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Improved social interaction, recognition and working memory with cannabidiol treatment in a prenatal infection (poly I:C) rat model

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Abstract

Neuropsychiatric disorders such as schizophrenia are associated with cognitive impairment, including learning, memory and attention deficits. Antipsychotic drugs are limited in their efficacy to improve cognition; therefore, new therapeutic agents are required. Cannabidiol (CBD), the non-intoxicating component of cannabis, has anti-inflammatory, neuroprotective and antipsychotic-like properties; however, its ability to improve the cognitive deficits of schizophrenia remains unclear. Using a prenatal infection model, we examined the effect of chronic CBD treatment on cognition and social interaction. Time-mated pregnant Sprague-Dawley rats (n=16) were administered polyinosinic-polycytidilic acid (poly I:C) (POLY; 4 mg/kg) or saline (CONT) at gestation day 15. Male offspring (PN56) were injected twice daily with 10 mg/kg CBD (CONT+CBD, POLY+CBD; n=12 per group) or vehicle (VEH; CONT+VEH, POLY+VEH; n=12 per group) for 3 weeks. Body weight, food and water intake was measured weekly. The Novel Object Recognition and rewarded T-maze alternation tests assessed recognition and working memory, respectively, and the social interaction test assessed sociability. POLY+VEH offspring exhibited impaired recognition and working memory, and reduced social interaction compared to CONT+VEH offspring (pvsPOLY +VEH), did not affect total body weight gain, food or water intake, and had no effect in control animals (all p>0.05). In conclusion, chronic CBD administration can attenuate the social interaction and cognitive deficits induced by prenatal poly I:C infection. These novel findings present interesting implications for potential use of CBD in treating the cognitive deficits and social withdrawal of schizophrenia.

Keywords

interaction, improved, recognition, working, memory, social, cannabidiol, model, treatment, prenatal, infection, (poly, i:c), rat

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Title: Improved social interaction, recognition and working memory with cannabidiol treatment in a prenatal infection (poly I:C) rat model.

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Running Title: Cannabidiol improves poly I:C-induced behavioural deficits.

Abstract

Neuropsychiatric disorders such as schizophrenia are associated with cognitive impairment, including learning, memory and attention deficits. Antipsychotic drugs are limited in their efficacy to improve cognition; therefore, new therapeutic agents are required. Cannabidiol (CBD), the non-intoxicating component of cannabis, has anti-inflammatory, neuroprotective and antipsychotic-like properties, however, its ability to improve the cognitive deficits of schizophrenia remains unclear. Using a prenatal infection model, we examined the effect of chronic CBD treatment on cognition and social interaction. Time-mated pregnant Sprague-Dawley rats (n=16) were administered poly I:C (POLY; 4 mg/kg) or saline (CONT) at gestation day 15. Male offspring (PN56) were injected twice daily with 10 mg/kg CBD (CONT+CBD, POLY+CBD; n=12/group) or vehicle (VEH; CONT+VEH, POLY+VEH; n=12/group) for 3 weeks. Body weight, food and water intake was measured weekly. The Novel Object Recognition and rewarded T-maze alternation tests assessed recognition and working memory, respectively, and the social interaction test assessed sociability. POLY+VEH offspring exhibited impaired recognition and working memory and reduced social interaction compared to CONT+VEH offspring ($p<0.01$). CBD treatment significantly improved recognition, working memory and social interaction deficits in the poly I:C model ($p<0.01$ vs. POLY+VEH), did not affect total body weight gain, food or water intake, and had no effect in control animals (all $p>0.05$). In conclusion, chronic CBD administration can attenuate the social interaction and cognitive deficits induced by prenatal poly I:C infection. These novel findings present interesting implications for potential use of CBD in treating the cognitive deficits and social withdrawal of schizophrenia.

INTRODUCTION

Evidence suggests that exposure to a prenatal infection during the first or second trimester of pregnancy can disrupt neurodevelopment, increasing the risk of developing neuropsychiatric disorders such as schizophrenia (Meyer, 2013). Prenatal infection with polyinosinic-polycytidilic acid (poly I:C), a synthetic double-stranded RNA virus, is a well-documented rodent model of schizophrenia-like phenotypes (Meyer, 2014; Meyer and Feldon, 2012). Following administration to the pregnant dam, poly I:C binds to the toll-like receptor 3 and initiates a maternal immune response that raises pro-inflammatory cytokines in the placenta, amniotic fluid and foetal brain (Meyer, 2013; Meyer *et al*, 2009). Neuro-inflammation in the foetal brain activates microglia, causing white matter injury and neuronal apoptosis (Meyer, 2013). Consequently, behavioural (e.g. deficits in cognition, social interaction and sensorimotor gating), neurochemical (e.g. striatal dopaminergic hyperfunction) and structural (e.g. reduced hippocampal and prefrontal cortical volumes) brain changes are observed in poly I:C offspring, which reflect observations in schizophrenia patients (as reviewed in Meyer, (2014)). These alterations emerge in the offspring during early adulthood, reflecting the delayed onset of schizophrenia symptoms observed in the clinic (Meyer, 2013). Moreover, some of the behavioural and structural changes induced by poly I:C can be reversed with clozapine and risperidone administration (Piontkewitz *et al*, 2009, 2011). Therefore, the poly I:C model shows construct, face and predictive validity, making it a useful tool to investigate pharmacological interventions for the treatment of schizophrenia (Meyer, 2014).

