2019

Cannabidiol improves behavioural and neurochemical deficits in adult female offspring of the maternal immune activation (poly I:C) model of neurodevelopmental disorders

Ashleigh L. Osborne

University of Wollongong, alo649@uowmail.edu.au

Nadia Solowij

University of Wollongong, nadia@uow.edu.au

Ilijana Babic

University of Wollongong, ib171@uowmail.edu.au

Jeremy Lum

University of Wollongong, jlum@uow.edu.au

Xu-Feng Huang

University of Wollongong, xhuang@uow.edu.au

See next page for additional authors

Follow this and additional works at: <https://ro.uow.edu.au/ihmri>

Part of the [Medicine and Health Sciences Commons](#)

Recommended Citation

Osborne, Ashleigh L.; Solowij, Nadia; Babic, Ilijana; Lum, Jeremy; Huang, Xu-Feng; Newell, Kelly A.; and Weston-Green, Katrina, "Cannabidiol improves behavioural and neurochemical deficits in adult female offspring of the maternal immune activation (poly I:C) model of neurodevelopmental disorders" (2019). *Illawarra Health and Medical Research Institute*. 1452.
<https://ro.uow.edu.au/ihmri/1452>

Cannabidiol improves behavioural and neurochemical deficits in adult female offspring of the maternal immune activation (poly I:C) model of neurodevelopmental disorders

Abstract

Cognitive impairment is a major source of disability in schizophrenia and current antipsychotic drugs (APDs) have minimal efficacy for this symptom domain. Cannabidiol (CBD), the major non-intoxicating component of *Cannabis sativa* L., exhibits antipsychotic and neuroprotective properties. We recently reported the effects of CBD on cognition in male offspring of a maternal immune activation (polyinosinic-polycytidilic acid (poly I:C)) model relevant to the aetiology of schizophrenia; however, the effects of CBD treatment in females are unknown. Sex differences are observed in the onset of schizophrenia symptoms and response to APD treatment. Furthermore, the endogenous cannabinoid system, a direct target of CBD, is sexually dimorphic in humans and rodents. Therefore, the present work aimed to assess the therapeutic impact of CBD treatment on behaviour and neurochemical signalling markers in female poly I:C offspring. 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. From postnatal day 56, female offspring received CBD (10 mg/kg, i.p.) or vehicle treatment for approximately 3 weeks. Following 2 weeks of CBD treatment, offspring underwent behavioural testing, including the novel object recognition, rewarded alternation T-maze and social interaction tests to assess recognition memory, working memory and sociability, respectively. After 3 weeks of CBD treatment, the prefrontal cortex (PFC) and hippocampus (HPC) were collected to assess effects on endocannabinoid, glutamatergic and gamma-aminobutyric acid (GABA) signalling markers. CBD attenuated poly I:C-induced deficits in recognition memory, social interaction and glutamatergic N-methyl-D-aspartate receptor (NMDAR) binding in the PFC of poly I:C offspring. Working memory performance was similar between treatment groups. CBD also increased glutamate decarboxylase 67, the rate-limiting enzyme that converts glutamate to GABA, and parvalbumin protein levels in the HPC. In contrast to the CBD treatment effects observed in poly I:C offspring, CBD administration to control rats reduced social interaction, cannabinoid CB1 receptor and NMDAR binding density in the PFC, suggesting that CBD administration to healthy rats may have negative consequences on social behaviour and brain maturation in adulthood. Overall, the findings of this study support the therapeutic benefits of CBD on recognition memory and sociability in female poly I:C offspring, and provide insight into the neurochemical changes that may underlie the therapeutic benefits of CBD in the poly I:C model.

Disciplines

Medicine and Health Sciences

Publication Details

Osborne, A. L., Solowij, N., Babic, I., Lum, J. S., Huang, X., Newell, K. A. & Weston-Green, K. (2019). Cannabidiol improves behavioural and neurochemical deficits in adult female offspring of the maternal immune activation (poly I:C) model of neurodevelopmental disorders. *Brain, Behavior, and Immunity*, 81 574-587.

Authors

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

Title: Cannabidiol improves behavioural and neurochemical deficits in adult female offspring
of the maternal immune activation (poly I:C) model of **neurodevelopmental disorders**

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}, Xu-Feng Huang (DSc, PhD,
MBBS)^{b, c, e}, Kelly A. Newell (PhD)^{a, b, c}, Katrina Weston-Green (PhD)*^{a, b, c, e}

Affiliations:

^aNeuropharmacology and Molecular Psychiatry Laboratory, School of Medicine, University
of Wollongong, Wollongong, NSW, 2522, Australia

^bCentre for Translational Neuroscience, Illawarra Health and Medical Research Institute,
Wollongong, NSW, 2522, Australia

^cMolecular Horizons, Faculty of Science, Medicine and Health, University of Wollongong,
Wollongong, NSW, 2522, Australia

^dSchool of Psychology, Faculty of Social Sciences, University of Wollongong, and Illawarra
Health and Medical Research Institute, Wollongong, NSW, 2522, Australia

^eAustralian Centre for Cannabinoid Clinical and Research Excellence, New Lambton
Heights, NSW, 2305 Australia

^fIllawarra and Shoalhaven Local Health District, Wollongong, NSW, 2500, Australia

*Corresponding Author:

Dr Katrina Weston-Green

School of Medicine

Faculty of Science, Medicine and Health

University of Wollongong

26 Northfields Avenue, Wollongong, NSW 2522 Australia

27 Tel: +61 2 4252 8506

28 Email: katrina_green@uow.edu.au

29

30 Keywords: cannabidiol; cannabinoid; schizophrenia; maternal immune activation; brain;

31 glutamate; GABA; cognition

32

33 Word count: 8709 (excluding figure legends and references)

Abstract

Cognitive impairment is a major source of disability in schizophrenia and current antipsychotic drugs (APDs) have minimal efficacy for this symptom domain. Cannabidiol (CBD), the major non-intoxicating component of *Cannabis sativa* L., exhibits antipsychotic and neuroprotective properties. We recently reported the effects of CBD on cognition in male offspring of a maternal immune activation (polyinosinic-polycytidilic acid (poly I:C)) model relevant to the aetiology of schizophrenia; however, the effects of CBD treatment in females are unknown. Sex differences are observed in the onset of schizophrenia symptoms and response to APD treatment. Furthermore, the endogenous cannabinoid system, a direct target of CBD, is sexually dimorphic in humans and rodents. Therefore, the present work aimed to assess the therapeutic impact of CBD treatment on behaviour and neurochemical signalling markers in female poly I:C offspring. 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. From postnatal day 56, female offspring received CBD (10 mg/kg, i.p.) or vehicle treatment for approximately 3 weeks. Following 2 weeks of CBD treatment, offspring underwent behavioural testing, including the novel object recognition, rewarded alternation T-maze and social interaction tests to assess recognition memory, working memory and sociability, respectively. After 3 weeks of CBD treatment, the prefrontal cortex (PFC) and hippocampus (HPC) were collected to assess effects on endocannabinoid, glutamatergic and gamma-aminobutyric acid (GABA) signalling markers. CBD attenuated poly I:C-induced deficits in recognition memory, social interaction and glutamatergic *N*-methyl-D-aspartate receptor (NMDAR) binding in the PFC of poly I:C offspring. Working memory performance was similar between treatment groups. CBD also increased glutamate decarboxylase 67, the rate-limiting enzyme that converts glutamate to GABA, and parvalbumin protein levels in the HPC. In contrast to the CBD treatment effects observed in poly I:C offspring, CBD

administration to **control** rats reduced social interaction, cannabinoid CB1 receptor and NMDAR binding density in the PFC, **suggesting that CBD administration to healthy rats may have negative consequences on social behaviour and brain maturation in adulthood.** Overall, the findings of this study support the therapeutic benefits of CBD on recognition memory and sociability in female poly I:C offspring, and provide insight into the neurochemical **changes that may underlie** the therapeutic benefits of CBD in the poly I:C model.

1 Introduction

People with schizophrenia experience a range of symptoms that can be characterised into positive (e.g. hallucinations and delusions) and negative symptoms (e.g. social withdrawal), as well as cognitive deficits (e.g. impairments in memory and attention). While the incidence of schizophrenia does not significantly differ between men and women, several studies have reported sex differences in age of onset, symptomatology, treatment response and clinical outcomes (Aleman *et al.* 2003; Ochoa *et al.* 2012; Holloway *et al.* 2013). Schizophrenia symptoms can be more severe in men and appear during late adolescence, while symptoms do not appear until early adulthood in women, with a second peak in symptom onset observed during menopause (Abel *et al.* 2010). Antipsychotic drugs (APDs) are the primary line of treatment and are generally effective at controlling the positive symptoms, while the negative symptoms and cognitive deficits are largely resistant to existing medications (Goff *et al.*, 2011; Lindenmayer *et al.*, 2013). Furthermore, APDs can cause adverse side effects, such as motor disturbances and body weight gain, which are mainly attributable to the first (typical) and second (atypical) generation drugs, respectively (Weston-Green *et al.*, 2013). While female patients tend to respond better to APD treatment (Smith, 2010), they also experience more severe side effects compared to men (Iversen *et al.*, 2018), highlighting the need for new therapeutic options that are effective in both sexes, with less adverse side effects.

Epidemiological evidence shows that maternal immune activation (MIA) during pregnancy can increase the risk of the child developing **neurodevelopmental disorders, including** schizophrenia, **autism spectrum and bipolar disorder** later in life (Khandaker *et al.*, 2013). MIA can be modelled in rodents by the administration of polyinosinic-polycytidilic (poly I:C) acid, a synthetic double-stranded RNA virus, to pregnant dams. Offspring develop behavioural perturbations that resemble a spectrum of schizophrenia-like symptoms,

including deficits in sensorimotor gating, working memory, latent inhibition and social interaction, and sensitivity to amphetamine (reviewed in Reisinger *et al*, 2015). This data is mainly obtained from male offspring; however, there is evidence to suggest that there are sex-specific effects in these behavioural profiles (Bitanirwe *et al.*, 2010; Hui *et al.*, 2018; Meehan *et al.*, 2017; O’Leary *et al.*, 2014; Piontkewitz *et al.*, 2011; Ratnayake *et al.*, 2014; Ruskin *et al.*, 2017; Xuan and Hampson, 2014; Zhang *et al.*, 2012). Studies examining female offspring are minimal, with investigations mainly limited to juvenile or late adult time points (Deslauriers *et al.*, 2013; Harvey and Boksa, 2012; Labouesse *et al.*, 2015; Meyer *et al.*, 2008; Nyffeler *et al.*, 2006; Rahman *et al.*, 2017). We recently characterised the behavioural (Osborne *et al.*, 2017a) and neurobiological (unpublished findings) changes in male poly I:C offspring compared to controls. Therefore, this study examined the behavioural and neurochemical effects of maternal poly I:C exposure in female offspring during early adulthood, a critical period of brain maturation, and when psychotic symptoms emerge in people with schizophrenia.

Cannabidiol (CBD) is a major non-intoxicating compound of the cannabis plant that exerts beneficial effects on cognitive impairment across a range of pathological conditions, including Alzheimer’s disease and stroke (Osborne *et al.*, 2017b). Recent clinical trials report therapeutic effects of CBD on positive and negative symptoms in unmedicated (Leweke *et al.*, 2012) and APD-treated schizophrenia patients, as well as a mild improvement in cognition in a male-dominated cohort (McGuire *et al.*, 2018). We recently reported that CBD treatment improved working memory, recognition memory and social interaction deficits in male poly I:C offspring (Osborne *et al.*, 2017a); however, the response of female poly I:C offspring to CBD treatment is unknown. Furthermore, female rats tend to have greater sensitivity to APD-induced metabolic dysfunction (Weston-Green *et al.*, 2010), but it is

unknown whether CBD causes weight gain side effects in female poly I:C offspring. Therefore, this study evaluated the effects of CBD treatment on cognition and sociability, as well as body weight, food and water intake in female poly I:C offspring.

CBD interacts with the endogenous cannabinoid (eCB) system, which regulates homeostatic processes in the brain and plays an important role in synaptic plasticity, mood, neuroprotection and cognition (Lu and Mackie, 2016). Dysregulation of the eCB system has been reported in schizophrenia, including dysfunction in the most abundant cannabinoid receptor in the CNS, the cannabinoid CB1 receptor (CB1R) (Dalton et al., 2011; Dean et al., 2001; Eggan et al., 2010, 2008), as well as its endogenous ligand anandamide (AEA) (Giuffrida et al., 2004; Koethe et al., 2009; Leweke et al., 2007; Muguruza et al., 2013). Importantly, the eCB system modulates the release of the major excitatory (glutamate) and inhibitory (GABA) to maintain optimal brain function (Lu and Mackie, 2016). These signalling systems are also implicated in schizophrenia pathophysiology, including hypofunction of the glutamatergic *N*-methyl-*D*-aspartate receptor (NMDAR) (Cohen et al., 2015), and a loss of inhibitory tone, including dysfunction in the ionotropic GABA_A receptor (GABA_AR), GABA synthesising enzyme glutamate decarboxylase 67 (GAD₆₇), as well as a subset of GABAergic neurons that express the calcium binding protein parvalbumin (PV) (reviewed in Jonge et al., 2017). While CBD may interact with the eCB system to exert its therapeutic benefits (Campos et al., 2017), there is limited understanding of the effects of CBD on the major excitatory and inhibitory neurotransmitter systems in the brain, glutamate and gamma-aminobutyric acid (GABA), respectively. Therefore, in the present study, the effects of CBD on markers of eCB, NMDAR and GABAergic signalling were analysed in the prefrontal cortex (PFC) and hippocampus (HPC) of female offspring due to the central role of these regions in schizophrenia neuropathology (Godsil et al., 2013). Overall, this study aimed

to characterise the behavioural and neurochemical effects of CBD treatment in female poly I:C offspring, which may have implications for the use of CBD as a novel therapeutic for females with schizophrenia.

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 Institute (NHMRC), Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 2013 (NHMRC, 2013). All efforts were made to minimise the number and suffering of animals. Animal experiments have been reported in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (Kilkenny et al., 2010).

2.2 Animals and Treatment

The methods for modelling MIA using poly I:C administration were conducted as previously reported by our laboratory (Osborne et al., 2017a). Briefly, time-mated pregnant Sprague-Dawley rats (gestational day (GD) 8, n=16; Animal Resource Centre, Perth, Australia) were housed individually and allowed to habituate to their surroundings for one week. On GD 15, dams were injected with either poly I:C (4 mg/kg, n = 8; **P1530, Lot Number: 114M4028V**; Sigma-Aldrich, Castle Hill, NSW Australia) or sterile saline (n=8) into the lateral tail vein. After postnatal day (PN) 21, female offspring were weaned and pair-housed with same treatment littermates. Offspring were maintained on a reversed 12 hour light–dark cycle (lights on 19:00h) with *ad libitum* access to food and water. From PN56, which is equivalent

to late adolescence/early adulthood in humans (Kilb, 2012; Semple et al., 2013), offspring were administered CBD (10 mg/kg, i.p.; n = 12/group, 6 independent litters; **THC-1440G-1, CAS: 13956-29-1; THC Pharm GmbH, Frankfurt, Germany**) or vehicle (5 ml/kg; n = 12/group, 6 independent litters). Treatments were administered for approximately 3 weeks, twice a day, at 12 hourly intervals (07:00h, 19:00h), based on the half-life of CBD in the rat brain (Deiana et al., 2011). The CBD dosage and treatment duration was based on our previous study (Osborne et al., 2017a) and others that report CBD exerts a therapeutic effect on cognitive deficits in inflammation-based rodent models (Barichello et al., 2012; Cassol-Jr et al., 2010; Fagherazzi et al., 2011; Schiavon et al., 2014). Body weight, food and water intake (for each cage) were recorded weekly during the treatment period.

2.3 Behavioural Testing

After two weeks of treatment, offspring behaviour was examined using the Novel Object Recognition (NOR), rewarded T-maze alternation and social interaction tests to assess recognition memory, working memory and social withdrawal, respectively. All behavioural experiments were performed between 09:00 and 18:00h during the active (dark) phase of the light-dark cycle, and testing was counterbalanced across treatment groups. After each test, the equipment was cleaned with 70% ethanol between trials to eliminate olfactory cues. The behavioural tests were performed in order of invasiveness to minimise stress effects on subsequent behavioural tasks and there was a 24 hour rest period between tests. Rat behaviour was recorded using standard commercial cameras (Logitech Pty Ltd, Alexandria, Australia) and de-identified video recordings were later analysed.

2.3.1 Novel Object Recognition (NOR) test

The NOR test was conducted using methods previously described by our laboratory (Babic et al., 2018; Osborne et al., 2017a). Briefly, plastic building blocks were placed in the home cages for 24-hour familiarisation. In the first trial, a rat was placed in a black, square arena (60cm x 60cm x 60cm, with an even lighting of 20 lux) to habituate for 5 min. Two familiar objects were placed in opposite corners of the upper half of the area and the rat was positioned in the centre of the lower wall, facing away from the objects. After 5 min of exploration, the rat was returned to its home cage for 1 h. In the second trial, the rat was returned to the arena, but this time one of the familiar objects was replaced with a novel object (toy figurine), and the rat was allowed to explore for 3 min. Object interaction was scored using the criteria previously described by our laboratory (Osborne et al., 2017a) and a discrimination index was calculated for each rat, expressed as: $T_N - T_F / (T_F + T_N)$, where T_N = time spent exploring the novel object (secs) and T_F = time spent exploring the familiar object (secs). Values for the discrimination index ranged from -1 to 1, where a positive score indicated preference for the novel object and a negative score indicated preference for the familiar object (Bevins and Besheer, 2006).

2.3.2 Rewarded T-maze Test

The T-maze test was conducted using methods previously detailed by our laboratory (Babic et al., 2018; Osborne et al., 2017a). Briefly, rats were habituated to a black, T-shaped maze (50 cm long x 10 cm wide, 30 cm high walls with an even lighting of 20 lux) that contained chocolate pellet rewards in the left and right arms of the T-maze for 5 min. Rats were trained over 3 consecutive days to alternate entry in the left and right arms of the T-maze. Trials consisted of (1) a 'forced' run, where one arm of the T-maze was closed by a removable divider and the reward was placed in the open arm, and (2) a 'choice' run, where the divider was removed (allowing the rat access to both arms of the T-maze) and the reward was placed

in the newly opened arm. Training days consisted of 8 trials, each comprised of 4 forced and 4 choice runs, with randomised alternation of the divider position in each forced run. The test day consisted of 10 trials (5 forced runs, 5 choice runs) per rat with the same alternation procedures used as above. Each trial was limited to 3 mins. A correct response was defined as a first entry into the correct arm. The percentage of correct responses and the latency to first entry (secs) were recorded for each rat.

2.3.3 Social Interaction Test

The social interaction test was conducted using methods described by our laboratory (De Santis et al., 2016; Du Bois et al., 2008; Osborne et al., 2017a). Two unfamiliar rats from the same treatment group were placed in opposite corners of a black square arena (described in section 2.3.1), allowed to freely roam the arena for 7 mins and were then returned to their home cage. The amount of time that rats spent interacting with each other (defined as time spent sniffing, following, grooming, climbing each other) was recorded (secs) for each pair of rats. Interaction times for each pair of rats were used for statistical analysis.

2.4 Neurochemical Analysis

After the last treatment (~10-12 hours), adult rats were sacrificed by carbon dioxide asphyxiation followed by rapid decapitation between 09:00 and 11:30h to reduce the potential confound of circadian rhythm on neurochemical analyses (Jasinska and Pyza, 2017). Whole brains were removed, immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

2.4.1 Histological Procedures

A cryostat (Jung CM 3000, Leica Instruments GmbH, Nussloch, Germany) was used to

collect alternating 14 μ M or 500 μ M coronal sections of rat brain tissue (n = 8 per group) that contained the PFC (prelimbic and infralimbic cortices; Bregma level: 4.2 mm to 2.56 mm) or HPC (- 4.36 mm to - 6.00 mm). Consecutive sections collected for receptor autoradiography experiments (14 μ M; 2-3 sections per region, per rat) were thaw-mounted on to PolysineTM slides (Sigma-Aldrich, Castle Hill, NSW, Australia) and stored at -20°C until further use. Sections collected for Western blot experiments (500 μ M; 2-3 sections per region, per rat) were mounted on glass slides, microdissected using a micropuncture kit and stored at -80°C until further use. This dissection protocol allowed simultaneous investigation of receptor binding and protein levels from the same animals.

2.4.2 Receptor Autoradiography

2.4.2.1 CB1R binding

CB1R binding density was examined using methods previously reported by our laboratory (Weston-Green et al., 2012; Yu et al., 2013). Briefly, slides were air-dried and pre-incubated in 50 mM Tris-HCl buffer (pH 7.4) with 0.1% bovine serum albumin (BSA) for 15 min at room temperature. Sections were incubated in 50 mM Tris-HCl buffer (with 0.1% BSA) containing 10 nM [³H]SR141716A (CB1R selective antagonist) (43 Ci/mmol; PerkinElmer TM Life and Analytical Sciences, Boston, USA) for 60 min at room temperature to determine total binding. Additional sections were incubated in 10 nM [³H]SR141716A in the presence of 100 μ M CP-55940 (cannabinoid receptor agonist) to determine non-specific binding. After incubation, slides were washed twice for 30 min in ice-cold buffer, rinsed in cold milliQ water and air-dried.

2.4.2.2 NMDAR Binding

NMDAR binding density was examined using 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 μ M glycine, 100 μ M glutamate, 1 mM ethylenediaminetetraacetic acid (EDTA) and 20 nM [3 H]MK-801 (NMDAR antagonist) (specific activity 22.5 Ci/mmol, PerkinElmer, Boston, USA) for 2.5 hours at room temperature to determine total binding. Additional sections were incubated in the same buffer containing [3 H]MK-801 in the presence of 200 μ M unlabelled MK-801 to determine non-specific binding. 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.

