBD), the main non-intoxicating phytocannabinoid found in the cannabis plant, has the potential to alleviate symptoms across a range of pathological conditions, including

epilepsy, chronic pain, anxiety and movement disorders (e.g. Parkinson's disease) (Crippa et al., 2018; Osborne et al., 2017b). However, there are limited studies that have investigated the chronic effects of CBD treatment in schizophrenia. CBD (800 mg, 4 weeks) significantly improved symptoms (measured on the Positive and Negative Syndrome Scale (PANSS)) in acute paranoid schizophrenia patients in a manner comparable to amisulpride, but had a more favourable side effect profile (e.g. less body weight gain) (Leweke et al., 2012). Recent clinical trials have explored the therapeutic potential of CBD as an adjunct to existing APD medications of stable schizophrenia outpatients. After 6 weeks of treatment, PANSS scores of the CBD-treated group (1000 mg/day, 6 weeks) significantly improved compared to placebo, while cognition improved from baseline (assessed using Brief Assessment of Cognition in Schizophrenia), but fell short of statistical significance against placebo ($p = 0.068$) (McGuire et al., 2018). In contrast, a similar trial that used a lower dose of CBD (600 mg/day, 6 weeks) found no improvement on PANSS or the MATRICS Consensus Cognitive Battery compared to baseline (Boggs et al., 2018), suggesting that dosage may be a critical factor for the efficacy of CBD in schizophrenia, particularly in APD-treated patients. Our laboratory recently reported that CBD treatment improved cognition and social interaction in adult male rat offspring exposed to maternal immune activation (MIA) using polyinosinic-polycytidilic (poly I:C) acid (Osborne et al., 2017a), which mimics some of the positive and negative symptoms, and cognitive deficits observed in schizophrenia (Meyer and Feldon, 2012). However, the mechanisms by which CBD improves negative and cognitive phenotypes in poly I:C offspring is unknown.

The endogenous cannabinoid (eCB) system plays an important role in various physiological functions, including neuroprotection, synaptic plasticity, memory and reward processing. The cannabinoid CB1 receptor (CB1R) is the main receptor of the eCB system in the brain with

high expression levels in regions involved in cognitive function, including the PFC and HPC (Lu and Mackie, 2016). Multiple studies have implicated eCB system dysregulation in the pathophysiology of schizophrenia, including alterations in CB1R expression (reviewed in Ferretjans et al., 2012), its endogenous ligand anandamide (AEA) (De Marchi et al., 2003; Giuffrida et al., 2004; Koethe et al., 2009; Leweke et al., 1999), and fatty acid amide hydrolase (FAAH), the enzyme primarily responsible for the degradation of AEA (Bioque et al., 2013; Takata et al., 2013). The eCB system is functionally linked to the major excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmitter systems in the brain, as endogenous cannabinoids bind to the CB1R and dampen presynaptic glutamate and/or GABA release (Viveros et al., 2012). An imbalance in glutamatergic and GABAergic signalling in the brain has been implicated in the cognitive deficits of schizophrenia (Gonzalez-Burgos and Lewis, 2012). Post-mortem schizophrenia studies report alterations in the ionotropic glutamatergic *N*-methyl-D-aspartate receptor (NMDAR) and its obligatory GluN1 subunit (Catts et al., 2016), and gamma-aminobutyric acid alpha receptor ($GABA_AR$) (Gonzalez-Burgos and Lewis, 2008), as well as a reduction in glutamate decarboxylase 67 (GAD_{67}; the rate-limiting enzyme that converts glutamate to GABA) and the calcium binding protein parvalbumin (PV), expressed on GABAergic interneurons (Cohen et al., 2015). The interaction of CBD with the eCB, glutamatergic and GABAergic systems is not well understood. CBD blocks the effects of CB1R/CB2 combined receptor agonists (McPartland et al., 2015; Pertwee, 2008; Thomas et al., 2007), acting as a CB1R negative allosteric modulator (NAM) *in vitro* (Laprairie et al., 2015) and may increase AEA levels by inhibiting FAAH activity (Leweke et al., 2012). Studies show that CBD can prevent behavioural and neurochemical deficits induced by NMDAR antagonism (Gomes et al., 2015a; Gururajan et al., 2012), and can act as a positive allosteric modulator of the $GABA_AR$ to increase inhibitory tone (Bakas et al., 2017). Therefore, the aim of this study was to examine the

effects of CBD treatment on eCB (CB1R binding density and FAAH protein levels), as well as glutamatergic (NMDAR binding and subunit levels) and GABAergic ($GABA_AR$ binding density, GAD_{67} and PV protein levels) markers in the brains of poly I:C offspring that exhibit cognitive deficits.

2. Experimental Procedures

2.1 Ethics Statement

Experimental procedures were approved by the Animal Ethics Committee of the University of Wollongong, NSW, Australia (AE15/05) and complied with the National Health and Medical Research Council (NHMRC), Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). All efforts were made to minimise the number and suffering of animals.

2.2 Animal Experiments

The detailed methods used for the animal experiments have been reported previously (Osborne et al., 2017a) in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (Kilkenny et al., 2010). Briefly, 16 time-mated pregnant Sprague-Dawley rats (12 weeks old, gestational day (GD) 15; Animal Resources Centre, Perth, WA) were administered poly I:C (4 mg/kg, i.v; Sigma-Aldrich, Castle Hill, Australia) or saline (control) to the lateral tail vein. Offspring were not cross-fostered and were maintained with their respective dams until weaning. After weaning (postnatal day (PN) 21), male offspring were pair-housed with same-treatment littermates. Offspring were administered CBD (10 mg/kg; i.p.; THC-Pharm GmbH, Frankfurt, Germany) dissolved in

Tween 80 and saline (vehicle, 1:16 (v/v); Chem Supply, Gillman, Australia), or vehicle (control), twice daily at 12 hourly intervals from PN 56 to PN 80, equating to late adolescence/early adulthood (Osborne et al., 2017a). The dose and duration of CBD administration was based on studies that report improvement in models with cognitive impairment following CBD treatment (Cassol et al., 2010; Barichello et al., 2012; Fagherazzi et al., 2012; Schiavon et al., 2014). During the second week of treatment (i.e. PN72), offspring underwent behavioural testing using the Novel Object Recognition, T-maze and Social Interaction tests as reported previously (Osborne et al., 2017a). The day after the last drug treatment, offspring were euthanased using carbon dioxide asphyxiation followed by rapid decapitation, between 09:00 and 11:30 h to minimise the potential effects of circadian rhythm variation on protein expression (Jasinska and Pyza, 2017). Whole brains were removed, immediately frozen in liquid nitrogen and stored at -80ºC until further analysis.

2.3 Histological Procedures

Rat brains (n = 8 per group) were sectioned coronally into alternating 14 µM or 500 µM sections using a cryostat (-17°C; Jung CM 3000, Leica Instruments GmbH, Nussloch, Germany). Sections were collected from the PFC (containing the prelimbic and infralimbic cortices; Bregma level: 4.2 mm to 2.56 mm) and HPC (containing the dorsal and ventral subregions, Bregma level: - 4.36 mm to - 6.00 mm). Consecutive sections (2-3 per region, per rat) were collected for receptor autoradiography experiments (14 µM) and thaw-mounted onto Polysine^{TM} slides (Sigma-Aldrich, Castle Hill, NSW, Australia) then stored at -20ºC until further analysis. Sections (2-3 per region, per rat) collected for Western blot experiments (500 µM) were mounted on glass slides and regions of interest were microdissected using a micropuncture kit, then stored at -80ºC until further use. This

dissection protocol allowed investigation of receptor binding and protein levels from the same animals.

2.4 *In situ* Receptor Autoradiography

2.4.1 CB1R binding

CB1R binding density was examined using *in situ* receptor autoradiography methods previously reported by our laboratory (Weston-Green et al., 2012a; Yu et al., 2013). Briefly, slides (3 slides per rat, per treatment group) were air-dried and incubated in 50 mM Tris-HCl buffer (pH 7.4) with 0.1% bovine serum albumin (BSA) for 15 min at room temperature. To determine total binding, sections were incubated in 50 mM Tris-HCl buffer (with 0.1% BSA) containing 10 nM [^{3}H]SR141716A (43 Ci/mmol; PerkinElmer TM Life and Analytical Sciences, Boston, USA), a selective inverse agonist for the CB1R, for 60 min at room temperature. Non-specific binding was determined by incubating additional sections in 10 nM [^{3}H]SR141716A in the presence of 100 µM CP-55940 (Sapphire Bioscience, Redfern, NSW, Australia), a non-selective agonist of cannabinoid receptors. After incubation, slides were washed twice for 30 min in ice-cold buffer, rinsed in cold milliQ water and air-dried overnight.

2.4.2 NMDAR binding

NMDAR binding density was examined based on methods previously described by our laboratory (du Bois et al., 2009; Newell et al., 2007). Briefly, sections were incubated in 30 mM N-2-hydroxyethyl piperazine-N′-2-ethanesulphonic acid (HEPES) buffer pH 7.5, containing 100 µM glycine, 100 µM glutamate, 1 mM ethylenediaminetetraacetic acid (EDTA) and 20 nM [^{3}H]MK-801 (specific activity 22.5 Ci/mmol, PerkinElmer, Boston,

USA) for 2.5 hours at room temperature (RT). Non-specific binding was determined by incubating sections in the same buffer containing [^{3}H]MK-801 in the presence of 200 µM unlabelled MK-801 (Sigma-Aldrich, Castle Hill, NSW, Australia). After incubation, sections were washed twice in ice-cold 30 mM HEPES buffer containing 1 mM EDTA (pH 7.5) for 20 min each, rinsed in cold milliQ water and air-dried overnight.

2.4.3 $GABA_AR$ binding

$GABA_A$ receptor binding density was examined based on methods previously described by our laboratory and others (du Bois et al., 2009; Xia and Haddad, 1992; Yu et al., 2013). Briefly, sections were pre-incubated three times in 50 mM Tris-citrate (pH 7.0) buffer at 4ºC for 5 mins. Sections were incubated in 50 mM Tris-citrate buffer containing 50 nM [^{3}H] muscimol (specific activity 29.5 Ci/mmol, PerkinElmer, Boston, USA), a selective agonist for the $GABA_AR$, for 45 min at 4ºC. Non-specific binding was determined by incubating sections in 50 mM Tris-citrate (pH 7.0) buffer containing [^{3}H] muscimol and 100 µM GABA (Sigma-Aldrich, Castle Hill, NSW, Australia). After incubation, sections were rinsed four times for 2 sec each in 50 mM Tris-citrate buffer (4°C), rinsed in cold milliQ water and air-dried overnight.

2.4.4 Quantification

Slides from CB1R, NMDAR and $GABA_AR$ binding experiments were exposed to Amersham Hyperfilm ECL (GE Healthcare Life Sciences, Parramatta, NSW, Australia) for 5 (NMDAR and $GABA_AR$) or 8 weeks (CB1R) with a set of Amersham tritium standards. After development, autoradiography films were de-identified, scanned using a GS-800 Imaging Densitometer (Bio-Rad, Hercules, California, USA), and quantified (left and right hemispheres) with Image J software (https://imagej.nih.gov/ij). Images were calibrated based

on the Rodbard curve obtained from the tritium standards to produce nCi/mg tissue equivalent (TE) values. Specific binding density was estimated by subtracting non-specific binding from total binding values. Values were converted to fmoles [^{3}H] ligand per mg TE taking into account the specific activity of the ligand, as we have previously reported (Weston-Green et al., 2012a, 2012c, 2008). Tissue sections that were damaged were excluded from analysis. Anatomical structures were confirmed using a standard rat brain atlas (Paxinos and Watson, 2007).

