1-1-2018

The effects of antipsychotics on the density of cannabinoid receptors in selected brain regions of male and female adolescent juvenile rats

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Abstract

Antipsychotic drugs have been increasingly prescribed to children and adolescents for treating various mental disorders, such as childhood-onset schizophrenia. The abnormality of endocannabinoid system is involved in the pathophysiology of these disorders in juveniles. This study investigated the effect of antipsychotics on the cannabinoid (CB) receptors in the brain of both male and female juvenile rats. The postnatal rats (PD23±1) were administered aripiprazole (1 mg/kg), olanzapine (1 mg/kg), risperidone (0.3 mg/kg) or vehicle (control) for 3 weeks. Quantitative autoradiography was used to investigate the binding densities of [3 H]CP-55940 (an agonist for CB1R and CB2R) and [3 H]SR141716A (a selective CB1R antagonist) in the rat brains. Risperidone significantly upregulated the [3 H]CP55940 and [3 H]SR141716A bindings in the prefrontal cortex (PFC), nucleus accumbens core (NAcC), nucleus accumbens shell (NAcS), cingulate cortex (Cg), and the caudate putamen (CPu) in male rats. Moreover, aripiprazole significantly elevated the [3 H]SR141716A binding in the Cg and NAcS of female rats. Furthermore, there is an overall higher [3 H]SR141716A binding level in the brain of female rats than male rats. Therefore, treatment with aripiprazole, olanzapine and risperidone could induce differential and gender specific effects on the binding density of cannabinoid receptors in the selected brain regions of childhood/adolescent rats.

Publication Details

Lian, J. & Deng, C. (2018). The effects of antipsychotics on the density of cannabinoid receptors in selected brain regions of male and female adolescent juvenile rats. Psychiatry Research, 266 317-322.

Psychiatry Research 266 (2018) 317–322

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

The effects of antipsychotics on the density of cannabinoid receptors in selected brain regions of male and female adolescent juvenile rats

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ARTICLE INFO

Keywords:
Aripiprazole
Risperidone
Olanzapine
Cannabinoid receptors
Adolescent rat

ABSTRACT

Antipsychotic drugs have been increasingly prescribed to children and adolescents for treating various mental disorders, such as childhood-onset schizophrenia. The abnormality of endocannabinoid system is involved in the pathophysiology of these disorders in juveniles. This study investigated the effect of antipsychotics on the cannabinoid (CB) receptors in the brain of both male and female juvenile rats. The postnatal rats (PD23 ± 1) were administered aripiprazole (1 mg/kg), olanzapine (1 mg/kg), risperidone (0.3 mg/kg) or vehicle (control) for 3 weeks. Quantitative autoradiography was used to investigate the binding densities of [^{3}H]CP-55940 (an agonist for CB1R and CB2R) and [^{3}H]SR141716A (a selective CB1R antagonist) in the rat brains. Risperidone significantly upregulated the [^{3}H]CP55940 and [^{3}H]SR141716A bindings in the prefrontal cortex (PFC), nucleus accumbens core (NAcC), nucleus accumbens shell (NAcS), cingulate cortex (Cg), and the caudate putamen (CPu) in male rats. Moreover, aripiprazole significantly elevated the [^{3}H]SR141716A binding in the Cg and NAcS of female rats. Furthermore, there is an overall higher [^{3}H]SR141716A binding level in the brain of female rats than male rats. Therefore, treatment with aripiprazole, olanzapine and risperidone could induce differential and gender specific effects on the binding density of cannabinoid receptors in the selected brain regions of childhood/adolescent rats.

1. Introduction

In recent years, prescriptions of atypical antipsychotic drugs (APDs) (mostly off-label use, particularly olanzapine, risperidone and aripiprazole), in children and adolescents have been dramatically increased for treating various mental disorders, such as childhood-onset schizophrenia, bipolar disorder, autism, attention deficit hyperactivity disorder, and Tourette's disorder (Ronsley et al., 2013). These APDs target multiple neurotransmission receptors, particularly dopamine D2 and serotonin (5-HT)2 receptors to achieve their therapeutic action (Ginovart and Kapur, 2012; Meltzer and Massey, 2011). Olanzapine and risperidone are antagonists at these neurotransmission receptors (Correll, 2010), while aripiprazole is a partial agonist of the dopamine D2 and 5-HT1A receptors, a partial antagonist of the 5-HT2A receptor, and is sometimes defined as a D2 receptor (D2R) functional selective drug (Di Sciascio and Riva, 2015; Levoyer et al., 2007; Mailman, 2007; Mailman and Murthy, 2010). It is important that those receptors also play crucial roles in neurodevelopment and almost all of the core brain functions (Ginovart and Kapur, 2012; Meltzer and Massey, 2011).

