2019

Early antipsychotic exposure affects NMDA and GABAA receptor binding in the brains of juvenile rats

Jiamei Lian
University of Wollongong, jlian@uow.edu.au

Chao Deng
University of Wollongong, chao@uow.edu.au

Publication Details

Lian, J. & Deng, C. (2019). Early antipsychotic exposure affects NMDA and GABAA receptor binding in the brains of juvenile rats. Psychiatry Research, 273 739-745.

Early antipsychotic exposure affects NMDA and GABAA receptor binding in the brains of juvenile rats

Abstract
Antipsychotics were developed to treat schizophrenia in adults; however they have been increasingly prescribed in children and adolescents. The NMDA and GABAA receptors are involved in neurodevelopment and the pathophysiology of various mental disorders in children and adolescents. Male and female juvenile rats were treated orally with risperidone (0.3 mg/kg, 3 times/day), aripiprazole (1 mg/kg), olanzapine (1 mg/kg) or vehicle (control), starting from postnatal day (PD) 23 (±1 day) for 3 weeks (corresponding to the childhood-adolescent period in humans). Quantitative autoradiography was used to detect the binding density of [^{3}H]MK-801 (an NMDA receptor antagonist) and [^{3}H]muscimol (a selective GABAA receptor agonist). Aripiprazole elevated the [^{3}H]MK801 binding levels in the NAcC of male rats, and the NAcS and CPu of female rats. Risperidone increased [^{3}H]MK801 levels in the CPu of female rats, and the NAcS of male rats. Aripiprazole upregulated [^{3}H]muscimol binding levels in the CPu and NAcC of male rats, while it elevated the [^{3}H]muscimol levels in the PFC of female rats, compared to controls. These results suggest that early treatment with these antipsychotics modulates NMDA and GABAA neurotransmission in juveniles, which may play a role in their clinical efficacy in the control of mental disorders in children and adolescents.

Disciplines
Medicine and Health Sciences

Publication Details
Lian, J. & Deng, C. (2019). Early antipsychotic exposure affects NMDA and GABAA receptor binding in the brains of juvenile rats. Psychiatry Research, 273 739-745.

Early antipsychotic exposure affects NMDA and $GABA_A$ receptor bindings in the brain of juvenile rats

Authors: Jiamei Lian[1,2], Chao Deng[1,2,*]

1. Antipsychotic Research Laboratory, Illawarra Health and Medical Research Institute, Wollongong, 2522, NSW, Australia
2. School of Medicine, University of Wollongong, Wollongong, 2522, NSW, Australia

***Corresponding Author:**

Professor Chao Deng, Illawarra Health and Medical Research Institute, Wollongong, 2522, NSW, Australia

E-mail: chao@uow.edu.au, Tel: (+61 2) 4221 4934, Fax: (+61 2) 4221 8130

Abstract

Antipsychotics were developed to treat schizophrenia in adults; however they have been increasingly prescribed in children and adolescents. The NMDA and $GABA_A$ receptors are involved in neurodevelopment and the pathophysiology of various mental disorders in children and adolescents. Male and female juvenile rats were treated orally with risperidone (0.3 mg/kg, 3 times/day), aripiprazole (1 mg/kg), olanzapine (1 mg/kg) or vehicle (control), starting from postnatal day (PD) 23 (±1 day) for 3 weeks (corresponding to the childhood-adolescent period in humans). Quantitative autoradiography was used to detect the binding density of [^{3}H]MK-801 (an NMDA receptor antagonist) and [^{3}H]muscimol (a selective $GABA_A$ receptor agonist). Aripiprazole elevated the [^{3}H]MK801 binding levels in the NAcC of male rats, and the NAcS and CPu of female rats. Risperidone increased [^{3}H]MK801 levels in the CPu of female rats, and the NAcS of male rats. Aripiprazole upregulated [^{3}H]muscimol binding levels in the CPu and NAcC of male rats, while it elevated the [^{3}H]muscimol levels in the PFC of female rats, compared to controls. These results suggest that early treatment with these antipsychotics modulates NMDA and $GABA_A$ neurotransmission in juveniles, which may play a role in their clinical efficacy in the control of mental disorders in children and adolescents.

Key Words: aripiprazole; risperidone; olanzapine; NMDA receptor, $GABA_A$ receptor; adolescent rat

Introduction

Since approximately one fifth of children and adolescents have been diagnosed with mental illness, antipsychotic prescriptions (mostly off-label) have increased rapidly for children and adolescents over the past decades (Ji and Findling, 2015; Olfson et al., 2014; Rettew et al., 2015). Atypical antipsychotic drugs, such as risperidone, aripiprazole, and olanzapine, have been used widely for treating a range of childhood mental disorders, such as autism, bipolar disorder, attention-deficit/hyperactively disorder (ADHD), and childhood-onset schizophrenia (Daviss et al., 2016; Fraguas et al., 2011), while typical antipsychotics (such as haloperidol) are now used less frequently in children and adolescents due to their serious extrapyramidal side effects (Karanges et al., 2014). Since children/adolescents are in a critical period of brain development, and more sensitive to the effects of antipsychotics than adults (Caccia et al., 2013), it is vital to investigate the neuropharmacological effects of antipsychotics in children/adolescents in order to improve antipsychotic medication in patients of this age group.

The glutamatergic N-methyl-D-aspartate (NMDA) receptor plays a key role in neurodevelopment and other neuronal functions including synaptic transmission, neuronal migration, excitability, plasticity and long-term potentiation (Naaijen et al., 2017). Thus, the alteration of NMDA receptor neurotransmission is involved in various neuropathological processes (Niciu et al., 2012). Gamma-aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the brain, and binds to two GABA receptors, $GABA_A$ and $GABA_B$ (Naaijen et al., 2017). The interactions between glutamate and GABA neurotransmission play an important role in brain development and functioning in the frontostriatal circuits (Keunen et al., 2015; Wu and Sun, 2015). Abnormalities of both the NMDA and $GABA_A$ receptors have been found in the brain of juvenile patients with mental

disorders such as schizophrenia, autism, bipolar disorder, and ADHD (Deng and Huang, 2006; Edden et al., 2012; Lakhan et al., 2013; MacMaster et al., 2003; Naaijen et al., 2017; Panaccione et al., 2013; Schmidt and Mirnics, 2015). The genes encoding the NMDA and GABA receptors and transporters have been identified as candidate genes for several neuropsychiatric disorders, including autism, ADHD and schizophrenia (Purkayastha et al., 2015; Williams et al., 2002). For example, a recent report illustrated that the genes encoding subunits of the $GABA_A$ receptor, including GABRB3 (rs2081648 and rs1426217), GABRA5 (rs35586628), and GABRG3 (rs208129) are involved in the pathogenesis of autistic spectrum disorders, which play a key role in the symptom-based and developmental deficits in Chinese Han Children and adolescents with autism spectrum disorders (Yang et al., 2017).

