UNIVERSITY
OF WOLLONGONG
AUSTRALIA

University of Wollongong
Research Online

Illawarra Health and Medical Research Institute | Faculty of Science, Medicine and Health

2018

Chronic antipsychotic treatment differentially modulates protein kinase A- and glycogen synthase kinase 3 beta-dependent signaling pathways, N-methyl-D-aspartate receptor and γ-aminobutyric acid A receptors in nucleus accumbens of juvenile rats

Bo Pan
University of Wollongong, *Yangzhou University*, bp355@uowmail.edu.au

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

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

Publication Details

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 γ-aminobutyric acid A receptors in nucleus accumbens of juvenile rats. Journal of Psychopharmacology, 32 (11), 1252-1263.

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au

Chronic antipsychotic treatment differentially modulates protein kinase A- and glycogen synthase kinase 3 beta-dependent signaling pathways, N-methyl-D-aspartate receptor and γ-aminobutyric acid A receptors in nucleus accumbens of juvenile rats

Abstract

Background: Antipsychotics are developed to treat mental disorders in adults; however, the prescription (mostly "off-label") of antipsychotics for children/adolescents has been constantly increasing over years. The influences of antipsychotics on juveniles requires investigation to validate their clinic use. Antipsychotics mainly exert their effects via several receptors and signaling pathways.

Aims: This study examined the effects of aripiprazole, olanzapine, and risperidone on selected signaling pathways, N-methyl-D-aspartate, and γ-aminobutyric acid A receptors in juveniles.

Methods: Rats were orally administered aripiprazole (1 mg/kg), olanzapine (1 mg/kg), risperidone (0.3 mg/kg), or vehicle three times/day from postnatal day 23 (±1 day) for three weeks. The effects of antipsychotics in the nucleus accumbens and caudate putamen were measured by Western blots.

Results: In the nucleus accumbens, all three drugs differentially increased N-methyl-D-aspartate and γ-aminobutyric acid A receptor expression. Additionally, all three antipsychotics differentially elevated the phosphorylation of glycogen synthase kinase 3 beta, β-catenin, and cAMP-responsive element-binding protein 1. In the caudate putamen, olanzapine increased β-catenin phosphorylation; and aripiprazole and olanzapine elevated γ-aminobutyric acid A receptor levels. Correlation analysis indicated that antipsychotics might modulate N-methyl-D-aspartate receptors via glycogen synthase kinase 3 beta-β-catenin signaling and/or cAMP-responsive element-binding protein 1 activation.

Conclusions: These findings suggest that antipsychotics can affect protein kinase A- and glycogen synthase kinase 3 beta-dependent signaling pathways in juveniles; and their modulation on N-methyl-D-aspartate and γ-aminobutyric acid A receptors is probably through glycogen synthase kinase 3 beta-β-catenin signaling and/or cAMP-responsive element-binding protein 1 activation.

Disciplines

Medicine and Health Sciences

Publication Details

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 γ-aminobutyric acid A receptors in nucleus accumbens of juvenile rats. Journal of Psychopharmacology, 32 (11), 1252-1263.

This journal article is available at Research Online: https://ro.uow.edu.au/ihmri/1324

Chronic antipsychotic treatment differentially modulates protein kinase A- and glycogen synthase kinase 3 beta-dependent signalling pathways, N-methyl-D-aspartate receptor and γ-aminobutyric acid A receptors in nucleus accumbens of juvenile rats

Bo Pan[1,-4], Jiamei Lian[3,4], *Chao Deng[3,4]

---

[1] The Key Laboratory of Syndrome Differentiation and Treatment of Gastric Cancer of the State Administration of Traditional Chinese Medicine, Yangzhou University Medical College, Yangzhou, China

[2]Department of Pharmacy, Yangzhou University Medical College, Yangzhou, China

[3]Antipsychotic Research Laboratory, Illawarra Health and Medical Research Institute, Australia

[4]School of Medicine, University of Wollongong, Australia

Corresponding Author:

Prof. Chao Deng, Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, 2522, NSW, Australia. E-mail: chao@uow.edu.au

Abstract

Background: Antipsychotics are developed to treat mental disorders in adults; however, the prescription (mostly "off-label") of antipsychotics for children/adolescents has been constantly increasing over years. The influences of antipsychotics on juveniles requires investigation to validate their clinic use. Antipsychotics mainly exert their effects via several receptors and signalling pathways.