2.4.2.3 GABA_AR Binding

GABA_AR binding density was examined using 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 room temperature for 5 mins. Sections were incubated in 50 mM Tris-citrate buffer containing 50 nM [3 H]muscimol (GABA_AR agonist) (specific activity 29.5 Ci/mmol, PerkinElmer, Boston, USA) for 45 min at 4°C to determine total binding. Additional sections were incubated in 50 mM Tris-citrate (pH 7.0) buffer containing [3 H]Muscimol and 100 μ M GABA (GABA receptor agonist) to determine non-specific binding. After incubation, sections were rinsed four times for 2 secs each in 50 mM Tris-citrate buffer (4°C), rinsed in cold milliQ water and air-dried.

2.5.4 Quantification

Slides from CB1R, NMDAR and GABA_AR binding experiments were exposed to Amersham Hyperfilm ECL (GE Healthcare Life Sciences, Parramatta, NSW, Australia) with a set of Amersham tritium standards, in X-ray film cassettes (De Santis et al., 2016). After 5 weeks (NMDAR and GABA_AR) or 8 weeks (CB1R), autoradiographs were developed, scanned using a GS-800 Imaging Densitometer (Bio-Rad, Hercules, California, USA) and analysed using Image J software (<https://imagej.nih.gov/ij>). Two sections were quantified per brain region, per rat to obtain total binding density values. Non-specific binding was subtracted from total binding to estimate specific binding. Values were converted to fmoles [³H] ligand per mg tissue equivalent (TE) taking into account the specific activity of the ligand. Anatomical structures were confirmed using a rat brain atlas (Paxinos and Watson, 2007).

2.6 Western blot

Briefly, brain samples were homogenised as previously described (Lum et al., 2016) and a DC assay kit was used to determine total protein concentration following the manufacturer's instructions (Bio-Rad, Australia). Protein samples (10 µg, within the linear range of detection of the primary antibodies) were separated in CriterionTM TGX Stain-FreeTM 4-20% gels (Bio-Rad, Australia), gels were activated (GelDoc XR+ imaging system; Bio-Rad, Australia) (Colella et al., 2012; Gürtler et al., 2013) and then proteins were transferred on to a PVDF membrane (Bio-Rad, Australia). Membranes were imaged (GelDoc XR+ imaging system; Bio-Rad, Australia) to capture total protein in each lane (Colella et al., 2012; Gürtler et al., 2013). To detect the proteins of interest, membranes were cut and incubated overnight at 4°C with the following primary antibodies: anti-FAAH1 (PFC: 1:2500, HPC: 1:5000 ; #ab54615, Abcam), anti-GluN1 (1:10000; #MAB363, Millipore), anti-GAD₆₇ (1:7500 #MAB5406, Millipore) and anti-parvalbumin (1:10000; #Ab11427, Abcam). Membranes were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5000;

#AP307P or #AP308P, Millipore). Bands were visualised using 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) and an internal control sample (containing equal amounts of each sample) to account for inter-gel variability. Samples were run in duplicate.

2.7 Statistical Analyses

Statistical analyses were conducted using SPSS (Version 21.0, IBM, Illinois, USA). Data were within 2SD of the group mean and were tested for normality using Shapiro Wilk tests. Analyses of variance (ANOVAs; factors: prenatal infection, offspring treatment) were used to analyse normally distributed data and pairwise comparisons were made between treatment groups (with Bonferroni's adjustment). If a significant difference was observed between CBD and vehicle-treated poly I:C offspring, then an additional comparison (using an independent t-test) was made to determine if CBD treatment in poly I:C offspring restored the parameter to control levels. Where data remained non-normally distributed following transformation, or there was unequal variance between groups (given by a significant result on Levene's Test of Equality of Error Variances), Mann-Whitney U tests were used to compare groups (indicated in Results section). A one-sample *t*-test was used to determine whether preference for the novel object (NOR test) and the percentage of correct entries (T-maze test) were above chance levels (50%). Repeated measures mixed ANOVA (between-subjects factors: prenatal infection, offspring treatment; within-subjects factor: time) was used to analyse average body weight (with baseline body weight as a covariate) and the degrees of freedom were adjusted

357 using the Greenhouse-Geisser correction (reported in Results section). Significance was set to
358 $p < 0.05$. Data are presented as mean $\pm$ SEM.

3. Results

3.1 Effect of cannabidiol on negative and cognitive symptom phenotypes

In the present study, we examined whether poly I:C exposure at mid to late gestation elicited recognition memory, working memory and social interaction deficits in adult female offspring, and assessed the therapeutic potential of chronic CBD treatment to attenuate poly I:C-induced deficits. First, we examined the effects of CBD treatment on recognition memory in the NOR test in control and poly I:C female offspring. A two-way ANOVA revealed no significant main effects of prenatal infection ($F_{(1, 38)} = 1.865$, $p = 0.180$) or offspring treatment ($F_{(1, 38)} = 0.581$, $p = 0.451$) on the discrimination index; however, there was a significant prenatal infection x offspring treatment interaction ($F_{(1, 38)} = 5.344$, $p = 0.026$). Pairwise comparisons revealed that the POLY+VEH group had a lower mean discrimination index compared to the CONT+VEH group ($p = 0.013$) (Figure 1A), indicating that maternal poly I:C exposure impaired recognition memory in adult offspring. Furthermore, CBD treatment significantly improved the discrimination index of poly I:C offspring (POLY+VEH vs. POLY+CBD, $p = 0.036$), restoring to control-like levels (POLY+CBD vs. CONT+VEH, $p = 0.578$) (Figure 1A). There was no significant difference in the discrimination index between the CONT+VEH and CONT+CBD group ($p = 0.280$) (Figure 1A), indicating that CBD administration did not affect recognition memory of healthy rats. Moreover, when the percentage of time spent interacting with the novel object was considered, all groups except the POLY+VEH group displayed a significant preference for the novel object that was above chance levels (CONT+VEH, $t(10) = 7.157$, $p < 0.001$; CONT+CBD, $t(9) = 4.055$, $p = 0.003$; POLY+VEH, $t(9) = 0.946$, $p = 0.369$; POLY+CBD, $t(10) = 5.698$, $p < 0.001$) (Figure 1B). Total object exploration times in the NOR test did not significantly differ between groups

383 (Figure 1C) (prenatal infection: $F_{(1, 37)} = 0.798$, $p = 0.377$; offspring treatment: $F_{(1, 37)} =$
384 1.443 , $p = 0.237$; prenatal infection x offspring treatment: $F_{(1, 37)} = 0.001$, $p = 0.976$).

Figure 1: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on the performance of female control (CONT) and poly I:C (POLY) offspring in the Novel Object Recognition test: (A) Discrimination index (calculated as $T_N - T_F / (T_F + T_N)$), (B) Preference for the novel object (against chance levels i.e. 50% indicated by the dashed line), and (C) Total object exploration time (s). Data expressed as mean $\pm$ SEM. * $p < 0.05$ vs. CONT+VEH. # $p < 0.05$ vs. POLY+VEH. ‘++’ $p < 0.01$, ‘+++’ $p < 0.001$ vs. chance (i.e. 50%). $n = 10$ -12 rats per group; **5-6 independent litters**. T_F : total time spent exploring familiar object; T_N : total time spent exploring novel object. Data expressed as mean $\pm$ SEM.

Working memory of female rat offspring was examined using the rewarded alternation T-maze test; however, results indicated these parameters were not altered in any of the female offspring treatment groups. Mann-Whitney tests showed that the percentage of correct entries in the T-maze test was comparable across groups (CONT+VEH vs. POLY+VEH, $p = 0.950$; POLY+VEH vs. POLY+CBD, $p = 0.724$; CONT+VEH vs. CONT+CBD, $p = 0.519$) and all groups performed above chance levels in the T-maze test (CONT+VEH, $t(10) = 3.862$, $p = 0.003$; CONT+CBD, $t(10) = 4.217$, $p = 0.002$; POLY+VEH, $t(11) = 2.779$, $p = 0.018$; POLY+CBD, $t(11) = 2.716$, $p = 0.020$) (Figure 2A). Similarly, maternal poly I:C exposure and CBD treatment did not effect latency to first arm entry in the T-maze test (prenatal infection: $F_{(1, 43)} = 1.044$, $p = 0.313$; offspring treatment: $F_{(1, 43)} = 2.259$, $p = 0.140$; prenatal infection x offspring treatment: $F_{(1, 43)} = 0.930$, $p = 0.340$) (Figure 2B).

Figure 2: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on the performance of female control (CONT) and poly I:C (POLY) offspring in the rewarded alternation T-maze test. (A) Percentage of correct entries and (B) latency to first entry (s) in the T-maze test. ‘+’ $p < 0.05$, ‘++’ $p < 0.01$ preference for the correct entry vs. chance (i.e. 50%, dashed line); $n = 10-12$ rats per group, **5-6 independent litters**. Data expressed as mean $\pm$ SEM.

The social interaction test was used to assess the sociability of control and poly I:C offspring following chronic CBD treatment. A two-way ANOVA revealed a significant main effect of prenatal infection ($F_{(1, 18)} = 10.975, p = 0.004$) and a prenatal infection x offspring treatment interaction ($F_{(1, 18)} = 26.798, p < 0.001$) on total interaction time. Vehicle-treated poly I:C offspring spent significantly less time interacting compared to controls (-50.78%; POLY+VEH vs. CONT+VEH, $p < 0.001$), indicating that maternal poly I:C exposure impaired social interaction in adult female offspring (Figure 3). CBD treatment significantly increased total interaction time in poly I:C offspring (+76.85%; POLY+CBD vs. POLY+VEH, $p < 0.001$), restoring control-like levels (POLY+CBD vs. CONT+VEH, $p = 0.130$) (Figure 3). Interestingly, female CONT+CBD offspring had a significantly lower interaction time compared to CONT+VEH offspring (-24.09%; $p = 0.014$), suggesting that CBD administration impaired social interaction in healthy rats (Figure 3).

Figure 3: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on the performance of female control (CONT) and poly I:C (POLY) offspring in the social interaction test. * $p < 0.05$, *** $p < 0.001$ vs. CONT+VEH; ### $p < 0.001$ vs. POLY+VEH; $n = 5-6$ pairs of rats per group. Data expressed as mean $\pm$ SEM.

3.2 Effect of cannabidiol on body weight, food and water intake

Body weight, food intake and water intake of female offspring were monitored over the treatment period. A two-way ANOVA revealed a significant main effect of prenatal infection on baseline body weight (i.e. Week 0 of treatment) ($F_{(1, 42)} = 37.168, p < 0.001$), whereby female offspring from poly I:C-treated dams were significantly heavier compared to offspring from control-treated dams (+14.39%, POLY vs. CONT) (Figure 4A). In order to examine the effect of CBD treatment on body weight over the treatment period, baseline body weight was included as a covariate for further analyses. Repeated measures mixed ANOVA revealed a significant prenatal infection x offspring treatment x time (weeks) interaction on the body weight of offspring ($F_{(1.701, 69.731)} = 4.455, p = 0.02$). All treatment groups gained weight over the treatment period ($p < 0.001$); however, there was no significant difference in average body weight between treatment groups at any of the time points (Week 1, 2 and 3; all $p > 0.05$) (Figure 4A). A two-way ANOVA revealed no significant effect of prenatal infection ($F_{(1, 20)} = 2.214, p = 0.152$) or offspring treatment ($F_{(1, 20)} = < 0.001, p = 0.988$) on average food intake over the treatment period, and no interaction between the factors ($F_{(1, 20)} = 0.541, p = 0.470$) (Figure 4B). In contrast, there was a significant main effect of prenatal infection ($F_{(1, 20)} = 13.849, p = 0.001$) on water intake, whereby poly I:C offspring consumed more water than control offspring over the treatment period (+23.86%) (Figure 4C). There was no significant effect of offspring treatment ($F_{(1, 20)} = 0.876, p = 0.360$) on average water intake and no interaction between the factors ($F_{(1, 20)} = 1.193, p = 0.288$).

Figure 4: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on body weight, food and water intake in female control (CONT) and poly I:C (POLY) offspring. **(A)** Average body weight of offspring (g) over the treatment period. *** $p < 0.001$ CONT vs. POLY offspring (significant effect of prenatal infection i.e. CONT+VEH and CONT+CBD groups vs. POLY+VEH and POLY+CBD groups) at baseline. $n = 11-12$ rats/group; **5-6 independent litters.** **(B)** Average food intake (g) and **(C)** average water intake (mL) over the treatment period. *** $p < 0.001$ CONT vs. POLY offspring (significant effect of prenatal

infection i.e. CONT+VEH and CONT+CBD groups vs. POLY+VEH + POLY+CBD groups).
 $n = 6$ pairs of rats/group. Data expressed as mean $\pm$ SEM.

3.2 Effect of cannabidiol on endocannabinoid markers

To evaluate CBD treatment effects on eCB neurotransmission, CB1R binding density and FAAH protein levels were examined in the PFC and HPC. Examples of [3 H]SR141716A binding to CB1Rs in the PFC and HPC are shown in Figure 5. Mann-Whitney tests showed no significant difference in [3 H]SR141716A binding density in the PFC of poly I:C offspring compared to controls ($p = 0.600$, POLY+VEH vs. CONT+VEH), and CBD treatment did not affect [3 H]SR141716A binding density in poly I:C offspring (POLY+CBD: 215.37 ± 18.24 vs. POLY+VEH: 244.58 ± 22.46 , $p = 0.699$) (Figure 6A). Interestingly, CBD administration significantly reduced [3 H]SR141716A binding density in the PFC of control offspring (-31.41% ; CONT+CBD vs. CONT+VEH, $p = 0.005$) (Figure 6A). In contrast, [3 H]SR141716A binding density in the HPC was similar between treatment groups (Figure 6B) (prenatal infection: $F_{(1, 25)} = 2.266$, $p = 0.145$; offspring treatment: $F_{(1, 25)} = 0.351$, $p = 0.559$; prenatal infection x offspring treatment: $F_{(1, 25)} = 1.076$, $p = 0.309$). FAAH protein levels in the PFC (Figure 6C) and HPC (Figure 6D) did not significantly differ between treatment groups ([PFC: prenatal infection: $F_{(1, 28)} = 2.051$, $p = 0.163$; offspring treatment: $F_{(1, 28)} = 0.019$, $p = 0.892$; prenatal infection x offspring treatment: $F_{(1, 28)} = 0.149$, $p = 0.702$]; [HPC: prenatal infection: $F_{(1, 27)} = 1.466$, $p = 0.237$; offspring treatment: $F_{(1, 27)} = 0.480$, $p = 0.494$; prenatal infection x offspring treatment: $F_{(1, 27)} = 1.742$, $p = 0.198$]).

	$[^3\text{H}]\text{SR141716A}$	$[^3\text{H}]\text{MK-801}$	$[^3\text{H}]\text{Muscimol}$
<p>PFC</p>				Total Binding
	Non-specific Binding
<p>HPC</p>				Total Binding
	Non-specific Binding

Figure 5: Representative autoradiographs of $[^3\text{H}]\text{SR141716A}$, $[^3\text{H}]\text{MK-801}$, and $[^3\text{H}]\text{Muscimol}$ binding density in the female rat brain. Schematic diagrams adapted from a rat brain atlas (Paxinos and Watson, 2007) showing the Bregma level quantified for the prefrontal cortex (PFC): 2.52 mm (containing the prelimbic and infralimbic cortices) and the hippocampus (HPC): - 4.92 mm (shaded). Total binding as well as non-specific binding for each ligand in the PFC and HPC are shown. Autoradiographs are typical examples of binding observed in $n = 6-8$ vehicle-treated control offspring.

Figure 6: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on endocannabinoid markers in the brains of female control (CONT) and poly I:C (POLY) offspring. Cannabinoid CB1 receptor ($[^3\text{H}]\text{SR141617A}$) binding density in the (A) prefrontal cortex (PFC) and (B) hippocampus (HPC). Fatty acid amide hydrolase (FAAH) protein levels in the (C) PFC and (D) HPC normalised to total protein signal. Example FAAH immunoblots shown. ** $p < 0.01$ vs. CONT+VEH group. $n = 6-8$ rats per group; 4-5 independent litters. Data expressed as mean $\pm$ SEM. TE = tissue equivalent.

3.3. Effect of cannabidiol on the glutamatergic NMDAR

To assess whether CBD treatment influenced excitatory glutamatergic neurotransmission, NMDAR binding density and protein levels of its obligatory GluN1 subunit were measured in the brains of offspring. Examples of [³H]MK-801 binding to NMDARs in the PFC and HPC are presented in Figure 5. A two-way ANOVA revealed no significant main effects of prenatal infection ($F_{(1, 25)} = 0.108$, $p = 0.745$) or offspring treatment ($F_{(1, 25)} = 0.241$, $p = 0.628$) on [³H]MK-801 binding density in the PFC, but there was a significant interaction between the two factors ($F_{(1, 25)} = 12.087$, $p = 0.002$). Pairwise comparisons showed that [³H]MK-801 binding density was significantly lower in POLY+VEH offspring compared to controls (-16.47%; POLY+VEH vs. CONT+VEH: $p = 0.033$) (Figure 7A). CBD treatment significantly increased [³H]MK-801 binding density in poly I:C offspring (+24.85%; POLY+CBD vs. POLY+VEH: $p = 0.011$) to control-like levels (POLY+CBD vs. CONT+VEH, $p = 0.600$) (Figure 7A). Interestingly, CBD administration significantly reduced [³H]MK-801 binding density in the PFC in control offspring (-15.62%; CONT+CBD vs. CONT+VEH: $p = 0.042$), in a similar manner to maternal poly I:C exposure (Figure 7A). In the HPC, [³H]MK-801 binding density was similar between groups (Figure 7B) (prenatal infection: $F_{(1, 23)} = 0.002$, $p = 0.961$; offspring treatment: $F_{(1, 23)} = 0.025$, $p = 0.875$; prenatal infection x offspring treatment: $F_{(1, 23)} = 0.022$, $p = 0.883$). In contrast to the [³H]MK-801 binding density results in the PFC, Mann-Whitney tests showed that protein levels of the obligatory GluN1 subunit in the PFC did not differ between treatment groups (all $p > 0.05$) (Figure 7C). Similarly, in the HPC there were no significant main effects of prenatal infection ($F_{(1, 27)} = 1.374$, $p = 0.251$) or offspring treatment ($F_{(1, 27)} = 3.023$, $p = 0.093$) and no interaction between the factors ($F_{(1, 27)} = 0.504$, $p = 0.484$) (Figure 7D).

Figure 7: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on the glutamatergic *N*-methyl-*D*-aspartate receptor (NMDAR) in control (CONT) and poly I:C (POLY) offspring. NMDAR ($[^3\text{H}]\text{MK-801}$) binding density in the (A) prefrontal cortex (PFC) and (B) hippocampus (HPC). Normalised protein levels of the obligatory GluN1 subunit of the NMDAR in the (C) PFC and (D) HPC (normalised to total protein signal). * $p < 0.05$ vs. CONT+VEH group, # $p < 0.05$ vs. POLY+VEH group. Data expressed as mean $\pm$ SEM. $n = 6-8$ rats per group; from 4-5 independent litters. TE = tissue equivalent.

3.4 Effect of cannabidiol on GABAergic markers

To assess whether markers of GABAergic signalling in the PFC and HPC were altered by CBD treatment, we measured GABA_AR binding density, as well as protein levels of GAD₆₇ (the rate-limiting enzyme that converts glutamate to GABA), and PV (a calcium-binding protein present on a subset of GABAergic interneurons). Examples of [³H]muscimol binding to the GABA_AR in the PFC and HPC are presented in Figure 5. [³H]Muscimol binding density was comparable between treatment groups in both the PFC (Figure 8A) and HPC (Figure 8B) (i.e. no significant main effects of prenatal infection (PFC: $F_{(1, 22)} = 0.665$, $p = 0.424$; HPC: $F_{(1, 25)} = 2.406$, $p = 0.133$) or offspring treatment (PFC: $F_{(1, 22)} = 1.985$, $p = 0.173$; HPC: $F_{(1, 25)} = 0.034$, $p = 0.856$) and no significant interactions (PFC: $F_{(1, 22)} = 1.050$, $p = 0.317$; HPC: $F_{(1, 25)} = 0.427$, $p = 0.519$)). In the PFC, there was no significant effect of prenatal infection ($F_{(1, 27)} = 0.054$, $p = 0.817$) or offspring treatment ($F_{(1, 27)} = 0.001$, $p = 0.977$) on GAD₆₇ protein levels, and no significant interaction between the factors (Figure 8C) ($F_{(1, 27)} = 0.149$, $p = 0.702$). In contrast, in the HPC there was a significant main effect of offspring treatment ($F_{(1, 25)} = 6.045$, $p = 0.021$), whereby CBD significantly increased GAD₆₇ protein levels relative to vehicle treatment (+17.36 %; CBD: 1.13 ± 0.059 vs. VEH: 0.962 ± 0.017) (Figure 8D). Poly I:C exposure did not affect GAD₆₇ protein levels (prenatal infection: $F_{(1, 25)} = 0.036$, $p = 0.850$), and there was no significant interaction between the factors ($F_{(1, 25)} = 0.508$, $p = 0.483$). There was no significant effect of prenatal infection ($F_{(1, 25)} = 1.514$, $p = 0.229$) or offspring treatment ($F_{(1, 25)} = 0.257$, $p = 0.616$) on PV protein levels in the PFC, and no interaction between factors (Figure 8E) ($F_{(1, 25)} = 0.364$, $p = 0.551$). However, there was a main effect of offspring treatment ($F_{(1, 22)} = 8.431$, $p = 0.008$) on PV protein expression in the HPC. CBD-treated rats had higher PV protein levels relative to vehicle-treated offspring (+28.98%; CBD: 1.21 ± 0.092 vs. VEH: 0.94 ± 0.043) (Figure 8F). No

significant effect of prenatal infection ($F_{(1, 22)} = 0.100, p = 0.755$) nor an interaction between the factors ($F_{(1, 22)} = 2.335, p = 0.141$) was observed.