The therapeutic effects of antipsychotics are attributed to their antagonism at the dopaminergic D2 and serotonergic 5-HT2 receptors (Ginovart and Kapur, 2012; Meltzer and Massey, 2011). Recent studies reported that early antipsychotic treatment altered dopaminergic and serotonergic neurotransmission (De Santis et al., 2018; De Santis et al., 2016; Lian et al., 2016). There is interaction between dopamine and NMDA neurotransmission in various brain regions in the nigrostriatal and mesostriatal circuits (Gardoni and Bellone, 2015). Furthermore, NMDA and $GABA_A$ receptors both modulate release of dopamine and 5-HT in the subcortical systems (Celada et al., 2013). On the other hand, the D1 and D2 receptors contribute to mediate the depolarisation-evoked release of GABA in the striatum (Arias-Montano et al., 2007; Tritsch and Sabatini, 2012). Previous research demonstrated that the reduced glutamate and GABA levels were observed in the nucleus accumbens (NAc) after three weeks' of olanzapine treatment in adolescent male rats (Xu et al., 2015). Another study illustrated that, after 3 weeks' of risperidone administration to juvenile male rats, risperidone significantly reduced NMDA receptor bindings in the NAc

and caudate putamen (CPu), while it elevated the AMPA receptor bindings in the medial prefrontal cortex (PFC) and CPu of male juvenile rats (Choi et al., 2009). Recent studies in our group have reported that aripiprazole and haloperidol could up-regulate the levels of NMDA NR1 and NR2A subunits via the D_2 receptor downstream protein kinase B (Akt)-glycogen synthase kinase 3β (GSK3β**)** pathway in the NAc of adult rats, while these antipsychotics mediate $GABA_A$ receptors via the cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) pathway (Pan et al., 2016a; Pan et al., 2016b). However, limited studies have examined the effects of early antipsychotic treatment on NMDA and GABA receptor neurotransmission in children and adolescents. Therefore, the effects of early antipsychotic exposure on NMDA and $GABA_A$ receptor binding levels have been examined in the brains of both male and female juvenile rats.

Methods

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 and water under a light (07:00 to 19:00) and dark (19:00 to 7:00) cycle, and temperature control (22ºC) 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 24 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 0.3 g cookie dough without drug twice a day during PD18-21. The postnatal male and female rats (PD23±1) were then randomly assigned to one of four treatment groups

as follows: (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-adolescent period in humans) (Andersen, 2003). These antipsychotics have been chosen because they are widely prescribed to children and adolescent (Caccia, 2013; Karanges et al., 2014). The dosages used in this study were translated based on the recommended dosages for the psychiatric treatment of paediatric patients based on body surface area, according to the US Food and Drug Administration (FDA) guideline for clinical trials (FDA, 2005; Reagan-Shaw et al., 2008; Taylor et al., 2009; Zuddas et al., 2011). It has also been previously reported that, at these dosages, aripiprazole treatment reaches above 90% DA D_2 receptor occupancy rates in the rat brains (Natesan et al., 2006), while olanzapine and risperidone reaches between 65-80% DA D2 receptor occupancy (Kapur et al., 2003; Natesan et al., 2006). Drugs were prepared in advance by mixing with cookie dough pellets and droplets of water, and were administered 3 times per day at 7:00, 15:00, and 21:00 (8±1 hour intervals) orally for 3 weeks (Deng et al., 2012; Lian et al., 2014). The rats in the control group received an equivalent pellet without drugs. 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).

Histological procedures

The rats were sacrificed 48 hours after the last drug treatment, the brain tissue was removed and frozen in liquid nitrogen, and then stored at -80 ºC until analysis. Brains were coronally sectioned at -18 ºC into 14 μm sections using a cryostat (Leica CM1850, Leica Microsystem,

Germany) for receptor autoradiography. Sections were thaw-mounted onto Polysine™ Microscope Slides (Menzel GmbH & Co. KG, Braunchweig, Germany) and stored at -20 ºC.

NMDA receptor binding using [^{3}H]MK-801

The NMDA receptor binding using [^{3}H]MK-801 (a potent, selective and noncompetitive antagonist of the NMDA receptor) was performed as previously described (Wang et al., 2014). Briefly, brain sections containing the PFC, NAc and CPu were thawed at room temperature, then incubated with 20 nM [^{3}H]MK-801 (specific activity: 22.5 Ci/mmol; PerkinElmer, USA) in 30 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH=7.45), containing 100 μM glycine, 100 μM glutamate, and 1 mM ethylenediaminetetraacetic acid (EDTA) for 2.5 h at room temperature to determine total binding of the NMDA receptor. Non-specific binding was determined by incubating the next sequential sections with 20 nM [^{3}H]MK-801 incubation buffer, and the addition of 20 μM MK-801 (Sigma Pharmaceuticals, Australia). Slides were washed twice for 20 min in ice-cold 30 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer containing 1 mM EDTA, and then dried under a stream of cool air to remove excess buffer salts (Wang et al., 2014).

$GABA_A$ receptor binding using [^{3}H]muscimol

In brief, binding of [^{3}H]muscimol (specific activity: 22.46 Ci/mmol, PerkinElmer, USA) to $GABA_A$ receptors using [^{3}H]muscimol was performed based on procedures previously described (Deng and Huang, 2006; Ling and Caspary, 2013). In brief, sections were pre-incubated in 50 mM Tris-Citrate buffer (pH=7.0), three times each for 5 min at 4 ºC. Sections were then incubated for 45 min at 4 ºC in the same buffer containing 3 nM [^{3}H]muscimol (specific activity: 22.46 Ci/mmol; Perkin-Elmer, USA) for the total binding. Non-specific binding was determined with the addition of 100 mM GABA. After incubation, the sections

were washed in ice-cold buffer (4 × 2 s), dipped in distilled water and air dried (Deng and Huang, 2006; Ling and Caspary, 2013).

Autoradiography and quantification of receptor bindings

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 was 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).

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 repeated ANOVAs (Treatment × Gender). Dunnett-T tests were followed for comparison between groups; and the Mann-Whitney U test was applied to the data with abnormal distribution. All data are expressed as mean ± SEM, and statistical significance will be accepted when $p<0.05$.

Results

NMDA receptor binding using [^{3}H]MK-801

Examples of [^{3}H]MK-801 binding to NMDA receptors are presented in Figure 1 (A' and B'). Two-way ANOVAs revealed significance in the Treatment factor in the NAcC ($F_{3,40}$=3.701,

p=0.019), NAcS ($F_{3,40}$=2.945, p=0.044), and CPu ($F_{3,40}$=2.760, p=0.055). There are borderline significant effects of the Gender factor in the CPu ($F_{1,40}$=3.970, p=0.053), and the interaction between Treatment and Gender factors in the CPu ($F_{3,40}$=2.760, p=0.055). However, there were no significant effects of these factors in [^{3}H]MK-801 binding in the PFC ($F_{3,40}$=1.012, p>0.05). Further analysis revealed that overall aripiprazole treatment significantly elevated the NMDA receptor binding level in the NAcC, NAcS and CPu (all p<0.05), while risperidone significantly increased the [^{3}H]MK-801 binding level in the NAcC (p<0.05).

In the male rats, aripiprazole treatment significantly increased the [^{3}H]MK-801 binding levels in the NAcC (p<0.05) compared to the control (Figure 2C). Risperidone treatment elevated the [^{3}H]MK-801 receptor binding density with a borderline significance in the NAcC (p=0.075) (Figure 2C). In the female rats, aripiprazole treatment significantly increased the [^{3}H]MK-801 receptor binding density in the NAcS (p<0.05), while the [^{3}H]MK-801 binding density in the CPu was also elevated with a borderline significance by aripiprazole (p=0.082) and risperidone (p=0.075) compared to the control (Figure 2B and C). Furthermore, risperidone treatment led to higher [^{3}H]MK-801 binding levels in the NAcC of female rats, compared to male rats (p=0.083).