Aims: This study examined the effects of aripiprazole, olanzapine, and risperidone on selected signalling pathways, N-methyl-D-aspartate, and γ-aminobutyric acid A receptors in juveniles.

Methods: Rats were orally administered aripiprazole (1mg/kg), olanzapine (1mg/kg), risperidone (0.3mg/kg), or vehicle 3 times/day from postnatal day 23 (±1 day) for 3 weeks. The effects of antipsychotics in the nucleus accumbens and caudate putamen were measured by Western Blots.

Results: In the nucleus accumbens, all three drugs differentially increased N-methyl-D-aspartate and γ-aminobutyric acid A receptor expression. Additionally, all three antipsychotics differentially elevated the phosphorylation of glycogen synthase kinase 3 beta, β-catenin, and cAMP-responsive

element-binding protein 1. In the caudate putamen, olanzapine increased β-catenin phosphorylation; and aripiprazole and olanzapine elevated γ-aminobutyric acid A receptor levels. Correlation analysis indicated that antipsychotics might modulate N-methyl-D-aspartate receptors via glycogen synthase kinase 3 beta -β-catenin signalling

and/or cAMP-responsive element-binding protein 1 activation.

Conclusions: These findings suggest that antipsychotics can affect protein kinase A- and glycogen synthase kinase 3 beta -dependent signalling pathways in juveniles; and their modulation on N-methyl-D-aspartate and γ-aminobutyric acid A receptors is probably through glycogen synthase kinase 3 beta -β-catenin signalling and/or cAMP-responsive element-binding protein 1 activation.

Declaration of interest/Finding: None of the authors has a conflict of interest. This work was supported by the National Health and Medical Research Council (APP1104184), Australia.

Keywords:

Antipsychotics, protein kinase A, glycogen synthase kinase 3 beta, N-methyl-D-aspartate receptor, γ-aminobutyric acid A receptor, juvenile animals, aripiprazole, olanzapine, risperidone

Introduction

Over the past decade, the prescription of antipsychotic drugs (mostly off-label use) in children and adolescents has increased markedly (Ronsley et al., 2013; Caccia, 2013). Particularly, risperidone (neuroscience-based nomenclature (NbN): dopamine D_2, serotonin 5-HT_2, noradrenaline NE α-2 receptor antagonist (Nutt and Blier, 2016)) is accounted for ~70% of total antipsychotic prescriptions (Olfson et al., 2010; Karanges et al., 2014). These antipsychotics are mostly prescribed to treat mental disorders, such as childhood-onset schizophrenia, depression, bipolar disorder, and autism (Schneider et al., 2014). Clinical studies have shown that children/adolescents are more likely to be affected by antipsychotics than adults, especially by the side-effects (Vitiello et al., 2009). Since the pharmacodynamic sensitivity to antipsychotics in children/adolescents is different from that in adults (Caccia, 2013), understanding the pharmacological mechanisms of antipsychotics in children/adolescents is required and might provide important evidence for the prescription of antipsychotics for children/adolescents in clinics.