2.5 Western Blot and Quantification

Brain samples containing the PFC and HPC (left and right hemispheres combined) were homogenised in buffer containing 0.1 M Tris-HCl, 2 mM EDTA, 10% glycerol, 2% SDS, 0.5 mM PMSF, Protease Inhibitor Cocktail (P8340; Sigma-Aldrich, Australia) and Phosphatase Inhibitor Cocktail 2 (P5726; Sigma-Aldrich, Australia) as previously described (Lum et al., 2016). A DC assay kit was used to determine total protein concentration following the manufacturer's instructions (Bio-Rad, Australia). Crude homogenates (10 µg protein; within the linear range of detection of the primary antibodies, see Supplementary Figure 1) were loaded into Criterion™ TGX Stain-Free™ 4-20% gels (Bio-Rad, Australia) and underwent electrophoresis in SDS buffer at 180V for 1 h. Gels were activated (GelDoc XR+ imaging system; Bio-Rad, Australia) (Colella et al., 2012; Gürtler et al., 2013), proteins were transferred on to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Australia) via electrophoresis for 1 h at 100 V, and imaged (GelDoc XR+ imaging system; Bio-Rad, Australia) to capture total protein in each lane (Colella et al., 2012; Gürtler et al., 2013). Membranes were blocked in 5% milk (w/v) in Tris Buffered Saline with Tween 20® (TBST) for 1 h at room temperature. To detect the proteins of interest, membranes were incubated overnight at 4ºC in the following primary antibodies: anti-FAAH (PFC: 1:2500, HPC: 1:5000

; #ab54615, Abcam), anti-GluN1 (1:10 000; #MAB363, Millipore), anti-GAD_{67} (1:7500 #MAB5406, Millipore), anti-PV (1:10 000; #Ab11427, Abcam). Membranes were washed in TBST (5 x 5 min) and incubated at room temperature for 1 h with either horseradish peroxidase (HRP)-conjugated goat anti-rabbit (1:5000; #AP307P, Millipore) or anti-mouse secondary antibody (1:5000; #AP308P, Millipore). After washing in TBST (3 x 5 min), membranes were incubated in Enhanced Chemiluminescence (ECL) reagents (Bio-strategy Laboratory Products, Tingalpa, QLD Australia) and scanned using a Gel Imager (Amersham 600RB, GE Healthcare, Parramatta, NSW Australia). De-identified band signals were quantified using Image Lab software (ver 6, Bio-Rad Laboratories Inc, California, USA). The values for each signal were normalised to total protein in the respective lane to account for loading variability (Colella et al., 2012; Gürtler et al., 2013). Values were then normalised to an internal control sample (consisting of equal amounts of sample pooled together) to account for inter-gel variability. Samples were assayed in duplicate at a minimum.

2.6 Statistical Analysis

Statistical analyses were performed using SPSS (Version 21.0, IBM Inc., Illinois, USA). Data points that were ± 2SD from the mean were considered outliers and removed from analysis. Data were tested for normality using Shapiro-Wilk tests. Two-way ANOVAs were used to examine the effects of PRENATAL INFECTION (poly I:C vs. vehicle) and OFFSPRING TREATMENT (CBD vs. vehicle) on receptor binding density and protein levels. Pairwise comparisons (with Bonferroni adjustment) were used to examine differences between groups. Data that were not normally distributed were transformed, retested for normality using Shapiro-Wilk tests, and analysed using parametric testing. Variables that remained non-normally distributed following transformation or that violated Levene's Test for Equality of Variances were analysed using non-parametric Mann-Whitney U tests (indicated in the

Results section). Relevant comparisons were made between vehicle-treated control and poly I:C offspring (CONT+VEH vs. POLY+VEH), vehicle and CBD-treated poly I:C offspring (POLY+VEH vs. POLY+CBD), as well as vehicle and CBD-treated control offspring (CONT+VEH vs. CONT+CBD). Where significant differences between the POLY+VEH and POLY+CBD groups were observed, an additional comparison was made between the CONT+VEH and POLY+CBD groups to determine if CBD restored the parameter to control levels. *P* values less than 0.05 were considered statistically significant. Data are presented as mean ± standard error of the mean (SEM).

3. Results

To determine the potential mechanisms underlying the beneficial effects of CBD, eCB, NMDAR and GABAergic markers were analysed in the brains of control and poly I:C offspring. Representative autoradiographs of CB1R ([^{3}H]SR141716A), NMDAR ([^{3}H]MK-801) and $GABA_AR$ ([^{3}H]Muscimol) total binding density (as well as non-specific binding for the respective ligands) in the PFC and HPC are shown (Figure 1). There was no difference in receptor binding density along the dorsal-ventral axis of the HPC (Supplementary Figure 2); therefore, the subregions were combined for analysis. Representative immunoblots showing the expected bands for FAAH (Figure 2C, 2D), the obligatory GluN1 subunit of the NMDAR (Figure 3C, 3D), GAD_{67} (Figure 4C, 4D) and PV (Figure 4E, 4F) are displayed with their respective graphs.

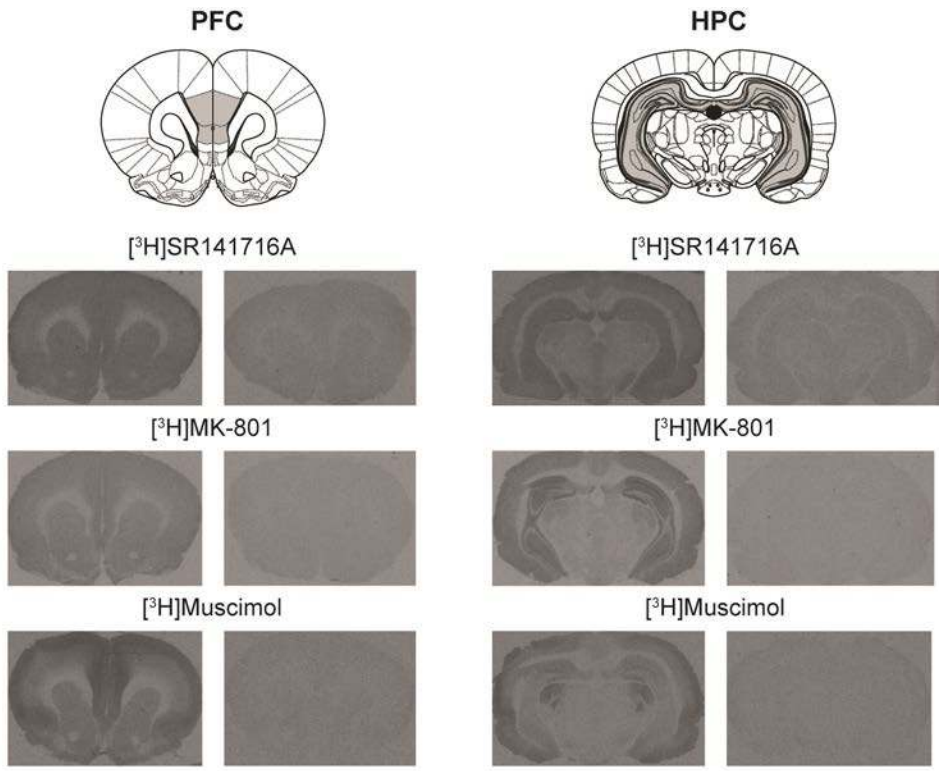


Figure 1: Example autoradiographs showing cannabinoid CB1 receptor (CB1R), *N*-methyl-*D*-aspartate receptor (NMDAR) and gamma-aminobutyric acid A receptor ($GABA_AR$) binding in the adult rat brain. Schematic diagram adapted from a rat brain atlas (Paxinos and Watson, 2007) showing the approximate Bregma level quantified for the prefrontal cortex (PFC): 2.52 mm (containing the prelimbic and infralimbic cortices; shaded) and the hippocampus (HPC): - 4.92 mm (shaded). Example autoradiographs demonstrate [^{3}H]SR141716A binding to CB1Rs, [^{3}H]MK-801 binding to NMDARs and [^{3}H]muscimol binding to $GABA_ARs$ in the PFC and HPC. Autoradiographs show total binding (left) and non-specific binding (right) for each ligand. Example autoradiographs are from vehicle-treated control offspring.

3.1 Effect of cannabidiol on endocannabinoid markers

CB1R binding density and FAAH protein levels were examined in the PFC and HPC to determine if maternal poly I:C exposure could result in persistent alterations to eCB markers, and whether chronic CBD treatment could reverse any changes. In the PFC, there was a

significant main effect of OFFSPRING TREATMENT ($F_{(1, 26)} = 6.481$, $p = 0.017$) and a tendency for a PRENATAL INFECTION x OFFSPRING TREATMENT interaction ($F_{(1, 26)} = 2.993$, $p = 0.095$), but no main effect of PRENATAL INFECTION ($F_{(1, 26)} = 1.679$, $p = 0.206$). Although the interaction did not reach statistical significance, visual inspection of the graph showed that poly I:C offspring had lower CB1R binding density in the PFC compared to control counterparts (-24.62%; POLY+VEH vs. CONT+VEH, $p = 0.042$) (Figure 2A). CBD treatment restored CB1R binding deficits in poly I:C offspring (+46.16%; POLY+CBD vs. POLY+VEH, $p = 0.006$) to control-like levels (POLY+CBD: 247.85 ± 15.19 vs. CONT+VEH: 224.97 ± 13.03; $p = 0.275$) (Figure 2A). CBD administration did not significantly alter CB1R binding density in the PFC of control offspring (CONT+CBD: 239.91 ± 19.92 vs. CONT+VEH: 224.97 ± 13.03; $p = 0.569$) (Figure 2A). The reduction in CB1R binding density in poly I:C offspring appeared to be specific to the PFC, since there were no significant effects of maternal poly I:C exposure or CBD treatment on CB1R binding density in the HPC of offspring (PRENATAL INFECTION: $F_{(1, 23)} = 0.326$, $p = 0.574$; OFFSPRING TREATMENT: $F_{(1, 23)} = 0.790$, $p = 0.383$; PRENATAL INFECTION x OFFSPRING TREATMENT: $F_{(1, 23)} = 0.321$, $p = 0.576$) (Figure 2B). FAAH protein levels in the PFC did not differ significantly between treatment groups (PRENATAL INFECTION: $F_{(1, 27)} = 0.112$, $p = 0.740$; OFFSPRING TREATMENT: $F_{(1, 27)} = 1.610$, $p = 0.215$; PRENATAL INFECTION x OFFSPRING TREATMENT: $F_{(1, 27)} = 0.690$, $p = 0.413$) (Figure 2C). In line with the findings in the PFC, a two-way ANOVA showed no significant effects on FAAH protein levels in the HPC (PRENATAL INFECTION: $F_{(1, 28)} = 0.066$, $p = 0.800$, OFFSPRING TREATMENT: $F_{(1, 28)} = 0.356$, $p = 0.556$, PRENATAL INFECTION x OFFSPRING TREATMENT: $F_{(1, 28)} = 0.185$, $p = 0.671$) (Figure 2D).