$GABA_A$ receptor binding using [^{3}H]muscimol

Examples of [^{3}H]muscimol binding to $GABA_A$ receptors are presented in Figure 1 (A'' and B''). There is a significant effect of the gender factor on $GABA_A$ receptors in the PFC, with higher [^{3}H]muscimol bindings observed in male rats than female rats ($F_{1,40}$=6.933, p=0.012). In male rats, aripiprazole treatment increased the [^{3}H]muscimol binding level significantly in the NAcC (p<0.05) (Figure 3C), and with a borderline significance in the CPu (p=0.061; Figure 3B). For the female rats, aripiprazole led to a higher [^{3}H]muscimol binding in the PFC

compared with the control (p=0.078) (Figure 3A). In terms of the gender differences, aripiprazole treatment significantly increased [^{3}H]muscimol binding in the NAcC of male but not female juvenile rats (p<0.05) (Figure 3C). However, there were no significant alterations in the [^{3}H]muscimol binding levels in these brain regions of both male and female juvenile rats treated with olanzapine or risperidone compared with the controls (all p>0.05) (Figure 3).

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]MK801 for NMDA and [^{3}H]muscimol for $GABA_A$ receptors in the brain nuclei of both male and female adolescent rats. Our results indicate that early exposure to the three antipsychotics have different effects on the NMDA and $GABA_A$ receptor bindings in various brain regions of male and female juvenile rats, which may be implicated in their therapeutic effects in children and adolescents.

In this study, aripiprazole treatment (1mg/kg, orally 3 times per day) elevated [^{3}H]MK-801 binding density on NMDA receptors in the NAcC of juvenile male rats, while it increased NMDA receptor binding in the NAcS and CPu of female rats. The result is consistent with recent reports that aripiprazole increased the expression of NMDA NR1 and NR2 in the NAc of male adult and adolescent rats after chronic drug treatment (Pan et al., 2016a; Pan et al., 2018). The elevated expression of NMDA NR1 and NR2A was also observed in the cortical and hippocampal brain regions of adult rats after aripiprazole administration, while a decreased NR2D were observed in the cortical areas (Schmitt et al., 2003; Segnitz et al.,

2011). Aripiprazole treatment (10 mg/kg/day for 4 months or 40mg/kg/day for 4 weeks via drinking water) has also been reported to increase [^{3}H]MK-801 bindings in the hippocampal and limbic area (Segnitz et al., 2011). While a reduction of [^{3}H]MK-801 bindings in the PFC was observed in adult rats with 4 weeks' treatment with 40mg/kg/day (via drinking water), but not in rats with 4 weeks' treatment with 4mg/kg once daily (Segnitz et al., 2011), this is consistent with the findings in this study that aripiprazole (3mg/kg, once daily for 3 weeks) did not affect [^{3}H]MK-801 bindings in the PFC.

In this study, olanzapine (1mg/kg, orally 3 times per day) had no effect on [^{3}H]MK-801 binding in the PFC, NAc and CPu, compared with control juvenile rats. Consistent with our results, a previous study reported that 4 weeks' olanzapine treatment (5mg/kg/day by osmotic minipump diffusion) did not affect [^{3}H]MK-801 binding density in the PFC and NAc of adult male rats, however it reduced [^{3}H]MK-801 binding in the CPu (Tarazi et al., 2003). Similarly, no altered mRNA expression of NMDA receptor subunits in the rat stratum was reported from chronic olanzapine treatment (Tascedda et al., 2001). The conflicting results in the CPu may be attributed to a higher dosage and the use of adult rats in Tarazi's study compared to this study. In this study, risperidone treatment (0.3mg/kg, orally 3 times per day) significantly increased the NMDA receptor binding density in the NAcC in males and in the CPu of female juvenile rats. Recently, it has been reported that risperidone treatment at the same dosage increased the expression of NMDA NR1 subunit in the NAc of male adolescent rats (Pan et al., 2018). It has been previously reported that risperidone at a lower dosage (0.3 mg/kg, i.p. injection once daily) didn't change the [^{3}H]MK-801 binding levels in the PFC, NAc and CPu of male juvenile rats, while higher dosages (at 1 and 3 mg/kg, i.p. injection daily) decreased the [^{3}H]MK-801 binding in the NAc and CPu, but not in the PFC (Choi et al., 2009). A study in adult male rats showed that a higher dose of risperidone (3mg/kg/day by osmotic

minipump diffusion) decreased [^{3}H]MK-801 binding in the CPu, but not in the PFC and NAc (Tarazi et al., 2003). Therefore, to date, all studies showed that risperidone did not affect [^{3}H]MK-801 binding in the PFC, however it had a dosage dependent effect in the NAc or CPu.

In terms of [^{3}H]muscimol binding to $GABA_A$ receptors, as far as we know this is the first study to investigate the effects of antipsychotic treatment on $GABA_A$ receptor binding in the brain of juvenile rats. Our results showed that aripiprazole upregulated the [^{3}H]muscimol binding level in the PFC, NAcC and CPu of juvenile rats. The results were consistent with the previous papers from our group that both short-term and chronic treatment of aripiprazole increased the expression of $GABA_A$ receptor (β-1 subunit) in the NAc of male adult and adolescent rats (Pan et al., 2016a; Pan et al., 2016b). Previously, it has been reported that 1 week treatment with haloperidol (1.5mg/kg/day in drinking water) and olanzapine (7mg/kg/day in drinking water) increased the [^{3}H]muscimol binding in the PFC of the male adult rats, while this increase was not observed in rats with 2 week or 4 week treatment with these drugs (Skilbeck et al., 2007). The result was consistent with this study that 3 weeks' olanzapine treatment did not affect [^{3}H]muscimol binding in the PFC of juvenile rats. The brain region specific effects of antipsychotics on $GABA_A$ receptor binding have also been shown in another study, where 6-month treatment with clozapine and haloperidol increased [^{3}H]muscimol binding in the NAc and CPu, and decreased the binding in the anterior cingulate and infralimbic cortex, but had no effects in the PFC of adult male rats (Zink et al., 2004). On the other hand, a time-dependent effect of antipsychotics on $GABA_A$ receptor binding has also previously been reported – [^{3}H]muscimol binding in the striatum of adult male rats was decreased by 7-month olanzapine treatment (0.1mg/kg/day in drinking water) and clozapine (0.1mg or 1mg/kg/day in drinking water), but not by 1- or 3-month treatment.