Cognitive dysfunction, including impairments to memory, attention and executive functioning, is experienced by 75-85% of people with schizophrenia (Barch and Ceaser,

2012). Cognitive deficits often precede the onset of other symptoms and are associated with poor medication compliance and a higher tendency for relapse in first episode psychosis (Meyer *et al*, 2011b). In fact, cognitive deficits are thought to be a better prognostic indicator in patients than other symptom domains, as earlier disease onset correlates with the severity of cognitive dysfunction (Gray and Roth, 2007) and cognitive dysfunction predicts clinical course and future functional outcomes (Green, 2006). The negative symptoms, which include alogia (lack of speech), avolition (lack of motivation), anhedonia (inability to feel pleasure) and social withdrawal, are frequently associated with disturbances to psychosocial functioning, relationships, employment and overall quality of life (Lindenmayer *et al*, 2013). While the positive symptoms of schizophrenia (hallucinations and delusions) are usually controlled by antipsychotic drug (APD) treatment, the drugs have minimal efficacy to improve the cognitive symptoms (Gray and Roth, 2007) and one-third of patients who experience negative symptoms are APD-resistant (Lindenmayer *et al*, 2013). Furthermore, APDs can cause side effects, particularly motor and metabolic (obesity and type 2 diabetes mellitus) disturbances (Weston-Green *et al*, 2013). Therefore, there is a requirement to improve the pharmacological treatment of schizophrenia.

A growing body of evidence demonstrates that cannabidiol (CBD), a component of *Cannabis sativa*, prevents hallucinations and cognitive impairment induced by cannabis and delta-9-tetrahydrocannabinol (Δ9-THC) administration (reviewed in Schubart *et al,* (2014)). Studies reported that CBD limited the positive and negative schizophrenia-like phenotypes in the MK-801 model, suggesting that CBD has antipsychotic potential (reviewed in Schubart *et al,* (2014)). These findings translate to the clinic, where CBD treatment improved positive and negative symptoms in schizophrenia patients,

performing comparably to a current APD, amisulpride, with fewer side effects (Leweke *et al*, 2012). A number of preclinical models of cognitive impairment suggest that CBD improves learning and memory (as reviewed in Osborne *et al*, (2016)); however, the effect of CBD on the cognitive deficits of schizophrenia is unclear. For example, acute CBD did not improve selective attention in schizophrenia outpatients (Hallak *et al*, 2010), or MK-801-induced social recognition memory deficits in rats (Deiana *et al*, 2015). CBD was protective against MK-801-induced object recognition memory impairment at high doses (Gomes *et al*, 2015); its effect on other cognitive domains impaired in schizophrenia (i.e. working memory) is unknown. A disadvantage of pharmacological models is that they do not incorporate neurodevelopmental or genetic approaches, which underlie the aetiology of schizophrenia in humans (Mouri *et al*, 2013). Therefore, the aim of this study was to determine whether chronic CBD treatment could improve recognition and working memory impairment and social withdrawal in a neurodevelopmental model of schizophrenia-like phenotypes. Body weight, food and water intake was also measured to determine whether CBD induces weight gain or hyperphagia.

MATERIALS AND METHODS

This study is reported in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (Kilkenny *et al*, 2010). The completed 'ARRIVE Guidelines Checklist' for reporting animal data in this manuscript is included in the Supplementary Information (Figure S1).

Ethics Statement

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

Animals and Drug Treatment

Sixteen time-mated pregnant (gestation day 8) Sprague-Dawley rats (12 weeks old) were obtained from the Animal Resources Centre (Perth, Australia), housed at the Animal Research Facility, University of Wollongong and habituated to the new environment for one week. Rats were housed individually on corncob bedding with plastic tunnels and nesting material for environmental enrichment, under a light-dark cycle (photophase 19:00-07:00 h) at 22ºC and allowed *ad libitum* access to standard laboratory chow (3.9 kcal/g; 10% fat, 74% carbohydrate, 16% protein) and water. On gestation day 15 (GD15; at 09:00 h), dams received a single intravenous injection (1 ml/kg) of either poly I:C (4 mg/kg, Sigma-Aldrich, Sydney, Australia; n=8) or saline (control; n=8) to the lateral tail vein. The dose, route of administration and timing of poly I:C administration was based on previous studies, in which behavioural impairments observed in the offspring mimic negative/cognitive schizophrenia-like phenotypes (Meyer and Feldon, 2012). Following birth, pups were culled to approximately 10 pups per litter and offspring were maintained with their respective dams until weaning on postnatal day 21 (PN21). Offspring were then pair-housed with littermates, with *ad libitum* access to standard laboratory chow and water. Pairs of male offspring were assigned to receive intraperitoneal (i.p) injections of either CBD (10 mg/kg; THC-Pharm GmbH, Frankfurt, Germany) dissolved in Tween 80 and saline