Childhood-adolescence is a critical period of neural development, with sculpting of the neuronal pruning, apoptosis and myelination (Schneider, 2008). Substances, such as APD treatment, during this crucial neurodevelopmental period may impact brain maturation and plasticity in brain neurotransmission (Ronsley et al., 2013; Schneider, 2008). Cannabinoid receptor mediates physiological and behavioural effects of natural and synthetic cannabinoids (Herkenham et al., 1991). It has been shown that developmental exposure to cannabinoids may induce subtle and long-lasting neuro-functional alterations (Trezza and Vanderschuren, 2008). Moreover, it has been indicated that the endocannabinoid CB1 receptors (CB1R) and their endogenous ligands are expressed in the early brain development period in rodents (Vitalis et al., 2008). The CB1R are widely distributed in the cerebral cortical brain regions of both humans and rats, including the prefrontal cortex (PFC), cingulate cortex (Cg), caudate putamen (CPu), and nucleus accumbens (NAc) (Breivogel and Childers, 1998; Derbenev et al., 2004). These brain regions are associated with the pathophysiology of

Abbreviation: 5-HT, serotonin; ADHD, attention deficit hyperactivity disorder; ANOVA, analysis of variance; APDs, antipsychotic drugs; BSA, bovine serum albumin; cAMP, cyclic adenosine monophosphate; Cg, cingulate cortex; CPu, caudate putamen; CB1R, cannabinoids 1 receptor; CB2R, cannabinoids 2 receptor; D2R, D2 receptor; NAc, nucleus accumbens; NAcC, nucleus accumbens core; NAcS, nucleus accumbens shell; PFC, prefrontal cortex; PD, postnatal day; RT, room temperature; SEM, standard error of mean

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https://doi.org/10.1016/j.psychres.2018.03.030
Received 30 August 2017; Received in revised form 5 January 2018; Accepted 8 March 2018
Available online 16 March 2018
0165-1781/

childhood schizophrenia, bipolar disorder, autism, and attention deficit hyperactivity disorder (ADHD) (Almeida et al., 2014; Zavitsanou et al., 2004), as well as the action of APDs (Cheng et al., 2008), but not involved directly in body weight regulation (Theisen et al., 2007). The elevation of CB1R binding was observed in the prefrontal area of brains from schizophrenic and other mental disorder patients (Ceccarini, 2014; Dalton et al., 2011; Jenko et al., 2012) Therefore, the endocannabinoid system plays a vital role in brain development and the pathology of various mental disorders (Renard et al., 2014), and contributes to the efficacy of APDs (Berk et al., 1999; Sundram et al., 2005).

Except for the endocannabinoid CB1R, the brain regions, including the PFC, Cg, CPu, and NAc also contain high levels of dopamine and 5-HT receptors; in these brain regions there are interactions between the monoamine neurotransmitters and the endocannabinoid system (Hermann et al., 2002; Wiley et al., 2008). The dopamine and 5-HT receptors are located in the mesocortical, mesolimbic and nigrostriatal pathways, which contribute to the effects of APDs on the function of cognition, affect and motor, respectively (Glass et al., 1997; Wiley et al., 2008). A number of studies have suggested the interaction between cannabinoid receptors and dopamine/5-HT receptors. As a selective CB1R antagonist, SR141716A upregulated the expression of dopamine, 5-HT, and noradrenaline in the PFC of rats (Tzavara et al., 2003). In the primary cell culture of striatal neurons, both the CB1R agonist HU210 and the D2R agonist quinpirole inhibited forskolin-stimulated cyclic adenosine monophosphate (cAMP) accumulation when applied separately, while HU210 and quinpirole in combination augmented cAMP accumulation, which was blocked by the CB1R antagonist SR141716A or the D2R antagonist sulphide (Glass et al., 1997). One previous study in the rat brain showed that the D2R level could be reduced by SR141716A through the CB1R in the extrapyramidal system (Alonso et al., 1999). On the other hand, it was reported that neurological and psychiatric disorders are often associated with long-term impairment of serotonergic and endocannabinoid control of synaptic plastic in the PFC (Ferreira et al., 2012). This is partially attributed to the highly overlapping distribution pattern of the cannabinoid CB1R and 5-HT receptors in the brain (Hermann et al., 2002). The previous study from our group reported that early treatment with various APDs had different effects on the 5-HT and dopamine receptors with some gender-dependent changes (Lian et al., 2016). It has been suggested that APDs increased CB1R availability in schizophrenia patients (Ranganathan et al., 2016). Therefore, APDs may regulate cannabinoid receptors through dopamine or serotonin neurotransmission.

It is worth noting that the onset of schizophrenia and bipolar disorder often occurs during late adolescence; therefore, evaluation of APD interaction with the endocannabinoid system in adolescents is indispensable (Wiley et al., 2008). Thus, to determine the effects of the APDs olanzapine, risperidone and aripiprazole on the endogenous cannabinoid system in the adolescent rat brain, the binding density of the cannabinoid CBR in the PFC, CPu, and NAc of adolescent rats was measured after 3 weeks' APD treatment.