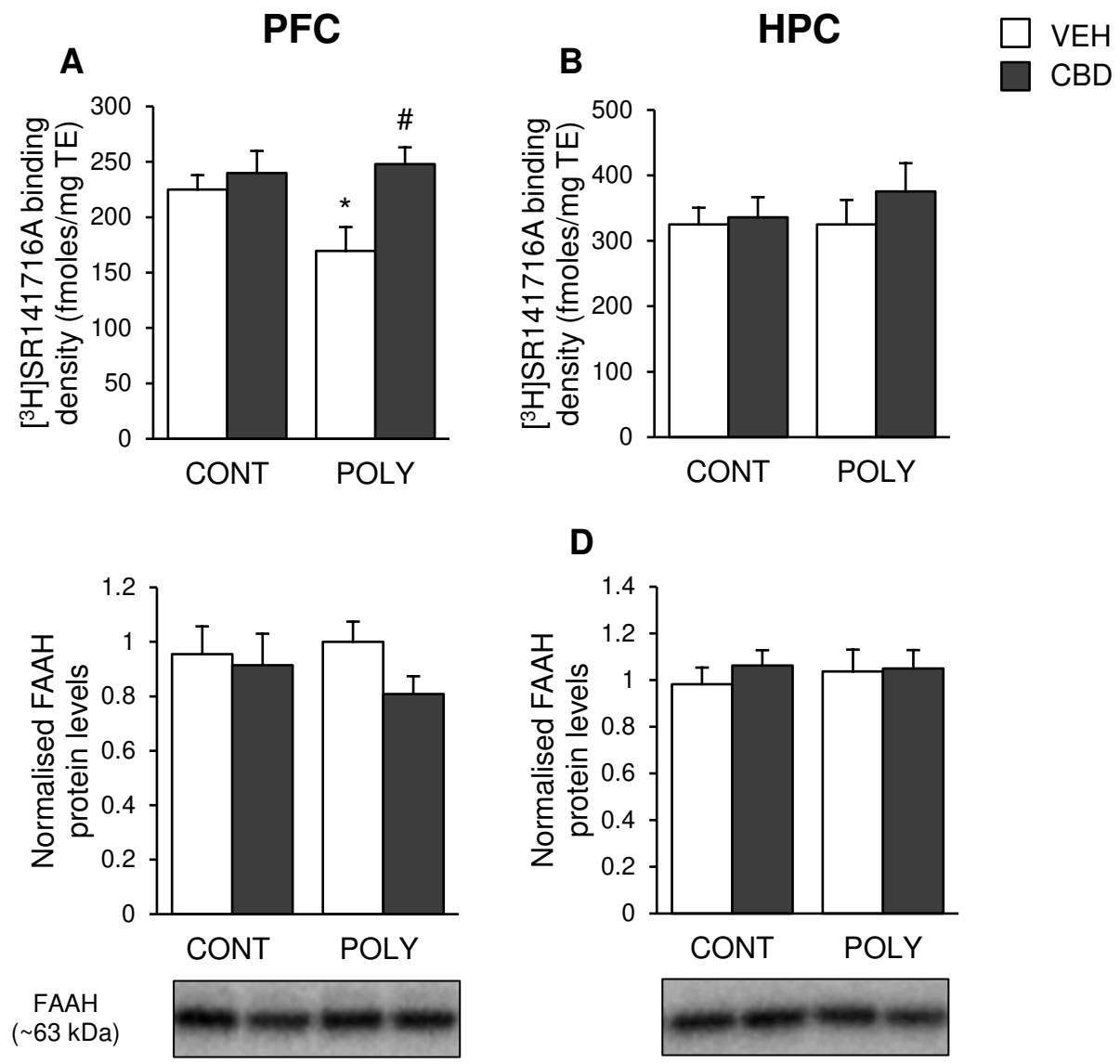


Figure 2: Cannabidiol (CBD) attenuated deficits in cannabinoid CB1R binding density in the prefrontal cortex (PFC) of male offspring exposed to maternal poly I:C infection. CB1R ([^{3}H]SR141716A) binding density in the (**A**) PFC and (**B**) hippocampus (HPC) of offspring treatment groups. Normalised fatty acid amide hydrolase (FAAH) protein levels in the (**C**) PFC and (**D**) HPC with typical immunoblots for each treatment group shown underneath the graphs (normalised to total protein levels in the respective lanes). Data expressed as mean ± SEM. n = 6-8 rats per group. *p<0.05 vs. CONT+VEH, #p<0.05 vs. POLY+VEH group. TE = tissue equivalent.

3.2 Effect of cannabidiol on the glutamatergic NMDAR

The effect of CBD treatment on NMDAR binding density and the obligatory GluN1 subunit of the NMDAR were examined in the PFC and HPC of offspring. A two-way ANOVA showed no significant effects of PRENATAL INFECTION ($F_{(1, 25)} = 0.054$, $p = 0.818$) or OFFSPRING TREATMENT ($F_{(1, 25)} = 0.430$, $p = 0.518$) on NMDAR binding density in the PFC, and no significant interaction between the factors ($F_{(1, 25)} = 0.224$, $p = 0.640$) (Figure 3A). A similar pattern was also observed in the HPC (PRENATAL INFECTION: $F_{(1, 23)} = 0.120$, $p = 0.732$; OFFSPRING TREATMENT: $F_{(1, 23)} = 2.256$, $p = 0.147$; PRENATAL INFECTION x OFFSPRING TREATMENT: $F_{(1, 23)} = 0.712$, $p = 0.408$) (Figure 3B). Reflecting the lack of change in binding density, GluN1 protein levels were not significantly altered between treatment groups in the PFC (Figure 3C) or HPC (Figure 3D) (PFC: PRENATAL INFECTION: $F_{(1, 27)} = 0.045$, $p = 0.833$; OFFSPRING TREATMENT: $F_{(1, 27)} = 1.763$, $p = 0.195$; PRENATAL INFECTION x OFFSPRING TREATMENT: $F_{(1, 27)} = 1.524$, $p = 0.228$. HPC (Mann-Whitney U tests): POLY+VEH vs. CONT+VEH, $p = 0.600$; POLY+CBD vs. POLY+VEH, $p = 0.916$; CONT+CBD vs. CONT+VEH, $p = 0.999$).

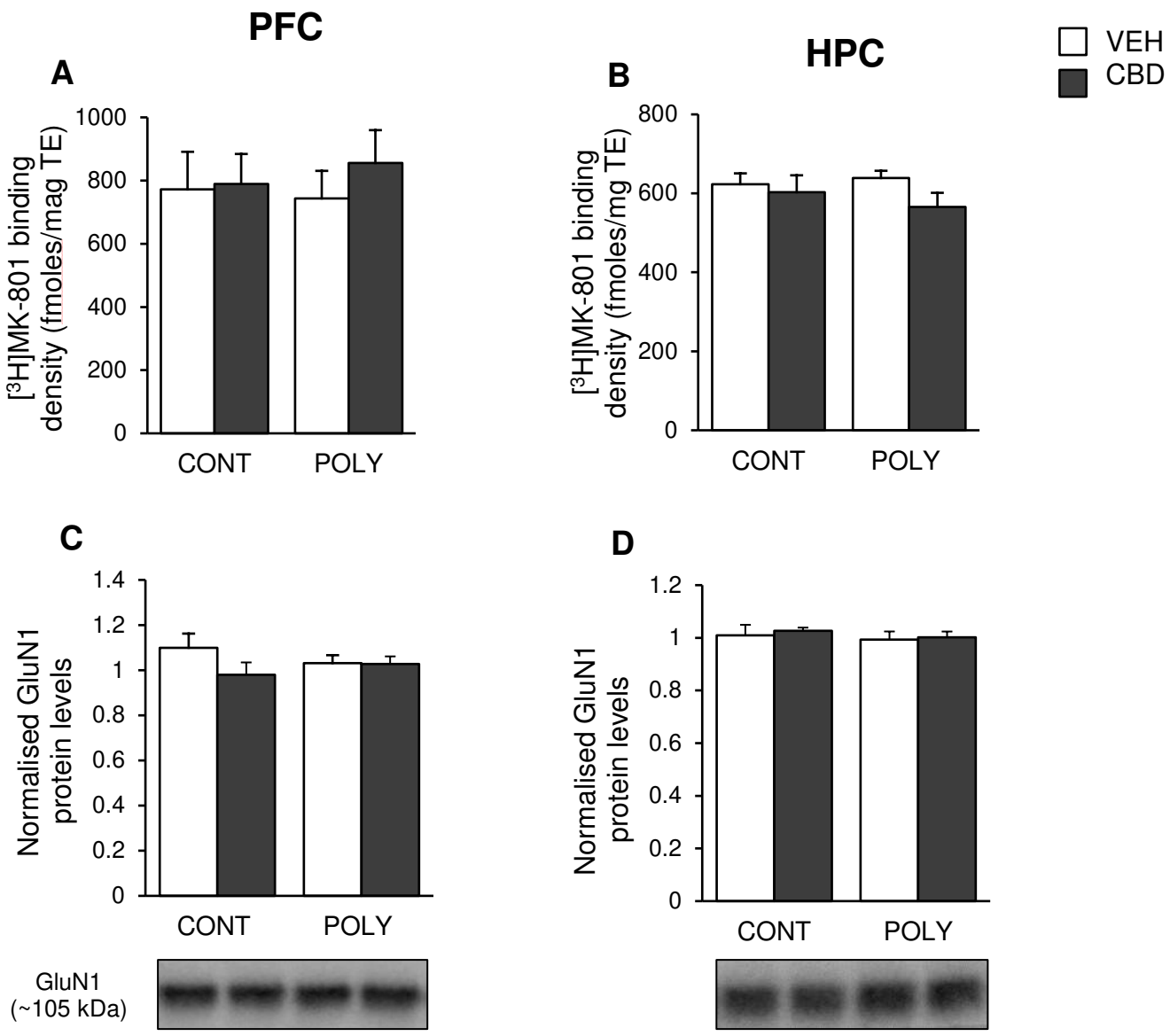


Figure 3: Cannabidiol (CBD) did not alter glutamatergic *N*-methyl-D-aspartate receptor (NMDAR) binding density or GluN1 protein levels in select brain regions of control (CONT) and poly I:C (POLY) offspring. NMDAR ([^{3}H]MK801) binding density in the (**A**) PFC and (**B**) HPC of offspring. Normalised GluN1 subunit protein levels (normalised to total protein levels in the respective lane) in the (**C**) PFC and (**D**) HPC, with typical GluN1 immunoblots (~105 kDa) shown underneath each graph. Data expressed as mean ± SEM. *n* = 6-8 rats per group.

449

450 3.3 Effect of cannabidiol on GABAergic markers

451 $GABA_AR$ binding density, as well as GAD_{67} and PV protein levels were examined in the
452 PFC and HPC to determine whether CBD treatment could attenuate any changes in
453 GABAergic markers following maternal poly I:C exposure. Mann-Whitney U tests revealed
454 that poly I:C exposure did not significantly alter $GABA_AR$ binding density in the PFC
455 (POLY+VEH vs. CONT+VEH: p = 0.568), and there was no significant impact of CBD
456 treatment on $GABA_AR$ binding density in poly I:C (POLY+CBD vs. POLY+VEH: p =
457 0.668) or control offspring (CONT+CBD vs. CONT+VEH: p = 0.317) (Figure 4A).
458 Similarly, no significant changes in $GABA_AR$ binding density were observed in the HPC
459 (PRENATAL INFECTION: $F_{(1, 22)} = 0.382$, $p = 0.543$; OFFSPRING TREATMENT: $F_{(1, 22)}$
460 = 1.186, p = 0.288; PRENATAL INFECTION x OFFSPRING TREATMENT: $F_{(1, 22)}$ =
461 1.814, p = 0.192) (Figure 4B). In the PFC, there was no significant difference in GAD_{67}
462 protein levels between groups (PRENATAL INFECTION: $F_{(1, 28)} = 0.244$, $p = 0.625$;
463 OFFSPRING TREATMENT: $F_{(1, 28)} = 0.149$, $p = 0.703$; PRENATAL INFECTION x
464 OFFSPRING TREATMENT: $F_{(1, 28)} = 0.364$, $p = 0.551$) (Figure 4C). Conversely, a two-way
465 ANOVA revealed significant main effects of PRENATAL INFECTION ($F_{(1, 27)} = 5.987$, p =
466 0.021) and OFFSPRING TREATMENT ($F_{(1, 27)} = 9.253$, $p = 0.005$) on relative GAD_{67}
467 protein levels in the HPC. There was no significant PRENATAL INFECTION x
468 OFFSPRING TREATMENT interaction ($F_{(1, 27)} = 0.600$, $p = 0.445$); however, visual
469 inspection of the means suggested individual group differences. Exploratory pairwise
470 comparisons showed a significant reduction in GAD_{67} protein levels in poly I:C offspring (-
471 15.50%; POLY+VEH vs. CONT+VEH, p = 0.028), that was attenuated by CBD treatment
472 (+17.85%; POLY+CBD vs. POLY+VEH, p = 0.013), and did not differ from controls
473 (POLY+CBD: 1.04 ±0.05 vs. CONT+VEH: 1.01±0.05, p = 0.108) (Figure 4D). In the PFC,

Mann-Whitney tests showed no significant difference in PV protein levels between groups (CONT+VEH vs. CONT+CBD, $p = 0.208$; CONT+VEH vs. POLY+VEH, $p = 0.247$; POLY+VEH vs. POLY+CBD, $p = 0.180$) (Figure 4E). In the HPC however, CBD treatment significantly increased PV protein levels (+21.09 %, OFFSPRING TREATMENT: $F_{(1, 26)} = 8.610$, $p = 0.007$) relative to vehicle treatment (CBD: 1.04±0.05 vs. VEH: 0.82±0.06) (Figure 4F). There was no main effect of PRENATAL INFECTION ($F_{(1, 26)} = 0.314$, $p = 0.580$) and no significant interaction between the factors ($F_{(1, 26)} = 0.509$, $p = 0.482$).