(vehicle; 1:16 (*v*/*v*); Sigma-Aldrich, Sydney, Australia), or vehicle alone (injection volume of 5 ml/kg), with an even spread of litters in each treatment group in order to control for potential litter effects. Drug solutions were prepared immediately prior to each drug administration, which occurred from PN 56, equating to late adolescence/early adulthood in humans (Andersen and Navalta, 2004). Treatments were administered for 3 weeks, twice a day, at 12 hourly intervals (07:00 h, 19:00 h), based on the half-life of CBD in the rat brain following i.p. injection (Deiana *et al*, 2011). The CBD dosage and treatment duration was based on previous studies that reported positive effects of CBD on cognition in inflammation-based rodent models (Barichello *et al*, 2012; Cassol-Jr *et al*, 2010; Fagherazzi *et al*, 2011; Schiavon *et al*, 2014). The experimental design resulted in 4 conditions: (1) offspring of control dams administered vehicle (CONT+VEH; n=12), (2) offspring of control dams administered CBD (CONT+CBD; n=12), (3) offspring of poly I:C dams administered vehicle (POLY+VEH; n=12), and (4) offspring of poly I:C dams administered CBD (POLY+CBD; n=12). Body weight, food and water intake (per cage i.e. 2 rats) were recorded weekly during the 3 week treatment period.

Behavioural Testing

After 2 weeks of CBD or vehicle treatment, rat offspring underwent a series of behavioural tests to examine social interaction, learning and memory (Figure 1A). Behavioural tests were conducted during the active phase (09:00 and 18:00 h) with a 24-hour rest period between tests to minimise stress. The time of daily testing was counterbalanced across the experimental groups. Rat behaviour was recorded using standard commercial cameras (Logitech Pty Ltd, Alexandria, Australia) and de-identified video recordings were later analysed. Equipment was cleaned with 70%

ethanol between trials to eliminate olfactory cues. The behavioural tests were performed in order of invasiveness as described below.

Novel Object Recognition (NOR) Test

The NOR test was performed as described previously, with minor modifications (Bevins and Besheer, 2006). Two objects (plastic building blocks) were placed in the home cages for 24-hour familiarisation. In the first trial (familiarisation), a rat was placed in an arena (black square arena 60cm x 60cm x 60cm, with even lighting of 20 lux) to habituate for 5 min then returned to its home cage. The two familiar objects were placed in opposite corners of the upper half of the arena and the rat was positioned in the centre of the far wall (i.e. the lower half of the arena), facing away from the objects (Figure 1B). The rat was left to explore the arena for 5 min then returned to its home cage for 1 h. In the second trial, one of the familiar objects was replaced with a novel object (toy figurine) in the arena and the rat was allowed to explore for 3 min. Time spent interacting with the objects was recorded, with interaction defined as direct contact with the object, including touching an object with the mouth, nose or paw. Accidental contact (i.e. bumping into the object in passing) and contact with the object as a means to explore other aspects of the arena (i.e. rearing) were not scored as object interaction (Bevins and Besheer, 2006). A discrimination ratio was calculated for each rat expressed as: $T_N / (T_F + T_N)$, where T_N = time spent exploring the novel object (secs) and T_F = time spent exploring the familiar object (secs). Values for the discrimination ratio ranged from 0 to 1, where a score closer to 1 indicated greater preference for the novel object, while a score closer to 0 indicated preference for the familiar object.

T-maze Test

Working memory was assessed using the T-maze rewarded alternation method described previously (Deacon and Rawlins, 2006). The apparatus consisted of a black, T-shaped maze (50 cm long x 10 cm wide, 30 cm high walls with even lighting of 20 lux), containing removable dividers to control access into the left and right arms. A central partition was used to limit access to only one arm of the T-maze at a time and increase alternation rate (Deacon and Rawlins, 2006) (Figure 1C). Prior to testing, rats were acclimatised to a reward stimulus (chocolate pellets) in their home cages. Food was restricted to 5g/100g body weight overnight prior to the habituation, training and testing days. In the habituation trial, the chocolate pellet rewards were placed at the end of the left and right arms of the T-maze and the dividers were removed. A rat was placed at the start arm and allowed to freely explore the maze for 5 min. After the habituation trial, the rat was returned to its home cage. Habituation was considered successful when rats consumed reward pellets from both arms of the T-maze (Deacon and Rawlins, 2006). As rats completed the habituation trial successfully, no further habituation trials were required. Rats then underwent training where they were taught to alternate entry into the left and right arms of the T-maze using (1) a 'forced' run: one arm of the T-maze was closed by a divider and the reward stimulus placed in the open arm, and (2) a 'choice' run: divider was removed opening both arms of the T-maze and the reward stimulus was positioned in the newly opened arm. Training took place over 3 consecutive training days and consisted of 8 trials (4 forced and 4 choice runs) per day, with randomised alternation of the divider position for each forced run. Training was considered successful when control rats achieved an 80% trial accuracy, as described by Deacon and Rawlins (2006). On the test day, the same alternation procedures were conducted as described above, with a total of 10 trials (5 forced runs, 5 choice runs) per rat. The time delay between the forced and choice runs was limited to 30 seconds and

the total trial time was 3 mins. A correct response was accepted as first entry into the correct arm. Trials were excluded from analysis if the rat jumped out of the maze. The percentage of correct responses and the latency to correct entry (secs) were recorded for each rat.