2. Method

2.1. Animals, diet and experimental procedures

Timed pregnant Sprague Dawley rats (at gestation day 16) were obtained from the Animal Resources Centre (Perth, WA, Australia). They were housed in individual cages and allowed *ad-libitum* access to standard laboratory chow diet (3.9 kcal/g: 10% fat, 74% carbohydrate, 16% protein) and water under a light (07:00 to 19:00) and dark (19:00 to 7:00) cycle and temperature control (22 °C) throughout the experiment (Deng et al., 2012; Lian et al., 2014). Day of birth was recognised as postnatal day (PD) 0. Pups were sexed on PD14, and 24 male and female rats were weaned on PD21 and housed in individual cages.

Before the treatment procedures, rats were trained for self-administration of the drug by feeding them using 0.3 g cookie dough (including 30.9% cornstarch, 30.9% sucrose, 6.3% gelatine, 15.5% casein, 6.4% fibre, 8.4% minerals and 1.6% vitamins) without drug twice a day during PD18-21. There were four treatment groups: (1) Aripiprazole (1 mg/kg, 3 times/day, Otsuka, Japan; n = 6), (2) Olanzapine (1 mg/kg,3 times/day, Eli Lilly, USA; n = 6), (3) Risperidone (0.3 mg/kg, 3 times/day, Janssen, USA; n = 6) or (4) Vehicle (control; n = 6) for 3 weeks (a period corresponding to the childhood-adolescence period in humans) (Andersen, 2003). Drugs were prepared in advance by mixing with cookie dough pellets and droplets of water, and were administered 3 times per day (8 ± 1 h intervals) orally for 3 weeks (Lian et al., 2014). The rats in the control group received an equivalent pellet without drug. Rats were observed throughout the experiment to ensure all cookie dough pellets were consumed. This study was approved by the Animal Ethics Committee, University of Wollongong, Australia (AE12/20); and all the procedures complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

2.2. Histological procedures

Forty eight hours after the last drug treatment, the rats were sacrificed, their brain tissue was removed and frozen in liquid nitrogen, and then stored at −80 °C until analysis. Brains were coronally sectioned at −18 °C into 14 μm using a cryostat (Leica CM1850, Leica Microsystem, Germany). Sections were thaw-mounted onto Polysine™ Microscope Slides (Menzel GmbH & Co. KG, Braunchweig, Germany) and stored at −20 °C. A set of sections from each animal was stained with 0.5% cresyl violet solution (Nissl staining) and used to confirm anatomical structures.

2.3. [^{3}H]CP-55940 binding

The cannabinoid receptor binding was performed using [^{3}H]CP-55940 (an agonist to CB1R and CB2R) as previously described (Deng et al., 2007). Briefly, brain sections containing the PFC, Cg, NAc and CPu were thawed at room temperature (RT), and after 30 min of pre-incubation in 50 mM Tris buffer (pH 7.4) containing 5% bovine serum albumin (BSA), they were incubated with 10 nM[^{3}H]CP-55940 (specific activity: 67 Ci/mmol; ki: 1.07 nM; PerkinElmer, USA) in 50 mM Tris buffer containing 5% BSA for 2 h at RT to determine total binding. Non-specific binding was determined by incubating the next sequential sections with 10 nM [^{3}H]CP-55940 incubation buffer, with the addition of 10 μM CP-55940 (Sigma Pharmaceuticals, Australia). Slides were washed for 5 min in ice-cold buffer, dipped in ice-cold distilled water, and then dried under a stream of cool air to remove excess buffer salts (Weston-Green et al., 2008).

2.4. [^{3}H]SR141716A binding

In brief, binding of [^{3}H]SR141716A (a CB1R selective antagonist, specific activity: 20.8 Ci/mmol, PerkinElmer, USA) using [^{3}H] SR141716A was performed based on procedures previously described (Deng et al., 2007). In brief, sections were pre-incubated in 50 nM Tris buffer including 5%BSA (pH 7.4) for 15 min at RT. Sections were then incubated for 1 h at RT in the same buffer containing 1.5 nM [^{3}H] SR141716A (specific activity: 67 Ci/mmol; ki: 2.04 nM; Amersham, UK) and 0.1% bovine serum albumin (BSA) for the total binding. Non-specific binding was determined with the addition of 100 μM HU210. After incubation, the sections were washed in ice-cold buffer containing 0.1% BSA (2 × 30 min), dipped in distilled water and air dried (Deng et al., 2007).