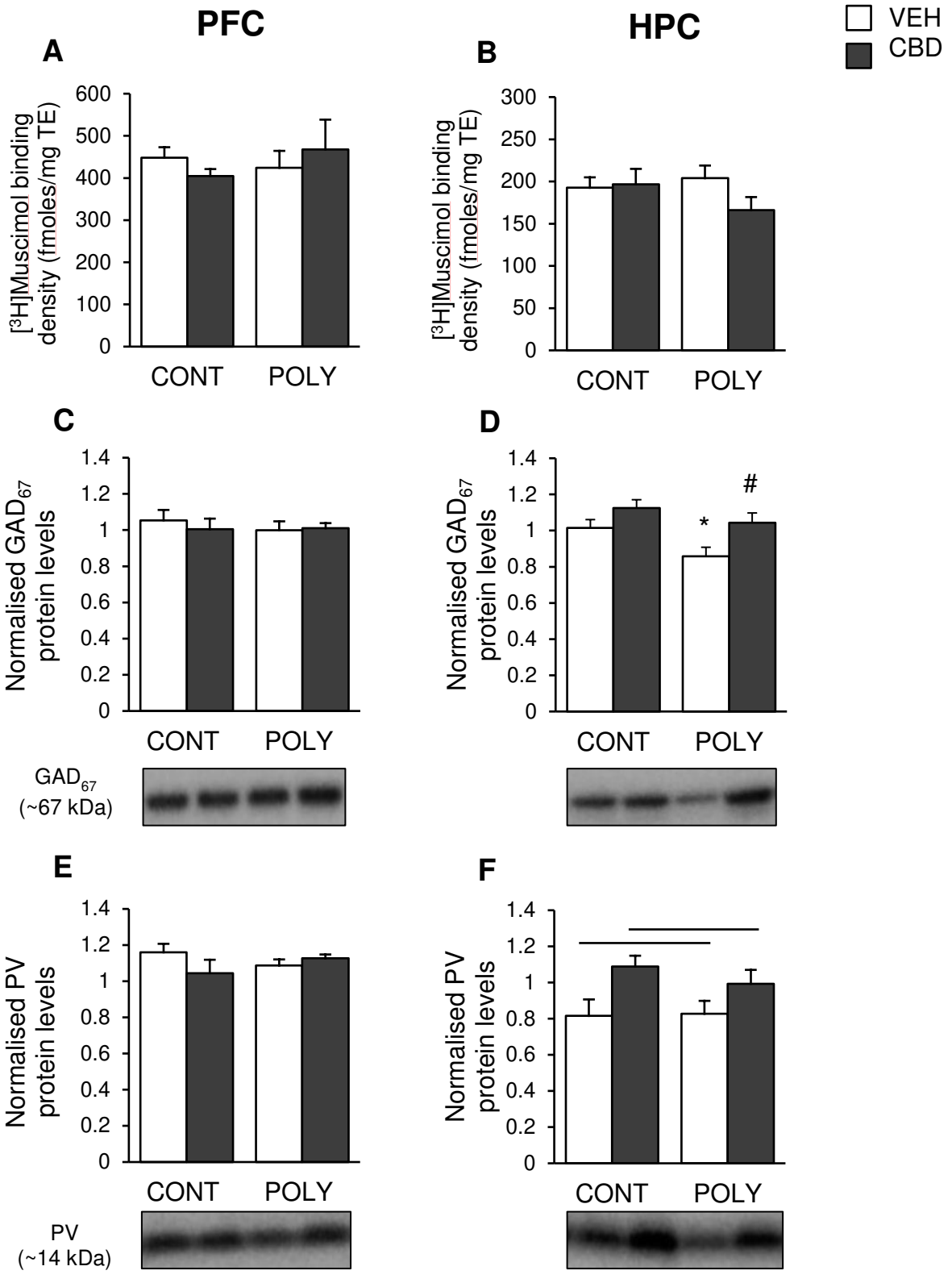


Figure 4: Cannabidiol (CBD) treatment altered glutamate decarboxylase 67 (GAD_{67}) and pavalbumin (PV) protein levels in the hippocampus (HPC) of control (CONT) and poly I:C (POLY) offspring. $GABA_AR$ ([^{3}H]Muscimol) binding density in the (**A**) prefrontal cortex (PFC) and (**B**) hippocampus (HPC) of offspring. Normalised glutamate decarboxylase 67

525 (GAD_{67}) (**C**, **D**) and parvalbumin (PV) (**E**, **F**) protein levels (normalised to total protein
526 levels in the respective lanes) in the PFC and HPC with representative immunoblots shown
527 underneath the graphs. *p < 0.05 vs. CONT+VEH group, #p < 0.05 vs. POLY+VEH group;
528 lines indicate offspring treatment effect (p < 0.01 CBD vs. VEH). Data expressed as mean ±
529 SEM. n = 6-8 rats per group.
530
531
532 **4. Discussion**
533
534 We have previously shown that CBD treatment restored working and recognition memory, as
535 well as social interaction deficits in male poly I:C offspring (Osborne et al., 2017a), however,
536 the effects of CBD treatment on brain neurochemistry had not been characterised. The
537 present study examined the effect of CBD treatment on eCB, glutamatergic and GABAergic
538 markers in the PFC and HPC of male poly I:C offspring. We have shown that CBD treatment
539 restored poly I:C-induced deficits in CB1R binding density in the PFC and hippocampal
540 GAD_{67} levels, and increased PV protein levels in the HPC regardless of whether offspring
541 were exposed to poly I:C in utero. This study provides the first evidence for the potential
542 molecular mechanisms underlying the therapeutic effects of CBD in the poly I:C model, and
543 may have implications for schizophrenia treatment.
544
545 In the present study, we found that CB1R binding density was reduced in the PFC, but not the
546 HPC of male poly I:C offspring that exhibit social interaction, recognition and working
547 memory deficits (Osborne et al., 2017a). In line with our findings, post-mortem studies of
548 predominantly male schizophrenia cohorts report alterations in CB1R expression in the PFC
549 (Eggan et al., 2010, 2008; Urigüen et al., 2009), but not the HPC (Dean et al., 2001).
550 Similarly, in other neurodevelopmental rodent models of schizophrenia, studies report
551 alterations in CB1R protein and mRNA expression in the PFC following gestational

552 methylazoxymethanol (MAM) administration (Stark et al., 2019; Gomes et al., 2018), with
553 no change in CB1R binding, protein or mRNA expression in the HPC of male
554 lipopolysaccharide- or MAM-exposed (in utero) offspring (Stark et al., 2019; Gomes et al.,
555 2018; Zavitsanou et al., 2013). However, this is the first study to report a corresponding
556 reduction in CB1R binding density in the PFC, a region critical to memory and social
557 behaviour, in male poly I:C offspring that also exhibit negative and cognitive phenotypes.
558 Our findings are similar to a recent study that reported social interaction and recognition
559 memory deficits in male offspring following gestational methylazoxymethanol (MAM)
560 exposure, with concurrent alterations in CB1R protein and mRNA expression (albeit
561 increases) in the PFC, but not the HPC of male offspring (Stark et al., 2019). It is unknown
562 how CBD rescues changes in CB1R expression in neurodevelopmental models. Recent *in*
563 *vitro* evidence suggests that CBD has an alternative mechanism of action to other
564 phytocannabinoids (e.g. THC), instead acting as a negative allosteric modulator of the CB1R
565 (Laprairie et al., 2015; Straiker et al., 2018; Tham et al., 2018). However, whether negative
566 allosteric modulation of the CB1R by CBD is responsible for the therapeutic effects observed
567 in the present study and others (e.g. Stark et al., 2019) is yet to be elucidated.
568
569 Deficits in GABAergic signalling are thought to underlie the pathophysiology of
570 schizophrenia, with post-mortem studies consistently reporting a reduction in GAD_{67} and PV
571 expression in the cortex and HPC of patients (Guidotti et al., 2000; Zhang et al., 2002;
572 Thompson et al., 2011; Kimoto et al., 2014). The reduction in hippocampal GAD_{67} levels
573 observed in the present study aligns with previous poly I:C studies that report reduced GAD_{67}
574 protein and mRNA expression, mainly in the dorsal HPC (Dickerson et al., 2014; Luoni et al.,
575 2017; Richetto et al., 2013), with no changes in the medial PFC (Dickerson et al., 2014).
576 Conversely, this is the first study to show that CBD can reverse deficits in hippocampal

577 GAD_{67} protein levels in poly I:C offspring. GAD_{67} is the rate-limiting enzyme responsible for
578 approximately 90% of GABA synthesis in the brain and can provide an indication of GABA
579 levels (Lazarus et al., 2015). Indeed, previous *in vitro* studies have reported that CBD can
580 elevate GABA levels in cortical (Banerjee et al., 1975) and striatal membranes (Sagredo et
581 al., 2007) by inhibiting GABA reuptake. While not impaired in poly I:C offspring in the
582 present study, PV protein levels in the HPC were significantly increased by CBD treatment.
583 The effect of CBD on hippocampal PV+ interneurons has not been reported in maternal
584 manipulation models of schizophrenia previously; however, CBD attenuated a reduction in
585 PV+ cells in the medial PFC following chronic administration of the NMDAR antagonist
586 MK-801 (Gomes et al., 2015a). Importantly, PV expression is activity-dependent, therefore,
587 taken together with recent *in vitro* findings that CBD can enhance inhibition elicited by PV+
588 and CCK+ GABAergic neurons (Khan et al., 2018), the results of the present study suggest
589 that CBD may increase inhibitory tone within the hippocampus.
590
591 In the present study, CBD treatment did not alter FAAH protein levels in the PFC or HPC.
592 FAAH is the primary enzyme responsible for the intracellular hydrolysis of AEA in neurons
593 (Lu and Mackie, 2016). AEA may play a protective role in schizophrenia, especially in the
594 early stages of the disorder (Giuffrida et al., 2004; Koethe et al., 2009); therefore, compounds
595 that limit AEA degradation (e.g. FAAH inhibitors) may be beneficial. Previous *in* vitro
596 investigations have identified CBD as a FAAH inhibitor (Elmes et al., 2015; Leweke et al.,
597 2012; Bisogno et al., 2001; De Petrocellis et al., 2011). Although we did not detect any
598 changes in FAAH protein levels following CBD treatment in the present study, we cannot
599 discount changes in enzymatic activity. Alternatively, CBD could interact with fatty acid
600 binding proteins, which are responsible for transporting AEA across the membrane for
601 intracellular hydrolysis by FAAH (Elmes et al., 2015). Further examination of AEA transport