Figure 8: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on GABAergic signalling markers in female control (CONT) and poly I:C (POLY) offspring. GABA_AR ([³H]Muscimol) binding density in the (A) prefrontal cortex (PFC) and (B) hippocampus (HPC). Normalised glutamate decarboxylase (GAD₆₇) and parvalbumin (PV) protein levels (normalised to total protein signal) in the (C, E) PFC and (D, F) HPC of female offspring. #*p* < 0.05, ##*p* < 0.01 CBD vs. VEH-treated offspring (main effect of offspring treatment). Data expressed as mean ± SEM. *n* = 6-8 rats per group; **from 4-5 independent litters**. TE: tissue equivalent.

4. Discussion

The present study demonstrates that CBD treatment in late adolescence/early adulthood can attenuate recognition memory and social interaction deficits in female poly I:C offspring. These findings align with results in male offspring (Osborne et al., 2017a); however, unlike males (Osborne et al., 2017a), female poly I:C offspring did not exhibit working memory impairment. CBD treatment also restored NMDAR binding deficits in the PFC of poly I:C offspring, and increased GAD₆₇ and PV protein levels in the HPC relative to vehicle administration. On the other hand, CBD administration in healthy offspring reduced social interaction, NMDAR and CB1R binding density in the PFC, which may have implications for CBD exposure in healthy females during a critical period of brain maturation. Overall, this study provides the first insight into the behavioural and neurochemical changes that occur following CBD treatment in female poly I:C offspring and suggests that CBD may be an efficacious treatment for the negative symptoms and certain cognitive domains in female patients with schizophrenia.

As cognitive impairment is a major source of disability in schizophrenia, the effects of CBD treatment on cognitive function were examined using two well-validated behavioural paradigms, the NOR and T-maze tests to measure recognition (Bevins and Besheer, 2006) and working memory (Deacon and Rawlins, 2006), respectively. In line with our previous study in males (Osborne et al., 2017a), we observed a recognition memory deficit in female poly I:C offspring that was restored by CBD treatment. These findings align with previous studies that show recognition memory deficits in people with schizophrenia (Calkins et al., 2003) and poly I:C offspring (Ozawa et al., 2006; Ratnayake et al., 2014, 2012) of both sexes. However, this is the first study to show that CBD treatment can improve recognition memory in a female model of schizophrenia-like phenotypes. This result coincides with the limited

literature showing beneficial effects of CBD on recognition memory in female rodents using other preclinical models of cognitive impairment, including hypoxic ischaemia (Pazos et al., 2012) and cerebral malaria (Campos et al., 2015). Our results have implications for the treatment of recognition memory deficits in schizophrenia; however, clinical trials are needed to determine if the positive findings in poly I:C rats translate to patients.

In the present study, female poly I:C offspring performed similarly to controls in the T-maze test, contrasting with our finding in poly I:C males that exhibited working memory impairment (Osborne et al., 2017a). It is unclear whether this finding reflects clinical observations in females with schizophrenia due to the limited number of studies conducted, with one study from our group reporting greater working memory impairment in males with chronic schizophrenia compared to females (Han et al., 2012). In addition, other human studies have reported that while healthy male controls perform better on some working memory tasks compared to female controls, this sex difference was not apparent in people with schizophrenia (Longenecker et al., 2010; Zhang et al., 2017). The majority of poly I:C studies evaluate males only or combine male and female data for statistical analysis. A recent study reported that both male and female poly I:C offspring were impaired on a delayed non-match to position task where rats were rewarded for choosing a novel stimulus (Meehan et al., 2017). Similarly, neonatal poly I:C exposure resulted in spontaneous alternation deficits in the Y-maze test in males, with no deficits in females when tested during early adulthood (Monte et al., 2017). On the other hand, when tested later in adulthood (>PN90) deficits in Y-maze performance have been reported in female poly I:C rats that were not apparent in males (O’Leary et al., 2014). Sex differences have also been reported in other cognitive domains in poly I:C offspring. For example, male poly I:C offspring (PN67) displayed executive function deficits and abnormal cognitive switching/flexibility in a reversal learning task, while female

poly I:C offspring of the same age were not impaired (Zhang et al., 2012). These findings suggest that male and female poly I:C offspring may present with different age-related behavioural deficits, possibly due to the timing of their neuropsychiatric trajectories. Indeed, one longitudinal study showed that male poly I:C offspring had latent inhibition deficits from early adulthood (PN70), but these deficits were delayed in female rats and did not present until almost three weeks later (PN90) (Piontkewitz et al., 2011). Therefore, it is possible that working memory deficits also appear in female poly I:C offspring later in adulthood, but further longitudinal studies that examine behaviour in the same rodent cohort are required.

The social interaction test was used to assess social withdrawal of offspring and can be used as an indicator of the negative symptoms of schizophrenia (Wilson and Koenig, 2014). Female poly I:C offspring spent significantly less time interacting with control counterparts and this deficit was restored to control levels by CBD treatment. Social interaction deficits are present in people with schizophrenia (Lindenmayer et al., 2013) and poly I:C offspring regardless of sex (Aavani et al., 2015; B. K. Bitanirwe et al., 2010; Xuan and Hampson, 2014; Zhu et al., 2014). To our knowledge, this is the first study to investigate the effects of CBD treatment on social interaction in females. In male rodents, CBD improved social interaction deficits induced by the NMDAR antagonist, MK-801 (Gomes et al., 2015b; Gururajan et al., 2012, 2011; Long et al., 2012) and the CB1R agonist, Δ^9 -THC (Malone et al., 2009), as well as in rodent models of MIA (Osborne et al., 2017a) and Dravet syndrome (Kaplan et al., 2017). Conversely, CBD treatment had no effect on social interaction in spontaneous hypertensive rats, a strain that exhibits positive and negative schizophrenia-like phenotypes (Almeida et al., 2013). In the clinic, CBD significantly improved negative symptom scores on the Positive and Negative Syndrome Scale in male-dominated cohorts of people with acute paranoid schizophrenia (Leweke et al., 2012) and APD-medicated

outpatients (McGuire et al., 2018). Taken together, these findings suggest that CBD improves negative symptoms in schizophrenia patients and preclinical models in both males and females.

Second generation (atypical) APDs, such as olanzapine and clozapine have a high weight gain liability in patients that can lead to chronic conditions, including obesity, diabetes mellitus type 2 and cardiovascular disease (Weston-Green et al., 2013). These adverse side effects can also result in medication non-compliance, symptom relapse and can exacerbate cognitive impairment (Weston-Green et al., 2013). Female patients tend to be more sensitive to APD-induced metabolic side effects than males, particularly in relation to weight gain (Seeman, 2009). This heightened sensitivity to APD-induced weight gain is also evident in female rats (Weston-Green et al., 2010). Body weight, food and water intake were recorded throughout the treatment period in female rats in the present study. Offspring from poly I:C-treated dams were significantly heavier than controls prior to the start of treatment. This finding is consistent with evidence of an association between sex and obesity in people with mental illness, particularly schizophrenia, with a higher prevalence of obesity in women compared to men (Gurpegui et al., 2012; Jonikas et al., 2016). In the present study, poly I:C offspring also consumed significantly more water over the treatment period. Interestingly, polydipsia or excessive water drinking is observed in animal models of schizophrenia-like phenotypes (Hawken et al., 2013; Hawken and Beninger, 2014), as well as people with schizophrenia (reviewed in de Leon *et al*, 1994); however, polydipsia could be an underlying symptom of the disease and/or a side effect of APD treatment (Bersani *et al*, 2007). Polydipsia is also a characteristic symptom of diabetes (Bhaskar et al., 2010); however, in the present study other metabolic parameters (e.g. glucose and insulin sensitivity) were not assessed. **Interestingly, a study that did assess metabolic dysfunction in male poly I:C**

mice reported altered food and water intake, as well as impaired glycaemic regulation in periadolescent mice; parameters that appeared to normalise in adulthood. On the other hand, a change in fat mass deposition (without a change in overall body weight) was observed in male mice during adulthood (Pacheco-López et al., 2013). Therefore, longitudinal examination of these metabolic parameters in female poly I:C offspring should also be examined. Importantly, CBD treatment did not significantly affect body weight gain, food or water intake of female adult poly I:C rats in the present study. The effects of CBD on body weight have not been reported previously in females; however, we found a similar result in male poly I:C offspring (Osborne et al., 2017a) that was also observed in a predominantly male cohort of patients with acute schizophrenia (Leweke et al., 2012). Further clinical research is needed to confirm if the low weight gain liability of CBD in rodents translates to female patients with schizophrenia.

In the present study, CBD treatment did not significantly alter CB1R binding density or protein levels of the AEA degradation enzyme, FAAH, in the brains of female poly I:C offspring. One explanation could be that these signalling components are not involved in the behavioural alterations observed in treated poly I:C offspring. Indeed, CBD has a low affinity for the orthosteric binding site of the CB1R (McPartland et al., 2015), but studies have shown that CBD is a potent inhibitor of CB1R agonists (e.g. CP55940) (Thomas et al., 2007), suggesting that CBD does exert effects on the CB1R. A second explanation is that CBD does affect the eCB system to influence behaviour, but without causing an imbalance in CB1R binding density. For example, instead of targeting the CB1R as an orthosteric ligand, CBD can act as a negative allosteric modulator of the CB1R (e.g. in the presence of Δ^9 -THC and the endogenous cannabinoid, 2-arachidonoylglycerol (2-AG)) (Laprairie et al., 2015; Tham et al., 2018). Alternatively, evidence suggests that CBD can alter AEA metabolism by inhibiting

FAAH enzymatic activity (Bisogno et al., 2001; De Petrocellis et al., 2011; Leweke et al., 2012). The present study showed that FAAH protein levels remain unchanged following CBD treatment; however, enzymatic activity was not investigated. Therefore, the effects of CBD on FAAH enzymatic activity require further investigation in poly I:C offspring. Also, investigating upstream processes such as the intracellular transport of AEA via fatty acid binding proteins (Elmes et al., 2015) may provide further insight into the influence of CBD treatment on AEA metabolism.

In the present study, we also examined CBD treatment effects on the glutamatergic NMDAR and its obligatory GluN1 subunit, as well as GABAergic markers, including the GABA_AR, GAD₆₇ and PV protein levels. CBD attenuated poly I:C-induced NMDAR binding deficits in the PFC, while protein levels of the obligatory GluN1 subunit were unchanged. In addition, CBD had no effect on NMDAR binding density in the HPC; however, poly I:C offspring did not exhibit deficits in this region, which is consistent with a previous poly I:C study in females (Rahman *et al.*, 2017). Although evidence for a direct interaction between CBD and the NMDAR is sparse, **interestingly, CBD can reverse behavioural (i.e. sensorimotor gating) and neurochemical deficits (i.e. NMDAR GluN1 subunit gene expression) induced by NMDAR antagonism (Gomes et al., 2015a; Gururajan et al., 2012), suggesting that CBD may modulate glutamatergic signalling via the NMDAR.** It is unclear whether the up-regulation in [³H]MK-801 binding in the PFC is due to indirect effects of CBD on the NMDAR (e.g. an alteration in glutamate levels) or if CBD alters the affinity of the phencyclidine binding site (i.e. where MK-801 binds) for the ligand. Given the differential results in NMDAR binding density and GluN1 protein levels, it is more likely that CBD alters NMDAR affinity; however, further studies are required to confirm. On the other hand, CBD significantly increased GAD₆₇ and PV protein levels in the HPC, regardless

of whether offspring were born to control or poly I:C-treated mothers. In line with the increase in GAD₆₇ protein levels, a previous study reported that CBD reversed deficits in GABA levels following 3-nitropropionic acid administration (a model of Huntington's disease); however, this study examined the rat striatum (Sagredo et al., 2007). On the other hand, a recent study reported that CBD upregulated the activity of hippocampal PV+ interneurons (Khan et al., 2018), which may explain the CBD-induced increase in PV protein levels observed in the present study. Taken together, these findings suggest that CBD **may enhance** inhibitory tone in the HPC. CBD was also recently identified as a positive allosteric modulator of the GABA_AR, *in vitro* (Bakas et al., 2017), **which may have therapeutic implications for schizophrenia**. However, CBD treatment did not affect GABA_AR binding density in the PFC or HPC of female offspring. This finding could suggest that CBD does not alter GABA_AR expression in poly I:C offspring that do not exhibit underlying GABA_AR dysfunction. Alternatively, CBD could influence GABA_AR activity in the brains of offspring, not detectable using the methods in the present study. Future studies using electrophysiology techniques may shed light on the effects of CBD treatment on GABA_AR function.

Interestingly, in the present study CBD administration impaired social interaction of healthy female control offspring, coupled with a decrease in CB1R and NMDAR binding density in the PFC. However, we previously found that CBD did not alter social interaction of control male offspring (Osborne et al., 2017a). Sexual dimorphism in the eCB system has been reported previously (for review see Rubino and Parolaro (2011)). The differential response of female control rats may be due to innate biological differences in drug absorption rates, metabolic enzyme activity, fat mass and renal clearance that could influence the metabolism of CBD (Narimatsu et al., 1993). In addition, sex hormones such as oestrogen, are known to influence both CB1R (Cooper and Craft, 2018) and NMDAR expression/signalling

(McRoberts et al., 2007). The timing of CBD administration (PN56-80) in the present study corresponds to late adolescence/early adulthood in humans, which is a vulnerable period of neurodevelopment (Semple et al., 2013). During this period, female rodents are particularly sensitive to effects of cannabinoids (e.g. Δ^9 -THC) (Macúchová et al., 2016) and sensitivity can vary depending on the stage of the oestrus cycle (Cooper and Craft, 2018). Although beyond the scope of the present study, future investigations could examine whether fluctuations in sex hormones during oestrus influence CBD treatment response in female rats. The findings of a deficit in social interaction and neurochemical signalling in female control offspring highlight the possibility that CBD could have detrimental sex-specific effects when used during a vulnerable period of neurodevelopment in non-pathological conditions.

Our findings show that CBD has beneficial effects on recognition memory and social interaction in female poly I:C offspring, which may be partly due to an upregulation in hippocampal GABAergic tone and glutamatergic signalling in the PFC. Interestingly, CBD increased GABAergic markers in the HPC of both control and poly I:C offspring. However, CBD had opposite effects on NMDAR binding density in the PFC of control and poly I:C offspring. On the other hand, it is unclear from the neurochemical data whether CBD exerts its effects in the HPC, which then impacts glutamatergic input to the PFC, consequently altering NMDAR expression; future studies are needed to address this question. **There are several limitations to the present study. Firstly, transportation of rats between supplier and research facility during pregnancy could be a potential confound when comparing these findings to other poly I:C studies. In addition, recent reports suggest batch to batch variation of poly I:C (reviewed in Kentner et al., 2018) and this study is limited by a lack of immunological response data in the pregnant dams. However, a previous dose-response study showed that administration of poly I:C using the same dose,**

administration route and gestational timing as the present study significantly raised pro-inflammatory cytokines levels in the pregnant dams (Missault et al., 2014). Furthermore, the estrous cycle was not measured in the present study and should be considered for future studies examining female rodents; however, the low data variability in our study suggests that the estrous cycle did not affect results. Overall, there is limited literature that examines the response of female offspring to maternal poly I:C exposure or CBD treatment. Sex differences in the eCB system have been extensively reported in humans and rodents (see Craft et al., (2013) for review). Therefore, it is important that preclinical studies evaluate both sexes when investigating the therapeutic potential of cannabinoids. Our findings show that CBD can improve behavioural and neurochemical deficits in female poly I:C offspring, without body weight gain side effects, suggesting that CBD may be a promising treatment option for female patients with schizophrenia.

Funding and Disclosure

This study was supported by the University of Wollongong utilising funding from a Faculty of Science, Medicine and Health Advancement Grant (2015/SPGA-S/02) awarded to KWG and XFH; and funding from Molecular Horizons awarded to KWG. These sources had no further role in the study design, decision to publish, or preparation of the manuscript. ALO and JSL were supported by an Australian Government Research Training Program Scholarship from the University of Wollongong and a Daniel Beck Memorial Award for Schizophrenia Research from the Beck Family and Neuroscience Research Australia. NS was supported by an Australian Research Council Future Fellowship (FT110100752). IB is supported by a Postgraduate Research Scholarship from the University of Wollongong and Illawarra Shoalhaven Local Health District. JSL is supported by Australian Rotary Health in the form of an Ian Scott Scholarship. The authors have no conflicts of interest to declare.

Acknowledgements

We would like to thank Cree de Clouett, Martin Engel, Ashleigh Gorak, Michael Green, Paul Le Mesurier, Luisa Lufe, Nicole Miles, Jessica Nealon, Anita-Louise Obeid, David Osborne, Tereza Pejovska, Dominic Sellers, Alexander Stamenkovic, Erika Svenson for their technical assistance during the animal experiments.

Contributors

KWG, ALO, NS and XFH designed the study; KAN and XFH contributed some of the reagents; ALO, IB, JSL and KWG performed the experiments; ALO analysed the data and wrote the first draft of the manuscript; KWG, JSL, 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

- Aavani, T., Rana, S.A., Hawkes, R., Pittman, Q.J., 2015. Maternal Immune Activation Produces Cerebellar Hyperplasia and Alterations in Motor and Social Behaviors in Male and Female Mice. *The Cerebellum* 14, 491–505. <https://doi.org/10.1007/s12311-015-0669-5>
- Abel, K.M., Drake, R., Goldstein, J.M., 2010. Sex differences in schizophrenia. *Int. Rev. Psychiatry* 22, 417–428. <https://doi.org/10.3109/09540261.2010.515205>
- Aleman, A., Kahn, R.S., Selten, J.-P., 2003. Sex Differences in the Risk of Schizophrenia: Evidence From Meta-analysis. *Arch. Gen. Psychiatry* 60, 565–571. <https://doi.org/10.1001/archpsyc.60.6.565>
- Almeida, V., Levin, R., Peres, F.F., Niigaki, S.T., Calzavara, M.B., Zuardi, A.W., Hallak, J.E., Crippa, J.A., Abílio, V.C., 2013. Cannabidiol exhibits anxiolytic but not antipsychotic property evaluated in the social interaction test. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 41, 30–35. <https://doi.org/10.1016/j.pnpbp.2012.10.024>
- Babic, I., Gorak, A., Engel, M., Sellers, D., Else, P., Osborne, A.L., Pai, N., Huang, X.-F., Nealon, J., Weston-Green, K., 2018. Liraglutide prevents metabolic side-effects and improves recognition and working memory during antipsychotic treatment in rats. *J. Psychopharmacol. Oxf. Engl.* 32, 578–590. <https://doi.org/10.1177/0269881118756061>

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

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

883 Bersani, G., Pesaresi, L., Orlandi, V., Gherardelli, S., Pancheri, P., 2007. Atypical antipsychotics and
884 polydipsia: a cause or a treatment? *Hum. Psychopharmacol.* 22, 103–107.
885 <https://doi.org/10.1002/hup.825>

886 Bevins, R.A., Besheer, J., 2006. Object recognition in rats and mice: a one-trial non-matching-to-
887 sample learning task to study “recognition memory.” *Nat. Protoc.* 1, 1306–1311.
888 <https://doi.org/10.1038/nprot.2006.205>

889 Bhaskar, M.E., Sowmya, G., Moorthy, S., Kumar, N.S., Praveena, R., Kumar, V., 2010. Presenting
890 Features of Diabetes Mellitus. *Indian J. Community Med. Off. Publ. Indian Assoc. Prev. Soc.*
891 *Med.* 35, 523–525. <https://doi.org/10.4103/0970-0218.74371>

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

897 Bitanhirwe, B.K., Peleg-Raibstein, D., Mouttet, F., Feldon, J., Meyer, U., 2010. Late Prenatal Immune
898 Activation in Mice Leads to Behavioral and Neurochemical Abnormalities Relevant to the
899 Negative Symptoms of Schizophrenia. *Neuropsychopharmacology* 35, 2462–2478.
900 <https://doi.org/10.1038/npp.2010.129>

901 Bitanhirwe, B.K.Y., Weber, L., Feldon, J., Meyer, U., 2010. Cognitive impairment following prenatal
902 immune challenge in mice correlates with prefrontal cortical AKT1 deficiency. *Int. J.*
903 *Neuropsychopharmacol.* 13, 981–996. <https://doi.org/10.1017/S1461145710000192>

904 Calkins, M.E., Gur, R.C., Ragland, J.D., Gur, R.E., 2003. Face recognition memory deficits in
905 schizophrenia patients and their relatives: Comparison with visual object memory
906 performance. *Schizophr. Res., Abstracts of the IXth International Congress on Schizophrenia*
907 *Research* 60, 128. [https://doi.org/10.1016/S0920-9964\(03\)80906-4](https://doi.org/10.1016/S0920-9964(03)80906-4)

908 Campos, A.C., Brant, F., Miranda, A.S., Machado, F.S., Teixeira, A.L., 2015. Cannabidiol Increases
909 Survival and Promotes Rescue of Cognitive Function. *Neuroscience* 289, 166–180.
910 <https://doi.org/10.1016/j.neuroscience.2014.12.051>

911 Campos, A.C., Fogaça, M.V., Scarante, F.F., Joca, S.R.L., Sales, A.J., Gomes, F.V., Sonogo, A.B.,
912 Rodrigues, N.S., Galve-Roperh, I., Guimarães, F.S., 2017. Plastic and Neuroprotective
913 Mechanisms Involved in the Therapeutic Effects of Cannabidiol in Psychiatric Disorders.
914 *Front. Pharmacol.* 8, 269. <https://doi.org/10.3389/fphar.2017.00269>

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

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

923 Colella, A.D., Chegenii, N., Tea, M.N., Gibbins, I.L., Williams, K.A., Chataway, T.K., 2012. Comparison
924 of Stain-Free gels with traditional immunoblot loading control methodology. *Anal. Biochem.*
925 430, 108–110. <https://doi.org/10.1016/j.ab.2012.08.015>