2.5. Autoradiography and quantification of cannabinoid receptor binding

All of the receptor binding slides were exposed to Kodak BioMax MR

film for 3 months, together with autoradiographic standards ([^{3}H]microscales from Amersham), in X-ray film cassettes. This is allowed by the analysis of binding images using the Multi-analyst image analysis system (Bio-Rad, USA). The specific binding was calculated by deducting nonspecific binding from total binding. A set of sections from each animal were stained with 0.5% cresyl violet solution (Nissl staining) and used to confirm anatomical structures. Specific brain regions in this project were identified by reference to the Nissl-stained sections and a standard rat brain atlas (Paxinos and Watson, 2007).

2.6. Statistical analysis

Statistical analysis was performed using SPSS (IBM version 21.0, SPSS Inc., NY, USA). The Kolmogorov-Smirnov test was used to examine the distribution of data from all experiments. The receptor binding density in relevant rat brain regions was analysed by two-way ANOVAs (Treatment × Gender). Post-hoc Dunnett-T tests allowed for comparison between groups and the Mann-Whitney U test was applied to the data without abnormal distribution. All data are expressed as mean ± SEM, and statistical significance will be accepted when $p < 0.05$.

3. Results

3.1. [^{3}H]CP-55940 binding

Examples of [^{3}H]CP-55940 binding to cannabinoid receptors are presented in Fig. 1 (C and D). Two-way ANOVAs revealed the significant effects of the Treatment factor on [^{3}H]CP-55940 binding in the PFC ($F_{3,40} = 3.863$, $p = 0.016$), Cg ($F_{3,40} = 3.682$, $p = 0.020$), NAc core (NAcC) ($F_{3,40} = 3.771$, $p = 0.018$), NAc shell (NAcS) ($F_{3,40} = 2.875$, $p = 0.048$), and CPu ($F_{3,40} = 3.721$, $p = 0.019$). Although there was no significant effect of the Gender factor in these brain nuclei, there was significant interaction between the two factors in the PFC ($F_{3,40} = 4.543$, $p = 0.008$). In the male rats, risperidone treatment significantly increased the levels of [^{3}H]CP-55940 binding density (Fig. 1). Post hoc analysis identified a significant increase in [^{3}H]CP-55940 binding density in the PFC, NAcC ($p < 0.01$) and Cg, CPu and NAcS ($p < 0.05$) in the rats receiving the risperidone treatment (Fig. 2). However, there was no significant difference between treatment groups in female rats (all $p > 0.05$) (Fig. 2).

3.2. [^{3}H] SR141716A binding

Examples of [^{3}H]SR141716A binding to CB1R are presented in Fig. 1 (E and F). Overall, female adolescent rats had a higher CB1R binding density in the Cg ($F_{1,40} = 12.488$, $p = 0.001$), NAcC ($F_{1,40} = 10.297$, $p = 0.003$), NAcS ($F_{1,40} = 9.786$, $p = 0.003$), and CPu ($F_{1,40} = 8.825$, $p = 0.005$) than the males. The significant effect of the Treatment factor was also observed in the PFC ($F_{3,40} = 4.082$, $p = 0.013$). Risperidone significantly increased [^{3}H]SR141716A binding in the PFC, NAcC, NAcS ($p < 0.05$), and CPu ($p < 0.01$) (Fig. 3). Aripiprazole treatment significantly upregulated the [^{3}H]SR141716A binding in the Cg and NAcS ($p < 0.05$) of female rats. There were significant differences between male and female rats by [^{3}H]SR141716A binding in the Cg, PFC, NAcC, NAcS and CPu (all