602 and degradation may provide additional insight into the mechanisms underlying the
603 therapeutic benefits of CBD in the poly I:C model.
604
605 CBD treatment did not alter NMDAR (or protein levels of its obligatory GluN1 subunit) and
606 $GABA_AR$ binding, which are primarily responsible for excitation and inhibition in the mature
607 brain, respectively. Our findings contrast with post-mortem studies in schizophrenia patients
608 that report a modest down-regulation in NMDAR binding (Catts et al., 2016), with a
609 corresponding up-regulation in $GABA_AR$ binding density (Benes et al., 1996a, 1996b;
610 Verdurand et al., 2013). Alterations in individual receptor subunits have also been reported,
611 including down-regulation of the GluN1 subunit of the NMDAR (Catts et al., 2015; Weickert
612 et al., 2013), as well as specific α subunits of the $GABA_AR$ in patients (Beneyto et al., 2011;
613 Volk et al., 2002) and poly I:C offspring (Meyer et al., 2008; Richetto et al., 2014). This is
614 the first study to report that CBD treatment does not alter NMDAR and $GABA_AR$ binding
615 density in male poly I:C offspring that do not exhibit binding deficits in these receptors.
616 Literature investigating the effects of CBD treatment on these neurochemical markers in
617 other preclinical schizophrenia models is limited. In line with the present study, CBD did not
618 alter NMDAR or $GABA_AR$ density in a genetic mouse model of schizophrenia-like
619 phenotypes (Neuregulin 1 transmembrane domain heterozygous mutant mice) that did not
620 exhibit binding deficits (Long et al., 2012), but did restore GluN1 gene expression in the PFC
621 of rodents exposed to NMDAR antagonism (MK-801) (Gomes et al., 2015a). Overall, the
622 findings of the present study suggest that CBD may not exert its therapeutic effects via the
623 NMDAR and $GABA_ARs$ in poly I:C offspring. However, we cannot discount changes in
624 other subunits of the NMDAR (e.g. GluN2A or GluN2B) or $GABA_AR$ (e.g. α subunits).
625 Alternatively, CBD could alter receptor activity or expression on specific neuronal

populations (e.g. PV+ interneurons, pyramidal cells) not detectable in the present study, which could be addressed by future investigations.

Although further research is needed to characterise the neurochemical changes observed following CBD treatment, this study provides the first insight into the potential mechanisms underlying the beneficial effects of CBD in a MIA model of schizophrenia. Following on from the previous behavioural investigation (Osborne et al., 2017a), the present study is limited by the use male offspring only. The eCB system is known to exhibit sexual dimorphism, particularly in CB1R expression and functionality (Rubino and Parolaro, 2011), therefore, future studies are needed to determine if female poly I:C offspring show a similar response to CBD treatment. Additionally, it is unclear whether the downregulation in CB1R binding and GAD_{67} levels in poly I:C offspring are a delayed result of the poly I:C stimulus; GABAergic and eCB signalling is present in the brain from early prenatal development (Danglot et al., 2006; Harkany et al., 2007), playing an important role in neuronal migration and proliferation, and is vulnerable to neuroinflammation (Di Marzo et al., 2015). Given that some behavioural phenotypes show delayed onset in the poly I:C model (reviewed in Meyer and Feldon, 2012), tracking the neurochemical profile of offspring would elucidate whether the deficits in CB1R binding and GAD_{67} levels are present from gestation (i.e. after the immune stimulus is delivered), or if the poly I:C insult primes the brain for altered maturation during adolescence. Nevertheless, CBD treatment during adolescence/early adulthood was able to rectify the behavioural (Osborne et al., 2017a) and neurochemical deficits observed in poly I:C offspring.

Conclusions

In the present study, CBD treatment reversed deficits in CB1R binding density in the PFC and hippocampal GAD_{67} protein levels in male poly I:C offspring. CBD also increased hippocampal PV levels regardless of in utero poly I:C exposure. CBD had no effect on FAAH protein levels, NMDAR or $GABA_AR$ binding density in either brain region examined, however, poly I:C offspring did not exhibit deficits in these markers. This is the first study to characterise the neurochemical changes that occur following CBD treatment in a MIA model of schizophrenia. Overall, our findings implicate eCB and GABAergic signalling markers in the therapeutic effects of CBD in male poly I:C offspring, and this may have important implications for schizophrenia treatment.

Role of Funding Source

This work was supported by a University of Wollongong Faculty of Science, Medicine and Health Advancement Grant (2015/SPGA-S/02) awarded to KWG and XFH, Centre for Medical and Molecular Biosciences funding awarded to KWG, and a Daniel Beck Memorial Award from Neuroscience Research Australia and the Beck family awarded to ALO. ALO and JSL were supported by an Australian Government Research Training Program Scholarship from the University of Wollongong. IB is supported by a Postgraduate Research Scholarship from the University of Wollongong and the Illawarra and Shoalhaven Local Health District. JSL is supported by Australian Rotary Health, in the form of an Ian Scott Scholarship. The funding sources had no further role in the study design, in the collection, analysis and interpretation of data, in the writing of the report, or the decision to submit the paper for publication.

Contributors

ALO, KWG, NS and XFH designed the study; ALO, JSL, IB and KWG performed the experiments; XFH and KAN provided some experimental reagents; ALO analysed the data and wrote the first draft of the manuscript; JSL, KWG, KAN, NS contributed to the interpretation of the data and final manuscript. All authors have approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

References

Bakas, T., van Nieuwenhuijzen, P.S., Devenish, S.O., McGregor, I.S., Arnold, J.C., Chebib, M., 2017. The direct actions of cannabidiol and 2-arachidonoyl glycerol at GABAA receptors. Pharmacol. Res. 119, 358–370. https://doi.org/10.1016/j.phrs.2017.02.022

Banerjee, S.P., Snyder, S.H., Mechoulam, R., 1975. Cannabinoids: influence on neurotransmitter uptake in rat brain synaptosomes. J. Pharmacol. Exp. Ther. 194, 74–81.

Barch, D.M., Ceaser, A., 2012. Cognition in schizophrenia: core psychological and neural mechanisms. Trends Cogn. Sci. 16, 27-34. https://doi.org/10.1016/j.tics.2011.11.015

Barichello, T., Ceretta, R.A., Generoso, J.S., Moreira, A.P., Simões, L.R., Comim, C.M., Quevedo, J., Vilela, M.C., Zuardi, A.W., Crippa, J.A., Teixeira, A.L., 2012. Cannabidiol reduces host immune response and prevents cognitive impairments in Wistar rats submitted to pneumococcal meningitis. Eur. J. Pharmacol. 697, 158–164. https://doi.org/10.1016/j.ejphar.2012.09.053

Benes, F.M., Khan, Y., Vincent, S.L., Wickramasinghe, R., 1996a. Differences in the subregional and cellular distribution of GABAA receptor binding in the hippocampal formation of schizophrenic brain. Synap. 22, 338–349. https://doi.org/10.1002/(SICI)1098-2396(199604)22:4<338::AID-SYN5>3.0.CO;2-C

Benes, F.M., Vincent, S.L., Marie, A., Khan, Y., 1996b. Up-regulation of GABAA receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. Neuroscience 75, 1021–1031.

Beneyto, M., Abbott, A., Hashimoto, T., Lewis, D.A., 2011. Lamina-specific alterations in cortical GABAA receptor subunit expression in schizophrenia. Cereb. Cortex 21, 999–1011. https://doi.org/10.1093/cercor/bhq169

Bioque, M., García-Bueno, B., MacDowell, K.S., Meseguer, A., Saiz, P.A., Parellada, M., Gonzalez-Pinto, A., Rodriguez-Jimenez, R., Lobo, A., Leza, J.C., Bernardo, M., 2013.

Peripheral endocannabinoid system dysregulation in first-episode psychosis. Neuropsychopharmacology 38, 2568–2577. https://doi.org/10.1038/npp.2013.165

Bisogno, T., Hanus, L., De Petrocellis, L., Tchilibon, S., Ponde, D.E., Brandi, I., Moriello, A.S., Davis, J.B., Mechoulam, R., Di Marzo, V., 2001. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br. J. Pharmacol. 134, 845–852. https://doi.org/10.1038/sj.bjp.0704327

Boggs, D.L., Surti, T., Gupta, A., Gupta, S., Niciu, M., Pittman, B., Schnakenberg Martin, A.M., Thurnauer, H., Davies, A., D'Souza, D.C., Ranganathan, M., 2018. The effects of cannabidiol (CBD) on cognition and symptoms in outpatients with chronic schizophrenia a randomized placebo controlled trial. Psychopharmacology (Berl.) 235, 1923–1932. https://doi.org/10.1007/s00213-018-4885-9

Broyd, S.J., van Hell, H.H., Beale, C., Yücel, M., Solowij, N., 2016. Acute and chronic effects of cannabinoids on human cognition-a systematic review. Biol. Psychiatry 79, 557–567. https://doi.org/10.1016/j.biopsych.2015.12.002

Cassol, O.J., Comim, C.M., Silva, B.R., Hermani, F.V., Constantino, L.S., Felisberto, F., Petronilho, F., Hallak, J.E.C., De Martinis, B.S., Zuardi, A.W., Crippa, J.A.S., Quevedo, J., Dal-Pizzol, F., 2010. Treatment with cannabidiol reverses oxidative stress parameters, cognitive impairment and mortality in rats submitted to sepsis by cecal ligation and puncture. Brain Res. 1348, 128–138. https://doi.org/10.1016/j.brainres.2010.06.023

Catts, V.S., Derminio, D.S., Hahn, C.-G., Weickert, C.S., 2015. Postsynaptic density levels of the NMDA receptor NR1 subunit and PSD-95 protein in prefrontal cortex from people with schizophrenia. NPJ Schizophr. 1, 15037. https://doi.org/10.1038/npjschz.2015.37

Catts, V.S., Lai, Y.L., Weickert, C.S., Weickert, T.W., Catts, S.V., 2016. A quantitative review of the postmortem evidence for decreased cortical N-methyl-d-aspartate receptor expression levels in schizophrenia: how can we link molecular abnormalities to mismatch negativity deficits? Biol. Psychol. 116, 57–67. https://doi.org/10.1016/j.biopsycho.2015.10.013

Christensen, R., Kristensen, P.K., Bartels, E.M., Bliddal, H., Astrup, A., 2007. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. The Lancet 370, 1706–1713. https://doi.org/10.1016/S0140-6736(07)61721-8

Cohen, S.M., Tsien, R.W., Goff, D.C., Halassa, M.M., 2015. The impact of NMDA Receptor hypofunction on GABAergic interneurons in the pathophysiology of schizophrenia. Schizophr. Res. 167, 98–107. https://doi.org/10.1016/j.schres.2014.12.026

Colella, A.D., Chegenii, N., Tea, M.N., Gibbins, I.L., Williams, K.A., Chataway, T.K., 2012. Comparison of Stain-Free gels with traditional immunoblot loading control methodology. Anal. Biochem. 430, 108–110. https://doi.org/10.1016/j.ab.2012.08.015

Crippa J.A., Guimaraes F.S., Campos A.C., Zuardi A.W., 2018. Translational investigation of the therapeutic potential of cannabidiol (CBD): toward a new age. Front Immunol. 9: 2009, doi: 10.3389/fimmu.2018.02009