Social Interaction Test

The social interaction test was used to assess the effects of CBD on sociability based on methods previously described by our laboratory (De Santis *et al*, 2016; Du Bois *et al*, 2008). This test capitalises on the natural tendency of rats to interact with each other; therefore, a lack of interaction can be considered as social withdrawal, reflecting the negative symptoms of schizophrenia (Wilson and Koenig, 2014). Two rats from the same treatment group that had not been exposed to each other previously (i.e. unfamiliar), were placed in opposite corners of a black square arena (as described in the NOR methods). Rats were allowed to freely roam the arena for 7 mins and were then returned to their home cage. The amount of time that the 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 were then collated for statistical analysis and the average interaction time per treatment group.

Statistical Analysis

All statistical analyses were conducted using SPSS (Version 21.0, IBM, Illinois, USA). Data were tested for normality using Shapiro Wilk tests. Data points that were 2 SD outside the mean were considered as outliers and removed from further analysis. A two-way ANOVA was used to analyse normally distributed mean total body weight gain, accumulated food and water intake, NOR and social interaction test data for main

effects of 'prenatal infection' and 'offspring treatment'. Where significant interactions were observed, pairwise comparisons between groups were made with Bonferonni's adjustment for multiple comparisons. T-maze data remained non-normally distributed following log transformation; data was therefore analysed using Mann-Whitney *U* tests for comparisons between groups, with Bonferroni's correction. Comparisons were made between groups to assess the impact of prenatal infection (CONT+VEH vs. POLY+VEH), the efficacy of CBD treatment in the model (POLY+VEH vs. POLY+CBD), the effect of CBD administration in control offspring (CONT+VEH vs. CONT+CBD) and to determine whether CBD treatment restored behaviour in the poly I:C model to control-like levels (POLY+CBD vs. CONT+VEH). One-sample *t*-tests were used to determine whether NOR performance and percentage of correct entries on the T-maze test, were above chance levels (50%). Significance was set to $p<0.05$.

RESULTS

Novel Object Recognition Test

The discrimination ratio data revealed a significant interaction between 'prenatal infection' and 'offspring treatment' ($F_{(1, 37)} = 10.39$, $p=0.003$). POLY+VEH offspring had a lower discrimination ratio than CONT+VEH offspring ($p=0.001$) (Figure 2A), indicating a recognition memory deficit following maternal poly I:C exposure. CBD treatment significantly improved discrimination ratio scores of poly I:C offspring (POLY+CBD vs. POLY+VEH, $p=0.003$) (Figure 2A). There was no significant difference between the discrimination ratios of vehicle and CBD-treated control rats (CONT+VEH vs. CONT+CBD, $p=0.205$), indicating that CBD administration had no effect on recognition memory in healthy rats (Figure 2A). In fact, all groups except the

POLY+VEH group showed a significant preference for the novel object that was above chance levels (CONT+VEH, $t(9) = 12.03$, $p<0.001$; CONT+CBD, $t(9) = 4.90$, $p=0.001$; POLY+VEH, $t(9) = 1.37$, $p=0.205$; POLY+CBD, $t(9) = 5.23$, $p=0.001$; n=10/group). There was no effect of 'prenatal infection' ($F_{(1, 36)} = 2.729$, $p=0.107$) or 'offspring treatment' ($F_{(1, 36)} = 0.072$, $p=0.790$), nor an interaction between the two factors ($F_{(1, 36)} = 0.003$, $p=0.958$) on total object exploration time (Figure 2B).

Rewarded Alternation T-maze Test

Offspring exposed to prenatal infection had a significantly lower percentage of correct entries compared to control counterparts (POLY+VEH vs. CONT+VEH, $p=0.005$) (Figure 3A), indicating that exposure to prenatal infection impaired aspects of working memory. CBD treatment attenuated the working memory deficits in poly I:C offspring (POLY+VEH vs. POLY+CBD, $p=0.009$) (Figure 3A), restoring T-maze performance to control-like levels (POLY+CBD vs. CONT+VEH, $p=0.561$). CBD administration did not affect the working memory performance of control offspring (CONT+VEH vs. CONT+CBD, $p=0.686$). When the percentage of correct entries was compared to chance, all groups except the POLY+VEH group preferred to enter the correct arm (CONT+VEH, $t(9) = 5.40$, $p<0.001$, n=10; CONT+CBD, $t(10) = 7.08$, $p<0.001$, n=11; POLY+VEH, $t(11) = 0.69$, $p=0.503$, n=12; POLY+CBD, $t(10) = 5.64$, $p<0.001$; n=11). Additionally, there were no significant differences between treatment groups for the latency to first entry in the T-maze test (all $p>0.05$) (Figure 3B).