Consistent with our findings that aripiprizole and risperidone had different effects on the NAcC and NAcS, previous studies have revealed both anatomical and functional differences in cognitive processing between the two NAc subnuclei (Salgado and Kaplitt, 2015). Although both NAcC and NAcS receive direct glutamatergic projections from various brain regions, there are differences between origins of projections to these subnuclei that, for example, the dorsal prelimbic, anterior cingulate, and perirhinal cortices projecting mainly to the NAcC, but the infralimbic and posterior piriform cortices projecting preferably to the NAcS (Li et al., 2018; Salgado and Kaplitt, 2015). In particular, glutamatergic projections from the PFC to NAcS has been reported to play a role in the reinstatement of drug-seeking behaviour (Bossert et al., 2012; Salgado and Kaplitt, 2015). On the other hand, $GABA_A$ receptors are preferentially located in the NAcC (Salgado and Kaplitt, 2015). Many psychiatric disorders in children, such as Autism, ADHD and childhood-onset schizophrenia, have impairments in behavioural flexibility with deficits in rewarding and learning (Bissonette and Roesch, 2016; Peters-Scheffer et al., 2013). Although the PFC has largely been involved in behavioural flexibility, recent findings suggest differential roles of the NAcC and NAcS in behavioural flexibility (West and Carelli, 2016). Therefore, different effects of these antipsychotics on NMDA and GABAA receptors might be suggested to be a potential mechanism for the therapeutic effects of these drugs in treating these paediatric psychiatric disorders. Further studies are necessary to investigate the effects of antipsychotics on NAcC and NAcS in animal models for specific psychiatric disorders in children.

One limitation of this study is that just one dosage for each drug and one time (3 week) point have been used, therefore further studies using multiple dosages and several time points are important to fully reveal their antipsychotic effects on $GABA_A$ and NMDA receptors. With

regards to the gender difference, this study found a lower [^{3}H]muscimol binding in the NAcC, NAcS and PFC in female rats than those in male rats. Antipsychotics also showed different effects on $GABA_A$ and NMDA receptors between the male and female rats, although we could not completely explain these gender differences. Previously early exposure to aripiprazole, olanzapine and risperidone has been reported to have similar gender differences in the dopamine D1, D2, 5-HT2A/2C, and cannabinoid receptors in juvenile rats (Lian and Deng, 2018; Lian et al., 2016). Juvenile treatment with these drugs also showed a more severe effect on adult locomotor activity, anxiety-like, and depressive-like behaviours in male rats than in female rats (De Santis et al., 2016). Since the gender difference also occurs in children and adolescents with mental disorders (Rapado-Castro et al., 2015; Rucklidge, 2010), it is worth paying attention to the potential clinical differences between male and female juvenile patients when prescribing these antipsychotics.

Since antipsychotics do not directly bind with NMDA and $GABA_A$ receptors (Correll, 2010), the modulation of these receptor observed in this study is possibly through other signalling pathways. For instance, aripiprazole is a partial agonist of D2 and 5-HT1A receptors, as well as a partial antagonist of 5-HT2A receptors with regionally differential effects on dopaminergic and 5-HTergic neurotransmission (Di Sciascio and Riva, 2015; Han et al., 2009a; Han et al., 2009b). There is evidence that, at therapeutic doses, aripiprazole exhibits low levels of 5-HT1A and 5-HT2A receptor occupancy and activity, and acts predominantly on dopamine D2 receptors (Mamo et al., 2007; Wood and Reavill, 2007). Therefore, aripiprazole-induced alteration of NMDA receptor expression may be regulated via acting on dopaminergic D2, such as through D2 receptor downstream protein kinase B (Akt)-glycogen synthase kinase 3 beta (GSK3β) and protein kinase A (PKA) signalling pathways (Beaulieu and Gainetdinov, 2011; Pan et al., 2016a). It has also been reported that the $GABA_A$ receptor

could be regulated by aripiprazole via D2 receptor downstream PKA signalling (Connelly et al., 2013; Pan et al., 2016b). On the other hand, risperidone is a potent D2 antagonist and also 5-HT2A/C antagonist (Correll, 2010). It was further reported that risperidone increased the 5-HT1A receptor levels, but decreased the 5-HT2A and D1 receptor levels in the frontal cortex of the rat brain (Choi et al., 2010; Lian et al., 2016; Tarazi et al., 2002). While olanzapine is also a 5-HT2A/C and D2 antagonist, it has less binding affinity to D2 receptors than both aripiprazole and risperidone (Correll, 2010). The different pharmacological profiles of these antipsychotics may explain their different effects on NMDA and $GABA_A$ receptor bindings in the brain.

In summary, this present study investigated the effects of early antipsychotics exposure on NMDA and $GABA_A$ receptor binding levels. Overall, the current study identified that aripiprazole and risperidone elevated the NMDA receptors binding in these therapeutic effect-related brain regions, but with gender difference. Furthermore, aripiprazole also increased the GABA receptor binding levels in these brain regions. Since abnormal NMDA and $GABA_A$ receptor neurotransmission is associated with various mental disorders, the result of this study suggests that these antipsychotics may have these therapeutic effects for treating mental disorders in childhood-adolescence via modulating NMDA and $GABA_A$ receptors. It is of note that healthy animals were used in this study; further studies should be conducted to investigate the effects of antipsychotics in the juvenile animal models for mental disorders, which may improve the antipsychotic treatment of children/adolescents.

Acknowledgements

This study was funded by the Australian National Health and Medical Research Council (NHMRC) Project Grant (APP 1104184) to CD and JL. J. Lian was also supported by an NHMRC Early Career Fellowship Award (APP1125937). The funding body had no further role in the study design, decision to publish or preparation of manuscript.

Author contributions

CD and JL designed the experiments. JL performed the experiments. JL and CD analysed the data. JL prepared the initial draft of the manuscript. CD revised the manuscript. Both authors commented on the final draft.

Conflict of interest

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

References

Andersen, S.L., 2003. Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci. Biobehav. Rev. 27 (1–2), 3-18.

Arias-Montano, J.A., Floran, B., Floran, L., Aceves, J., Young, J.M., 2007. Dopamine D(1) receptor facilitation of depolarization-induced release of gamma-amino-butyric acid in rat striatum is mediated by the cAMP/PKA pathway and involves P/Q-type calcium channels. Synapse 61 (5), 310-319.

Beaulieu, J.-M., Gainetdinov, R.R., 2011. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol. Rev. 63 (1), 182.

Bissonette, G.B., Roesch, M.R., 2016. Editorial: neural circuitry of behavioral flexibility: dopamine and related systems. Front. Behav. Neurosci. 10, 6.

Bossert, J.M., Stern, A.L., Theberge, F.R., Marchant, N.J., Wang, H.L., Morales, M., Shaham, Y., 2012. Role of projections from ventral medial prefrontal cortex to nucleus accumbens shell in context-induced reinstatement of heroin seeking. J. Neurosci. 32 (14), 4982-4991.

Caccia, S., 2013. Safety and pharmacokinetics of atypical antipsychotics in children and adolescents. Paediatr. Drugs 15 (3), 217-233.

Caccia, S., Invernizzi, R.W., Nobili, A., Pasina, L., 2013. A new generation of antipsychotics: pharmacology and clinical utility of cariprazine in schizophrenia. Ther. Clin. Risk Manag. 9, 319-328.

Celada, P., Puig, M.V., Artigas, F., 2013. Serotonin modulation of cortical neurons and networks. Front. Integr. Neurosci. 7, 25.

Choi, Y.K., Gardner, M.P., Tarazi, F.I., 2009. Effects of risperidone on glutamate receptor subtypes in developing rat brain. Eur. Neuropsychopharmacol. 19 (2), 77-84.

Choi, Y.K., Moran-Gates, T., Gardner, M.P., Tarazi, F.I., 2010. Effects of repeated risperidone exposure on serotonin receptor subtypes in developing rats. Eur. Neuropsychopharmacol. 20 (3), 187-194.

Connelly, W.M., Errington, A.C., Di Giovanni, G., Crunelli, V., 2013. Metabotropic regulation of extrasynaptic GABAA receptors. Front. Neural Circuits 7, 171.