Our previous evidence demonstrates that various antipsychotics influence G-protein dependent protein kinase A (PKA) signalling and G-protein independent protein kinase B (Akt)-glycogen synthase kinase 3 beta (GSK3β) signalling pathways to exert their effects in the striatum of adult animals (Pan et al., 2016b; Pan et al., 2016a; Pan et al., 2016c; Pan et al., 2015). However, to our knowledge, there is no study that investigates antipsychotic effects on these signalling pathways during the childhood-adolescent period. In addition, it has been widely accepted that antipsychotics are able to exert their effects on several other signalling pathways or substrates. For example, the

93 dishevelled(Dvl)-GSK3β-β-catenin pathway has been reported to be modulated by
94 various antipsychotics (including aripiprazole (NbN: dopamine D2, serotonin 5-HT1A
95 receptor partial agonist (Nutt and Blier, 2016)), olanzapine (NbN: dopamine D2,
96 serotonin 5-HT2 receptor antagonist (Nutt and Blier, 2016)), risperidone, etc.) in adults
97 (Alimohamad et al., 2005b; Alimohamad et al., 2005a; Sutton and Rushlow, 2011; Seo et
98 al., 2015; Pan et al., 2016a; Pan et al., 2016b). Antipsychotics (e.g. aripiprazole) might
99 also exert therapeutic effects via cAMP-responsive element-binding protein 1 (CREB1)
100 in adult animals (Mavrikaki et al., 2014; Pan et al., 2016c; Pan et al., 2016b). Whether
101 these pathways and substrates are involved in the regulation of antipsychotics in
102 children/adolescents is not clear.

103 N-methyl-D-aspartate (NMDA) and $GABA_A$(γ-aminobutyric acid) receptor signalling
104 play key roles in neurodevelopment and the formation of brain core functions, and
105 deficits in these receptors have been considered to be associated with various mental
106 disorders in children/adolescents (Panaccione et al., 2013; Schmidt and Mirnics, 2015;
107 Mouri et al., 2007; Sakamoto et al., 2011). Previous studies have shown that the $GABA_A$
108 receptor can be regulated by various antipsychotics in adults through dopamine D_2
109 receptor (D_2R)-downstream PKA signalling, which was also suggested by our recent
110 studies (Skilbeck et al., 2007; Zink et al., 2004; Pan et al., 2016c; Pan et al., 2016b);
111 similarly, modulation of antipsychotics on NMDA receptors (NMDARs) in adults has
112 also been well documented (Schmitt et al., 2003; Segnitz et al., 2011; Pan et al., 2016b);
113 antipsychotics might regulate NMDARs via D_2R-mediated GSK3β and CREB1
114 signalling in adult rat brains (Pan et al., 2016b). Both NMDA and $GABA_A$ receptors are
115 in an immature from during the postnatal developmental period which may cause animals

to be more sensitive to antipsychotic treatment (Fritschy et al., 1994; Lopez-Tellez et al., 2004; Sheng et al., 1994). Although these antipsychotics target multiple receptors such as dopamine D_2 and 5-HT_{2A} receptors, D_2Rs play a critical role in their therapeutic effects (Ginovart and Kapur, 2012). Both risperidone and aripiprazole have very high affinity with D_2Rs (Correll, 2010). Unfortunately, there are very limited studies that have systematically examined the effects of early treatment with these antipsychotics on the D_2R-mediated signalling pathways and substrates during childhood-adolescence, which is the key issue that needs to be addressed in the present study. Furthermore, the striatum, which mainly contains the nucleus accumbens (NAc) and caudate putamen (CPu), is a key brain region that is associated with the pathophysiology of various mental disorders in children/adolescents, such as schizophrenia, autism, depression, and bipolar disorder (DelBello et al., 2006; James et al., 2016; Langen et al., 2009; Gabbay et al., 2013). Therefore, in this study, we investigated the effects of oral treatment (3 times per day) of aripiprazole, olanzapine, and risperidone at a clinical equivalent dosage (a better mimicking of the clinical treatment paradigm) on the above mentioned PKA- and GSK3β-dependent signalling pathways as well as $GABA_A$ and NMDA receptors in the NAc and CPu of juvenile rats.