- Cooper, Z.D., Craft, R.M., 2018. Sex-Dependent Effects of Cannabis and Cannabinoids: A Translational Perspective. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 43, 34–51. <https://doi.org/10.1038/npp.2017.140>
- Craft, R.M., Marusich, J.A., Wiley, J.L., 2013. Sex differences in cannabinoid pharmacology: A reflection of differences in the endocannabinoid system? *Life Sci.* 92, 476–481. <https://doi.org/10.1016/j.lfs.2012.06.009>
- Dalton, V.S., Long, L.E., Weickert, C.S., Zavitsanou, K., 2011. Paranoid schizophrenia is characterized by increased CB1 receptor binding in the dorsolateral prefrontal cortex. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 36, 1620–1630. <https://doi.org/10.1038/npp.2011.43>
- de Leon, J., Verghese, C., Tracy, J.I., Josiassen, R.C., Simpson, G.M., 1994. Polydipsia and water intoxication in psychiatric patients: a review of the epidemiological literature. *Biol. Psychiatry* 35, 408–419.
- De Petrocellis, L., Ligresti, A., Moriello, A.S., Allarà, 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>
- De Santis, M., Lian, J., Huang, X.-F., Deng, C., 2016. Early antipsychotic treatment in childhood/adolescent period has long-term effects on depressive-like, anxiety-like and locomotor behaviours in adult rats. *J. Psychopharmacol. (Oxf.)* 30, 204–214. <https://doi.org/10.1177/0269881115616383>
- Deacon, R.M.J., Rawlins, J.N.P., 2006. T-maze alternation in the rodent. *Nat Protoc.* 1, 7–12. <https://doi.org/10.1038/nprot.2006.2>
- 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.
- Deiana, S., Watanabe, A., Yamasaki, Y., Amada, N., Arthur, M., Fleming, S., Woodcock, H., Dorward, P., Pigliacampo, B., Close, S., Platt, B., Riedel, G., 2011. Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarin (CBDV), Δ^9 -tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive–compulsive behaviour. *Psychopharmacology (Berl.)* 219, 859–873. <https://doi.org/10.1007/s00213-011-2415-0>
- Deslauriers, J., Larouche, A., Sarret, P., Grignon, S., 2013. Combination of prenatal immune challenge and restraint stress affects prepulse inhibition and dopaminergic/GABAergic markers. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 45, 156–164. <https://doi.org/10.1016/j.pnpbp.2013.05.006>
- 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>
- Du Bois, T.M., Huang, X.-F., Deng, C., 2008. Perinatal administration of PCP alters adult behaviour in female Sprague–Dawley rats. *Behav. Brain Res.* 188, 416–419. <https://doi.org/10.1016/j.bbr.2007.11.017>
- 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 Δ^9 -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., 2011. 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>
- 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. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 29, 2108–2114. <https://doi.org/10.1038/sj.npp.1300558>
- Godsil, B.P., Kiss, J.P., Spedding, M., Jay, T.M., 2013. The hippocampal-prefrontal pathway: the weak link in psychiatric disorders? *Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol.* 23, 1165–1181. <https://doi.org/10.1016/j.euroneuro.2012.10.018>
- 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., 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>
- Gurpegui, M., Martínez-Ortega, J.M., Gutiérrez-Rojas, L., Rivero, J., Rojas, C., Jurado, D., 2012. Overweight and obesity in patients with bipolar disorder or schizophrenia compared with a non-psychiatric sample. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 37, 169–175. <https://doi.org/10.1016/j.pnpbp.2012.01.014>
- 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>
- Gururajan, A., Taylor, D.A., Malone, D.T., 2011. Effect of cannabidiol in a MK-801-rodent model of aspects of Schizophrenia. *Behav. Brain Res.* 222, 299–308. <https://doi.org/10.1016/j.bbr.2011.03.053>
- Han, M., Huang, X.-F., Chen, D.C., Xiu, M.H., Hui, L., Liu, H., Kosten, T.R., Zhang, X.Y., 2012. Gender differences in cognitive function of patients with chronic schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry, New Drugs of Abuse* 39, 358–363. <https://doi.org/10.1016/j.pnpbp.2012.07.010>
- Harvey, L., Boksa, P., 2012. A stereological comparison of GAD67 and reelin expression in the hippocampal stratum oriens of offspring from two mouse models of maternal inflammation during pregnancy. *Neuropharmacology* 62, 1767–1776. <https://doi.org/10.1016/j.neuropharm.2011.11.022>
- Hawken, E.R., Beninger, R.J., 2014. The amphetamine sensitization model of schizophrenia symptoms and its effect on schedule-induced polydipsia in the rat. *Psychopharmacology (Berl.)* 231, 2001–2008. <https://doi.org/10.1007/s00213-013-3345-9>

- Hawken, E.R., Delva, N.J., Beninger, R.J., 2013. Increased drinking following social isolation rearing: implications for polydipsia associated with schizophrenia. *PLoS One* 8, e56105. <https://doi.org/10.1371/journal.pone.0056105>
- Holloway, T., Moreno, J.L., Umali, A., Rayannavar, V., Hodes, G.E., Russo, S.J., Gonzalez-Maeso, J., 2013. Prenatal stress induces schizophrenia-like alterations of serotonin 2A and metabotropic glutamate 2 receptors in the adult offspring: role of maternal immune system. *J. Neurosci.* 33, 1088–1098. <https://doi.org/10.1523/JNEUROSCI.2331-12.2013>
- Hui, C.W., St-Pierre, A., El Hajj, H., Remy, Y., Hébert, S.S., Luheshi, G.N., Srivastava, L.K., Tremblay, M.-È., 2018. Prenatal Immune Challenge in Mice Leads to Partly Sex-Dependent Behavioral, Microglial, and Molecular Abnormalities Associated with Schizophrenia. *Front. Mol. Neurosci.* 11, 13. <https://doi.org/10.3389/fnmol.2018.00013>
- Iversen, T.S.J., Steen, N.E., Dieset, I., Hope, S., Mørch, R., Gardsjord, E.S., Jørgensen, K.N., Melle, I., Andreassen, O.A., Molden, E., Jönsson, E.G., 2018. Side effect burden of antipsychotic drugs in real life – Impact of gender and polypharmacy. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 82, 263–271. <https://doi.org/10.1016/j.pnpbp.2017.11.004>
- Jasinska, M., Pyza, E., 2017. Circadian Plasticity of Mammalian Inhibitory Interneurons. *Neural Plast.* 2017, 1–12. <https://doi.org/10.1155/2017/6373412>
- Jonge, D., C, J., Vinkers, C.H., Pol, H., E, H., Marsman, A., 2017. GABAergic Mechanisms in Schizophrenia: Linking Postmortem and In Vivo Studies. *Front. Psychiatry* 8. <https://doi.org/10.3389/fpsy.2017.00118>
- Jonikas, J.A., Cook, J.A., Razzano, L.A., Steigman, P.J., Hamilton, M.M., Swarbrick, M.A., Santos, A., 2016. Associations Between Gender and Obesity Among Adults with Mental Illnesses in a Community Health Screening Study. *Community Ment. Health J.* 52, 406–415. <https://doi.org/10.1007/s10597-015-9965-2>
- Kaplan, J.S., Stella, N., Catterall, W.A., Westenbroek, R.E., 2017. Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. *Proc. Natl. Acad. Sci.* 114, 11229–11234. <https://doi.org/10.1073/pnas.1711351114>
- Kentner, A.C., Bilbo, S.D., Brown, A.S., Hsiao, E.Y., McAllister, A.K., Meyer, U., Pearce, B.D., Pletnikov, M.V., Yolken, R.H., Bauman, M.D., 2018. Maternal immune activation: reporting guidelines to improve the rigor, reproducibility, and transparency of the model. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* <https://doi.org/10.1038/s41386-018-0185-7>
- 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.* <https://doi.org/10.1111/bph.14202>
- Khandaker, G.M., Zimbron, J., Lewis, G., Jones, P.B., 2013. Prenatal maternal infection, neurodevelopment and adult schizophrenia: a systematic review of population-based studies. *Psychol. Med.* 43, 239–257. <https://doi.org/10.1017/S0033291712000736>
- Kilb, W., 2012. Development of the GABAergic system from birth to adolescence. *Neurosci. Rev. J. Bringing Neurobiol. Neurol. Psychiatry* 18, 613–630. <https://doi.org/10.1177/1073858411422114>
- 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>
- 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 J. Ment. Sci.* 194, 371–372. <https://doi.org/10.1192/bjp.bp.108.053843>
- Labouesse, M.A., Dong, E., Grayson, D.R., Guidotti, A., Meyer, U., 2015. Maternal immune activation induces GAD1 and GAD2 promoter remodeling in the offspring prefrontal cortex. *Epigenetics* 10, 1143–1155. <https://doi.org/10.1080/15592294.2015.1114202>

- 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>
- Leweke, F.M., Giuffrida, A., Koethe, D., Schreiber, D., Nolden, B.M., Kranaster, L., Neatby, M.A., Schneider, M., Gerth, C.W., Hellmich, M., Klosterkötter, J., Piomelli, D., 2007. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. *Schizophr. Res.* 94, 29–36. <https://doi.org/10.1016/j.schres.2007.04.025>
- 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>
- 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>
- 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>
- Longenecker, J., Dickinson, D., Weinberger, D.R., Elvevåg, B., 2010. Cognitive differences between men and women: A comparison of patients with schizophrenia and healthy volunteers. *Schizophr. Res.* 120, 234–235. <https://doi.org/10.1016/j.schres.2009.12.009>
- 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>
- Macúchová, E., Ševčíková, M., Hřebíčková, I., Nohejlová, K., Šlamberová, R., 2016. How various drugs affect anxiety-related behavior in male and female rats prenatally exposed to methamphetamine. *Int. J. Dev. Neurosci. Off. J. Int. Soc. Dev. Neurosci.* 51, 1–11. <https://doi.org/10.1016/j.ijdevneu.2016.04.001>
- Malone, D.T., Jongejan, D., Taylor, D.A., 2009. Cannabidiol reverses the reduction in social interaction produced by low dose Δ^9 -tetrahydrocannabinol in rats. *Pharmacol. Biochem. Behav.* 93, 91–96. <https://doi.org/10.1016/j.pbb.2009.04.010>
- 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., Duncan, M., Di Marzo, V., Pertwee, R.G., 2015. Are cannabidiol and $\Delta(9)$ -tetrahydrocannabinol negative modulators of the endocannabinoid system? A systematic review. *Br. J. Pharmacol.* 172, 737–753. <https://doi.org/10.1111/bph.12944>
- McRoberts, J.A., Li, J., Ennes, H.S., Mayer, E.A., 2007. Sex-dependent differences in the activity and modulation of NMDA receptors in rat DRG neurons. *Neuroscience* 148, 1015–1020. <https://doi.org/10.1016/j.neuroscience.2007.07.006>
- Meehan, C., Harms, L., Frost, J.D., Barreto, R., Todd, J., Schall, U., Shannon Weickert, C., Zavitsanou, K., Michie, P.T., Hodgson, D.M., 2017. Effects of immune activation during early or late gestation on schizophrenia-related behaviour in adult rat offspring. *Brain. Behav. Immun., Perinatal Inflammation* 63, 8–20. <https://doi.org/10.1016/j.bbi.2016.07.144>

- 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>
- Missault, S., Van den Eynde, K., Vanden Berghe, W., Fransen, E., Weeren, A., Timmermans, J.P., Kumar-Singh, S., Dedeurwaerdere, S., 2014. The risk for behavioural deficits is determined by the maternal immune response to prenatal immune challenge in a neurodevelopmental model. *Brain. Behav. Immun.* 42, 138–146. <https://doi.org/10.1016/j.bbi.2014.06.013>
- Monte, A.S., Mello, B.S.F., Borella, V.C.M., da Silva Araujo, T., da Silva, F.E.R., Sousa, F.C.F. de, de Oliveira, A.C.P., Gama, C.S., Seeman, M.V., Vasconcelos, S.M.M., Lucena, D.F.D., Macêdo, D., 2017. Two-hit model of schizophrenia induced by neonatal immune activation and peripubertal stress in rats: Study of sex differences and brain oxidative alterations. *Behav. Brain Res.* 331, 30–37. <https://doi.org/10.1016/j.bbr.2017.04.057>
- Muguruza, C., Lehtonen, M., Aaltonen, N., Morentin, B., Meana, J.J., Callado, L.F., 2013. Quantification of endocannabinoids in postmortem brain of schizophrenic subjects. *Schizophr. Res.* 148, 145–150. <https://doi.org/10.1016/j.schres.2013.06.013>
- Narimatsu, S., Watanabe, K., Matsunaga, T., Yamamoto, I., Imaoka, S., Funae, Y., Yoshimura, H., 1993. Suppression of liver microsomal drug-metabolizing enzyme activities in adult female rats pretreated with cannabidiol. *Biol. Pharm. Bull.* 16, 428–430.
- 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. <https://doi.org/10.1007/s00702-007-0668-x>
- NHMRC, 2013. Australian code for the care and use of animals for scientific purposes.
- Nyffeler, M., Meyer, U., Yee, B.K., Feldon, J., Knuesel, I., 2006. Maternal immune activation during pregnancy increases limbic GABAA receptor immunoreactivity in the adult offspring: implications for schizophrenia. *Neuroscience* 143, 51–62. <https://doi.org/10.1016/j.neuroscience.2006.07.029>
- Ochoa, S., Usall, J., Cobo, J., Labad, X., Kulkarni, J., 2012. Gender differences in schizophrenia and first-episode psychosis: a comprehensive literature review. *Schizophr. Res. Treat.* 2012, 916198. <https://doi.org/10.1155/2012/916198>
- O’Leary, C., Desbonnet, L., Clarke, N., Petit, E., Tighe, O., Lai, D., Harvey, R., Waddington, J.L., O’Tuathaigh, C., 2014. Phenotypic effects of maternal immune activation and early postnatal milieu in mice mutant for the schizophrenia risk gene neuregulin-1. *Neuroscience* 277, 294–305. <https://doi.org/10.1016/j.neuroscience.2014.06.028>
- 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>
- Ozawa, K., Hashimoto, K., Kishimoto, T., Shimizu, E., Ishikura, H., Iyo, M., 2006. Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. *Biol. Psychiatry* 59, 546–554. <https://doi.org/10.1016/j.biopsych.2005.07.031>
- Pacheco-López, G., Giovanoli, S., Langhans, W., Meyer, U., 2013. Priming of Metabolic Dysfunctions by Prenatal Immune Activation in Mice: Relevance to Schizophrenia. *Schizophr. Bull.* 39, 319–329. <https://doi.org/10.1093/schbul/sbr178>
- Paxinos, G., Watson, C., 2007. *The Rat Brain Atlas in Stereotaxic Coordinates*, 6th ed. Elsevier Inc., UK.

- Pazos, M.R., Cinquina, V., Gomez, A., Layunta, R., Santos, M., Fernandez-Ruiz, J., Martinez-Orgado, J., 2012. Cannabidiol administration after hypoxia-ischemia to newborn rats reduces long-term brain injury and restores neurobehavioral function. *Neuropharmacology* 63, 776–783. <https://doi.org/10.1016/j.neuropharm.2012.05.034>
- Piontkewitz, Y., Arad, M., Weiner, I., 2011. Abnormal Trajectories of Neurodevelopment and Behavior Following In Utero Insult in the Rat. *Biol. Psychiatry, Genetic and Environmental Contributors to Disturbed Cortical Development in Developmental Disorders* 70, 842–851. <https://doi.org/10.1016/j.biopsych.2011.06.007>
- Rahman, T., Zavitsanou, K., Purves-Tyson, T., Harms, L.R., Meehan, C., Schall, U., Todd, J., Hodgson, D.M., Michie, P.T., Weickert, C.S., 2017. Effects of Immune Activation during Early or Late Gestation on N-Methyl-d-Aspartate Receptor Measures in Adult Rat Offspring. *Front. Psychiatry* 8, 77. <https://doi.org/10.3389/fpsy.2017.00077>
- Ratnayake, U., Quinn, T., LaRosa, D.A., Dickinson, H., Walker, D.W., 2014. Prenatal exposure to the viral mimetic poly I:C alters fetal brain cytokine expression and postnatal behaviour. *Dev. Neurosci.* 36, 83–94. <https://doi.org/10.1159/000362205>
- Ratnayake, U., Quinn, T.A., Castillo-Melendez, M., Dickinson, H., Walker, D.W., 2012. Behaviour and hippocampus-specific changes in spiny mouse neonates after treatment of the mother with the viral-mimetic Poly I:C at mid-pregnancy. *Brain. Behav. Immun.* 26, 1288–1299. <https://doi.org/10.1016/j.bbi.2012.08.011>
- Reisinger, S., Khan, D., Kong, E., Berger, A., Pollak, A., Pollak, D.D., 2015. The poly(I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery. *Pharmacol. Ther.* 149, 213–226. <https://doi.org/10.1016/j.pharmthera.2015.01.001>
- Rubino, T., Parolaro, D., 2011. Sexually Dimorphic Effects of Cannabinoid Compounds on Emotion and Cognition. *Front. Behav. Neurosci.* 5, 1–5. <https://doi.org/10.3389/fnbeh.2011.00064>
- Ruskin, D.N., Murphy, M.I., Slade, S.L., Masino, S.A., 2017. Ketogenic diet improves behaviors in a maternal immune activation model of autism spectrum disorder. *PLoS ONE* 12. <https://doi.org/10.1371/journal.pone.0171643>
- Sagredo, O., Ramos, J.A., Decio, A., Mechoulam, R., Fernández-Ruiz, J., 2007. Cannabidiol reduced the striatal atrophy caused 3-nitropropionic acid in vivo by mechanisms independent of the activation of cannabinoid, vanilloid TRPV1 and adenosine A2A receptors. *Eur. J. Neurosci.* 26, 843–851. <https://doi.org/10.1111/j.1460-9568.2007.05717.x>
- Schiavon, A.P., Soares, L.M., Bonato, J.M., Milani, H., Guimarães, F.S., Oliveira, R.M.W. de, 2014. Protective effects of cannabidiol against hippocampal cell death and cognitive impairment induced by bilateral common carotid artery occlusion in mice. *Neurotox. Res.* 26, 307–316. <https://doi.org/10.1007/s12640-014-9457-0>
- Seeman, M.V., 2009. Secondary effects of antipsychotics: women at greater risk than men. *Schizophr. Bull.* 35, 937–948. <https://doi.org/10.1093/schbul/sbn023>
- Semple, B.D., Blomgren, K., Gimlin, K., Ferriero, D.M., Noble-Haeusslein, L.J., 2013. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog. Neurobiol.* 0, 1–16. <https://doi.org/10.1016/j.pneurobio.2013.04.001>
- Tham, M., Yilmaz, O., Alaverdashvili, M., Kelly, M.E.M., Denovan-Wright, E.M., Laprairie, R.B., 2018. Allosteric and orthosteric pharmacology of cannabidiol and cannabidiol-dimethylheptyl at the type 1 and type 2 cannabinoid receptors. *Br. J. Pharmacol.* <https://doi.org/10.1111/bph.14440>
- Thomas, A., Baillie, G.L., Phillips, A.M., Razdan, R.K., Ross, R.A., Pertwee, R.G., 2007. Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br. J. Pharmacol.* 150, 613–623. <https://doi.org/10.1038/sj.bjp.0707133>
- Weston-Green, K., Huang, X.-F., Deng, C., 2013. Second generation antipsychotic-induced type 2 diabetes: a role for the muscarinic M3 receptor. *CNS Drugs* 27, 1069–1080. <https://doi.org/10.1007/s40263-013-0115-5>

- Weston-Green, K., Huang, X.-F., Deng, C., 2012. Alterations to melanocortinergic, gABAergic and cannabinoid neurotransmission associated with olanzapine-induced weight gain. *PLoS ONE* 7, 1–12. <https://doi.org/10.1371/journal.pone.0033548>
- Weston-Green, K., Huang, X.-F., Deng, C., 2010. Sensitivity of the female rat to olanzapine-induced weight gain--far from the clinic? *Schizophr. Res.* 116, 299–300. <https://doi.org/10.1016/j.schres.2009.09.034>
- Wilson, C.A., Koenig, J.I., 2014. Social interaction and social withdrawal in rodents as readouts for investigating the negative symptoms of schizophrenia. *Eur. Neuropsychopharmacol.*, Negative symptoms of schizophrenia: clinical characteristics and their measurement, experimental modelling, and opportunities for improved treatment 24, 759–773. <https://doi.org/10.1016/j.euroneuro.2013.11.008>
- Xia, Y., Haddad, G.G., 1992. Ontogeny and distribution of GABAA receptors in rat brainstem and rostral brain regions. *Neuroscience* 49, 973–989.
- Xuan, I.C.Y., Hampson, D.R., 2014. Gender-Dependent Effects of Maternal Immune Activation on the Behavior of Mouse Offspring. *PLOS ONE* 9, e104433. <https://doi.org/10.1371/journal.pone.0104433>
- 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>
- Zhang, B., Han, M., Tan, S., Yang, F., Tan, Y., Jiang, S., Zhang, X., Huang, X.-F., 2017. Gender differences measured by the MATRICS consensus cognitive battery in chronic schizophrenia patients. *Sci. Rep.* 7, 11821. <https://doi.org/10.1038/s41598-017-12027-w>
- Zhang, Y., Cazakoff, B.N., Thai, C.A., Howland, J.G., 2012. Prenatal exposure to a viral mimetic alters behavioural flexibility in male, but not female, rats. *Neuropharmacology, Schizophrenia* 62, 1299–1307. <https://doi.org/10.1016/j.neuropharm.2011.02.022>
- Zhu, F., Zheng, Y., Liu, Y., Zhang, X., Zhao, J., 2014. Minocycline alleviates behavioral deficits and inhibits microglial activation in the offspring of pregnant mice after administration of polyriboinosinic–polyribocytidilic acid. *Psychiatry Res.* 219, 680–686. <https://doi.org/10.1016/j.psychres.2014.06.046>

Highlights:

- The effects of CBD treatment were examined in a female poly I:C model of schizophrenia
- CBD treatment restored recognition memory, social interaction and NMDAR binding deficits in female poly I:C offspring, without causing weight gain side effects
- CBD increased GAD₆₇ and parvalbumin protein levels in the hippocampus of offspring regardless of maternal poly I:C exposure
- CBD administration impaired social interaction, NMDAR and CB1R binding density in control offspring.
- These findings suggest that CBD is an efficacious treatment for behavioural and neurochemical changes in a female rodent model relevant to schizophrenia

Title: Cannabidiol improves behavioural and neurochemical deficits in adult female offspring
of the maternal immune activation (poly I:C) model of neurodevelopmental disorders

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}, Xu-Feng Huang (DSc, PhD,
MBBS)^{b, c, e}, Kelly A. Newell (PhD)^{a, b, c}, Katrina Weston-Green (PhD)*^{a, b, c, e}

Affiliations:

^aNeuropharmacology and Molecular Psychiatry Laboratory, School of Medicine, University
of Wollongong, Wollongong, NSW, 2522, Australia

^bCentre for Translational Neuroscience, Illawarra Health and Medical Research Institute,
Wollongong, NSW, 2522, Australia

^cMolecular Horizons, Faculty of Science, Medicine and Health, University of Wollongong,
Wollongong, NSW, 2522, Australia

^dSchool of Psychology, Faculty of Social Sciences, University of Wollongong, and Illawarra
Health and Medical Research Institute, Wollongong, NSW, 2522, Australia

^eAustralian Centre for Cannabinoid Clinical and Research Excellence, New Lambton
Heights, NSW, 2305 Australia

^fIllawarra and Shoalhaven Local Health District, Wollongong, NSW, 2500, Australia

*Corresponding Author:

Dr Katrina Weston-Green

School of Medicine

Faculty of Science, Medicine and Health

University of Wollongong

26 Northfields Avenue, Wollongong, NSW 2522 Australia

27 Tel: +61 2 4252 8506

28 Email: katrina_green@uow.edu.au

29

30 Keywords: cannabidiol; cannabinoid; schizophrenia; maternal immune activation; brain;

31 glutamate; GABA; cognition

32

33 Word count: 8709 (excluding figure legends and references)

Abstract

Cognitive impairment is a major source of disability in schizophrenia and current antipsychotic drugs (APDs) have minimal efficacy for this symptom domain. Cannabidiol (CBD), the major non-intoxicating component of *Cannabis sativa* L., exhibits antipsychotic and neuroprotective properties. We recently reported the effects of CBD on cognition in male offspring of a maternal immune activation (polyinosinic-polycytidilic acid (poly I:C)) model relevant to the aetiology of schizophrenia; however, the effects of CBD treatment in females are unknown. Sex differences are observed in the onset of schizophrenia symptoms and response to APD treatment. Furthermore, the endogenous cannabinoid system, a direct target of CBD, is sexually dimorphic in humans and rodents. Therefore, the present work aimed to assess the therapeutic impact of CBD treatment on behaviour and neurochemical signalling markers in female poly I:C offspring. 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. From postnatal day 56, female offspring received CBD (10 mg/kg, i.p.) or vehicle treatment for approximately 3 weeks. Following 2 weeks of CBD treatment, offspring underwent behavioural testing, including the novel object recognition, rewarded alternation T-maze and social interaction tests to assess recognition memory, working memory and sociability, respectively. After 3 weeks of CBD treatment, the prefrontal cortex (PFC) and hippocampus (HPC) were collected to assess effects on endocannabinoid, glutamatergic and gamma-aminobutyric acid (GABA) signalling markers. CBD attenuated poly I:C-induced deficits in recognition memory, social interaction and glutamatergic *N*-methyl-D-aspartate receptor (NMDAR) binding in the PFC of poly I:C offspring. Working memory performance was similar between treatment groups. CBD also increased glutamate decarboxylase 67, the rate-limiting enzyme that converts glutamate to GABA, and parvalbumin protein levels in the HPC. In contrast to the CBD treatment effects observed in poly I:C offspring, CBD

administration to control rats reduced social interaction, cannabinoid CB1 receptor and NMDAR binding density in the PFC, suggesting that CBD administration to healthy rats may have negative consequences on social behaviour and brain maturation in adulthood. Overall, the findings of this study support the therapeutic benefits of CBD on recognition memory and sociability in female poly I:C offspring, and provide insight into the neurochemical changes that may underlie the therapeutic benefits of CBD in the poly I:C model.

1 Introduction

People with schizophrenia experience a range of symptoms that can be characterised into positive (e.g. hallucinations and delusions) and negative symptoms (e.g. social withdrawal), as well as cognitive deficits (e.g. impairments in memory and attention). While the incidence of schizophrenia does not significantly differ between men and women, several studies have reported sex differences in age of onset, symptomatology, treatment response and clinical outcomes (Aleman *et al.* 2003; Ochoa *et al.* 2012; Holloway *et al.* 2013). Schizophrenia symptoms can be more severe in men and appear during late adolescence, while symptoms do not appear until early adulthood in women, with a second peak in symptom onset observed during menopause (Abel *et al.* 2010). Antipsychotic drugs (APDs) are the primary line of treatment and are generally effective at controlling the positive symptoms, while the negative symptoms and cognitive deficits are largely resistant to existing medications (Goff *et al.*, 2011; Lindenmayer *et al.*, 2013). Furthermore, APDs can cause adverse side effects, such as motor disturbances and body weight gain, which are mainly attributable to the first (typical) and second (atypical) generation drugs, respectively (Weston-Green *et al.*, 2013). While female patients tend to respond better to APD treatment (Smith, 2010), they also experience more severe side effects compared to men (Iversen *et al.*, 2018), highlighting the need for new therapeutic options that are effective in both sexes, with less adverse side effects.

Epidemiological evidence shows that maternal immune activation (MIA) during pregnancy can increase the risk of the child developing neurodevelopmental disorders, including schizophrenia, autism spectrum and bipolar disorder later in life (Khandaker *et al.*, 2013). MIA can be modelled in rodents by the administration of polyinosinic-polycytidilic (poly I:C) acid, a synthetic double-stranded RNA virus, to pregnant dams. Offspring develop behavioural perturbations that resemble a spectrum of schizophrenia-like symptoms,

including deficits in sensorimotor gating, working memory, latent inhibition and social interaction, and sensitivity to amphetamine (reviewed in Reisinger *et al*, 2015). This data is mainly obtained from male offspring; however, there is evidence to suggest that there are sex-specific effects in these behavioural profiles (Bitanirwe *et al.*, 2010; Hui *et al.*, 2018; Meehan *et al.*, 2017; O’Leary *et al.*, 2014; Piontkewitz *et al.*, 2011; Ratnayake *et al.*, 2014; Ruskin *et al.*, 2017; Xuan and Hampson, 2014; Zhang *et al.*, 2012). Studies examining female offspring are minimal, with investigations mainly limited to juvenile or late adult time points (Deslauriers *et al.*, 2013; Harvey and Boksa, 2012; Labouesse *et al.*, 2015; Meyer *et al.*, 2008; Nyffeler *et al.*, 2006; Rahman *et al.*, 2017). We recently characterised the behavioural (Osborne *et al.*, 2017a) and neurobiological (unpublished findings) changes in male poly I:C offspring compared to controls. Therefore, this study examined the behavioural and neurochemical effects of maternal poly I:C exposure in female offspring during early adulthood, a critical period of brain maturation, and when psychotic symptoms emerge in people with schizophrenia.

Cannabidiol (CBD) is a major non-intoxicating compound of the cannabis plant that exerts beneficial effects on cognitive impairment across a range of pathological conditions, including Alzheimer’s disease and stroke (Osborne *et al.*, 2017b). Recent clinical trials report therapeutic effects of CBD on positive and negative symptoms in unmedicated (Leweke *et al.*, 2012) and APD-treated schizophrenia patients, as well as a mild improvement in cognition in a male-dominated cohort (McGuire *et al.*, 2018). We recently reported that CBD treatment improved working memory, recognition memory and social interaction deficits in male poly I:C offspring (Osborne *et al.*, 2017a); however, the response of female poly I:C offspring to CBD treatment is unknown. Furthermore, female rats tend to have greater sensitivity to APD-induced metabolic dysfunction (Weston-Green *et al.*, 2010), but it is

unknown whether CBD causes weight gain side effects in female poly I:C offspring. Therefore, this study evaluated the effects of CBD treatment on cognition and sociability, as well as body weight, food and water intake in female poly I:C offspring.

CBD interacts with the endogenous cannabinoid (eCB) system, which regulates homeostatic processes in the brain and plays an important role in synaptic plasticity, mood, neuroprotection and cognition (Lu and Mackie, 2016). Dysregulation of the eCB system has been reported in schizophrenia, including dysfunction in the most abundant cannabinoid receptor in the CNS, the cannabinoid CB1 receptor (CB1R) (Dalton et al., 2011; Dean et al., 2001; Eggan et al., 2010, 2008), as well as its endogenous ligand anandamide (AEA) (Giuffrida et al., 2004; Koethe et al., 2009; Leweke et al., 2007; Muguruza et al., 2013). Importantly, the eCB system modulates the release of the major excitatory (glutamate) and inhibitory (GABA) to maintain optimal brain function (Lu and Mackie, 2016). These signalling systems are also implicated in schizophrenia pathophysiology, including hypofunction of the glutamatergic *N*-methyl-*D*-aspartate receptor (NMDAR) (Cohen et al., 2015), and a loss of inhibitory tone, including dysfunction in the ionotropic GABA_A receptor (GABA_AR), GABA synthesising enzyme glutamate decarboxylase 67 (GAD₆₇), as well as a subset of GABAergic neurons that express the calcium binding protein parvalbumin (PV) (reviewed in Jonge et al., 2017). While CBD may interact with the eCB system to exert its therapeutic benefits (Campos et al., 2017), there is limited understanding of the effects of CBD on the major excitatory and inhibitory neurotransmitter systems in the brain, glutamate and gamma-aminobutyric acid (GABA), respectively. Therefore, in the present study, the effects of CBD on markers of eCB, NMDAR and GABAergic signalling were analysed in the prefrontal cortex (PFC) and hippocampus (HPC) of female offspring due to the central role of these regions in schizophrenia neuropathology (Godsil et al., 2013). Overall, this study aimed

to characterise the behavioural and neurochemical effects of CBD treatment in female poly I:C offspring, which may have implications for the use of CBD as a novel therapeutic for females with schizophrenia.

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 Institute (NHMRC), Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 2013 (NHMRC, 2013). All efforts were made to minimise the number and suffering of animals. Animal experiments have been reported in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (Kilkenny et al., 2010).

2.2 Animals and Treatment

The methods for modelling MIA using poly I:C administration were conducted as previously reported by our laboratory (Osborne et al., 2017a). Briefly, time-mated pregnant Sprague-Dawley rats (gestational day (GD) 8, n=16; Animal Resource Centre, Perth, Australia) were housed individually and allowed to habituate to their surroundings for one week. On GD 15, dams were injected with either poly I:C (4 mg/kg, n = 8; P1530, Lot Number: 114M4028V; Sigma-Aldrich, Castle Hill, NSW Australia) or sterile saline (n=8) into the lateral tail vein. After postnatal day (PN) 21, female offspring were weaned and pair-housed with same treatment littermates. Offspring were maintained on a reversed 12 hour light–dark cycle (lights on 19:00h) with *ad libitum* access to food and water. From PN56, which is equivalent

to late adolescence/early adulthood in humans (Kilb, 2012; Semple et al., 2013), offspring were administered CBD (10 mg/kg, i.p.; n = 12/group, 6 independent litters; THC-1440G-1, CAS: 13956-29-1; THC Pharm GmbH, Frankfurt, Germany) or vehicle (5 ml/kg; n = 12/group, 6 independent litters). Treatments were administered for approximately 3 weeks, twice a day, at 12 hourly intervals (07:00h, 19:00h), based on the half-life of CBD in the rat brain (Deiana et al., 2011). The CBD dosage and treatment duration was based on our previous study (Osborne et al., 2017a) and others that report CBD exerts a therapeutic effect on cognitive deficits in inflammation-based rodent models (Barichello et al., 2012; Cassol-Jr et al., 2010; Fagherazzi et al., 2011; Schiavon et al., 2014). Body weight, food and water intake (for each cage) were recorded weekly during the treatment period.

2.3 Behavioural Testing

After two weeks of treatment, offspring behaviour was examined using the Novel Object Recognition (NOR), rewarded T-maze alternation and social interaction tests to assess recognition memory, working memory and social withdrawal, respectively. All behavioural experiments were performed between 09:00 and 18:00h during the active (dark) phase of the light-dark cycle, and testing was counterbalanced across treatment groups. After each test, the equipment was cleaned with 70% ethanol between trials to eliminate olfactory cues. The behavioural tests were performed in order of invasiveness to minimise stress effects on subsequent behavioural tasks and there was a 24 hour rest period between tests. Rat behaviour was recorded using standard commercial cameras (Logitech Pty Ltd, Alexandria, Australia) and de-identified video recordings were later analysed.

2.3.1 Novel Object Recognition (NOR) test

The NOR test was conducted using methods previously described by our laboratory (Babic et al., 2018; Osborne et al., 2017a). Briefly, plastic building blocks were placed in the home cages for 24-hour familiarisation. In the first trial, a rat was placed in a black, square arena (60cm x 60cm x 60cm, with an even lighting of 20 lux) to habituate for 5 min. Two familiar objects were placed in opposite corners of the upper half of the area and the rat was positioned in the centre of the lower wall, facing away from the objects. After 5 min of exploration, the rat was returned to its home cage for 1 h. In the second trial, the rat was returned to the arena, but this time one of the familiar objects was replaced with a novel object (toy figurine), and the rat was allowed to explore for 3 min. Object interaction was scored using the criteria previously described by our laboratory (Osborne et al., 2017a) and a discrimination index was calculated for each rat, expressed as: $T_N - T_F / (T_F + T_N)$, where T_N = time spent exploring the novel object (secs) and T_F = time spent exploring the familiar object (secs). Values for the discrimination index ranged from -1 to 1, where a positive score indicated preference for the novel object and a negative score indicated preference for the familiar object (Bevins and Besheer, 2006).

2.3.2 Rewarded T-maze Test

The T-maze test was conducted using methods previously detailed by our laboratory (Babic et al., 2018; Osborne et al., 2017a). Briefly, rats were habituated to a black, T-shaped maze (50 cm long x 10 cm wide, 30 cm high walls with an even lighting of 20 lux) that contained chocolate pellet rewards in the left and right arms of the T-maze for 5 min. Rats were trained over 3 consecutive days to alternate entry in the left and right arms of the T-maze. Trials consisted of (1) a 'forced' run, where one arm of the T-maze was closed by a removable divider and the reward was placed in the open arm, and (2) a 'choice' run, where the divider was removed (allowing the rat access to both arms of the T-maze) and the reward was placed

in the newly opened arm. Training days consisted of 8 trials, each comprised of 4 forced and 4 choice runs, with randomised alternation of the divider position in each forced run. The test day consisted of 10 trials (5 forced runs, 5 choice runs) per rat with the same alternation procedures used as above. Each trial was limited to 3 mins. A correct response was defined as a first entry into the correct arm. The percentage of correct responses and the latency to first entry (secs) were recorded for each rat.

2.3.3 Social Interaction Test

The social interaction test was conducted using methods described by our laboratory (De Santis et al., 2016; Du Bois et al., 2008; Osborne et al., 2017a). Two unfamiliar rats from the same treatment group were placed in opposite corners of a black square arena (described in section 2.3.1), allowed to freely roam the arena for 7 mins and were then returned to their home cage. The amount of time that rats spent interacting with each other (defined as time spent sniffing, following, grooming, climbing each other) was recorded (secs) for each pair of rats. Interaction times for each pair of rats were used for statistical analysis.

2.4 Neurochemical Analysis

After the last treatment (~10-12 hours), adult rats were sacrificed by carbon dioxide asphyxiation followed by rapid decapitation between 09:00 and 11:30h to reduce the potential confound of circadian rhythm on neurochemical analyses (Jasinska and Pyza, 2017). Whole brains were removed, immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

2.4.1 Histological Procedures

A cryostat (Jung CM 3000, Leica Instruments GmbH, Nussloch, Germany) was used to

collect alternating 14 μ M or 500 μ M coronal sections of rat brain tissue (n = 8 per group) that contained the PFC (prelimbic and infralimbic cortices; Bregma level: 4.2 mm to 2.56 mm) or HPC (- 4.36 mm to - 6.00 mm). Consecutive sections collected for receptor autoradiography experiments (14 μ M; 2-3 sections per region, per rat) were thaw-mounted on to PolysineTM slides (Sigma-Aldrich, Castle Hill, NSW, Australia) and stored at -20°C until further use. Sections collected for Western blot experiments (500 μ M; 2-3 sections per region, per rat) were mounted on glass slides, microdissected using a micropuncture kit and stored at -80°C until further use. This dissection protocol allowed simultaneous investigation of receptor binding and protein levels from the same animals.

2.4.2 Receptor Autoradiography

2.4.2.1 CB1R binding

CB1R binding density was examined using methods previously reported by our laboratory (Weston-Green et al., 2012; Yu et al., 2013). Briefly, slides were air-dried and pre-incubated in 50 mM Tris-HCl buffer (pH 7.4) with 0.1% bovine serum albumin (BSA) for 15 min at room temperature. Sections were incubated in 50 mM Tris-HCl buffer (with 0.1% BSA) containing 10 nM [³H]SR141716A (CB1R selective antagonist) (43 Ci/mmol; PerkinElmerTM Life and Analytical Sciences, Boston, USA) for 60 min at room temperature to determine total binding. Additional sections were incubated in 10 nM [³H]SR141716A in the presence of 100 μ M CP-55940 (cannabinoid receptor agonist) to determine non-specific binding. After incubation, slides were washed twice for 30 min in ice-cold buffer, rinsed in cold milliQ water and air-dried.

2.4.2.2 NMDAR Binding

NMDAR binding density was examined using 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 μ M glycine, 100 μ M glutamate, 1 mM ethylenediaminetetraacetic acid (EDTA) and 20 nM [3 H]MK-801 (NMDAR antagonist) (specific activity 22.5 Ci/mmol, PerkinElmer, Boston, USA) for 2.5 hours at room temperature to determine total binding. Additional sections were incubated in the same buffer containing [3 H]MK-801 in the presence of 200 μ M unlabelled MK-801 to determine non-specific binding. 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.

2.4.2.3 GABA_AR Binding

GABA_AR binding density was examined using 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 room temperature for 5 mins. Sections were incubated in 50 mM Tris-citrate buffer containing 50 nM [3 H]muscimol (GABA_AR agonist) (specific activity 29.5 Ci/mmol, PerkinElmer, Boston, USA) for 45 min at 4°C to determine total binding. Additional sections were incubated in 50 mM Tris-citrate (pH 7.0) buffer containing [3 H]Muscimol and 100 μ M GABA (GABA receptor agonist) to determine non-specific binding. After incubation, sections were rinsed four times for 2 secs each in 50 mM Tris-citrate buffer (4°C), rinsed in cold milliQ water and air-dried.

2.5.4 Quantification

Slides from CB1R, NMDAR and GABA_AR binding experiments were exposed to Amersham Hyperfilm ECL (GE Healthcare Life Sciences, Parramatta, NSW, Australia) with a set of Amersham tritium standards, in X-ray film cassettes (De Santis et al., 2016). After 5 weeks (NMDAR and GABA_AR) or 8 weeks (CB1R), autoradiographs were developed, scanned using a GS-800 Imaging Densitometer (Bio-Rad, Hercules, California, USA) and analysed using Image J software (<https://imagej.nih.gov/ij>). Two sections were quantified per brain region, per rat to obtain total binding density values. Non-specific binding was subtracted from total binding to estimate specific binding. Values were converted to fmoles [³H] ligand per mg tissue equivalent (TE) taking into account the specific activity of the ligand. Anatomical structures were confirmed using a rat brain atlas (Paxinos and Watson, 2007).

2.6 Western blot

Briefly, brain samples were homogenised as previously described (Lum et al., 2016) and a DC assay kit was used to determine total protein concentration following the manufacturer's instructions (Bio-Rad, Australia). Protein samples (10 µg, within the linear range of detection of the primary antibodies) were separated in CriterionTM TGX Stain-FreeTM 4-20% gels (Bio-Rad, Australia), gels were activated (GelDoc XR+ imaging system; Bio-Rad, Australia) (Colella et al., 2012; Gürtler et al., 2013) and then proteins were transferred on to a PVDF membrane (Bio-Rad, Australia). Membranes were imaged (GelDoc XR+ imaging system; Bio-Rad, Australia) to capture total protein in each lane (Colella et al., 2012; Gürtler et al., 2013). To detect the proteins of interest, membranes were cut and incubated overnight at 4°C with the following primary antibodies: anti-FAAH1 (PFC: 1:2500, HPC: 1:5000 ; #ab54615, Abcam), anti-GluN1 (1:10000; #MAB363, Millipore), anti-GAD₆₇ (1:7500 #MAB5406, Millipore) and anti-parvalbumin (1:10000; #Ab11427, Abcam). Membranes were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5000;

#AP307P or #AP308P, Millipore). Bands were visualised using 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) and an internal control sample (containing equal amounts of each sample) to account for inter-gel variability. Samples were run in duplicate.

2.7 Statistical Analyses

Statistical analyses were conducted using SPSS (Version 21.0, IBM, Illinois, USA). Data were within 2SD of the group mean and were tested for normality using Shapiro Wilk tests. Analyses of variance (ANOVAs; factors: prenatal infection, offspring treatment) were used to analyse normally distributed data and pairwise comparisons were made between treatment groups (with Bonferroni's adjustment). If a significant difference was observed between CBD and vehicle-treated poly I:C offspring, then an additional comparison (using an independent t-test) was made to determine if CBD treatment in poly I:C offspring restored the parameter to control levels. Where data remained non-normally distributed following transformation, or there was unequal variance between groups (given by a significant result on Levene's Test of Equality of Error Variances), Mann-Whitney U tests were used to compare groups (indicated in Results section). A one-sample *t*-test was used to determine whether preference for the novel object (NOR test) and the percentage of correct entries (T-maze test) were above chance levels (50%). Repeated measures mixed ANOVA (between-subjects factors: prenatal infection, offspring treatment; within-subjects factor: time) was used to analyse average body weight (with baseline body weight as a covariate) and the degrees of freedom were adjusted

357 using the Greenhouse-Geisser correction (reported in Results section). Significance was set to
358 $p < 0.05$. Data are presented as mean $\pm$ SEM.