Social Interaction Test

A two-way ANOVA revealed a significant effect of 'prenatal infection' ($F_{(1, 18)} = 9.11$, $p=0.007$) and 'offspring treatment' ($F_{(1, 18)} = 10.14$, $p=0.005$) on total interaction

time and a significant interaction between the two factors ($F_{(1, 18)}$ = 9.21, p=0.007). Further investigation showed that POLY+VEH offspring spent significantly less time interacting compared to their CONT+VEH counterparts (p<0.001), indicating a social interaction deficit resulting from maternal poly I:C exposure (Figure 4). CBD treatment significantly increased total interaction time (POLY+CBD vs. POLY+VEH, p<0.001; n=6 pairs/group) (Figure 4). CBD treatment did not alter the social behaviour of control offspring (CONT+VEH vs. CONT+CBD, p=0.921; n=5 pairs/group) (Figure 4).

Body weight, food and water intake

There were no interactions between 'prenatal infection' or 'offspring treatment' on mean total body weight gain ($F_{(1, 43)}$ = 0.37, p=0.549) (Figure 5A), accumulated food intake ($F_{(1, 20)}$ = 0.21, p=0.651) (Figure 5B) or accumulated water intake ($F_{(1, 20)}$ = 0.001, p=0.970) (Figure 5C), indicating that poly I:C exposure and CBD administration did not alter basic metabolic parameters in this study.

DISCUSSION

To our knowledge, this is the first study to investigate the effect of chronic CBD treatment (10 mg/kg, twice-daily) on social interaction and cognitive deficits of adult male offspring exposed to prenatal infection. For the first time, this study has shown that CBD treatment restores working memory deficits in a model of schizophrenia-like phenotypes. This suggests that chronic CBD treatment may be beneficial in the treatment of cognitive and social interaction deficits associated with neuropsychiatric disorders in the clinical scenario. The animal model used in the present study is a well-described neurodevelopmental model that mimics aspects of the behavioural, chemical and structural brain alterations reported in schizophrenia patients (reviewed in Meyer

and Feldon (2012)). Prenatal poly I:C infection has also been used to model autism, which shares similar aetiology and some overlapping symptom domains with schizophrenia. Modelling of autism phenotypes primarily involves poly I:C administration to rodents and non-human primates during early to mid-gestation to elicit repetitive behaviour (measured in the marble-burying test), motor stereotypy (such as increased self-grooming) and decreased sociability in offspring (reviewed in Careaga et al., (2016)). In the present study, poly I:C was administered during mid to late gestation (GD15), which induces decreased sociability in offspring, and has been shown to model other negative and cognitive behavioural phenotypes relevant to schizophrenia (Meyer and Feldon, 2012). In addition, evidence demonstrates delayed onset of symptoms until early adulthood in these offspring (reviewed in Meyer (2014), which closely resembles schizophrenia rather than autism (Meyer *et al*, 2011a).

Social interaction test results confirmed the presence of a negative phenotype in male rat offspring exposed to poly I:C during mid to late gestation. Previous studies reporting social interaction deficits vary in the dose and gestational timing of poly I:C administration, as well as the social interaction test protocol used (Buschert *et al*, 2016; Ibi *et al*, 2009; Labouesse *et al*, 2015; Ratnayake *et al*, 2014; Richetto *et al*, 2015; Zhu *et al*, 2014). Overall, our study adds to the growing body of literature suggesting that social interaction deficits are a robust phenotype of the poly I:C model, regardless of the species, strain, dose and gestational timing of poly I:C administration. In the present study, CBD treatment rescued social interaction in poly I:C offspring, indicating an antipsychotic potential for CBD. This result coincides with a clinical trial where CBD significantly reduced negative symptoms (as assessed by the Positive and Negative Syndrome Scale) in schizophrenia patients after 2 weeks and continued to lower scores

until the end of treatment (4 weeks) (Leweke *et al*, 2012). On the contrary, a recent study investigated the effect of CBD on social interaction in the poly I:C model and reported no effect of low dose CBD treatment during peri-adolescence (1 mg/kg; from PN30 to PN60) on social interaction of mice (Peres *et al*, 2016). Importantly, that study also failed to model social interaction deficits in poly I:C offspring, possibly due to the low dose and gestational timing of poly I:C exposure (10 mg/kg on GD9) (Peres *et al*, 2016). Mid to late gestational poly I:C exposure is typically used to model negative behavioural phenotypes (Meyer and Feldon, 2012); however, early gestational exposure (GD9) can elicit social interaction deficits in mice (Buschert *et al*, 2016), but may require double the dose (20 mg/kg) that was used by Peres *et al*, (2016). Previous studies modelling negative behavioural phenotypes have reported a beneficial effect of CBD on social interaction in the MK-801 model (Gomes *et al*, 2015; Gururajan *et al*, 2011, 2012; Long *et al*, 2012) with no effect in spontaneously hypertensive rats (strain that presents with hyperlocomotion and social withdrawal phenotypes), while low dose CBD (1 mg/kg) enhanced social interaction of controls (Almeida *et al*, 2013). Taken together with the literature, our study suggests a therapeutic benefit of CBD on social interaction deficits in poly I:C rat offspring and aligns with the beneficial effects seen in schizophrenia patients and most preclinical models. Additional studies investigating other negative symptoms, such as anhedonia and avolition (assessed in rodents using intra-cranial self-stimulation) (Carlezon and Chartoff, 2007), would be useful to further confirm the therapeutic benefit of CBD on negative schizophrenia-like phenotypes.