Correll, C.U., 2010. From receptor pharmacology to improved outcomes: individualising the selection, dosing, and switching of antipsychotics. Eur. Psychiatry 25 Suppl 2, S12-21.

Daviss, W.B., Barnett, E., Neubacher, K., Drake, R.E., 2016. Use of antipsychotic medications for nonpsychotic children: risks and implications for mental health services. Psychiatr. Serv. 67 (3), 339-341.

De Santis, M., Huang, X.F., Deng, C., 2018. Early antipsychotic treatment in juvenile rats elicits long-term alterations to the adult serotonin receptors. Neuropsychiatr. Dis. Treat. 14, 1569-1583.

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. Psychopharm. 30 (2), 204-214.

Deng, C., Huang, X.F., 2006. Increased density of GABAA receptors in the superior temporal gyrus in schizophrenia. Exp. Brain Res. 168 (4), 587-590.

Deng, C., Lian, J., Pai, N., Huang, X.F., 2012. Reducing olanzapine-induced weight gain side-effect by betahistine: a study in the rat model. J. Psychopharm. 26 (9), 1291-1279.

Di Sciascio, G., Riva, M.A., 2015. Aripiprazole: from pharmacological profile to clinical use. Neuropsychiatr. Dis. Treat. 11, 2635-2647.

Edden, R.A., Crocetti, D., Zhu, H., Gilbert, D.L., Mostofsky, S.H., 2012. Reduced GABA concentration in attention-deficit/hyperactivity disorder. Arch. Gen. Psychiatry 69 (7), 750-753.

FDA, 2005. Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers, in: HHS, FDA, CDER (Eds.), Guidance for Industry, Rockville, Maryland, USA.

Fraguas, D., Correll, C.U., Merchan-Naranjo, J., Rapado-Castro, M., Parellada, M., Moreno, C., Arango, C., 2011. Efficacy and safety of second-generation antipsychotics in children and adolescents with psychotic and bipolar spectrum disorders: comprehensive review of prospective head-to-head and placebo-controlled comparisons. Eur. Neuropsychopharmacol. 21 (8), 621-645.

Gardoni, F., Bellone, C., 2015. Modulation of the glutamatergic transmission by Dopamine: a focus on Parkinson, Huntington and Addiction diseases. Front. Cell. Neurosci. 9, 25.

Ginovart, N., Kapur, S., 2012. Role of dopamine D(2) receptors for antipsychotic activity. Handb. Exp. Pharmacol.(212), 27-52.

Han, M., Huang, X.F., Deng, C., 2009a. Aripiprazole differentially affects mesolimbic and nigrostriatal dopaminergic transmission: implications for long-term drug efficacy and low extrapyramidal side-effects. Int. J. Neuropsychopharmacol. 12 (7), 941-952.

Han, M., Huang, X.F., du Bois, T.M., Deng, C., 2009b. The effects of antipsychotic drugs administration on 5-HT_{1A} receptor expression in the limbic system of the rat brain. Neuroscience 164 (4), 1754-1763.

Ji, N.Y., Findling, R.L., 2015. An update on pharmacotherapy for autism spectrum disorder in children and adolescents. Curr. Opin. Psychiatry 28 (2), 91-101.

Kapur, S., VanderSpek, S.C., Brownlee, B.A., Nobrega, J.N., 2003. Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. J. Pharmacol. Exp. Ther. 305 (2), 625-631.

Karanges, E.A., Stephenson, C.P., McGregor, I.S., 2014. Longitudinal trends in the dispensing of psychotropic medications in Australia from 2009-2012: focus on children, adolescents and prescriber specialty. Aust. N. Z. J. Psychiatry 48 (10), 917-931.

Keunen, K., van Elburg, R.M., van Bel, F., Benders, M.J., 2015. Impact of nutrition on brain development and its neuroprotective implications following preterm birth. Pediatr. Res. 77 (1-2), 148-155.

Lakhan, S.E., Caro, M., Hadzimichalis, N., 2013. NMDA receptor activity in neuropsychiatric disorders. Front. Psychiatry 4, 52.

Li, Z., Chen, Z., Fan, G., Li, A., Yuan, J., Xu, T., 2018. Cell-Type-Specific afferent innervation of the nucleus accumbens core and shell. Front. Neuroanat. 12 (84).

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 Res. 266, 317-322.

Lian, J., Huang, X.-F., Pai, N., Deng, C., 2014. Preventing olanzapine-induced weight gain using betahistine: a study in a rat model with chronic olanzapine treatment. PLoS One 9 (8), e104160.

Lian, J., Pan, B., Deng, C., 2016. Early antipsychotic exposure affects serotonin and dopamine receptor binding density differently in selected brain loci of male and female juvenile rats. Pharmacol. Rep. 68 (5), 1028-1035.

Ling, L., Caspary, D., 2013. Autoradiographic 3H-Gaboxadol Receptor Binding Protocol. Bio Protoc 3 (23), e989.

MacMaster, F.P., Carrey, N., Sparkes, S., Kusumakar, V., 2003. Proton spectroscopy in medication-free pediatric attention-deficit/hyperactivity disorder. Biol. Psychiatry 53 (2), 184-187.

Mamo, D., Graff, A., Mizrahi, R., Shammi, C.M., Romeyer, F., Kapur, S., 2007. Differential effects of aripiprazole on D(2), 5-HT(2), and 5-HT(1A) receptor occupancy in patients with schizophrenia: a triple tracer PET study. Am. J. Psychiatry 164 (9), 1411-1417.

Meltzer, H., Massey, B., 2011. The role of serotonin receptors in the action of atypical antipsychotic drugs. Curr. Opin. Pharmacol. 11 (1), 59-67.

Naaijen, J., Bralten, J., Poelmans, G., Glennon, J.C., Franke, B., Buitelaar, J.K., 2017. Glutamatergic and GABAergic gene sets in attention-deficit/hyperactivity disorder: association to overlapping traits in ADHD and autism. Transl Psychiatry 7 (1), e999.

Natesan, S., Reckless, G.E., Nobrega, J.N., Fletcher, P.J., Kapur, S., 2006. Dissociation between in vivo occupancy and functional antagonism of dopamine D2 receptors: comparing aripiprazole to other antipsychotics in animal models. Neuropsychopharmacology 31 (9), 1854-1863.

Niciu, M.J., Kelmendi, B., Sanacora, G., 2012. Overview of glutamatergic neurotransmission in the nervous system. Pharmacol. Biochem. Behav. 100 (4), 656-664.

Olfson, M., Blanco, C., Wang, S., Laje, G., Correll, C.U., 2014. National trends in the mental health care of children, adolescents, and adults by office-based physicians. JAMA Psychiatry 71 (1), 81-90.

Pan, B., Huang, X.F., Deng, C., 2016a. Chronic administration of aripiprazole activates GSK3beta-dependent signalling pathways, and up-regulates GABAA receptor expression and CREB1 activity in rats. Sci. Rep. 6, 30040.

Pan, B., Lian, J., Deng, C., 2018. Chronic antipsychotic treatment differentially modulates protein kinase A- and glycogen synthase kinase 3 beta-dependent signaling pathways, N-methyl-D-aspartate receptor and gamma-aminobutyric acid A receptors in nucleus accumbens of juvenile rats. J. Psychopharm. 32 (11), 1252-1263.