3. Results

3.1 Effect of cannabidiol on negative and cognitive symptom phenotypes

In the present study, we examined whether poly I:C exposure at mid to late gestation elicited recognition memory, working memory and social interaction deficits in adult female offspring, and assessed the therapeutic potential of chronic CBD treatment to attenuate poly I:C-induced deficits. First, we examined the effects of CBD treatment on recognition memory in the NOR test in control and poly I:C female offspring. A two-way ANOVA revealed no significant main effects of prenatal infection ($F_{(1, 38)} = 1.865$, $p = 0.180$) or offspring treatment ($F_{(1, 38)} = 0.581$, $p = 0.451$) on the discrimination index; however, there was a significant prenatal infection x offspring treatment interaction ($F_{(1, 38)} = 5.344$, $p = 0.026$). Pairwise comparisons revealed that the POLY+VEH group had a lower mean discrimination index compared to the CONT+VEH group ($p = 0.013$) (Figure 1A), indicating that maternal poly I:C exposure impaired recognition memory in adult offspring. Furthermore, CBD treatment significantly improved the discrimination index of poly I:C offspring (POLY+VEH vs. POLY+CBD, $p = 0.036$), restoring to control-like levels (POLY+CBD vs. CONT+VEH, $p = 0.578$) (Figure 1A). There was no significant difference in the discrimination index between the CONT+VEH and CONT+CBD group ($p = 0.280$) (Figure 1A), indicating that CBD administration did not affect recognition memory of healthy rats. Moreover, when the percentage of time spent interacting with the novel object was considered, all groups except the POLY+VEH group displayed a significant preference for the novel object that was above chance levels (CONT+VEH, $t(10) = 7.157$, $p < 0.001$; CONT+CBD, $t(9) = 4.055$, $p = 0.003$; POLY+VEH, $t(9) = 0.946$, $p = 0.369$; POLY+CBD, $t(10) = 5.698$, $p < 0.001$) (Figure 1B). Total object exploration times in the NOR test did not significantly differ between groups

383 (Figure 1C) (prenatal infection: $F_{(1, 37)} = 0.798$, $p = 0.377$; offspring treatment: $F_{(1, 37)} =$
384 1.443 , $p = 0.237$; prenatal infection x offspring treatment: $F_{(1, 37)} = 0.001$, $p = 0.976$).

Figure 1: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on the performance of female control (CONT) and poly I:C (POLY) offspring in the Novel Object Recognition test: (A) Discrimination index (calculated as $T_N - T_F / (T_F + T_N)$), (B) Preference for the novel object (against chance levels i.e. 50% indicated by the dashed line), and (C) Total object exploration time (s). Data expressed as mean $\pm$ SEM. * $p < 0.05$ vs. CONT+VEH. # $p < 0.05$ vs. POLY+VEH. ‘++’ $p < 0.01$, ‘+++’ $p < 0.001$ vs. chance (i.e. 50%). $n = 10-12$ rats per group; 5-6 independent litters. T_F : total time spent exploring familiar object; T_N : total time spent exploring novel object. Data expressed as mean $\pm$ SEM.

Working memory of female rat offspring was examined using the rewarded alternation T-maze test; however, results indicated these parameters were not altered in any of the female offspring treatment groups. Mann-Whitney tests showed that the percentage of correct entries in the T-maze test was comparable across groups (CONT+VEH vs. POLY+VEH, $p = 0.950$; POLY+VEH vs. POLY+CBD, $p = 0.724$; CONT+VEH vs. CONT+CBD, $p = 0.519$) and all groups performed above chance levels in the T-maze test (CONT+VEH, $t(10) = 3.862$, $p = 0.003$; CONT+CBD, $t(10) = 4.217$, $p = 0.002$; POLY+VEH, $t(11) = 2.779$, $p = 0.018$; POLY+CBD, $t(11) = 2.716$, $p = 0.020$) (Figure 2A). Similarly, maternal poly I:C exposure and CBD treatment did not effect latency to first arm entry in the T-maze test (prenatal infection: $F_{(1, 43)} = 1.044$, $p = 0.313$; offspring treatment: $F_{(1, 43)} = 2.259$, $p = 0.140$; prenatal infection x offspring treatment: $F_{(1, 43)} = 0.930$, $p = 0.340$) (Figure 2B).

Figure 2: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on the performance of female control (CONT) and poly I:C (POLY) offspring in the rewarded alternation T-maze test. **(A)** Percentage of correct entries and **(B)** latency to first entry (s) in the T-maze test. ‘+’ $p < 0.05$, ‘++’ $p < 0.01$ preference for the correct entry vs. chance (i.e. 50%, dashed line); $n = 10-12$ rats per group, 5-6 independent litters. Data expressed as mean $\pm$ SEM.

The social interaction test was used to assess the sociability of control and poly I:C offspring following chronic CBD treatment. A two-way ANOVA revealed a significant main effect of prenatal infection ($F_{(1, 18)} = 10.975, p = 0.004$) and a prenatal infection x offspring treatment interaction ($F_{(1, 18)} = 26.798, p < 0.001$) on total interaction time. Vehicle-treated poly I:C offspring spent significantly less time interacting compared to controls (-50.78%; POLY+VEH vs. CONT+VEH, $p < 0.001$), indicating that maternal poly I:C exposure impaired social interaction in adult female offspring (Figure 3). CBD treatment significantly increased total interaction time in poly I:C offspring (+76.85%; POLY+CBD vs. POLY+VEH, $p < 0.001$), restoring control-like levels (POLY+CBD vs. CONT+VEH, $p = 0.130$) (Figure 3). Interestingly, female CONT+CBD offspring had a significantly lower interaction time compared to CONT+VEH offspring (-24.09%; $p = 0.014$), suggesting that CBD administration impaired social interaction in healthy rats (Figure 3).

Figure 3: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on the performance of female control (CONT) and poly I:C (POLY) offspring in the social interaction test. * $p < 0.05$, *** $p < 0.001$ vs. CONT+VEH; ### $p < 0.001$ vs. POLY+VEH; $n = 5-6$ pairs of rats per group. Data expressed as mean $\pm$ SEM.

3.2 Effect of cannabidiol on body weight, food and water intake

Body weight, food intake and water intake of female offspring were monitored over the treatment period. A two-way ANOVA revealed a significant main effect of prenatal infection on baseline body weight (i.e. Week 0 of treatment) ($F_{(1, 42)} = 37.168, p < 0.001$), whereby female offspring from poly I:C-treated dams were significantly heavier compared to offspring from control-treated dams (+14.39%, POLY vs. CONT) (Figure 4A). In order to examine the effect of CBD treatment on body weight over the treatment period, baseline body weight was included as a covariate for further analyses. Repeated measures mixed ANOVA revealed a significant prenatal infection x offspring treatment x time (weeks) interaction on the body weight of offspring ($F_{(1.701, 69.731)} = 4.455, p = 0.02$). All treatment groups gained weight over the treatment period ($p < 0.001$); however, there was no significant difference in average body weight between treatment groups at any of the time points (Week 1, 2 and 3; all $p > 0.05$) (Figure 4A). A two-way ANOVA revealed no significant effect of prenatal infection ($F_{(1, 20)} = 2.214, p = 0.152$) or offspring treatment ($F_{(1, 20)} = < 0.001, p = 0.988$) on average food intake over the treatment period, and no interaction between the factors ($F_{(1, 20)} = 0.541, p = 0.470$) (Figure 4B). In contrast, there was a significant main effect of prenatal infection ($F_{(1, 20)} = 13.849, p = 0.001$) on water intake, whereby poly I:C offspring consumed more water than control offspring over the treatment period (+23.86%) (Figure 4C). There was no significant effect of offspring treatment ($F_{(1, 20)} = 0.876, p = 0.360$) on average water intake and no interaction between the factors ($F_{(1, 20)} = 1.193, p = 0.288$).

Figure 4: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on body weight, food and water intake in female control (CONT) and poly I:C (POLY) offspring. **(A)** Average body weight of offspring (g) over the treatment period. *** $p < 0.001$ CONT vs. POLY offspring (significant effect of prenatal infection i.e. CONT+VEH and CONT+CBD groups vs. POLY+VEH and POLY+CBD groups) at baseline. $n = 11-12$ rats/group; 5-6 independent litters. **(B)** Average food intake (g) and **(C)** average water intake (mL) over the treatment period. *** $p < 0.001$ CONT vs. POLY offspring (significant effect of prenatal infection i.e.

CONT+VEH and CONT+CBD groups vs. POLY+VEH + POLY+CBD groups). $n = 6$ pairs of rats/group. Data expressed as mean $\pm$ SEM.

3.2 Effect of cannabidiol on endocannabinoid markers

To evaluate CBD treatment effects on eCB neurotransmission, CB1R binding density and FAAH protein levels were examined in the PFC and HPC. Examples of [3 H]SR141716A binding to CB1Rs in the PFC and HPC are shown in Figure 5. Mann-Whitney tests showed no significant difference in [3 H]SR141716A binding density in the PFC of poly I:C offspring compared to controls ($p = 0.600$, POLY+VEH vs. CONT+VEH), and CBD treatment did not affect [3 H]SR141716A binding density in poly I:C offspring (POLY+CBD: 215.37 ± 18.24 vs. POLY+VEH: 244.58 ± 22.46 , $p = 0.699$) (Figure 6A). Interestingly, CBD administration significantly reduced [3 H]SR141716A binding density in the PFC of control offspring (-31.41% ; CONT+CBD vs. CONT+VEH, $p = 0.005$) (Figure 6A). In contrast, [3 H]SR141716A binding density in the HPC was similar between treatment groups (Figure 6B) (prenatal infection: $F_{(1, 25)} = 2.266$, $p = 0.145$; offspring treatment: $F_{(1, 25)} = 0.351$, $p = 0.559$; prenatal infection x offspring treatment: $F_{(1, 25)} = 1.076$, $p = 0.309$). FAAH protein levels in the PFC (Figure 6C) and HPC (Figure 6D) did not significantly differ between treatment groups ([PFC: prenatal infection: $F_{(1, 28)} = 2.051$, $p = 0.163$; offspring treatment: $F_{(1, 28)} = 0.019$, $p = 0.892$; prenatal infection x offspring treatment: $F_{(1, 28)} = 0.149$, $p = 0.702$]; [HPC: prenatal infection: $F_{(1, 27)} = 1.466$, $p = 0.237$; offspring treatment: $F_{(1, 27)} = 0.480$, $p = 0.494$; prenatal infection x offspring treatment: $F_{(1, 27)} = 1.742$, $p = 0.198$]).

	$[^3\text{H}]\text{SR141716A}$	$[^3\text{H}]\text{MK-801}$	$[^3\text{H}]\text{Muscimol}$
<p>PFC</p>				Total Binding
	Non-specific Binding
<p>HPC</p>				Total Binding
	Non-specific Binding

Figure 5: Representative autoradiographs of $[^3\text{H}]\text{SR141716A}$, $[^3\text{H}]\text{MK-801}$, and $[^3\text{H}]\text{Muscimol}$ binding density in the female rat brain. Schematic diagrams adapted from a rat brain atlas (Paxinos and Watson, 2007) showing the Bregma level quantified for the prefrontal cortex (PFC): 2.52 mm (containing the prelimbic and infralimbic cortices) and the hippocampus (HPC): - 4.92 mm (shaded). Total binding as well as non-specific binding for each ligand in the PFC and HPC are shown. Autoradiographs are typical examples of binding observed in n = 6-8 vehicle-treated control offspring.

Figure 6: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on endocannabinoid markers in the brains of female control (CONT) and poly I:C (POLY) offspring. Cannabinoid CB1 receptor ([³H]SR141617A) binding density in the (A) prefrontal cortex (PFC) and (B) hippocampus (HPC). Fatty acid amide hydrolase (FAAH) protein levels in the (C) PFC and (D) HPC normalised to total protein signal. Example FAAH immunoblots shown. ** $p < 0.01$ vs. CONT+VEH group. $n = 6-8$ rats per group; 4-5 independent litters. Data expressed as mean $\pm$ SEM. TE = tissue equivalent.

3.3. Effect of cannabidiol on the glutamatergic NMDAR

To assess whether CBD treatment influenced excitatory glutamatergic neurotransmission, NMDAR binding density and protein levels of its obligatory GluN1 subunit were measured in the brains of offspring. Examples of [³H]MK-801 binding to NMDARs in the PFC and HPC are presented in Figure 5. A two-way ANOVA revealed no significant main effects of prenatal infection ($F_{(1, 25)} = 0.108$, $p = 0.745$) or offspring treatment ($F_{(1, 25)} = 0.241$, $p = 0.628$) on [³H]MK-801 binding density in the PFC, but there was a significant interaction between the two factors ($F_{(1, 25)} = 12.087$, $p = 0.002$). Pairwise comparisons showed that [³H]MK-801 binding density was significantly lower in POLY+VEH offspring compared to controls (-16.47%; POLY+VEH vs. CONT+VEH: $p = 0.033$) (Figure 7A). CBD treatment significantly increased [³H]MK-801 binding density in poly I:C offspring (+24.85%; POLY+CBD vs. POLY+VEH: $p = 0.011$) to control-like levels (POLY+CBD vs. CONT+VEH, $p = 0.600$) (Figure 7A). Interestingly, CBD administration significantly reduced [³H]MK-801 binding density in the PFC in control offspring (-15.62%; CONT+CBD vs. CONT+VEH: $p = 0.042$), in a similar manner to maternal poly I:C exposure (Figure 7A). In the HPC, [³H]MK-801 binding density was similar between groups (Figure 7B) (prenatal infection: $F_{(1, 23)} = 0.002$, $p = 0.961$; offspring treatment: $F_{(1, 23)} = 0.025$, $p = 0.875$; prenatal infection x offspring treatment: $F_{(1, 23)} = 0.022$, $p = 0.883$). In contrast to the [³H]MK-801 binding density results in the PFC, Mann-Whitney tests showed that protein levels of the obligatory GluN1 subunit in the PFC did not differ between treatment groups (all $p > 0.05$) (Figure 7C). Similarly, in the HPC there were no significant main effects of prenatal infection ($F_{(1, 27)} = 1.374$, $p = 0.251$) or offspring treatment ($F_{(1, 27)} = 3.023$, $p = 0.093$) and no interaction between the factors ($F_{(1, 27)} = 0.504$, $p = 0.484$) (Figure 7D).

Figure 7: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on the glutamatergic *N*-methyl-*D*-aspartate receptor (NMDAR) in control (CONT) and poly I:C (POLY) offspring. NMDAR ([³H]MK-801) binding density in the (A) prefrontal cortex (PFC) and (B) hippocampus (HPC). Normalised protein levels of the obligatory GluN1 subunit of the NMDAR in the (C) PFC and (D) HPC (normalised to total protein signal). **p* < 0.05 vs. CONT+VEH group, #*p* < 0.05 vs. POLY+VEH group. Data expressed as mean ± SEM. *n* = 6-8 rats per group; from 4-5 independent litters. TE = tissue equivalent.

3.4 Effect of cannabidiol on GABAergic markers

To assess whether markers of GABAergic signalling in the PFC and HPC were altered by CBD treatment, we measured GABA_AR binding density, as well as protein levels of GAD₆₇ (the rate-limiting enzyme that converts glutamate to GABA), and PV (a calcium-binding protein present on a subset of GABAergic interneurons). Examples of [³H]muscimol binding to the GABA_AR in the PFC and HPC are presented in Figure 5. [³H]Muscimol binding density was comparable between treatment groups in both the PFC (Figure 8A) and HPC (Figure 8B) (i.e. no significant main effects of prenatal infection (PFC: $F_{(1, 22)} = 0.665$, $p = 0.424$; HPC: $F_{(1, 25)} = 2.406$, $p = 0.133$) or offspring treatment (PFC: $F_{(1, 22)} = 1.985$, $p = 0.173$; HPC: $F_{(1, 25)} = 0.034$, $p = 0.856$) and no significant interactions (PFC: $F_{(1, 22)} = 1.050$, $p = 0.317$; HPC: $F_{(1, 25)} = 0.427$, $p = 0.519$)). In the PFC, there was no significant effect of prenatal infection ($F_{(1, 27)} = 0.054$, $p = 0.817$) or offspring treatment ($F_{(1, 27)} = 0.001$, $p = 0.977$) on GAD₆₇ protein levels, and no significant interaction between the factors (Figure 8C) ($F_{(1, 27)} = 0.149$, $p = 0.702$). In contrast, in the HPC there was a significant main effect of offspring treatment ($F_{(1, 25)} = 6.045$, $p = 0.021$), whereby CBD significantly increased GAD₆₇ protein levels relative to vehicle treatment (+17.36 %; CBD: 1.13 ± 0.059 vs. VEH: 0.962 ± 0.017) (Figure 8D). Poly I:C exposure did not affect GAD₆₇ protein levels (prenatal infection: $F_{(1, 25)} = 0.036$, $p = 0.850$), and there was no significant interaction between the factors ($F_{(1, 25)} = 0.508$, $p = 0.483$). There was no significant effect of prenatal infection ($F_{(1, 25)} = 1.514$, $p = 0.229$) or offspring treatment ($F_{(1, 25)} = 0.257$, $p = 0.616$) on PV protein levels in the PFC, and no interaction between factors (Figure 8E) ($F_{(1, 25)} = 0.364$, $p = 0.551$). However, there was a main effect of offspring treatment ($F_{(1, 22)} = 8.431$, $p = 0.008$) on PV protein expression in the HPC. CBD-treated rats had higher PV protein levels relative to vehicle-treated offspring (+28.98%; CBD: 1.21 ± 0.092 vs. VEH: 0.94 ± 0.043) (Figure 8F). No

significant effect of prenatal infection ($F_{(1, 22)} = 0.100, p = 0.755$) nor an interaction between the factors ($F_{(1, 22)} = 2.335, p = 0.141$) was observed.

Figure 8: The effect of cannabidiol (CBD) or vehicle (VEH) treatment on GABAergic signalling markers in female control (CONT) and poly I:C (POLY) offspring. GABA_AR ([³H]Muscimol) binding density in the (A) prefrontal cortex (PFC) and (B) hippocampus (HPC). Normalised glutamate decarboxylase (GAD₆₇) and parvalbumin (PV) protein levels (normalised to total protein signal) in the (C, E) PFC and (D, F) HPC of female offspring. #*p* < 0.05, ##*p* < 0.01 CBD vs. VEH-treated offspring (main effect of offspring treatment). Data expressed as mean ± SEM. *n* = 6-8 rats per group; from 4-5 independent litters. TE: tissue equivalent.

4. Discussion

The present study demonstrates that CBD treatment in late adolescence/early adulthood can attenuate recognition memory and social interaction deficits in female poly I:C offspring. These findings align with results in male offspring (Osborne et al., 2017a); however, unlike males (Osborne et al., 2017a), female poly I:C offspring did not exhibit working memory impairment. CBD treatment also restored NMDAR binding deficits in the PFC of poly I:C offspring, and increased GAD₆₇ and PV protein levels in the HPC relative to vehicle administration. On the other hand, CBD administration in healthy offspring reduced social interaction, NMDAR and CB1R binding density in the PFC, which may have implications for CBD exposure in healthy females during a critical period of brain maturation. Overall, this study provides the first insight into the behavioural and neurochemical changes that occur following CBD treatment in female poly I:C offspring and suggests that CBD may be an efficacious treatment for the negative symptoms and certain cognitive domains in female patients with schizophrenia.

As cognitive impairment is a major source of disability in schizophrenia, the effects of CBD treatment on cognitive function were examined using two well-validated behavioural paradigms, the NOR and T-maze tests to measure recognition (Bevins and Besheer, 2006) and working memory (Deacon and Rawlins, 2006), respectively. In line with our previous study in males (Osborne et al., 2017a), we observed a recognition memory deficit in female poly I:C offspring that was restored by CBD treatment. These findings align with previous studies that show recognition memory deficits in people with schizophrenia (Calkins et al., 2003) and poly I:C offspring (Ozawa et al., 2006; Ratnayake et al., 2014, 2012) of both sexes. However, this is the first study to show that CBD treatment can improve recognition memory in a female model of schizophrenia-like phenotypes. This result coincides with the limited

literature showing beneficial effects of CBD on recognition memory in female rodents using other preclinical models of cognitive impairment, including hypoxic ischaemia (Pazos et al., 2012) and cerebral malaria (Campos et al., 2015). Our results have implications for the treatment of recognition memory deficits in schizophrenia; however, clinical trials are needed to determine if the positive findings in poly I:C rats translate to patients.