The findings of the present study align with a growing body of evidence suggesting beneficial effects of CBD on cognitive deficits induced by numerous pharmacological and pathological factors (reviewed in Osborne *et al* (2016)). Preclinical studies show a

therapeutic benefit of CBD on recognition memory deficits in rodent models of Alzheimer's disease (Cheng *et al*, 2014a; Fagherazzi *et al*, 2011) and cerebral malaria (Campos *et al*, 2015), as well as working memory deficits induced by hepatic encephalopathy (Magen *et al*, 2009). In the present study, CBD treatment improved the working memory deficits of poly I:C offspring in the T-maze test. This is the first study to investigate the effect of CBD on working memory deficits in a preclinical model of schizophrenia phenotypes. The deficits caused by poly I:C coincide with Connor *et al.* (2012), who reported a reduced number of correct alternations in the T-maze test of offspring exposed to poly I:C during late gestation, indicating impaired working memory. In the present study, the latency to first entry did not differ between treatment groups in the T-maze test. The delay to first entry can be influenced by general motivation towards an incentive, arousal and attention (Pioli *et al*, 2014), providing an overall indication of the rodent's motivation to explore its environment and complete the alternation task (Deacon and Rawlins, 2006). Therefore, in the present study, the lower percentage of correct responses in the poly I:C group, coupled with no change in the latency to first entry in the present study, suggests that the outcome is due to working memory impairment and was not influenced by internal processes such as motivation.

This is the first study to investigate the effects of CBD treatment on recognition memory in a prenatal poly I:C infection model. The only other study to investigate the effect of CBD on recognition memory in schizophrenia reported that chronic, high dose CBD treatment (60 mg/kg) attenuated object recognition memory deficits induced by MK-801 administration (Gomes *et al*, 2015). MK-801 is a *N*-methyl-D-aspartate (NMDA) receptor antagonist that is used to model aspects of schizophrenia but, unlike

399 maternal poly I:C exposure, it does not mimic the developmental aspects of the disease
400 (Mouri *et al*, 2013). Recognition memory impairments have been reported previously in
401 the poly I:C model; however, the dose, frequency and timing of poly I:C administration,
402 as well as the age of the rodents during behavioural testing (adolescent vs. adulthood)
403 vary across studies (Ibi *et al*, 2009; Li *et al*, 2014; Ozawa *et al*, 2006; Ratnayake *et al*,
404 2014; Wolff *et al*, 2011). On the other hand, a previous study using a similar paradigm
405 (4 mg/kg poly I:C, administered at GD17) reported no recognition memory deficits in
406 adult male or female rat offspring following exposure to poly I:C in late gestation
407 (Howland *et al*, 2012). This contrast may be due to differences in the NOR test
408 protocols used, as the delay between the familiarisation and test trials was 24 hours
409 (Howland *et al*, 2012), compared to the 1 hour delay in the present study. The extended
410 delay may give rise to examination of long term memory as well (Antunes and Biala,
411 2012), whereas the present study design aimed to assess recognition memory using a
412 shorter inter-trial delay. In the present study, total object exploration times did not differ
413 between the treatment groups. Taken together, this suggests that the lower
414 discrimination ratio observed in poly I:C offspring was not due to lack of object
415 exploration (i.e. impaired motor activity) or an aversion to novel stimuli (i.e. a
416 tendency to spend more time with the familiar object indicated by a discrimination ratio
417 closer to 0) (as described in Deacon and Rawlins (2006)), but a lack of discrimination
418 between the familiar and novel objects.
419
420 The effects of current APDs on social interaction, recognition and working memory
421 have been reported previously in preclinical models. Studies examining social
422 behavioural deficits have shown an improvement (Calzavara et al, 2011; Deiana et al,
423 2015; Gururajan et al, 2012; Hołuj et al, 2015; Kamińska and Rogóż, 2015) or no