Danglot, L., Triller, A., Marty, S., 2006. The development of hippocampal interneurons in rodents. Hippocampus 16, 1032–1060. https://doi.org/10.1002/hipo.20225

de Bruin, N.M.W.J., Prickaerts, J., Lange, J.H.M., Akkerman, S., Andriambeloson, E., de Haan, M., Wijnen, J., van Drimmelen, M., Hissink, E., Heijink, L., Kruse, C.G., 2010. SLV330, a cannabinoid CB1 receptor antagonist, ameliorates deficits in the T-maze, object recognition and Social Recognition Tasks in rodents. Neurobiol. Learn. Mem. 93, 522–531. https://doi.org/10.1016/j.nlm.2010.01.010

De Marchi, N., De Petrocellis, L., Orlando, P., Daniele, F., Fezza, F., Di Marzo, V., 2003. Endocannabinoid signalling in the blood of patients with schizophrenia. Lipids Health Dis. 2, 5. https://doi.org/10.1186/1476-511X-2-5

De Petrocellis, L., Ligresti, A., Moriello, A.S., Allara, M., Bisogno, T., Petrosino, S., Stott, C.G., Di Marzo, V., 2011. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. Br. J. Pharmacol. 163, 1479–1494. https://doi.org/10.1111/j.1476-5381.2010.01166.x

Dean, B., Sundram, S., Bradbury, R., Scarr, E., Copolov, D., 2001. Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. Neuroscience 103, 9–15. https://doi.org/10.1016/S0306-4522(00)00552-2

Di Marzo, V., Stella, N., Zimmer, A., 2015. Endocannabinoid signalling and the deteriorating brain. Nat. Rev. Neurosci. 16, 30–42. https://doi.org/10.1038/nrn3876

Dickerson, D.D., Overeem, K.A., Wolff, A.R., Williams, J.M., Abraham, W.C., Bilkey, D.K., 2014. Association of aberrant neural synchrony and altered GAD67 expression following exposure to maternal immune activation, a risk factor for schizophrenia. Transl. Psychiatry 4, e418. https://doi.org/10.1038/tp.2014.64

Dietsche, B., Kircher, T., Falkenberg, I., 2017. Structural brain changes in schizophrenia at different stages of the illness: A selective review of longitudinal magnetic resonance imaging studies. Aust. N. Z. J. Psychiatry 51, 500–508. https://doi.org/10.1177/0004867417699473

D'Souza, D.C., Radhakrishnan, R., Sherif, M., Cortes-Briones, J., Cahill, J., Gupta, S., Skosnik, P.D., Ranganathan, M., 2016. Cannabinoids and psychosis. Curr. Pharm. Des. 22, 6380–6391. https://doi.org/10.2174/1381612822666160826105628

du Bois, T.M., Deng, C., Han, M., Newell, K.A., Huang, X.-F., 2009. Excitatory and inhibitory neurotransmission is chronically altered following perinatal NMDA receptor blockade. Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol. 19, 256–265. https://doi.org/10.1016/j.euroneuro.2008.12.002

Eggan, S.M., Hashimoto, T., Lewis, D.A., 2008. Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. Arch. Gen. Psychiatry 65, 772–784. https://doi.org/10.1001/archpsyc.65.7.772

Eggan, S.M., Stoyak, S.R., Verrico, C.D., Lewis, D.A., 2010. Cannabinoid CB1 receptor immunoreactivity in the prefrontal cortex: comparison of schizophrenia and major depressive disorder. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 35, 2060–2071. https://doi.org/10.1038/npp.2010.75

Elmes, M.W., Kaczocha, M., Berger, W.T., Leung, K., Ralph, B.P., Wang, L., Sweeney, J.M., Miyauchi, J.T., Tsirka, S.E., Ojima, I., Deutsch, D.G., 2015. Fatty acid-binding proteins (FABPs) are intracellular carriers for Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). J. Biol. Chem. 290, 8711–8721. https://doi.org/10.1074/jbc.M114.618447

Fagherazzi, E.V., Garcia, V.A., Maurmann, N., Bervanger, T., Halmenschlager, L.H., Busato, S.B., Hallak, J.E., Zuardi, A.W., Crippa, J.A., Schröder, N., 2012. Memory-rescuing effects of cannabidiol in an animal model of cognitive impairment relevant to neurodegenerative disorders. Psychopharmacology (Berl.) 219, 1133–1140. https://doi.org/10.1007/s00213-011-2449-3

Ferretjans, R., Moreira, F.A., Teixeira, A.L., Salgado, J.V., 2012. The endocannabinoid system and its role in schizophrenia: a systematic review of the literature. Rev. Bras. Psiquiatr. Sao Paulo Braz. 1999 34 Suppl 2, S163-177.

Giuffrida, A., Leweke, F.M., Gerth, C.W., Schreiber, D., Koethe, D., Faulhaber, J., Klosterkötter, J., Piomelli, D., 2004. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology 29, 2108–2114. https://doi.org/10.1038/sj.npp.1300558

Goff, D.C., Hill, M., Barch, D., 2011. The treatment of cognitive impairment in schizophrenia. Pharmacol. Biochem. Behav. 99, 245–253. https://doi.org/10.1016/j.pbb.2010.11.009

Gomes, F.V., Edelson, J.R., Volk, D.W., Grace, A.A., 2018. Altered brain cannabinoid 1 receptor mRNA expression across postnatal development in the MAM model of schizophrenia. Schizophr. Res. https://doi.org/10.1016/j.schres.2018.04.030

Gomes, F.V., Issy, A.C., Ferreira, F.R., Viveros, M.-P., Del Bel, E.A., Guimaraes, F.S., 2015a. Cannabidiol attenuates sensorimotor gating disruption and molecular changes induced by chronic antagonism of NMDA receptors in mice. Int. J. Neuropsychopharmacol. 18, 1–10. https://doi.org/10.1093/ijnp/pyu041

Gomes, F.V., Llorente, R., Del Bel, E.A., Viveros, M.-P., López-Gallardo, M., Guimarães, F.S., 2015b. Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol. Schizophr. Res. 164, 155–163. https://doi.org/10.1016/j.schres.2015.01.015

Gonzalez-Burgos, G., Lewis, D.A., 2012. NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr. Bull. 38, 950–957. https://doi.org/10.1093/schbul/sbs010

Gonzalez-Burgos, G., Lewis, D.A., 2008. GABA neurons and the mechanisms of network oscillations: implications for understanding cortical dysfunction in schizophrenia. Schizophr. Bull. 34, 944–961. https://doi.org/10.1093/schbul/sbn070

Guidotti, A., Auta, J., Davis, J.M., Di-Giorgi-Gerevini, V., Dwivedi, Y., Grayson, D.R., Impagnatiello, F., Pandey, G., Pesold, C., Sharma, R., Uzunov, D., Costa, E., DiGiorgi Gerevini, V., 2000. Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. Arch. Gen. Psychiatry 57, 1061–1069.

Guo, W., Liu, F., Chen, J., Wu, R., Li, L., Zhang, Z., Chen, H., Zhao, J., 2017. Hyperactivity of the default-mode network in first-episode, drug-naive schizophrenia at rest revealed by family-based case-control and traditional case-control designs. Medicine (Baltimore) 96, e6223. https://doi.org/10.1097/MD.0000000000006223

Gürtler, A., Kunz, N., Gomolka, M., Hornhardt, S., Friedl, A.A., McDonald, K., Kohn, J.E., Posch, A., 2013. Stain-Free technology as a normalization tool in Western blot analysis. Anal. Biochem. 433, 105–111. https://doi.org/10.1016/j.ab.2012.10.010

Gururajan, A., Taylor, D.A., Malone, D.T., 2012. Cannabidiol and clozapine reverse MK-801-induced deficits in social interaction and hyperactivity in Sprague–Dawley rats. J. Psychopharmacol. (Oxf.) 26, 1317–1332. https://doi.org/10.1177/0269881112441865

Harkany, T., Guzmán, M., Galve-Roperh, I., Berghuis, P., Devi, L.A., Mackie, K., 2007. The emerging functions of endocannabinoid signaling during CNS development. Trends Pharmacol. Sci. 28, 83–92. https://doi.org/10.1016/j.tips.2006.12.004

Hasanein, P., Teimuri Far, M., 2015. Effects of URB597 as an inhibitor of fatty acid amide hydrolase on WIN55, 212-2-induced learning and memory deficits in rats. Pharmacol. Biochem. Behav. 131, 130–135. https://doi.org/10.1016/j.pbb.2015.02.007

Hill, S.K., Bishop, J.R., Palumbo, D., Sweeney, J.A., 2010. Effect of second-generation antipsychotics on cognition: current issues and future challenges. Expert Rev. Neurother. 10, 43–57. https://doi.org/10.1586/ern.09.143

Jasinska, M., Pyza, E., 2017. Circadian plasticity of mammalian inhibitory interneurons. Neural Plast. 2017, 1-12. https://doi.org/10.1155/2017/6373412

Keefe RS, Bilder RM, Davis SM, Harvey PD, Palmer BW, Gold JM, et al. Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE Trial. *Arch Gen Psychiatry* (2007) 64:633–47. doi: 10.1001/archpsyc.64.6.633

Khan, A.A., Shekh-Ahmad, T., Khalil, A., Walker, M.C., Ali, A.B., 2018. Cannabidiol exerts antiepileptic effects by restoring hippocampal interneuron functions in a temporal lobe epilepsy model. Br. J. Pharmacol. 175, 2097-2115. https://doi.org/10.1111/bph.14202

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G., 2010. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 8, e1000412. https://doi.org/10.1371/journal.pbio.1000412

Kimoto, S., Bazmi, H.H., Lewis, D.A., 2014. Lower expression of glutamic acid decarboxylase 67 in the prefrontal cortex in schizophrenia: contribution of altered regulation by Zif268. Am. J. Psychiatry 171, 969–978. https://doi.org/10.1176/appi.ajp.2014.14010004

Koethe, D., Giuffrida, A., Schreiber, D., Hellmich, M., Schultze-Lutter, F., Ruhrmann, S., Klosterkötter, J., Piomelli, D., Leweke, F.M., 2009. Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. Br. J. Psychiatry 194, 371–372. https://doi.org/10.1192/bjp.bp.108.053843

Laprairie, R.B., Bagher, A.M., Kelly, M.E.M., Denovan-Wright, E.M., 2015. Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. Br. J. Pharmacol. 172, 4790–4805. https://doi.org/10.1111/bph.13250

Lazarus, M.S., Krishnan, K., Huang, Z.J., 2015. GAD67 deficiency in parvalbumin interneurons produces deficits in inhibitory transmission and network disinhibition in mouse prefrontal cortex. Cereb. Cortex 25, 1290–1296. https://doi.org/10.1093/cercor/bht322

Leweke, F.M., Giuffrida, A., Wurster, U., Emrich, H.M., Piomelli, D., 1999. Elevated endogenous cannabinoids in schizophrenia. Neuroreport 10, 1665–1669.