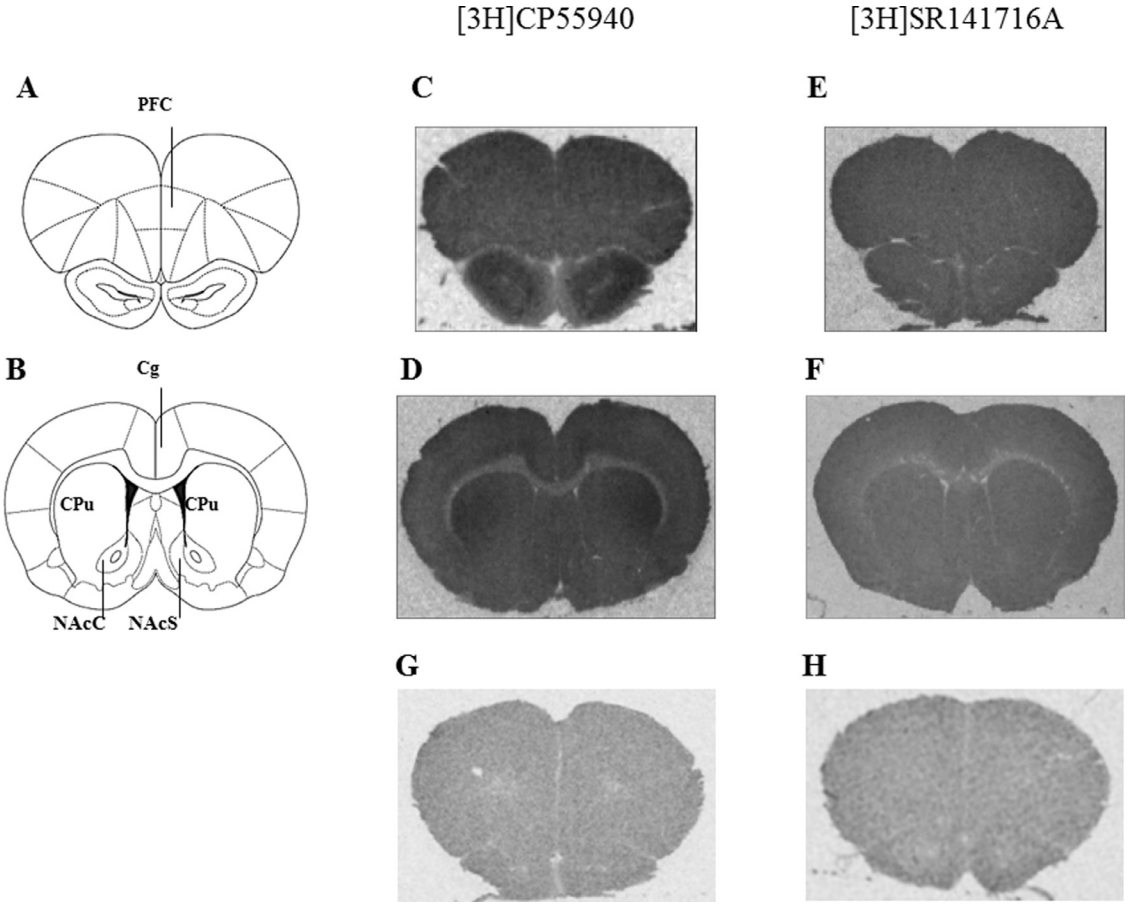

Fig. 1. Examples of [^{3}H]CP55940 and [^{3}H]SR141716A bindings in the rat brain. A-B, The schematic diagram is adapted from a rat brain atlas (Paxinos and Watson, 2007) showing the level of Bregma 4.68 mm (A) and 1.08 mm (B). C–D, examples of autoradiograms to show [^{3}H]CP5594 binding (from the non-treatment group of male rats). E–F, examples of [^{3}H]SR141716A binding (from the non-treatment group of female rats). G–H, examples of the non-specific bindings from male and female rats, respectively. Abbreviations: PFC, prefrontal cortex; Cg, cingulate cortex; CPu, caudate putamen; NAcC, nucleus accumbens core; NAcS, nucleus accumbens shell.