change (Deiana et al, 2015; Gao and Li, 2014; Hołuj et al, 2015; Snigdha and Neill, 2008) following administration with olanzapine, risperidone and aripiprazole. In addition, adolescent olanzapine administration impaired working memory of adult rats in the T-maze test (Milstein et al, 2013), while olanzapine and risperidone administration improved MK-801- and PCP-induced object recognition memory deficits in mice and rats (Li et al, 2016; Mutlu et al, 2011; Snigdha et al, 2010). Recognition and working memory impairments have been linked to dysfunction in the dopaminergic and glutamatergic/GABAergic systems, particularly in the hippocampus and cortical circuitry (Abi-Dargham and Moore, 2003; Antunes and Biala, 2012), and these systems are highly implicated in schizophrenia pathology (reviewed in Lewis (2012)). Hyperfunction in the dopaminergic mesolimbic pathway, which projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is implicated in the positive symptoms of schizophrenia (Brisch *et al*, 2014). Conversely, hypofunction in the dopaminergic mesocortical pathway, which projects from the VTA to the prefrontal cortex (PFC), is implicated in the negative and cognitive symptoms of schizophrenia (Goldman-Rakic *et al*, 2004). Dopamine also modulates glutamergic activity directly through dopamine D1 receptors located on pyramidal neurons or indirectly through dopamine D1/D2R co-localisation on GABAergic interneurons, indicating an interaction between the dopaminergic and glutamatergic/GABAergic systems in schizophrenia (Laruelle, 2014). Interestingly, poly I:C offspring also exhibit altered dopaminergic signalling (Luchicchi *et al*, 2016; Meyer *et al*, 2008) and decreased expression of glutamatergic (e.g. NR1 subunit of the NMDAR) and GABAergic markers (e.g. GAD_{67} and the calcium-binding protein parvalbumin) in the PFC and hippocampus (Forrest *et al*, 2012; Ibi *et al*, 2009; Meyer *et al*, 2008), reflecting some of the molecular changes observed in schizophrenia patients.

There is limited literature investigating the mechanisms underlying the therapeutic benefits of CBD in neuropsychiatric disorders (Osborne *et al*, 2016); however, recent studies have suggested that CBD interacts with the dopaminergic system (Renard *et al*, 2016; Seeman, 2016). For example, CBD inhibited binding of the selective dopamine D2R antagonist, domperidone, in rat striatal tissue in the same manner as the APD, aripiprazole. In contrast to other atypical APDs (such as olanzapine) that antagonise the D2R, CBD is hypothesised to act as a partial agonist at D2Rs to normalise dopaminergic signalling (Seeman, 2016). In addition, CBD administered into the NAc attenuated amphetamine-induced dopaminergic hyperfunction (via the mammalian target of rapamycin (mTOR) signalling) in the VTA of rats (Renard *et al*, 2016). Given that CBD attenuates dopaminergic hyperfunction in the mesolimbic pathway and shows partial agonistic activity at D2Rs, it is possible that CBD may also normalise dopaminergic hypofunction in the mesocortical pathway to improve negative and cognitive symptoms of schizophrenia. Limited evidence suggests that CBD also interacts with the glutamatergic and GABAergic systems. For example, CBD restored MK-801-induced deficits in parvalbumin expression and GRIN1 mRNA expression (gene that encodes for the NR1 subunit of the NMDAR) in the PFC and hippocampus, respectively (Gomes *et al*, 2014). Further investigation into the impact of CBD on these major neurotransmitter systems implicated in schizophrenia pathology may shed light on the mechanisms underlying the improvement of social behaviour, recognition and working memory in poly I:C offspring in the present study.

It is important to note that while CBD improved social interaction, working and recognition memory deficits in poly I:C offspring in the present study, it had no effect

474 in control offspring. This observation is supported by previous preclinical studies
475 (Barichello *et al*, 2012; Campos *et al*, 2015; Cassol-Jr *et al*, 2010; Cheng *et al*, 2014a,
476 2014b; Fagherazzi *et al*, 2011; Long *et al*, 2012) and demonstrates a tendency for CBD
477 to improve deficits associated with pathological states, rather than alter behaviour in
478 healthy rodents. In the present study, CBD was administered twice-daily based on
479 evidence that the half-life of the drug is approximately 11 hours in the rat brain
480 following an intraperitoneal injection (Deiana *et al*, 2011). The dose of CBD used in the
481 present study (10mg/kg) was based on previous studies that reported improvement of
482 cognitive impairment in rodent models, without negative side-effects (Barichello *et al*,
483 2012; Cassol-Jr *et al*, 2010; Fagherazzi *et al*, 2011; Schiavon *et al*, 2014). Consideration
484 of the biphasic dose-response of CBD is important as high doses of CBD can lead to
485 negative effects such as sedation (600mg) and hyperprolactinemia (120-240 mg/kg) in
486 healthy humans and rats, respectively, which are side-effects also associated with APDs
487 (Zuardi, 2008). Further research into any long-term negative effects of CBD would be
488 prudent. Current second generation APDs such as olanzapine, clozapine and risperidone
489 are also associated with metabolic side effects including diabetes and obesity that can
490 hinder the treatment of schizophrenia patients (Weston-Green *et al*, 2013). We found no
491 effect of CBD on total body weight gain, food intake or water intake over the treatment
492 period. Using a female rodent model, our laboratory has shown that APD administration
493 increases body weight gain and food intake, and is associated with changes in metabolic
494 signalling pathways in the brain and periphery (Weston-Green *et al*, 2011, 2012a,
495 2012b). In humans, women are particularly sensitive to the metabolic side effects of
496 APDs (Seeman, 2009) and female rats mimic this sensitivity (Weston-Green *et al*,
497 2010), while male rats appear to confer a level of resistance to APD-induced obesity
498 (Minet-Ringuet *et al*, 2006; Weston-Green *et al*, 2010) but the mechanisms are unclear.