Leweke, F.M., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C.W., Hoyer, C., Klosterkötter, J., Hellmich, M., Koethe, D., 2012. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl. Psychiatry 2, e94. https://doi.org/10.1038/tp.2012.15

Li, M., Long, C., Yang, L., 2015. Hippocampal-prefrontal circuit and disrupted functional connectivity in psychiatric and neurodegenerative disorders. BioMed Res. Int. 2015, 1-10. https://doi.org/10.1155/2015/810548

Lindenmayer, J.-P., Nasrallah, H., Pucci, M., James, S., Citrome, L., 2013. A systematic review of psychostimulant treatment of negative symptoms of schizophrenia: challenges and therapeutic opportunities. Schizophr. Res. 147, 241–252. https://doi.org/10.1016/j.schres.2013.03.019

Long, L.E., Chesworth, R., Huang, X.-F., Wong, A., Spiro, A., McGregor, I.S., Arnold, J.C., Karl, T., 2012. Distinct neurobehavioural effects of cannabidiol in transmembrane domain neuregulin 1 mutant mice. PLOS ONE 7, e34129. https://doi.org/10.1371/journal.pone.0034129

Lu, H.-C., Mackie, K., 2016. An introduction to the endogenous cannabinoid system. Biol. Psychiatry 79, 516–525. https://doi.org/10.1016/j.biopsych.2015.07.028

Lum, J.S., Fernandez, F., Matosin, N., Andrews, J.L., Huang, X.-F., Ooi, L., Newell, K.A., 2016. Neurodevelopmental expression profile of dimeric and monomeric group 1 mGluRs: relevance to schizophrenia pathogenesis and treatment. Sci. Rep. 6, 34391. https://doi.org/10.1038/srep34391

Luoni, A., Richetto, J., Longo, L., Riva, M.A., 2017. Chronic lurasidone treatment normalizes GABAergic marker alterations in the dorsal hippocampus of mice exposed

to prenatal immune activation. Eur. Neuropsychopharmacol. 27, 170-179. https://doi.org/10.1016/j.euroneuro.2016.12.001

MacKenzie N.E., Kowalchuk C., Agarwal S.M., Costa-Dookhan K.A., Caravaggio, F., Gerretsen ,P., Chintoh, A., Remington, G.J, Taylor, V.H., Mueller, D.J., Graff-Guerrero, A., Hahn M.K., 2018. Antipsychotics, Metabolic Adverse Effects, and Cognitive Function in Schizophrenia. Front Psychiatry 5, 9: 622. doi: 10.3389/fpsyt.2018.00622.

McGuire, P., Robson, P., Cubala, W.J., Vasile, D., Morrison, P.D., Barron, R., Taylor, A., Wright, S., 2018. Cannabidiol (CBD) as an adjunctive therapy in schizophrenia: a multicenter randomized controlled trial. Am. J. Psychiatry 175, 225–231. https://doi.org/10.1176/appi.ajp.2017.17030325

McPartland, J.M., Duncan, M., Di Marzo, V., Pertwee, R.G., 2015. Are cannabidiol and Δ9-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br. J. Pharmacol. 172, 737–753. https://doi.org/10.1111/bph.12944

Meyer, U., Feldon, J., 2012. To poly(I:C) or not to poly(I:C): Advancing preclinical schizophrenia research through the use of prenatal immune activation models. Neuropharmacology 62, 1308–1321. https://doi.org/10.1016/j.neuropharm.2011.01.009

Meyer, U., Nyffeler, M., Yee, B.K., Knuesel, I., Feldon, J., 2008. Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice. Brain. Behav. Immun. 22, 469–486. https://doi.org/10.1016/j.bbi.2007.09.012

Morales, P., Goya, P., Jagerovic, N., Hernandez-Folgado, L., 2016. Allosteric modulators of the CB1 cannabinoid receptor: a structural update review. Cannabis Cannabinoid Res. 1, 22–30. https://doi.org/10.1089/can.2015.0005

Murphy, M., Mills, S., Winstone, J., Leishman, E., Wager-Miller, J., Bradshaw, H., Mackie, K., 2017. Chronic adolescent Δ9-tetrahydrocannabinol treatment of male mice leads to long-term cognitive and behavioral dysfunction, which are prevented by concurrent cannabidiol treatment. Cannabis Cannabinoid Res. 2, 235–246. https://doi.org/10.1089/can.2017.0034

Newell, K.A., Zavitsanou, K., Huang, X.-F., 2007. Short and long term changes in NMDA receptor binding in mouse brain following chronic phencyclidine treatment. J. Neural Transm. 114, 995–1001. https://doi.org/10.1007/s00702-007-0668-x

NHMRC, 2013. Australian code for the care and use of animals for scientific purposes.

Osborne, A.L., Solowij, N., Babic, I., Huang, X.-F., Weston-Green, K., 2017a. Improved social interaction, recognition and working memory with cannabidiol treatment in a prenatal infection (poly I:C) rat model. Neuropsychopharmacology 42, 1447–1457. https://doi.org/10.1038/npp.2017.40

Osborne, A.L., Solowij, N., Weston-Green, K., 2017b. A systematic review of the effect of cannabidiol on cognitive function: Relevance to schizophrenia. Neurosci. Biobehav. Rev. 72, 310–324. https://doi.org/10.1016/j.neubiorev.2016.11.012

Paxinos, G., Watson, C., 2007. The rat brain atlas in stereotaxic coordinates, 6th ed. Elsevier Inc., UK.

Pertwee, R.G., 2008. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin. Br. J. Pharmacol. 153, 199–215. https://doi.org/10.1038/sj.bjp.0707442

Richetto, J., Calabrese, F., Meyer, U., Riva, M.A., 2013. Prenatal versus postnatal maternal factors in the development of infection-induced working memory impairments in mice. Brain. Behav. Immun. 33, 190–200. https://doi.org/10.1016/j.bbi.2013.07.006

959 Richetto, J., Calabrese, F., Riva, M.A., Meyer, U., 2014. Prenatal immune activation induces
960 maturation-dependent alterations in the prefrontal GABAergic transcriptome.
961 Schizophr. Bull. 40, 351–361. https://doi.org/10.1093/schbul/sbs195
962 Richetto, J., Labouesse, M.A., Poe, M.M., Cook, J.M., Grace, A.A., Riva, M.A., Meyer, U.,
963 2015. Behavioral effects of the benzodiazepine-positive allosteric modulator SH-053-
964 2'F-S-CH_3 in an immune-mediated neurodevelopmental disruption model. Int. J.
965 Neuropsychopharmacol. 18, 1-11. https://doi.org/10.1093/ijnp/pyu055
966 Rubino, T., Parolaro, D., 2011. Sexually dimorphic effects of cannabinoid compounds on
967 emotion and cognition. Front. Behav. Neurosci. 5, 1-
968 5. https://doi.org/10.3389/fnbeh.2011.00064
969 Sagredo, O., Ramos, J.A., Decio, A., Mechoulam, R., Fernández-Ruiz, J., 2007. Cannabidiol
970 reduced the striatal atrophy caused 3-nitropropionic acid in vivo by mechanisms
971 independent of the activation of cannabinoid, vanilloid TRPV1 and adenosine A2A
972 receptors. Eur. J. Neurosci. 26, 843–851. https://doi.org/10.1111/j.1460-
973 9568.2007.05717.x
974 Schiavon, A.P., Soares, L.M., Bonato, J.M., Milani, H., Guimarães, F.S., Weffort de Oliveira,
975 R.M., 2014. Protective effects of cannabidiol against hippocampal cell death and
976 cognitive impairment induced by bilateral common carotid artery occlusion in mice.
977 Neurotox. Res. 26, 307–316. https://doi.org/10.1007/s12640-014-9457-0
978 Schneider, M., Schömig, E., Leweke, F.M., 2008. Acute and chronic cannabinoid treatment
979 differentially affects recognition memory and social behavior in pubertal and adult
980 rats. Addict. Biol. 13, 345–357. https://doi.org/10.1111/j.1369-1600.2008.00117.x
981 Solowij, N., Broyd, S.J., Beale, C., Prick, J.-A., Greenwood, L.-M., van Hell, H., Suo, C.,
982 Galettis, P., Pai, N., Fu, S., Croft, R.J., Martin, J.H., Yücel, M., 2018. Therapeutic
983 effects of prolonged cannabidiol treatment on psychological symptoms and cognitive
984 function in regular cannabis users: a pragmatic open-label clinical trial. Cannabis
985 Cannabinoid Res. 3, 21–34. https://doi.org/10.1089/can.2017.0043
986 Stark, T., Ruda-Kucerova, J., Iannotti, F.A., D'Addario, C., Di Marco, R., Pekarik, V.,
987 Drazanova, E., Piscitelli, F., Bari, M., Babinska, Z., Giurdanella, G., Di Bartolomeo,
988 M., Salomone, S., Sulcova, A., Maccarrone, M., Wotjak, C.T., Starcuk, Z., Drago, F.,
989 Mechoulam, R., Di Marzo, V., Micale, V., 2019. Peripubertal cannabidiol treatment
990 rescues behavioral and neurochemical abnormalities in the MAM model of
991 schizophrenia. Neuropharmacology 146, 212–221.
992 https://doi.org/10.1016/j.neuropharm.2018.11.035
993 Straiker, A., Dvorakova, M., Zimmowitch, A., Mackie, K.P., 2018. Cannabidiol inhibits
994 endocannabinoid signaling in autaptic hippocampal neurons. Mol. Pharmacol.
995 https://doi.org/10.1124/mol.118.111864
996 Takata, A., Iwayama, Y., Fukuo, Y., Ikeda, M., Okochi, T., Maekawa, M., Toyota, T.,
997 Yamada, K., Hattori, E., Ohnishi, T., Toyoshima, M., Ujike, H., Inada, T., Kunugi,
998 H., Ozaki, N., Nanko, S., Nakamura, K., Mori, N., Kanba, S., Iwata, N., Kato, T.,
999 Yoshikawa, T., 2013. A population-specific uncommon variant in GRIN3A
1000 associated with schizophrenia. Biol. Psychiatry, Genome and Phenome in
1001 Schizophrenia 73, 532–539. https://doi.org/10.1016/j.biopsych.2012.10.024
1002 Thomas, A., Baillie, G.L., Phillips, A.M., Razdan, R.K., Ross, R.A., Pertwee, R.G., 2007.
1003 Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2
1004 receptor agonists in vitro. Br. J. Pharmacol. 150, 613–623.
1005 https://doi.org/10.1038/sj.bjp.0707133

Urigüen, L., García-Fuster, M.J., Callado, L.F., Morentin, B., Harpe, R.L., Casadó, V., Lluis, C., Franco, R., García-Sevilla, J.A., Meana, J.J., 2009. Immunodensity and mRNA expression of A2A adenosine, D2 dopamine, and CB1 cannabinoid receptors in postmortem frontal cortex of subjects with schizophrenia: effect of antipsychotic treatment. Psychopharmacology (Berl.) 206, 313–324. https://doi.org/10.1007/s00213-009-1608-2

van Erp, T.G.M., Hibar, D.P., Rasmussen, J.M., Glahn, D.C., Pearlson, G.D., Andreassen, O.A., Agartz, I., Westlye, L.T., Haukvik, U.K., Dale, A.M., Melle, I., Hartberg, C.B., Gruber, O., Kraemer, B., Zilles, D., Donohoe, G., Kelly, S., McDonald, C., Morris, D.W., Cannon, D.M., Corvin, A., Machielsen, M.W.J., Koenders, L., Haan, L. de, Veltman, D.J., Satterthwaite, T.D., Wolf, D.H., Gur, R.C., Gur, R.E., Potkin, S.G., Mathalon, D.H., Mueller, B.A., Preda, A., Macciardi, F., Ehrlich, S., Walton, E., Hass, J., Calhoun, V.D., Bockholt, H.J., Sponheim, S.R., Shoemaker, J.M., Haren, N.E.M. van, Pol, H.E.H., Ophoff, R.A., Kahn, R.S., Roiz-Santiañez, R., Crespo-Facorro, B., Wang, L., Alpert, K.I., Jönsson, E.G., Dimitrova, R., Bois, C., Whalley, H.C., McIntosh, A.M., Lawrie, S.M., Hashimoto, R., Thompson, P.M., Turner, J.A., 2016. Subcortical brain volume abnormalities in 2028 individuals with schizophrenia and 2540 healthy controls via the ENIGMA consortium. Mol. Psychiatry 21, 547–553. https://doi.org/10.1038/mp.2015.63

Varvel, S.A., Hamm, R.J., Martin, B.R., Lichtman, A.H., 2001. Differential effects of Δ[sup 9] -THC on spatial reference and working memory in mice. Psychopharmacology (Berl.) 157, 142.