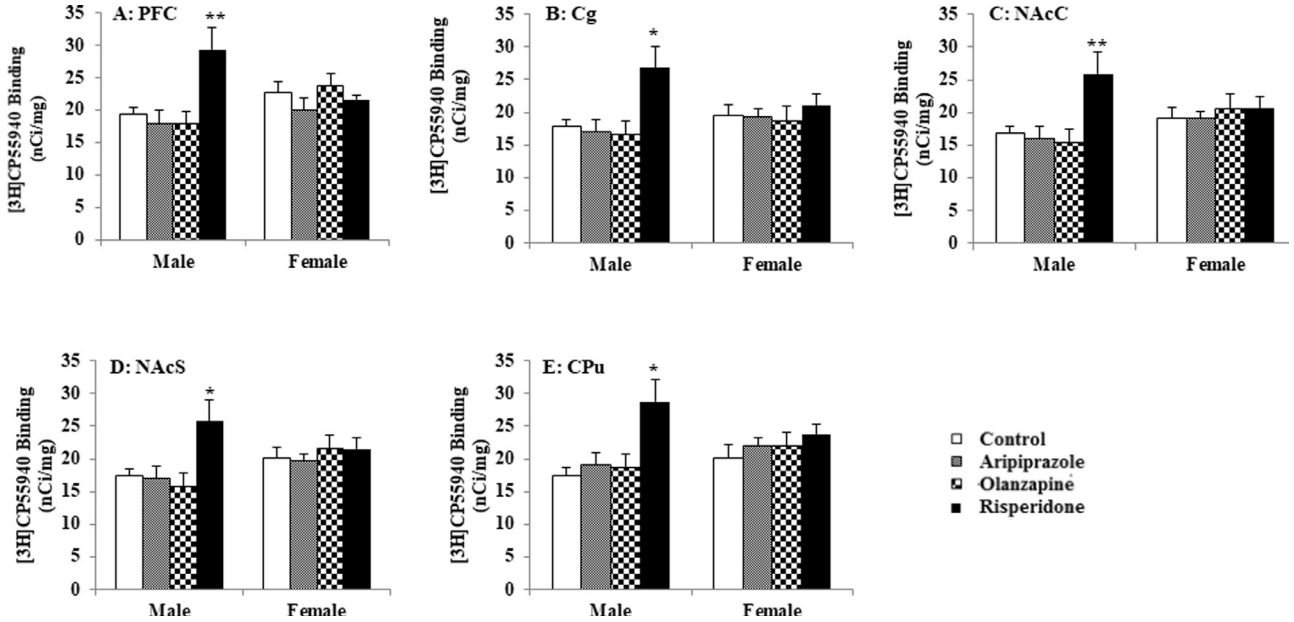

Fig. 2. The effects of aripiprazole, olanzapine, and risperidone treatment on [^{3}H]CP55940 binding (nCi/mg tissue) in (A) the prefrontal cortex (PFC), (B) cingulate cortex (Cg), (C) nucleus accumbens, core (NAcC), (D) nucleus accumbens, shell (NAcS) and (E) caudate putamen (CPu) of both male and female adolescent rats (n = 6/group). Data shown are the mean values ± SEM. *$p < 0.05$, **$p < 0.01$ *vs.* control.

$p < 0.05$) with aripiprazole treatment (Fig. 3), while the clear difference between males and females was observed in the Cg, NAcC, NAcS, and CPu with olanzapine treatment ($p < 0.05$) (Fig. 3).

4. Discussion

This study investigated the effects of olanzapine, risperidone and aripiprazole treatment during PD22-42 (a period corresponding to the childhood-adolescence period in humans) on the binding of [^{3}H]CP55940 and [^{3}H]SR141716A for cannabinoids receptors in selected brain regions of both male and female adolescent rats. To our knowledge, this is the first study to examine the effects of APDs on cannabinoid receptors in both adolescent male and female rats using the two cannabinoid ligands. The two cannabinoid receptor bindings showed different binding patterns in these brain regions.

The endocannabinoids CB1R and CB2R are present from the early stages of gestation and play a number of vital roles for the developing organism (Fride, 2004). The roles of endocannabinoid CB1R in the developing organism include embryonal implantation, neural development, neuroprotection, initiation of suckling in the newborn, while they also contribute to memory, motor and addictive behaviours and the pathogenesis of various mental disorders (Fride, 2004; Ujike and