A limitation of the present study is the use of males only; further studies investigating the effects of CBD on metabolic parameters, as well as CBD's ability to restore social and cognitive poly I:C-induced deficits in female rats are necessary. Nevertheless, the lack of weight gain change in CBD-treated poly I:C offspring aligns with findings from a clinical trial that reported no alterations in the body weight of schizophrenia patients (82% male), following one month of CBD treatment (Leweke *et al*, 2012). Overall, our finding adds to the limited literature suggesting that CBD may have low weight gain liability. The present study did not employ a cross-fostering paradigm. Previous studies have shown that some behavioural deficits (e.g. latent inhibition and conditioned fear) are present in control offspring cross-fostered to poly I:C dams (Meyer *et al*, 2006; Schwendener *et al*, 2008), suggesting that changes in maternal behaviour can influence the behavioural phenotype of offspring. Future studies could address the potential influence of maternal behavioural changes on the phenotypes observed in the present study.

Summary

This study has shown for the first time that CBD can improve recognition and working memory and social interaction deficits in a prenatal poly I:C infection model, reinforcing the potential of CBD to ameliorate the negative symptoms of schizophrenia. Chronic CBD treatment increased preference for the novel object in the NOR, improved working memory performance in the T-maze test and restored social interaction deficits in poly I:C offspring. Future studies investigating the efficacy of CBD to improve cognition in this model would benefit from considering additional cognitive domains that are impaired in neuropsychiatric disorders, including spatial learning and memory, attention or cognitive flexibility as outcome measures. Although the mechanisms of

action of CBD are not well understood, CBD may exert its therapeutic effects on cognition by targeting key neurotransmitter systems (e.g. dopaminergic, glutamatergic, GABAergic) implicated in cognitive function, but more research is needed to elucidate the underlying mechanisms. Nevertheless, these novel findings present interesting implications for the use of CBD to treat the social and cognitive deficits associated with disorders such as schizophrenia.

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Figure Legends

Figure 1: Experimental design and methods used to investigate the effects of cannabidiol (CBD) treatment on offspring behaviour in the poly I:C rat model. (a) The experimental timeline used in the present study with the age of offspring in postnatal (PN) days. (b) A schematic of the Novel Object Recognition test used to assess recognition memory of offspring. (c) A schematic of the rewarded T-maze alternation test used to assess working memory performance of offspring.

Figure 2: The effect of cannabidiol (CBD) or vehicle (VEH) administration on recognition memory of control (CONT) and poly I:C (POLY) offspring in the Novel Object Recognition test. (a) Maternal poly I:C exposure significantly reduced discrimination ratio scores compared to control male offspring, which was attenuated by CBD treatment (n=10 rats/group). (b) All groups showed comparable total object exploration times (n=9-11 rats/group). Data presented as mean ± SEM. $^{**}p<0.01$ vs. CONT+VEH rats, $^{\#\#}p<0.01$ vs. POLY+VEH rats.

Figure 3: The effect of cannabidiol (CBD) or vehicle (VEH) administration on working memory of control (CONT) and poly I:C (POLY) offspring in the rewarded T-maze alternation test. (a) CBD treatment attenuated poly I:C-induced working memory deficits in male offspring (n=10-12 rats/group). (b) The latency to first entry did not differ between treatment groups (n=10-12 rats/group). Data presented as mean ± SEM. $^{**}p<0.01$ compared to CONT+VEH rats, $^{\#\#}p<0.01$ compared to POLY+VEH rats.

Figure 4: Effect of cannabidiol (CBD) or vehicle (VEH) administration on mean total interaction time of control (CONT) and poly I:C (POLY) offspring in the social interaction test. The data are presented as mean ± SEM, for pairs of rats (n=5 pairs/control group; n=6 pairs/poly I:C group). ***p<0.001 vs. CONT+VEH rats, $^{###}p$<0.001 vs. POLY+VEH rats.

Figure 5: The effect of cannabidiol (CBD; 10 mg/kg) or vehicle (VEH) administration on basic metabolic parameters in control (CONT) and poly I:C (POLY) male offspring. (a) Maternal poly I:C exposure and CBD treatment had no effect on mean total body weight gain. (b) All groups showed comparable mean accumulated food and (c) water intake over the course of the study. Data presented as mean ± SEM, n=12 rats/group (except the CONT+CBD group, where n=11) for body weight gain and n=6 pairs of rats/group for mean accumulated food and water intake.