Verdurand, M., Fillman, S.G., Weickert, C.S., Zavitsanou, K., 2013. Increases in [3H]muscimol and [3H]flumazenil binding in the dorsolateral prefrontal cortex in schizophrenia are linked to α4 and γ2S mRNA levels respectively. PloS One 8, 1–12. https://doi.org/10.1371/journal.pone.0052724

Volk, D.W., Pierri, J.N., Fritschy, J.-M., Auh, S., Sampson, A.R., Lewis, D.A., 2002. Reciprocal alterations in pre- and postsynaptic inhibitory markers at chandelier cell inputs to pyramidal neurons in schizophrenia. Cereb. Cortex 12, 1063–1070.

Weickert, C.S., Fung, S.J., Catts, V.S., Schofield, P.R., Allen, K.M., Moore, L.T., Newell, K.A., Pellen, D., Huang, X.-F., Catts, S.V., Weickert, T.W., 2013. Molecular evidence of N-methyl-D-aspartate receptor hypofunction in schizophrenia. Mol. Psychiatry 18, 1185–1192. https://doi.org/10.1038/mp.2012.137

Weston-Green, K., Huang, X.-F., Deng, C., 2012b. Alterations to melanocortinergic, GABAergic and cannabinoid neurotransmission associated with olanzapine-induced weight gain. PloS One 7, e33548. https://doi.org/10.1371/journal.pone.0033548

Weston-Green, K., Huang, X.-F., Han, M., Deng, C., 2008. The effects of antipsychotics on the density of cannabinoid receptors in the dorsal vagal complex of rats: implications for olanzapine-induced weight gain. Int. J. Neuropsychopharmacol. 11, 827–835. https://doi.org/10.1017/S1461145708008560

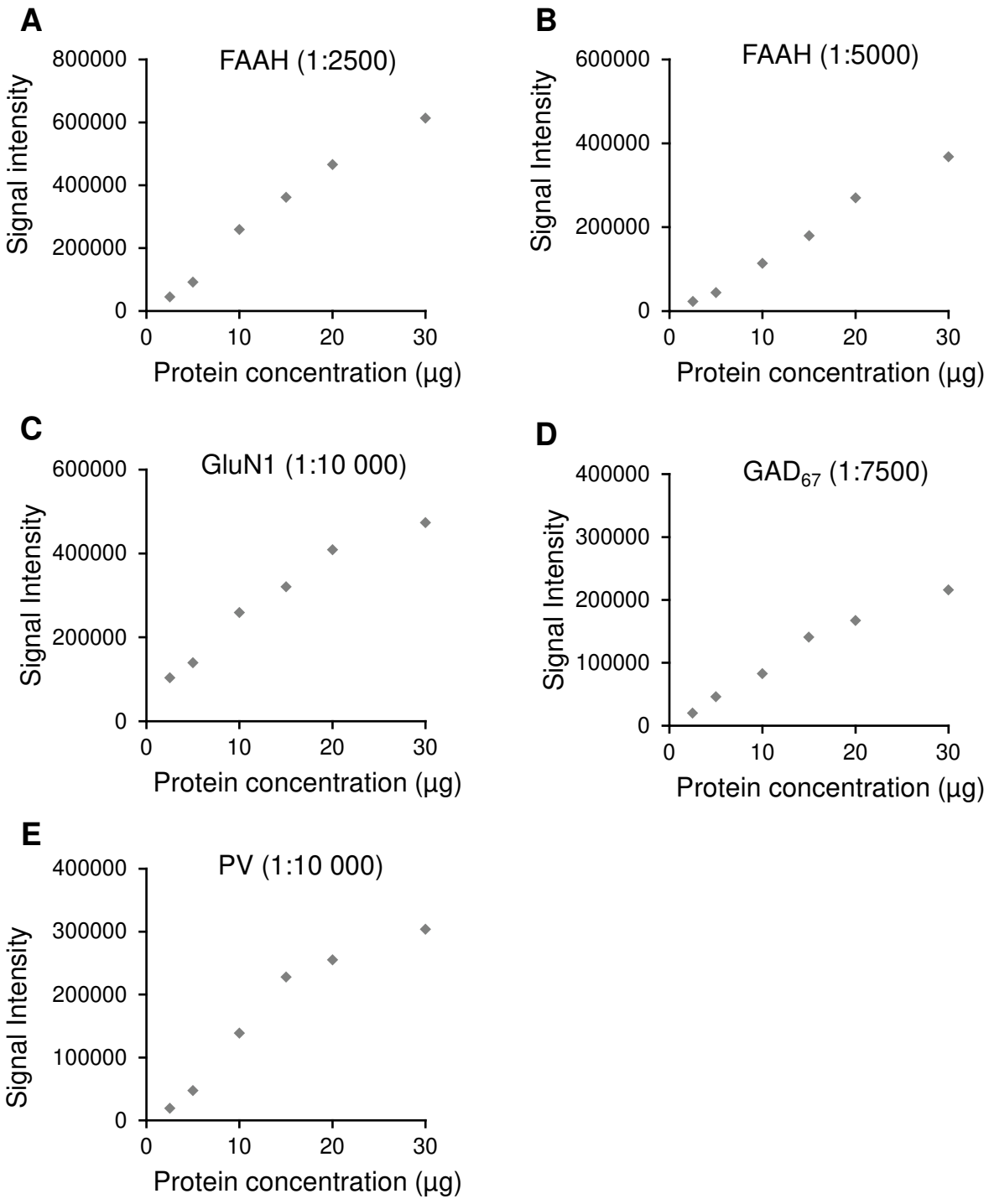
Weston-Green, K., Huang, X.-F., Lian, J., Deng, C., 2012b. Effects of olanzapine on muscarinic M3 receptor binding density in the brain relates to weight gain, plasma insulin and metabolic hormone levels. Eur. Neuropsychopharmacol. 22, 364–373. https://doi.org/10.1016/j.euroneuro.2011.09.003

Xia, Y., Haddad, G.G., 1992. Ontogeny and distribution of GABAA receptors in rat brainstem and rostral brain regions. Neuroscience 49, 973–989.

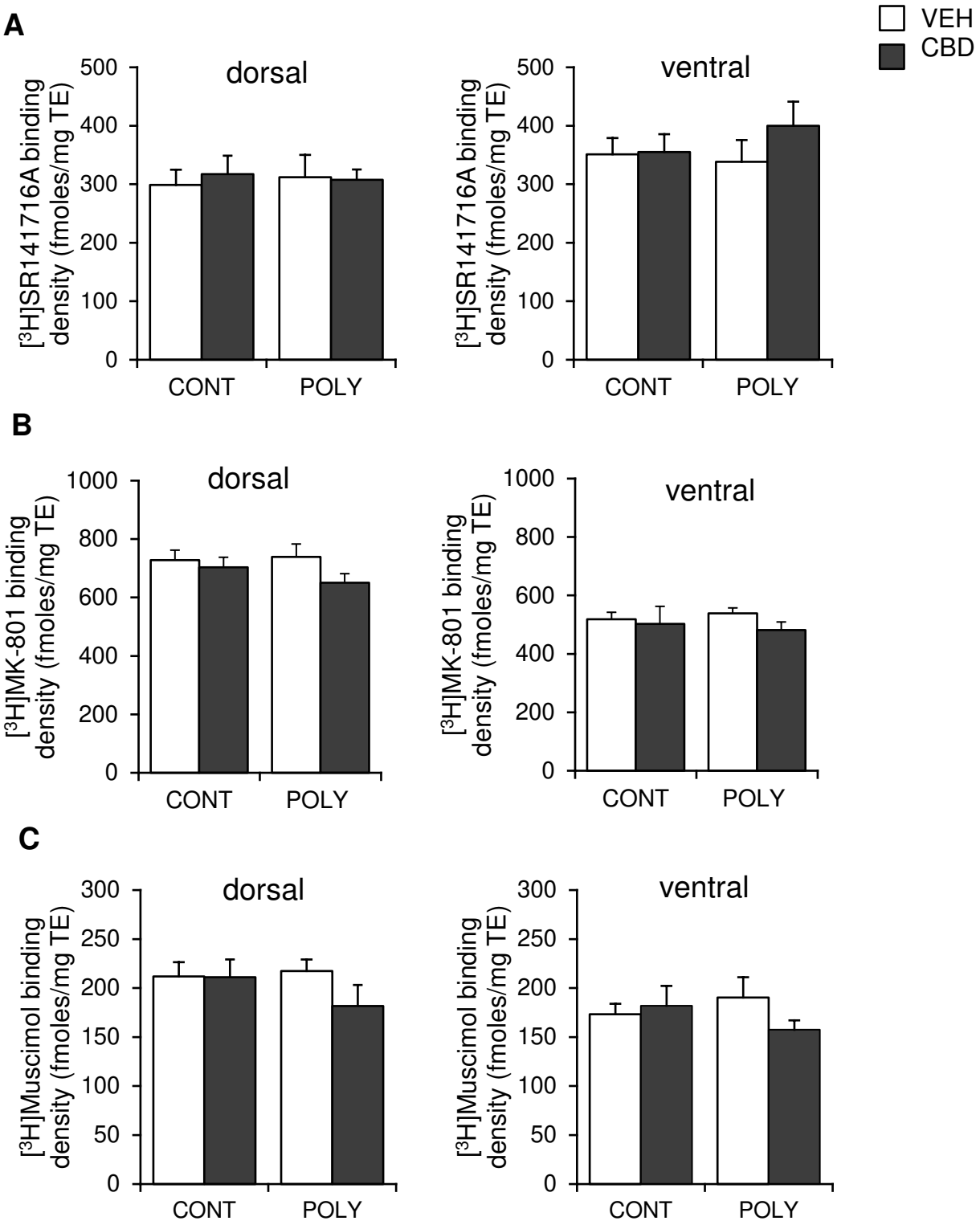
Yu, Y., Wu, Y., Patch, C., Wu, Z., Szabo, A., Li, D., Huang, X.-F., 2013. DHA prevents altered 5-HT1A, 5-HT2A, CB1 and GABAA receptor binding densities in the brain of male rats fed a high-saturated-fat diet. J. Nutr. Biochem. 24, 1349–1358. https://doi.org/10.1016/j.jnutbio.2012.11.002

Zavitsanou, K., Dalton, V.S., Walker, A.K., Weickert, C.S., Sominsky, L., Hodgson, D.M., 2013. Neonatal lipopolysaccharide treatment has long-term effects on monoaminergic and cannabinoid receptors in the rat. Synap. 67, 290–299. https://doi.org/10.1002/syn.21640

Supplementary Material



Supplementary Figure 1: Crude rat brain homogenate was prepared at increasing concentrations of total protein (2.5, 5, 10, 15, 20 and 30 µg) and immunoblotted for the primary antibodies of interest, A, B) fatty acid amide hydrolase (FAAH), C) GluN1 subunit, D) glutamate decarboxylase (GAD_{67}) and E) parvalbumin (PV), as previously described in the original manuscript.



Supplementary Figure 2: No difference in (**A**) CB1R ([^{3}H]SR141716A), (**B**) NMDAR ([^{3}H]MK801) or (**C**) $GABA_AR$ ([^{3}H]Muscimol) binding density in the dorsal (left) and ventral (right) hippocampus of control (CONT) and poly I:C (POLY) offspring. Data expressed as mean ± SEM. *n* = 5-7 rats per group.