In the present study, female poly I:C offspring performed similarly to controls in the T-maze test, contrasting with our finding in poly I:C males that exhibited working memory impairment (Osborne et al., 2017a). It is unclear whether this finding reflects clinical observations in females with schizophrenia due to the limited number of studies conducted, with one study from our group reporting greater working memory impairment in males with chronic schizophrenia compared to females (Han et al., 2012). In addition, other human studies have reported that while healthy male controls perform better on some working memory tasks compared to female controls, this sex difference was not apparent in people with schizophrenia (Longenecker et al., 2010; Zhang et al., 2017). The majority of poly I:C studies evaluate males only or combine male and female data for statistical analysis. A recent study reported that both male and female poly I:C offspring were impaired on a delayed non-match to position task where rats were rewarded for choosing a novel stimulus (Meehan et al., 2017). Similarly, neonatal poly I:C exposure resulted in spontaneous alternation deficits in the Y-maze test in males, with no deficits in females when tested during early adulthood (Monte et al., 2017). On the other hand, when tested later in adulthood (>PN90) deficits in Y-maze performance have been reported in female poly I:C rats that were not apparent in males (O’Leary et al., 2014). Sex differences have also been reported in other cognitive domains in poly I:C offspring. For example, male poly I:C offspring (PN67) displayed executive function deficits and abnormal cognitive switching/flexibility in a reversal learning task, while female

poly I:C offspring of the same age were not impaired (Zhang et al., 2012). These findings suggest that male and female poly I:C offspring may present with different age-related behavioural deficits, possibly due to the timing of their neuropsychiatric trajectories. Indeed, one longitudinal study showed that male poly I:C offspring had latent inhibition deficits from early adulthood (PN70), but these deficits were delayed in female rats and did not present until almost three weeks later (PN90) (Piontkewitz et al., 2011). Therefore, it is possible that working memory deficits also appear in female poly I:C offspring later in adulthood, but further longitudinal studies that examine behaviour in the same rodent cohort are required.

The social interaction test was used to assess social withdrawal of offspring and can be used as an indicator of the negative symptoms of schizophrenia (Wilson and Koenig, 2014). Female poly I:C offspring spent significantly less time interacting with control counterparts and this deficit was restored to control levels by CBD treatment. Social interaction deficits are present in people with schizophrenia (Lindenmayer et al., 2013) and poly I:C offspring regardless of sex (Aavani et al., 2015; B. K. Bitanirwe et al., 2010; Xuan and Hampson, 2014; Zhu et al., 2014). To our knowledge, this is the first study to investigate the effects of CBD treatment on social interaction in females. In male rodents, CBD improved social interaction deficits induced by the NMDAR antagonist, MK-801 (Gomes et al., 2015b; Gururajan et al., 2012, 2011; Long et al., 2012) and the CB1R agonist, Δ^9 -THC (Malone et al., 2009), as well as in rodent models of MIA (Osborne et al., 2017a) and Dravet syndrome (Kaplan et al., 2017). Conversely, CBD treatment had no effect on social interaction in spontaneous hypertensive rats, a strain that exhibits positive and negative schizophrenia-like phenotypes (Almeida et al., 2013). In the clinic, CBD significantly improved negative symptom scores on the Positive and Negative Syndrome Scale in male-dominated cohorts of people with acute paranoid schizophrenia (Leweke et al., 2012) and APD-medicated

outpatients (McGuire et al., 2018). Taken together, these findings suggest that CBD improves negative symptoms in schizophrenia patients and preclinical models in both males and females.

Second generation (atypical) APDs, such as olanzapine and clozapine have a high weight gain liability in patients that can lead to chronic conditions, including obesity, diabetes mellitus type 2 and cardiovascular disease (Weston-Green et al., 2013). These adverse side effects can also result in medication non-compliance, symptom relapse and can exacerbate cognitive impairment (Weston-Green et al., 2013). Female patients tend to be more sensitive to APD-induced metabolic side effects than males, particularly in relation to weight gain (Seeman, 2009). This heightened sensitivity to APD-induced weight gain is also evident in female rats (Weston-Green et al., 2010). Body weight, food and water intake were recorded throughout the treatment period in female rats in the present study. Offspring from poly I:C-treated dams were significantly heavier than controls prior to the start of treatment. This finding is consistent with evidence of an association between sex and obesity in people with mental illness, particularly schizophrenia, with a higher prevalence of obesity in women compared to men (Gurpegui et al., 2012; Jonikas et al., 2016). In the present study, poly I:C offspring also consumed significantly more water over the treatment period. Interestingly, polydipsia or excessive water drinking is observed in animal models of schizophrenia-like phenotypes (Hawken et al., 2013; Hawken and Beninger, 2014), as well as people with schizophrenia (reviewed in de Leon *et al*, 1994); however, polydipsia could be an underlying symptom of the disease and/or a side effect of APD treatment (Bersani *et al*, 2007). Polydipsia is also a characteristic symptom of diabetes (Bhaskar et al., 2010); however, in the present study other metabolic parameters (e.g. glucose and insulin sensitivity) were not assessed. Interestingly, a study that did assess metabolic dysfunction in male poly I:C mice

reported altered food and water intake, as well as impaired glycaemic regulation in periadolescent mice; parameters that appeared to normalise in adulthood. On the other hand, a change in fat mass deposition (without a change in overall body weight) was observed in male mice during adulthood (Pacheco-López et al., 2013). Therefore, longitudinal examination of these metabolic parameters in female poly I:C offspring should also be examined. Importantly, CBD treatment did not significantly affect body weight gain, food or water intake of female adult poly I:C rats in the present study. The effects of CBD on body weight have not been reported previously in females; however, we found a similar result in male poly I:C offspring (Osborne et al., 2017a) that was also observed in a predominantly male cohort of patients with acute schizophrenia (Leweke et al., 2012). Further clinical research is needed to confirm if the low weight gain liability of CBD in rodents translates to female patients with schizophrenia.

In the present study, CBD treatment did not significantly alter CB1R binding density or protein levels of the AEA degradation enzyme, FAAH, in the brains of female poly I:C offspring. One explanation could be that these signalling components are not involved in the behavioural alterations observed in treated poly I:C offspring. Indeed, CBD has a low affinity for the orthosteric binding site of the CB1R (McPartland et al., 2015), but studies have shown that CBD is a potent inhibitor of CB1R agonists (e.g. CP55940) (Thomas et al., 2007), suggesting that CBD does exert effects on the CB1R. A second explanation is that CBD does affect the eCB system to influence behaviour, but without causing an imbalance in CB1R binding density. For example, instead of targeting the CB1R as an orthosteric ligand, CBD can act as a negative allosteric modulator of the CB1R (e.g. in the presence of Δ^9 -THC and the endogenous cannabinoid, 2-arachidonoylglycerol (2-AG)) (Laprairie et al., 2015; Tham et al., 2018). Alternatively, evidence suggests that CBD can alter AEA metabolism by inhibiting

FAAH enzymatic activity (Bisogno et al., 2001; De Petrocellis et al., 2011; Leweke et al., 2012). The present study showed that FAAH protein levels remain unchanged following CBD treatment; however, enzymatic activity was not investigated. Therefore, the effects of CBD on FAAH enzymatic activity require further investigation in poly I:C offspring. Also, investigating upstream processes such as the intracellular transport of AEA via fatty acid binding proteins (Elmes et al., 2015) may provide further insight into the influence of CBD treatment on AEA metabolism.

In the present study, we also examined CBD treatment effects on the glutamatergic NMDAR and its obligatory GluN1 subunit, as well as GABAergic markers, including the GABA_AR, GAD₆₇ and PV protein levels. CBD attenuated poly I:C-induced NMDAR binding deficits in the PFC, while protein levels of the obligatory GluN1 subunit were unchanged. In addition, CBD had no effect on NMDAR binding density in the HPC; however, poly I:C offspring did not exhibit deficits in this region, which is consistent with a previous poly I:C study in females (Rahman *et al*, 2017). Although evidence for a direct interaction between CBD and the NMDAR is sparse, interestingly, CBD can reverse behavioural (i.e. sensorimotor gating) and neurochemical deficits (i.e. NMDAR GluN1 subunit gene expression) induced by NMDAR antagonism (Gomes et al., 2015a; Gururajan et al., 2012), suggesting that CBD may modulate glutamatergic signalling via the NMDAR. It is unclear whether the up-regulation in [³H]MK-801 binding in the PFC is due to indirect effects of CBD on the NMDAR (e.g. an alteration in glutamate levels) or if CBD alters the affinity of the phencyclidine binding site (i.e. where MK-801 binds) for the ligand. Given the differential results in NMDAR binding density and GluN1 protein levels, it is more likely that CBD alters NMDAR affinity; however, further studies are required to confirm. On the other hand, CBD significantly increased GAD₆₇ and PV protein levels in the HPC, regardless of whether offspring were

born to control or poly I:C-treated mothers. In line with the increase in GAD₆₇ protein levels, a previous study reported that CBD reversed deficits in GABA levels following 3-nitropropionic acid administration (a model of Huntington's disease); however, this study examined the rat striatum (Sagredo et al., 2007). On the other hand, a recent study reported that CBD upregulated the activity of hippocampal PV⁺ interneurons (Khan et al., 2018), which may explain the CBD-induced increase in PV protein levels observed in the present study. Taken together, these findings suggest that CBD may enhance inhibitory tone in the HPC. CBD was also recently identified as a positive allosteric modulator of the GABA_AR, *in vitro* (Bakas et al., 2017), which may have therapeutic implications for schizophrenia. However, CBD treatment did not affect GABA_AR binding density in the PFC or HPC of female offspring. This finding could suggest that CBD does not alter GABA_AR expression in poly I:C offspring that do not exhibit underlying GABA_AR dysfunction. Alternatively, CBD could influence GABA_AR activity in the brains of offspring, not detectable using the methods in the present study. Future studies using electrophysiology techniques may shed light on the effects of CBD treatment on GABA_AR function.

Interestingly, in the present study CBD administration impaired social interaction of healthy female control offspring, coupled with a decrease in CB1R and NMDAR binding density in the PFC. However, we previously found that CBD did not alter social interaction of control male offspring (Osborne et al., 2017a). Sexual dimorphism in the eCB system has been reported previously (for review see Rubino and Parolaro (2011)). The differential response of female control rats may be due to innate biological differences in drug absorption rates, metabolic enzyme activity, fat mass and renal clearance that could influence the metabolism of CBD (Narimatsu et al., 1993). In addition, sex hormones such as oestrogen, are known to influence both CB1R (Cooper and Craft, 2018) and NMDAR expression/signalling

(McRoberts et al., 2007). The timing of CBD administration (PN56-80) in the present study corresponds to late adolescence/early adulthood in humans, which is a vulnerable period of neurodevelopment (Semple et al., 2013). During this period, female rodents are particularly sensitive to effects of cannabinoids (e.g. Δ^9 -THC) (Macúchová et al., 2016) and sensitivity can vary depending on the stage of the oestrus cycle (Cooper and Craft, 2018). Although beyond the scope of the present study, future investigations could examine whether fluctuations in sex hormones during oestrus influence CBD treatment response in female rats. The findings of a deficit in social interaction and neurochemical signalling in female control offspring highlight the possibility that CBD could have detrimental sex-specific effects when used during a vulnerable period of neurodevelopment in non-pathological conditions.

Our findings show that CBD has beneficial effects on recognition memory and social interaction in female poly I:C offspring, which may be partly due to an upregulation in hippocampal GABAergic tone and glutamatergic signalling in the PFC. Interestingly, CBD increased GABAergic markers in the HPC of both control and poly I:C offspring. However, CBD had opposite effects on NMDAR binding density in the PFC of control and poly I:C offspring. On the other hand, it is unclear from the neurochemical data whether CBD exerts its effects in the HPC, which then impacts glutamatergic input to the PFC, consequently altering NMDAR expression; future studies are needed to address this question. There are several limitations to the present study. Firstly, transportation of rats between supplier and research facility during pregnancy could be a potential confound when comparing these findings to other poly I:C studies. In addition, recent reports suggest batch to batch variation of poly I:C (reviewed in Kentner et al., 2018) and this study is limited by a lack of immunological response data in the pregnant dams. However, a previous dose-response study showed that administration of poly I:C using the same dose, administration route and

gestational timing as the present study significantly raised pro-inflammatory cytokines levels in the pregnant dams (Missault et al., 2014). Furthermore, the estrous cycle was not measured in the present study and should be considered for future studies examining female rodents; however, the low data variability in our study suggests that the estrous cycle did not affect results. Overall, there is limited literature that examines the response of female offspring to maternal poly I:C exposure or CBD treatment. Sex differences in the eCB system have been extensively reported in humans and rodents (see Craft et al., (2013) for review). Therefore, it is important that preclinical studies evaluate both sexes when investigating the therapeutic potential of cannabinoids. Our findings show that CBD can improve behavioural and neurochemical deficits in female poly I:C offspring, without body weight gain side effects, suggesting that CBD may be a promising treatment option for female patients with schizophrenia.

Funding and Disclosure

This study was supported by the University of Wollongong utilising funding from a Faculty of Science, Medicine and Health Advancement Grant (2015/SPGA-S/02) awarded to KWG and XFH; and funding from Molecular Horizons awarded to KWG. These sources had no further role in the study design, decision to publish, or preparation of the manuscript. ALO and JSL were supported by an Australian Government Research Training Program Scholarship from the University of Wollongong and a Daniel Beck Memorial Award for Schizophrenia Research from the Beck Family and Neuroscience Research Australia. NS was supported by an Australian Research Council Future Fellowship (FT110100752). IB is supported by a Postgraduate Research Scholarship from the University of Wollongong and Illawarra Shoalhaven Local Health District. JSL is supported by Australian Rotary Health in the form of an Ian Scott Scholarship. The authors have no conflicts of interest to declare.

Acknowledgements

We would like to thank Cree de Clouett, Martin Engel, Ashleigh Gorak, Michael Green, Paul Le Mesurier, Luisa Lufe, Nicole Miles, Jessica Nealon, Anita-Louise Obeid, David Osborne, Tereza Pejovska, Dominic Sellers, Alexander Stamenkovic, Erika Svenson for their technical assistance during the animal experiments.

Contributors

KWG, ALO, NS and XFH designed the study; KAN and XFH contributed some of the reagents; ALO, IB, JSL and KWG performed the experiments; ALO analysed the data and wrote the first draft of the manuscript; KWG, JSL, 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

- Aavani, T., Rana, S.A., Hawkes, R., Pittman, Q.J., 2015. Maternal Immune Activation Produces Cerebellar Hyperplasia and Alterations in Motor and Social Behaviors in Male and Female Mice. *The Cerebellum* 14, 491–505. <https://doi.org/10.1007/s12311-015-0669-5>
- Abel, K.M., Drake, R., Goldstein, J.M., 2010. Sex differences in schizophrenia. *Int. Rev. Psychiatry* 22, 417–428. <https://doi.org/10.3109/09540261.2010.515205>
- Aleman, A., Kahn, R.S., Selten, J.-P., 2003. Sex Differences in the Risk of Schizophrenia: Evidence From Meta-analysis. *Arch. Gen. Psychiatry* 60, 565–571. <https://doi.org/10.1001/archpsyc.60.6.565>
- Almeida, V., Levin, R., Peres, F.F., Niigaki, S.T., Calzavara, M.B., Zuardi, A.W., Hallak, J.E., Crippa, J.A., Abílio, V.C., 2013. Cannabidiol exhibits anxiolytic but not antipsychotic property evaluated in the social interaction test. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 41, 30–35. <https://doi.org/10.1016/j.pnpbp.2012.10.024>
- Babic, I., Gorak, A., Engel, M., Sellers, D., Else, P., Osborne, A.L., Pai, N., Huang, X.-F., Nealon, J., Weston-Green, K., 2018. Liraglutide prevents metabolic side-effects and improves recognition and working memory during antipsychotic treatment in rats. *J. Psychopharmacol. Oxf. Engl.* 32, 578–590. <https://doi.org/10.1177/0269881118756061>

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

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

883 Bersani, G., Pesaresi, L., Orlandi, V., Gherardelli, S., Pancheri, P., 2007. Atypical antipsychotics and
884 polydipsia: a cause or a treatment? *Hum. Psychopharmacol.* 22, 103–107.
885 <https://doi.org/10.1002/hup.825>

886 Bevins, R.A., Besheer, J., 2006. Object recognition in rats and mice: a one-trial non-matching-to-
887 sample learning task to study “recognition memory.” *Nat. Protoc.* 1, 1306–1311.
888 <https://doi.org/10.1038/nprot.2006.205>

889 Bhaskar, M.E., Sowmya, G., Moorthy, S., Kumar, N.S., Praveena, R., Kumar, V., 2010. Presenting
890 Features of Diabetes Mellitus. *Indian J. Community Med. Off. Publ. Indian Assoc. Prev. Soc.*
891 *Med.* 35, 523–525. <https://doi.org/10.4103/0970-0218.74371>

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

897 Bitanhirwe, B.K., Peleg-Raibstein, D., Mouttet, F., Feldon, J., Meyer, U., 2010. Late Prenatal Immune
898 Activation in Mice Leads to Behavioral and Neurochemical Abnormalities Relevant to the
899 Negative Symptoms of Schizophrenia. *Neuropsychopharmacology* 35, 2462–2478.
900 <https://doi.org/10.1038/npp.2010.129>

901 Bitanhirwe, B.K.Y., Weber, L., Feldon, J., Meyer, U., 2010. Cognitive impairment following prenatal
902 immune challenge in mice correlates with prefrontal cortical AKT1 deficiency. *Int. J.*
903 *Neuropsychopharmacol.* 13, 981–996. <https://doi.org/10.1017/S1461145710000192>

904 Calkins, M.E., Gur, R.C., Ragland, J.D., Gur, R.E., 2003. Face recognition memory deficits in
905 schizophrenia patients and their relatives: Comparison with visual object memory
906 performance. *Schizophr. Res., Abstracts of the IXth International Congress on Schizophrenia*
907 *Research* 60, 128. [https://doi.org/10.1016/S0920-9964\(03\)80906-4](https://doi.org/10.1016/S0920-9964(03)80906-4)

908 Campos, A.C., Brant, F., Miranda, A.S., Machado, F.S., Teixeira, A.L., 2015. Cannabidiol Increases
909 Survival and Promotes Rescue of Cognitive Function. *Neuroscience* 289, 166–180.
910 <https://doi.org/10.1016/j.neuroscience.2014.12.051>

911 Campos, A.C., Fogaça, M.V., Scarante, F.F., Joca, S.R.L., Sales, A.J., Gomes, F.V., Sonogo, A.B.,
912 Rodrigues, N.S., Galve-Roperh, I., Guimarães, F.S., 2017. Plastic and Neuroprotective
913 Mechanisms Involved in the Therapeutic Effects of Cannabidiol in Psychiatric Disorders.
914 *Front. Pharmacol.* 8, 269. <https://doi.org/10.3389/fphar.2017.00269>

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

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

923 Colella, A.D., Chegenii, N., Tea, M.N., Gibbins, I.L., Williams, K.A., Chataway, T.K., 2012. Comparison
924 of Stain-Free gels with traditional immunoblot loading control methodology. *Anal. Biochem.*
925 430, 108–110. <https://doi.org/10.1016/j.ab.2012.08.015>

- Cooper, Z.D., Craft, R.M., 2018. Sex-Dependent Effects of Cannabis and Cannabinoids: A Translational Perspective. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 43, 34–51. <https://doi.org/10.1038/npp.2017.140>
- Craft, R.M., Marusich, J.A., Wiley, J.L., 2013. Sex differences in cannabinoid pharmacology: A reflection of differences in the endocannabinoid system? *Life Sci.* 92, 476–481. <https://doi.org/10.1016/j.lfs.2012.06.009>
- Dalton, V.S., Long, L.E., Weickert, C.S., Zavitsanou, K., 2011. Paranoid schizophrenia is characterized by increased CB1 receptor binding in the dorsolateral prefrontal cortex. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 36, 1620–1630. <https://doi.org/10.1038/npp.2011.43>
- de Leon, J., Verghese, C., Tracy, J.I., Josiassen, R.C., Simpson, G.M., 1994. Polydipsia and water intoxication in psychiatric patients: a review of the epidemiological literature. *Biol. Psychiatry* 35, 408–419.
- De Petrocellis, L., Ligresti, A., Moriello, A.S., Allarà, 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>
- De Santis, M., Lian, J., Huang, X.-F., Deng, C., 2016. Early antipsychotic treatment in childhood/adolescent period has long-term effects on depressive-like, anxiety-like and locomotor behaviours in adult rats. *J. Psychopharmacol. (Oxf.)* 30, 204–214. <https://doi.org/10.1177/0269881115616383>
- Deacon, R.M.J., Rawlins, J.N.P., 2006. T-maze alternation in the rodent. *Nat Protoc.* 1, 7–12. <https://doi.org/10.1038/nprot.2006.2>
- 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.
- Deiana, S., Watanabe, A., Yamasaki, Y., Amada, N., Arthur, M., Fleming, S., Woodcock, H., Dorward, P., Pigliacampo, B., Close, S., Platt, B., Riedel, G., 2011. Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarin (CBDV), Δ^9 -tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive–compulsive behaviour. *Psychopharmacology (Berl.)* 219, 859–873. <https://doi.org/10.1007/s00213-011-2415-0>
- Deslauriers, J., Larouche, A., Sarret, P., Grignon, S., 2013. Combination of prenatal immune challenge and restraint stress affects prepulse inhibition and dopaminergic/GABAergic markers. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 45, 156–164. <https://doi.org/10.1016/j.pnpbp.2013.05.006>
- 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>
- Du Bois, T.M., Huang, X.-F., Deng, C., 2008. Perinatal administration of PCP alters adult behaviour in female Sprague–Dawley rats. *Behav. Brain Res.* 188, 416–419. <https://doi.org/10.1016/j.bbr.2007.11.017>
- 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>