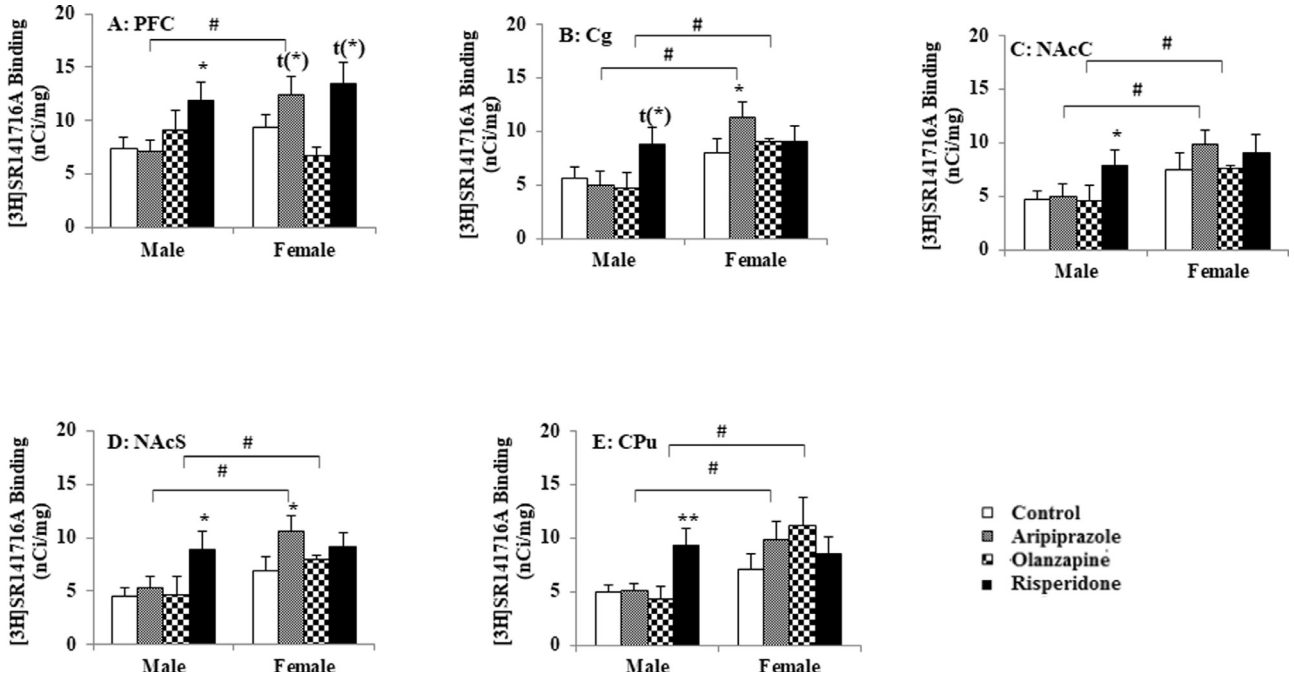

Fig. 3. The effects of aripiprazole, olanzapine, and risperidone treatment on [^{3}H]SR141716A binding (nCi/mg tissue) in (A) the prefrontal cortex (PFC), (B) cingulate cortex (Cg), (C) nucleus accumbens, core (NAcC), (D) nucleus accumbens, shell (NAcS) and (E) caudate putamen (CPu) of both male and female adolescent rats (n = 6/group). Data shown are the mean values ± SEM. *$p < 0.05$, **$p < 0.01$ *vs.* control. #$p < 0.05$, male vs. female. *t(*)*0.05 < $p < 0.1$ *vs.* control.

Morita, 2004). CB2Rs are primarily located on immune cells, and play important roles in cell-mediated and humoral immunity, and neuroinflammation, while they are also expressed in the brain and contribute to neuronal and functional expression (Cabral and Griffin-Thomas, 2009; Onaivi et al., 2006; Onaivi et al., 2012). One post-mortem study with a radioligand showed the differences in the levels of [^{3}H]CP55940 binding in the dorsolateral PFC, CPu and hippocampus of 14 schizophrenic patients compared with controls (Dalton et al., 2011). Therefore, it is important to investigate the modulation of early APD treatments on cannabinoid receptor development, which may be implicated in treatment for various mental disorders in adolescents, although APDs such as olanzapine exhibited negligible affinities to CB1R (Theisen et al., 2007).

This study suggest that risperidone administration significantly upregulated the [^{3}H]CP55940 bindings in all brain regions examined in male adolescent rats. In line with our findings, a previous study also revealed that risperidone treatment increased cannabinoid receptor bindings using [^{3}H]CP55940 in the NAc of male rats (Secher et al., 2010). It was also reported that increased binding by [^{3}H]CP55940 occurred in the NAc after prolonged clozapine treatment of rats (Sundram et al., 2005). Another previous study reported olanzapine enhanced the [^{3}H]CP55940 binding in the NAc of adult male rats (Sundram et al., 2005). Although the same olanzapine dosage was used in those studies (1 mg/kg), there was no effect of early olanzapine treatment on [^{3}H]CP55940 binding observed in any brain region of adolescent rats, which may be due to the different age of the rats (adolescent rats in this study vs. 6-weeks in Sundram's study), and administration method (mini-pump without drug washout period in Sundram's study, vs. cookie dough with drug washout period in this study). The above age difference result was also supported by the previous study, in that the change of cannabinoid CB1R in the brain is related to the age of the rats (Liu et al., 2003). It is interesting that, in this study, APDs did not affect [^{3}H]CP55940 binding in female rats. Although the [^{3}H]CP55940 binds to both CB1 and CB2 receptors, its binding affinity to the CB1R is approximately 100 times higher than that of the CB2R (Onaivi et al., 2006). This suggests risperidone elevated mainly CB1R in these brain regions; however, the roles of CB2R could not be completely excluded. Previous studies have reported that brain CB2R is involved in neurophysiological functions including modulating midbrain dopamine neuronal activity, dopamine-related behaviour, and hippocampal synaptic transmission (Li and Kim, 2016). Deletion of CB2R induced long-term memory deficit and schizophrenia-like behaviours in mice (Li and Kim, 2016; Ortega-Alvaro et al., 2011). There are also genetic associations between polymorphism of *CNR2* gene (encoding for CB2R) and neuropsychiatric disorders with increased risk of autism spectrum disorders, bipolar disorders, depression, drug abuse, schizophrenia (Ishiguro et al., 2010; Minocci et al., 2011; Onaivi et al., 2013). Therefore, it is important for further studies to investigate the effects of APDs on CB2R using selective CB2R ligands such as HU910, HU308 or JWH133 (Soethoudt et al., 2017).