Pan, B., Lian, J., Huang, X.F., Deng, C., 2016b. Aripiprazole increases the PKA signalling and expression of the GABAA receptor and CREB1 in the nucleus accumbens of rats. J. Mol. Neurosci. 59 (1), 36-47.

Panaccione, I., Napoletano, F., Forte, A.M., Kotzalidis, G.D., Del Casale, A., Rapinesi, C., Brugnoli, C., Serata, D., Caccia, F., Cuomo, I., Ambrosi, E., Simonetti, A., Savoja, V., De Chiara, L., Danese, E., Manfredi, G., Janiri, D., Motolese, M., Nicoletti, F., Girardi, P., Sani, G., 2013. Neurodevelopment in schizophrenia: the role of the wnt pathways. Curr. Neuropharmacol. 11 (5), 535-558.

Paxinos, G., Watson, C., 2007. The rat brain in sterotaxic coordinates, six ed. Academic Press.

Peters-Scheffer, N., Didden, R., Sigafoos, J., Green, V.A., Korzilius, H., 2013. Behavioral flexibility in children with autism spectrum disorder and intellectual disability. Res. Autism Spectr. Disord. 7 (6), 699-709.

Purkayastha, P., Malapati, A., Yogeeswari, P., Sriram, D., 2015. A Review on GABA/Glutamate Pathway for Therapeutic Intervention of ASD and ADHD. Curr. Med. Chem. 22 (15), 1850 - 1859.

Rapado-Castro, M., Bartholomeusz, C.F., Castro-Fornieles, J., Gonzalez-Pinto, A., Otero, S., Baeza, I., Moreno, C., Graell, M., Janssen, J., Bargallo, N., Pantelis, C., Desco, M., Arango, C., 2015. Gender effects on brain changes in early-onset psychosis. Eur. Child Adolesc. Psychiatry 24 (10), 1193-1205.

Reagan-Shaw, S., Nihal, M., Ahmad, N., 2008. Dose translation from animal to human studies revisited. FASEB J. 22 (3), 659-661.

Rettew, D.C., Greenblatt, J., Kamon, J., Neal, D., Harder, V., Wasserman, R., Berry, P., MacLean, C.D., Hogue, N., McMains, W., 2015. Antipsychotic medication prescribing in children enrolled in medicaid. Pediatrics 135 (4), 658-665.

Rucklidge, J.J., 2010. Gender differences in attention-deficit/hyperactivity disorder. Psychiatr. Clin. North Am. 33 (2), 357-373.

Salgado, S., Kaplitt, M.G., 2015. The nucleus accumbens: a comprehensive review. Stereotact. Funct. Neurosurg. 93 (2), 75-93.
Schmidt, M.J., Mirnics, K., 2015. Neurodevelopment, GABA system dysfunction, and schizophrenia. Neuropsychopharmacology 40 (1), 190-206.
Schmitt, W., Anson, C.E., Hill, J.P., Powell, A.K., 2003. Cation-pi binding of an alkali metal ion by pendant alpha,alpha-dimethylbenzyl groups within a dinuclear iron(III) structural unit. J. Am. Chem. Soc. 125 (37), 11142-11143.
Segnitz, N., Ferbert, T., Schmitt, A., Gass, P., Gebicke-Haerter, P.J., Zink, M., 2011. Effects of chronic oral treatment with aripiprazole on the expression of NMDA receptor subunits and binding sites in rat brain. Psychopharmacology (Berl) 217 (1), 127-142.
Skilbeck, K.J., O'Reilly, J.N., Johnston, G.A., Hinton, T., 2007. The effects of antipsychotic drugs on GABAA receptor binding depend on period of drug treatment and binding site examined. Schizophr. Res. 90 (1-3), 76-80.
Tarazi, F.I., Baldessarini, R.J., Kula, N.S., Zhang, K., 2003. Long-term effects of olanzapine, risperidone, and quetiapine on ionotropic glutamate receptor types: implications for antipsychotic drug treatment. J. Pharmacol. Exp. Ther. 306 (3), 1145-1151.
Tarazi, F.I., Zhang, K., Baldessarini, R.J., 2002. Long-term effects of olanzapine, risperidone, and quetiapine on serotonin 1A, 2A and 2C receptors in rat forebrain regions. Psychopharmacology (Berl.) 161 (3), 263-270.
Tascedda, F., Blom, J.M.C., Brunello, N., Zolin, K., Gennarelli, M., Colzi, A., Bravi, D., Carra, S., Racagni, G., Riva, M.A., 2001. Modulation of glutamate receptors in response to the novel antipsychotic olanzapine in rats. Biol. Psychiatry 50 (2), 117-122.
Taylor, D., Paton, C., Kapur, S., 2009. Maudsley prescribing guidelines, 10 ed. Informa Healthcare, New York.
Tritsch, N.X., Sabatini, B.L., 2012. Dopaminergic modulation of synaptic transmission in cortex and striatum. Neuron 76 (1), 33-50.
Wang, Q., Li, J., Wei, X., Liao, J., Xu, Y., Lu, T., Qin, B., Xie, J., Deng, C., Huang, X., 2014. Alterations of NMDA receptor binding in various brain regions among 6-hydroxydopamine-induced Parkinsonian rats. Int. J. Neurosci. 124 (6), 457-465.
West, E.A., Carelli, R.M., 2016. Nucleus accumbens core and shell differentially encode reward-associated cues after reinforcer devaluation. J. Neurosci. 36 (4), 1128-1139.
Williams, N.M., Bowen, T., Spurlock, G., Norton, N., Williams, H.J., Hoogendoorn, B., Owen, M.J., O'Donovan, M.C., 2002. Determination of the genomic structure and mutation screening in schizophrenic individuals for five subunits of the N-methyl-D-aspartate glutamate receptor. Mol. Psychiatry 7 (5), 508-514.
Wood, M., Reavill, C., 2007. Aripiprazole acts as a selective dopamine D2 receptor partial agonist. Expert Opin. Investig. Drugs 16 (6), 771-775.
Wu, C., Sun, D., 2015. GABA receptors in brain development, function, and injury. Metab. Brain Dis. 30 (2), 367-379.
Xu, S., Gullapalli, R.P., Frost, D.O., 2015. Olanzapine antipsychotic treatment of adolescent rats causes long term changes in glutamate and GABA levels in the nucleus accumbens. Schizophr. Res. 161 (2-3), 452-457.
Yang, S., Guo, X., Dong, X., Han, Y., Gao, L., Su, Y., Dai, W., Zhang, X., 2017. GABAA receptor subunit gene polymorphisms predict symptom-based and developmental deficits in Chinese Han children and adolescents with autistic spectrum disorders. Sci. Rep. 7 (1), 3290.
Zink, M., Schmitt, A., May, B., Muller, B., Demirakca, T., Braus, D.F., Henn, F.A., 2004. Differential effects of long-term treatment with clozapine or haloperidol on GABAA receptor binding and GAD67 expression. Schizophr. Res. 66 (2-3), 151-157.

Zuddas, A., Zanni, R., Usala, T., 2011. Second generation antipsychotics (SGAs) for non-psychotic disorders in children and adolescents: a review of the randomized controlled studies. Eur. Neuropsychopharmacol. 21 (8), 600-620.