976 Elmes, M.W., Kaczocha, M., Berger, W.T., Leung, K., Ralph, B.P., Wang, L., Sweeney, J.M., Miyauchi,
 977 J.T., Tsirka, S.E., Ojima, I., Deutsch, D.G., 2015. Fatty Acid-binding Proteins (FABPs) Are
 978 Intracellular Carriers for Δ^9 -Tetrahydrocannabinol (THC) and Cannabidiol (CBD). *J. Biol.*
 979 *Chem.* 290, 8711–8721. <https://doi.org/10.1074/jbc.M114.618447>
 980 Fagherazzi, E.V., Garcia, V.A., Maurmann, N., Bervanger, T., Halmenschlager, L.H., Busato, S.B.,
 981 Hallak, J.E., Zuardi, A.W., Crippa, J.A., Schröder, N., 2011. Memory-rescuing effects of
 982 cannabidiol in an animal model of cognitive impairment relevant to neurodegenerative
 983 disorders. *Psychopharmacology (Berl.)* 219, 1133–1140. [https://doi.org/10.1007/s00213-](https://doi.org/10.1007/s00213-011-2449-3)
 984 [011-2449-3](https://doi.org/10.1007/s00213-011-2449-3)
 985 Giuffrida, A., Leweke, F.M., Gerth, C.W., Schreiber, D., Koethe, D., Faulhaber, J., Klosterkötter, J.,
 986 Piomelli, D., 2004. Cerebrospinal anandamide levels are elevated in acute schizophrenia and
 987 are inversely correlated with psychotic symptoms. *Neuropsychopharmacol. Off. Publ. Am.*
 988 *Coll. Neuropsychopharmacol.* 29, 2108–2114. <https://doi.org/10.1038/sj.npp.1300558>
 989 Godsil, B.P., Kiss, J.P., Spedding, M., Jay, T.M., 2013. The hippocampal-prefrontal pathway: the weak
 990 link in psychiatric disorders? *Eur. Neuropsychopharmacol. J. Eur. Coll.*
 991 *Neuropsychopharmacol.* 23, 1165–1181. <https://doi.org/10.1016/j.euroneuro.2012.10.018>
 992 Goff, D.C., Hill, M., Barch, D., 2011. The treatment of cognitive impairment in schizophrenia.
 993 *Pharmacol. Biochem. Behav.* 99, 245–253. <https://doi.org/10.1016/j.pbb.2010.11.009>
 994 Gomes, F.V., Issy, A.C., Ferreira, F.R., Viveros, M.-P., Del Bel, E.A., Guimaraes, F.S., 2015a.
 995 Cannabidiol attenuates sensorimotor gating disruption and molecular changes induced by
 996 chronic antagonism of NMDA receptors in mice. *Int. J. Neuropsychopharmacol.* 18, 1–10.
 997 <https://doi.org/10.1093/ijnp/pyu041>
 998 Gomes, F.V., Llorente, R., Del Bel, E.A., Viveros, M.-P., López-Gallardo, M., Guimarães, F.S., 2015b.
 999 Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol.
 1000 *Schizophr. Res.* 164, 155–163. <https://doi.org/10.1016/j.schres.2015.01.015>
 1001 Gurpegui, M., Martínez-Ortega, J.M., Gutiérrez-Rojas, L., Rivero, J., Rojas, C., Jurado, D., 2012.
 1002 Overweight and obesity in patients with bipolar disorder or schizophrenia compared with a
 1003 non-psychiatric sample. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 37, 169–175.
 1004 <https://doi.org/10.1016/j.pnpbp.2012.01.014>
 1005 Gürtler, A., Kunz, N., Gomolka, M., Hornhardt, S., Friedl, A.A., McDonald, K., Kohn, J.E., Posch, A.,
 1006 2013. Stain-Free technology as a normalization tool in Western blot analysis. *Anal. Biochem.*
 1007 433, 105–111. <https://doi.org/10.1016/j.ab.2012.10.010>
 1008 Gururajan, A., Taylor, D.A., Malone, D.T., 2012. Cannabidiol and clozapine reverse MK-801-induced
 1009 deficits in social interaction and hyperactivity in Sprague–Dawley rats. *J. Psychopharmacol.*
 1010 *(Oxf.)* 26, 1317–1332. <https://doi.org/10.1177/0269881112441865>
 1011 Gururajan, A., Taylor, D.A., Malone, D.T., 2011. Effect of cannabidiol in a MK-801-rodent model of
 1012 aspects of Schizophrenia. *Behav. Brain Res.* 222, 299–308.
 1013 <https://doi.org/10.1016/j.bbr.2011.03.053>
 1014 Han, M., Huang, X.-F., Chen, D.C., Xiu, M.H., Hui, L., Liu, H., Kosten, T.R., Zhang, X.Y., 2012. Gender
 1015 differences in cognitive function of patients with chronic schizophrenia. *Prog.*
 1016 *Neuropsychopharmacol. Biol. Psychiatry, New Drugs of Abuse* 39, 358–363.
 1017 <https://doi.org/10.1016/j.pnpbp.2012.07.010>
 1018 Harvey, L., Boksa, P., 2012. A stereological comparison of GAD67 and reelin expression in the
 1019 hippocampal stratum oriens of offspring from two mouse models of maternal inflammation
 1020 during pregnancy. *Neuropharmacology* 62, 1767–1776.
 1021 <https://doi.org/10.1016/j.neuropharm.2011.11.022>
 1022 Hawken, E.R., Beninger, R.J., 2014. The amphetamine sensitization model of schizophrenia
 1023 symptoms and its effect on schedule-induced polydipsia in the rat. *Psychopharmacology*
 1024 *(Berl.)* 231, 2001–2008. <https://doi.org/10.1007/s00213-013-3345-9>

- Hawken, E.R., Delva, N.J., Beninger, R.J., 2013. Increased drinking following social isolation rearing: implications for polydipsia associated with schizophrenia. *PLoS One* 8, e56105. <https://doi.org/10.1371/journal.pone.0056105>
- Holloway, T., Moreno, J.L., Umali, A., Rayannavar, V., Hodes, G.E., Russo, S.J., Gonzalez-Maeso, J., 2013. Prenatal stress induces schizophrenia-like alterations of serotonin 2A and metabotropic glutamate 2 receptors in the adult offspring: role of maternal immune system. *J. Neurosci.* 33, 1088–1098. <https://doi.org/10.1523/JNEUROSCI.2331-12.2013>
- Hui, C.W., St-Pierre, A., El Hajj, H., Remy, Y., Hébert, S.S., Luheshi, G.N., Srivastava, L.K., Tremblay, M.-È., 2018. Prenatal Immune Challenge in Mice Leads to Partly Sex-Dependent Behavioral, Microglial, and Molecular Abnormalities Associated with Schizophrenia. *Front. Mol. Neurosci.* 11, 13. <https://doi.org/10.3389/fnmol.2018.00013>
- Iversen, T.S.J., Steen, N.E., Dieset, I., Hope, S., Mørch, R., Gardsjord, E.S., Jørgensen, K.N., Melle, I., Andreassen, O.A., Molden, E., Jönsson, E.G., 2018. Side effect burden of antipsychotic drugs in real life – Impact of gender and polypharmacy. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 82, 263–271. <https://doi.org/10.1016/j.pnpbp.2017.11.004>
- Jasinska, M., Pyza, E., 2017. Circadian Plasticity of Mammalian Inhibitory Interneurons. *Neural Plast.* 2017, 1–12. <https://doi.org/10.1155/2017/6373412>
- Jonge, D., C, J., Vinkers, C.H., Pol, H., E, H., Marsman, A., 2017. GABAergic Mechanisms in Schizophrenia: Linking Postmortem and In Vivo Studies. *Front. Psychiatry* 8. <https://doi.org/10.3389/fpsy.2017.00118>
- Jonikas, J.A., Cook, J.A., Razzano, L.A., Steigman, P.J., Hamilton, M.M., Swarbrick, M.A., Santos, A., 2016. Associations Between Gender and Obesity Among Adults with Mental Illnesses in a Community Health Screening Study. *Community Ment. Health J.* 52, 406–415. <https://doi.org/10.1007/s10597-015-9965-2>
- Kaplan, J.S., Stella, N., Catterall, W.A., Westenbroek, R.E., 2017. Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. *Proc. Natl. Acad. Sci.* 114, 11229–11234. <https://doi.org/10.1073/pnas.1711351114>
- Kentner, A.C., Bilbo, S.D., Brown, A.S., Hsiao, E.Y., McAllister, A.K., Meyer, U., Pearce, B.D., Pletnikov, M.V., Yolken, R.H., Bauman, M.D., 2018. Maternal immune activation: reporting guidelines to improve the rigor, reproducibility, and transparency of the model. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* <https://doi.org/10.1038/s41386-018-0185-7>
- 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.* <https://doi.org/10.1111/bph.14202>
- Khandaker, G.M., Zimbron, J., Lewis, G., Jones, P.B., 2013. Prenatal maternal infection, neurodevelopment and adult schizophrenia: a systematic review of population-based studies. *Psychol. Med.* 43, 239–257. <https://doi.org/10.1017/S0033291712000736>
- Kilb, W., 2012. Development of the GABAergic system from birth to adolescence. *Neurosci. Rev. J. Bringing Neurobiol. Neurol. Psychiatry* 18, 613–630. <https://doi.org/10.1177/1073858411422114>
- 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>
- 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 J. Ment. Sci.* 194, 371–372. <https://doi.org/10.1192/bjp.bp.108.053843>
- Labouesse, M.A., Dong, E., Grayson, D.R., Guidotti, A., Meyer, U., 2015. Maternal immune activation induces GAD1 and GAD2 promoter remodeling in the offspring prefrontal cortex. *Epigenetics* 10, 1143–1155. <https://doi.org/10.1080/15592294.2015.1114202>

- 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>
- Leweke, F.M., Giuffrida, A., Koethe, D., Schreiber, D., Nolden, B.M., Kranaster, L., Neatby, M.A., Schneider, M., Gerth, C.W., Hellmich, M., Klosterkötter, J., Piomelli, D., 2007. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. *Schizophr. Res.* 94, 29–36. <https://doi.org/10.1016/j.schres.2007.04.025>
- 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>
- 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>
- 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>
- Longenecker, J., Dickinson, D., Weinberger, D.R., Elvevåg, B., 2010. Cognitive differences between men and women: A comparison of patients with schizophrenia and healthy volunteers. *Schizophr. Res.* 120, 234–235. <https://doi.org/10.1016/j.schres.2009.12.009>
- 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>
- Macúchová, E., Ševčíková, M., Hřebíčková, I., Nohejlová, K., Šlamberová, R., 2016. How various drugs affect anxiety-related behavior in male and female rats prenatally exposed to methamphetamine. *Int. J. Dev. Neurosci. Off. J. Int. Soc. Dev. Neurosci.* 51, 1–11. <https://doi.org/10.1016/j.ijdevneu.2016.04.001>
- Malone, D.T., Jongejan, D., Taylor, D.A., 2009. Cannabidiol reverses the reduction in social interaction produced by low dose Δ^9 -tetrahydrocannabinol in rats. *Pharmacol. Biochem. Behav.* 93, 91–96. <https://doi.org/10.1016/j.pbb.2009.04.010>
- 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., Duncan, M., Di Marzo, V., Pertwee, R.G., 2015. Are cannabidiol and $\Delta(9)$ -tetrahydrocannabinol negative modulators of the endocannabinoid system? A systematic review. *Br. J. Pharmacol.* 172, 737–753. <https://doi.org/10.1111/bph.12944>
- McRoberts, J.A., Li, J., Ennes, H.S., Mayer, E.A., 2007. Sex-dependent differences in the activity and modulation of NMDA receptors in rat DRG neurons. *Neuroscience* 148, 1015–1020. <https://doi.org/10.1016/j.neuroscience.2007.07.006>
- Meehan, C., Harms, L., Frost, J.D., Barreto, R., Todd, J., Schall, U., Shannon Weickert, C., Zavitsanou, K., Michie, P.T., Hodgson, D.M., 2017. Effects of immune activation during early or late gestation on schizophrenia-related behaviour in adult rat offspring. *Brain. Behav. Immun., Perinatal Inflammation* 63, 8–20. <https://doi.org/10.1016/j.bbi.2016.07.144>

- 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>
- Missault, S., Van den Eynde, K., Vanden Berghe, W., Fransen, E., Weeren, A., Timmermans, J.P., Kumar-Singh, S., Dedeurwaerdere, S., 2014. The risk for behavioural deficits is determined by the maternal immune response to prenatal immune challenge in a neurodevelopmental model. *Brain. Behav. Immun.* 42, 138–146. <https://doi.org/10.1016/j.bbi.2014.06.013>
- Monte, A.S., Mello, B.S.F., Borella, V.C.M., da Silva Araujo, T., da Silva, F.E.R., Sousa, F.C.F. de, de Oliveira, A.C.P., Gama, C.S., Seeman, M.V., Vasconcelos, S.M.M., Lucena, D.F.D., Macêdo, D., 2017. Two-hit model of schizophrenia induced by neonatal immune activation and peripubertal stress in rats: Study of sex differences and brain oxidative alterations. *Behav. Brain Res.* 331, 30–37. <https://doi.org/10.1016/j.bbr.2017.04.057>
- Muguruza, C., Lehtonen, M., Aaltonen, N., Morentin, B., Meana, J.J., Callado, L.F., 2013. Quantification of endocannabinoids in postmortem brain of schizophrenic subjects. *Schizophr. Res.* 148, 145–150. <https://doi.org/10.1016/j.schres.2013.06.013>
- Narimatsu, S., Watanabe, K., Matsunaga, T., Yamamoto, I., Imaoka, S., Funae, Y., Yoshimura, H., 1993. Suppression of liver microsomal drug-metabolizing enzyme activities in adult female rats pretreated with cannabidiol. *Biol. Pharm. Bull.* 16, 428–430.
- 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. <https://doi.org/10.1007/s00702-007-0668-x>
- NHMRC, 2013. Australian code for the care and use of animals for scientific purposes.
- Nyffeler, M., Meyer, U., Yee, B.K., Feldon, J., Knuesel, I., 2006. Maternal immune activation during pregnancy increases limbic GABAA receptor immunoreactivity in the adult offspring: implications for schizophrenia. *Neuroscience* 143, 51–62. <https://doi.org/10.1016/j.neuroscience.2006.07.029>
- Ochoa, S., Usall, J., Cobo, J., Labad, X., Kulkarni, J., 2012. Gender differences in schizophrenia and first-episode psychosis: a comprehensive literature review. *Schizophr. Res. Treat.* 2012, 916198. <https://doi.org/10.1155/2012/916198>
- O’Leary, C., Desbonnet, L., Clarke, N., Petit, E., Tighe, O., Lai, D., Harvey, R., Waddington, J.L., O’Tuathaigh, C., 2014. Phenotypic effects of maternal immune activation and early postnatal milieu in mice mutant for the schizophrenia risk gene neuregulin-1. *Neuroscience* 277, 294–305. <https://doi.org/10.1016/j.neuroscience.2014.06.028>
- 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>
- Ozawa, K., Hashimoto, K., Kishimoto, T., Shimizu, E., Ishikura, H., Iyo, M., 2006. Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. *Biol. Psychiatry* 59, 546–554. <https://doi.org/10.1016/j.biopsych.2005.07.031>
- Pacheco-López, G., Giovanoli, S., Langhans, W., Meyer, U., 2013. Priming of Metabolic Dysfunctions by Prenatal Immune Activation in Mice: Relevance to Schizophrenia. *Schizophr. Bull.* 39, 319–329. <https://doi.org/10.1093/schbul/sbr178>
- Paxinos, G., Watson, C., 2007. *The Rat Brain Atlas in Stereotaxic Coordinates*, 6th ed. Elsevier Inc., UK.

- Pazos, M.R., Cinquina, V., Gomez, A., Layunta, R., Santos, M., Fernandez-Ruiz, J., Martinez-Orgado, J., 2012. Cannabidiol administration after hypoxia-ischemia to newborn rats reduces long-term brain injury and restores neurobehavioral function. *Neuropharmacology* 63, 776–783. <https://doi.org/10.1016/j.neuropharm.2012.05.034>
- Piontkewitz, Y., Arad, M., Weiner, I., 2011. Abnormal Trajectories of Neurodevelopment and Behavior Following In Utero Insult in the Rat. *Biol. Psychiatry, Genetic and Environmental Contributors to Disturbed Cortical Development in Developmental Disorders* 70, 842–851. <https://doi.org/10.1016/j.biopsych.2011.06.007>
- Rahman, T., Zavitsanou, K., Purves-Tyson, T., Harms, L.R., Meehan, C., Schall, U., Todd, J., Hodgson, D.M., Michie, P.T., Weickert, C.S., 2017. Effects of Immune Activation during Early or Late Gestation on N-Methyl-d-Aspartate Receptor Measures in Adult Rat Offspring. *Front. Psychiatry* 8, 77. <https://doi.org/10.3389/fpsy.2017.00077>
- Ratnayake, U., Quinn, T., LaRosa, D.A., Dickinson, H., Walker, D.W., 2014. Prenatal exposure to the viral mimetic poly I:C alters fetal brain cytokine expression and postnatal behaviour. *Dev. Neurosci.* 36, 83–94. <https://doi.org/10.1159/000362205>
- Ratnayake, U., Quinn, T.A., Castillo-Melendez, M., Dickinson, H., Walker, D.W., 2012. Behaviour and hippocampus-specific changes in spiny mouse neonates after treatment of the mother with the viral-mimetic Poly I:C at mid-pregnancy. *Brain. Behav. Immun.* 26, 1288–1299. <https://doi.org/10.1016/j.bbi.2012.08.011>
- Reisinger, S., Khan, D., Kong, E., Berger, A., Pollak, A., Pollak, D.D., 2015. The poly(I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery. *Pharmacol. Ther.* 149, 213–226. <https://doi.org/10.1016/j.pharmthera.2015.01.001>
- Rubino, T., Parolaro, D., 2011. Sexually Dimorphic Effects of Cannabinoid Compounds on Emotion and Cognition. *Front. Behav. Neurosci.* 5, 1–5. <https://doi.org/10.3389/fnbeh.2011.00064>
- Ruskin, D.N., Murphy, M.I., Slade, S.L., Masino, S.A., 2017. Ketogenic diet improves behaviors in a maternal immune activation model of autism spectrum disorder. *PLoS ONE* 12. <https://doi.org/10.1371/journal.pone.0171643>
- Sagredo, O., Ramos, J.A., Decio, A., Mechoulam, R., Fernández-Ruiz, J., 2007. Cannabidiol reduced the striatal atrophy caused 3-nitropropionic acid in vivo by mechanisms independent of the activation of cannabinoid, vanilloid TRPV1 and adenosine A2A receptors. *Eur. J. Neurosci.* 26, 843–851. <https://doi.org/10.1111/j.1460-9568.2007.05717.x>
- Schiavon, A.P., Soares, L.M., Bonato, J.M., Milani, H., Guimarães, F.S., Oliveira, R.M.W. de, 2014. Protective effects of cannabidiol against hippocampal cell death and cognitive impairment induced by bilateral common carotid artery occlusion in mice. *Neurotox. Res.* 26, 307–316. <https://doi.org/10.1007/s12640-014-9457-0>
- Seeman, M.V., 2009. Secondary effects of antipsychotics: women at greater risk than men. *Schizophr. Bull.* 35, 937–948. <https://doi.org/10.1093/schbul/sbn023>
- Semple, B.D., Blomgren, K., Gimlin, K., Ferriero, D.M., Noble-Haeusslein, L.J., 2013. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog. Neurobiol.* 0, 1–16. <https://doi.org/10.1016/j.pneurobio.2013.04.001>
- Tham, M., Yilmaz, O., Alaverdashvili, M., Kelly, M.E.M., Denovan-Wright, E.M., Laprairie, R.B., 2018. Allosteric and orthosteric pharmacology of cannabidiol and cannabidiol-dimethylheptyl at the type 1 and type 2 cannabinoid receptors. *Br. J. Pharmacol.* <https://doi.org/10.1111/bph.14440>
- Thomas, A., Baillie, G.L., Phillips, A.M., Razdan, R.K., Ross, R.A., Pertwee, R.G., 2007. Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br. J. Pharmacol.* 150, 613–623. <https://doi.org/10.1038/sj.bjp.0707133>
- Weston-Green, K., Huang, X.-F., Deng, C., 2013. Second generation antipsychotic-induced type 2 diabetes: a role for the muscarinic M3 receptor. *CNS Drugs* 27, 1069–1080. <https://doi.org/10.1007/s40263-013-0115-5>

- Weston-Green, K., Huang, X.-F., Deng, C., 2012. Alterations to melanocortinergic, gABAergic and cannabinoid neurotransmission associated with olanzapine-induced weight gain. *PLoS ONE* 7, 1–12. <https://doi.org/10.1371/journal.pone.0033548>
- Weston-Green, K., Huang, X.-F., Deng, C., 2010. Sensitivity of the female rat to olanzapine-induced weight gain--far from the clinic? *Schizophr. Res.* 116, 299–300. <https://doi.org/10.1016/j.schres.2009.09.034>
- Wilson, C.A., Koenig, J.I., 2014. Social interaction and social withdrawal in rodents as readouts for investigating the negative symptoms of schizophrenia. *Eur. Neuropsychopharmacol.*, Negative symptoms of schizophrenia: clinical characteristics and their measurement, experimental modelling, and opportunities for improved treatment 24, 759–773. <https://doi.org/10.1016/j.euroneuro.2013.11.008>
- Xia, Y., Haddad, G.G., 1992. Ontogeny and distribution of GABAA receptors in rat brainstem and rostral brain regions. *Neuroscience* 49, 973–989.
- Xuan, I.C.Y., Hampson, D.R., 2014. Gender-Dependent Effects of Maternal Immune Activation on the Behavior of Mouse Offspring. *PLOS ONE* 9, e104433. <https://doi.org/10.1371/journal.pone.0104433>
- 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>
- Zhang, B., Han, M., Tan, S., Yang, F., Tan, Y., Jiang, S., Zhang, X., Huang, X.-F., 2017. Gender differences measured by the MATRICS consensus cognitive battery in chronic schizophrenia patients. *Sci. Rep.* 7, 11821. <https://doi.org/10.1038/s41598-017-12027-w>
- Zhang, Y., Cazakoff, B.N., Thai, C.A., Howland, J.G., 2012. Prenatal exposure to a viral mimetic alters behavioural flexibility in male, but not female, rats. *Neuropharmacology, Schizophrenia* 62, 1299–1307. <https://doi.org/10.1016/j.neuropharm.2011.02.022>
- Zhu, F., Zheng, Y., Liu, Y., Zhang, X., Zhao, J., 2014. Minocycline alleviates behavioral deficits and inhibits microglial activation in the offspring of pregnant mice after administration of polyriboinosinic–polyribocytidilic acid. *Psychiatry Res.* 219, 680–686. <https://doi.org/10.1016/j.psychres.2014.06.046>