These results were confirmed by binding of a selective CB1R ligand [^{3}H]SR141716A. Consistently with the [^{3}H]CP55940 binding, risperidone also increased the [^{3}H]SR141716A binding level in the PFC, NAc, CPu and Cg of male rats. The elevated [^{3}H]SR141716A bindings was also observed in the NAcS, and PFC of female rats, compared to controls after aripiprazole administration. There were higher [^{3}H]SR141716A binding levels in the PFC, Cg, NAcC, NAcS, and CPu of female rats, than those of male rats, after aripiprazole treatment. To the best of our knowledge, this is the first study to investigate the effect of APDs on [^{3}H]SR141716A in adolescent subjects. Since [^{3}H]SR141716A is a specific CB1R ligand, this suggests that risperidone increases mainly the CB1R binding level in these brain regions. Although [^{3}H]CP55940 showed gender difference in the effect of risperidone treatment, gender difference was more obvious in [^{3}H]SR141716A bindings a higher [^{3}H]SR141716A binding in female rats. This may be attributed to the specific CB1R binding profile for [^{3}H]SR141716A, compared to [^{3}H]CP55940, which binds to both CB1R and CB2R. It is important to note that the gender difference has often been observed in children and adolescents with mental disorders (Rapado-Castro et al., 2015; Rucklidge, 2010; Society, 2017), as well as antipsychotic effects (Haack et al., 2009; Rubin et al., 2008). However, olanzapine had no effect on the [^{3}H]SR141716A binding for female rats, compared to controls.

The CB1Rs are present on serotonergic and dopaminergic neurons both in soma and synaptic terminals and modulate neurotransmission (Lau and Schloss, 2008). Therefore, APDs like risperidone and aripiprazole may modulate the endocannabinoid system *via* other neurotransmitters, such as dopamine and 5-HT. The cannabinoid ligands could modulate dopaminergic neurotransmission in the PFC, the striatum and the mesolimbic system (Patel and Hillard, 2003; Tzavara et al., 2003), which are associated with schizophrenia (Abi-Dargham and Moore, 2003; Madsen et al., 2006). It was reported that administration of the D2R antagonist raclopride increased anandamide levels, and led to compensatory upregulation of CB1R, which attenuated excessive dopamine signalling, indicating the therapeutic implication for APD treatment (Secher et al., 2010). Our previous study illustrated the dopamine receptor change by APD administration, in that aripiprazole administration significantly decreased dopamine D1R, but not D2R binding density in the PFC of male rats (Lian et al., 2016). The SR141716A, as the CB1R antagonist, was reported to increase the levels of serotonin and dopamine in the forebrain involved in the control of these monoamines by CB1R (Darmani et al., 2003). Furthermore, CB1R has also been reported to regulate 5-HT2A/C receptor mediated behaviours, possibly through serotonin regulation (Darmani et al., 2003; Secher et al., 2010). Therefore, APDs may indirectly modulate the CB1R *via* dopaminergic and 5-HTeric neurotransmission. Overall, it is proposed that APDs affecting either system might have indirect effects on the other.

In this study, rats were sacrificed 48 hours after last dose of APDs is to allow for further clearance of any residual drugs to avoid their direct interference with [3H]CP55940 and [3H]SR141716A binding. Due to the experimental limitation there were not enough brain samples to measure APD concentrations in this study, however it has been reported that the half-lives of olanzapine, risperidone, aripiprazole in rat brain are 5.1 h, 13.6 h, and 2 h, respectively, while the half-lives of these drugs in rat plasma are 2.5–3.5 h, 12.4 h, 2 h (Aravagiri and Marder, 2002; Aravagiri et al., 1999; Feltenstein et al., 2009; Liu et al., 2015). Based on their half-lives in the rat brain, after 48 hours drug wash-out period, very limited amount of APDs will be remained in the rat brain. Furthermore, these APDs have negligible affinities with CB receptors (Correll, 2010; Theisen et al., 2007). Therefore, the changes in CB1R binding observed in this study would not be the direct effect of APDs, but CB1R neuroadaptations after 3 weeks treatment with these drugs. It should also be aware that the other limitation of this study was without measuring the behavioural and physiological changes, it is important for further studies to investigate the relationships of these CB1R binding changes with the potential behavioural and physiological changes in rats.

In summary, this study observed that treatment with APDs including aripiprazole, olanzapine and risperidone induces differential expression of cannabinoids CB1R in the selected brain regions of childhood/adolescent rats with gender differences. This study showed the different effect of various APDs on [^{3}H]CP55940 and [^{3}H]SR141716A bindings in various brain regions, which suggest that early APD treatment modulates cannabinoid receptors, particularly CB1R in the brain of adolescents. Risperidone is the most frequently used APD (~70%) in children and adolescents, and its therapeutic efficacy to control symptoms of mental disorders in children/adolescents may be through modulating CB1R neurotransmission. Further studies should be conducted to investigate the regional and symptom-specific alterations of cannabinoid receptors in mental disorders, which may improve the treatment of APDs on children/adolescents.

Acknowledgement

This study was funded by the Australian National Health and Medical Research Council Project grant (NHMRC, APP 1104184) to C. Deng and J. Lian. J. Lian was also supported by an NHMRC Early Career Fellowship Award (APP1125937).

Author contributions

C.D. and J.L. designed the experiments. J.L. performed the experiments. J.L. and C.D. analysed the data. J.L. prepared the initial draft of the manuscript. C.D. revised the manuscript.

Conflict of interest

There is no conflict of interest in relation to this paper.

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