Figure Legends

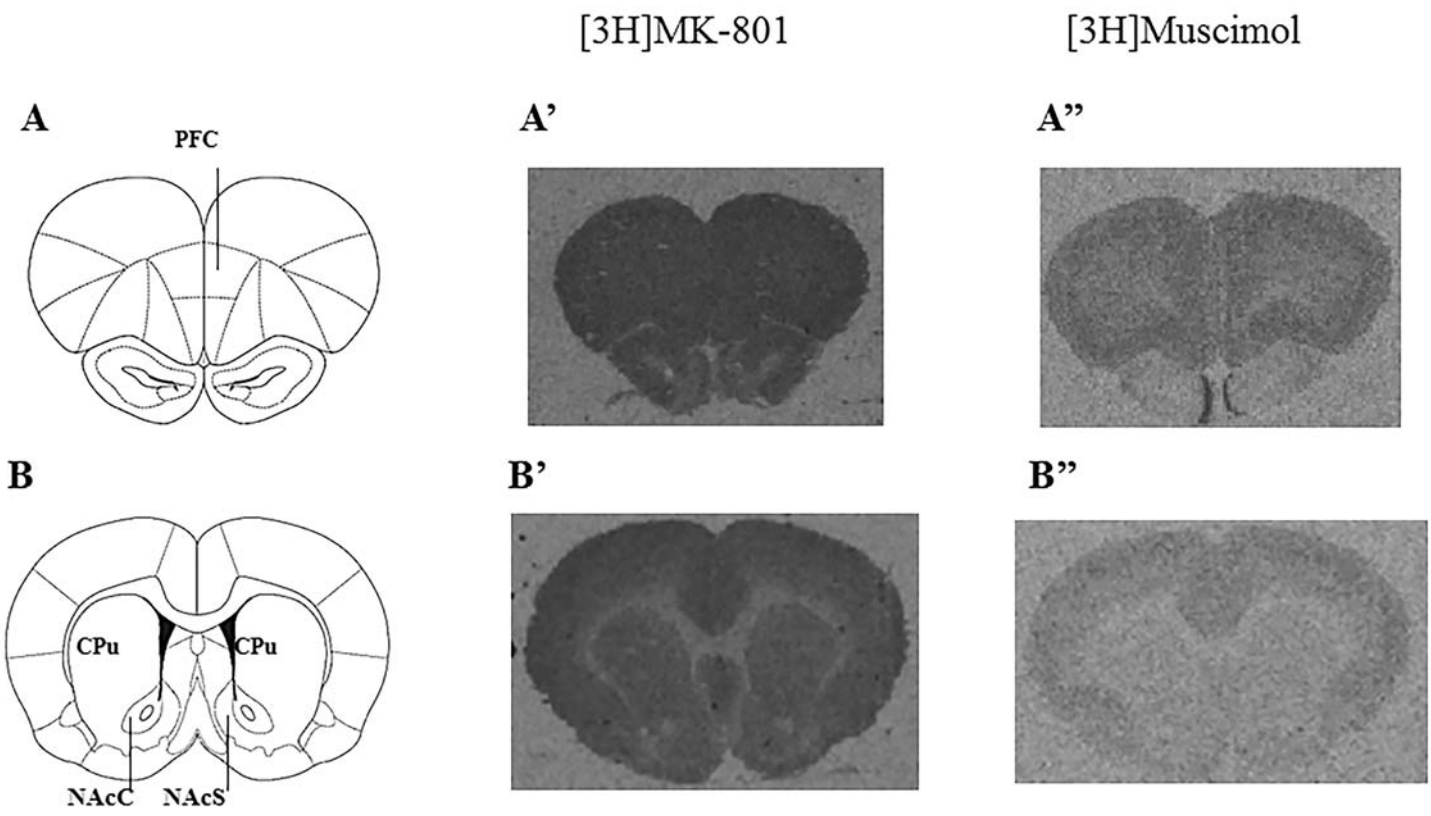
Figure 1. Examples of [^{3}H]MK-801 and [^{3}H]Muscimol bindings in the rat brain. A-B, The schematic diagram is adapted from a rat brain atlas (Paxinos and Watson, 2007) showing the level of Bregma 4.68 mm (A) and 1.08 mm (B). A'-B', examples of autoradiograms to show [^{3}H]MK-801 binding. A''-B'', examples of [^{3}H]Muscimol binding. Abbreviations: PFC, prefrontal cortex; CPu, caudate putamen; NAcC, nucleus accumbens core; NAcS, nucleus accumbens shell.

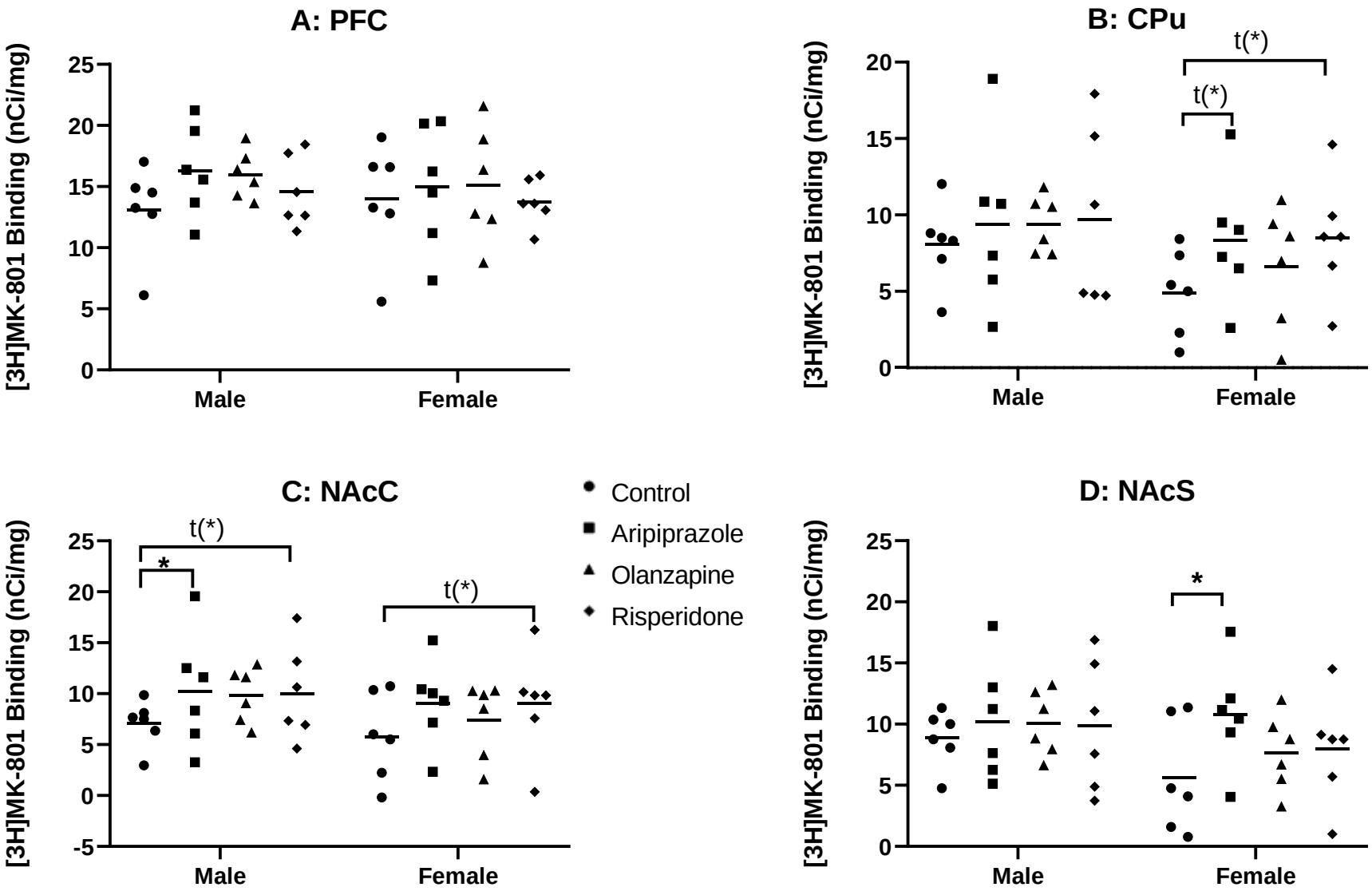
Figure 2. The effects of aripiprazole, olanzapine, and risperidone treatment on [^{3}H]MK801 binding (nCi/mg tissue) in (A) the prefrontal cortex (PFC), (B) caudate putamen (CPu), (C) nucleus accumbens, core (NAcC), and (D) nucleus accumbens, shell (NAcS) of both male and female juvenile rats (n=6/group). *p<0.05, **p<0.01 *vs.* control. *t(*)* 0.05<p<0.1 vs. control..

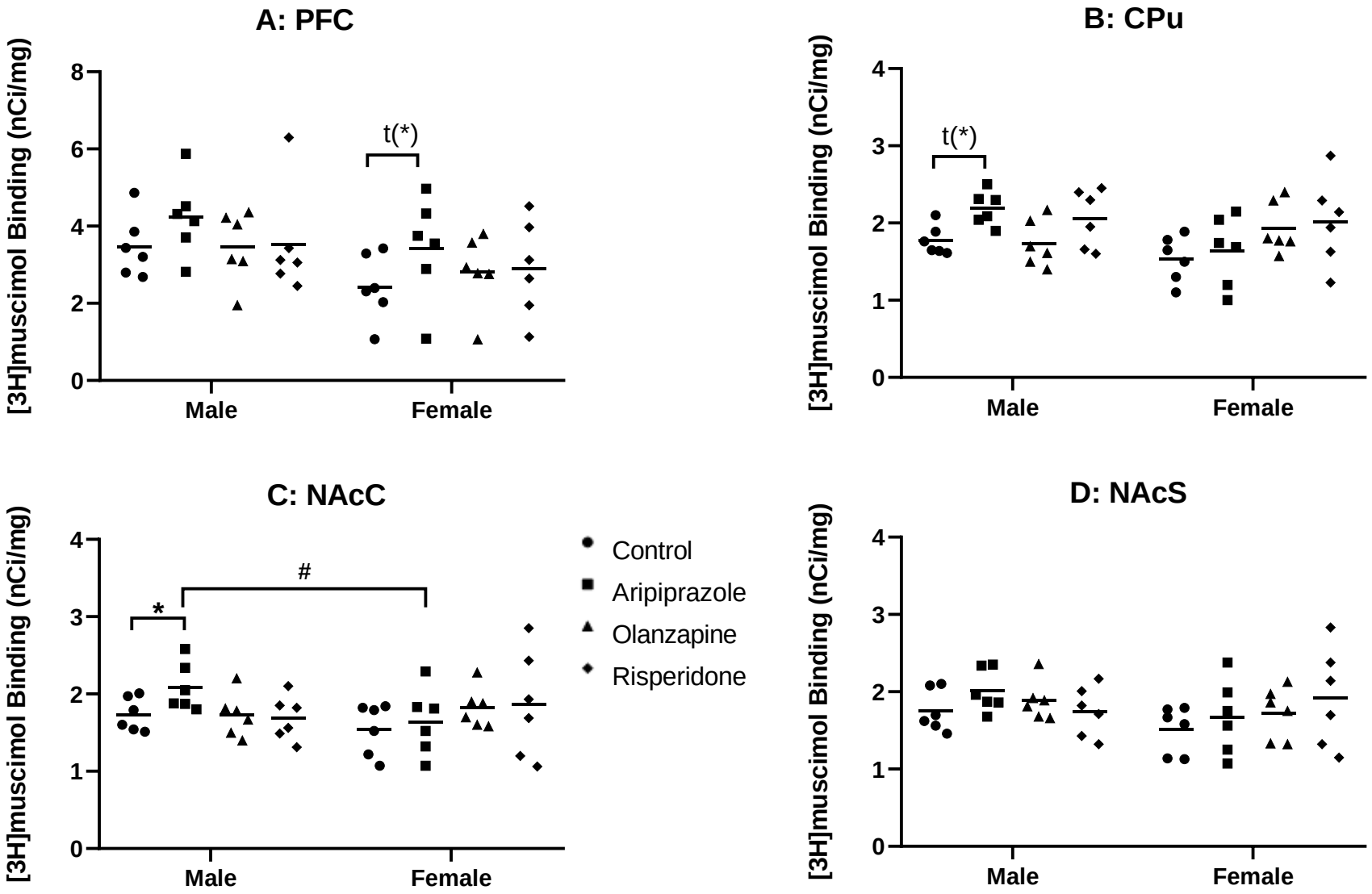
Figure 3. The effects of aripiprazole, olanzapine, and risperidone treatment on [^{3}H]Muscimol binding (nCi/mg tissue) in (A) the prefrontal cortex (PFC), (B) caudate putamen (CPu), (C) nucleus accumbens, core (NAcC) and (D) nucleus accumbens, shell (NAcS) of both male and female juvenile rats (n=6/group). *p<0.05, **p<0.01 *vs*. control. # p<0.05, male *vs.* female. *t(*)* 0.05<p<0.1 vs. control.

Abbreviation

5-HT	Serotonin
5-HT1A	Serotonin 5-HT1A receptor
5-HT2A	Serotonin 5-HT2A receptor
5-HT2C	Serotonin 5-HT2C receptor
ADHD	Attention Deficit Hyperactivity Disorder
AMPA	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid
ANOVA	Analysis of Variance
Akt	Protein Kinase B
cAMP	cyclic adenosine monophosphate
CPu	Caudate Putamen
D2	Dopamine D2 Receptor
EDTA	ethylenediaminetetraacetic acid
GABA	γ-Aminobutryric Acid
GABAA	GABAA receptor
GSK3□	glycogen synthase kinase 3□
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
MK-801	Dizocilpine
NAc	Nucleus Accumbens
NAcC	Nucleus accumbens core
NAcS	Nucleus accumbens shell
PFC	Prefrontal cortex
PKA	protein kinase A
PD	Postnatal Day


[3H]MK-801
[3H]Muscimol
A
PFC
A'
A"
B
CPu
CPu
NAcC
NAcS
B'
B"


A: PFC
B: CPu
C: NAcC
D: NAcS
[3H]MK-801 Binding (nCi/mg)
Male
Female
t(*)
*
Control
Aripiprazole
Olanzapine
Risperidone


A: PFC
B: CPu
C: NAcC
D: NAcS
[3H]muscimol Binding (nCi/mg)
t(*)
*
#
Male
Female
Control
Aripiprazole
Olanzapine
Risperidone