Methods

Animals and drug administration

Fourteen timed, pregnant Sprague-Dawley rats were obtained at gestation day 14 from the Animal Resource Centre (Perth, WA, Australia). They were housed individually at 22˚C, on a 12h light-dark cycle (lights on: 07:00 AM and light off: 7:00 PM), and allowed *ad libitum* access to water and standard laboratory chow diet throughout the

139 experiment (Lian et al., 2016). To avoid variations from potential interactions with sexual
140 hormones, twenty-four male pups born from these mother rats were used for this study.
141 The day of birth was considered as postnatal day (PN) 0. On PN21, young male rats (n =
142 6/group) were randomly assigned to one of the following treatments: aripiprazole (1.0
143 mg/kg, *t.i.d.*, Bristol-Myers Squibb, New York, USA), olanzapine (1.0 mg/kg, *t.i.d.*, Eli
144 Lilly, Indianapolis, IN, USA), risperidone (0.3 mg/kg, *t.i.d.*, Apotex, Macquarie Park,
145 NSW, Australia), or vehicle. Drug powders mixed with the cookie dough pellets was
146 delivered orally 3 times per day at 07:00 AM, 03:00 PM and 11:00 PM (Pan et al., 2016b;
147 Lian et al., 2016) from PN23 (±1 day). The treatment period was 3 weeks, which
148 corresponds to childhood-adolescence in humans (Brenhouse and Andersen, 2011).
149 Controls received equivalent pellets without drugs. All rats were sacrificed and the brains
150 were obtained two hours after the final dose of antipsychotics. The brains were
151 immediately frozen in liquid nitrogen and then stored under -80°C for future use.

152 The rats were administered antipsychotic drugs three times/day to ensure consistently
153 high concentrations to better mirror the human scenario of oral administration once per
154 day (Lian et al., 2016; Pan et al., 2016b). The dosages were based on the recommended
155 dosages in humans based on body surface area, according to the FDA guidelines for
156 clinical trials (FDA, 2005; Reagan-Shaw et al., 2008), all of which are within the
157 recommended dosage ranges for the psychiatric treatment of children/adolescents
158 (Fraguas et al., 2011; Greenaway and Elbe, 2009). It has been previously reported that, at
159 these used dosages, all these drug reaches 60-80% D_2 receptor occupancy rates in the rat
160 brain (Kapur et al., 2003; Natesan et al., 2006). All experimental procedures were
161 approved by the Animal Ethics Committee, University of Wollongong (AE12/20), and

complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, Australia, 2004).

Brain dissection

The discrete brain regions were collected using a brain microdissection puncture technique as described previously (Pan et al., 2015; Pan et al., 2016c; Pan et al., 2016b). Specifically, based on the brain atlas (Paxinos and Watson, 2005), three sections through the striatum (Bregma 1.00 to 2.20mm) were dissected for the NAc and CPu, respectively. Dissected tissue was kept at -80°C for future use.

Western blots

Frozen brain samples were homogenised in homogenising buffer containing NP-40 cell lysis buffer (Invitrogen, #FNN0021), Protease Inhibitor Cocktail (Sigma-Aldrich, #P8340), β-Glycerophosphate (Sigma-Aldrich, #G9422), and PMSF (Sigma-Aldrich, #P7626). Protein concentration of each sample was measured by the DC Protein Assay (Bio-Rad, #500-0111). Western blot experiments were performed as described previously (Pan et al., 2016b; Pan et al., 2016a; Pan et al., 2016c). Briefly, each sample containing 10μg of protein was denatured, and loaded into Criterion™ TGX™ Precast Gels (Bio-rad, #5671095). The proteins were separated in Criterion™ Vertical Electrophoresis Cells (Bio-rad, #1656001), and then electrophoretically transferred to a polyvinylidene difluoride membrane in Criterion™ Blotters (Bio-rad, #1704071). All membranes were then blocked by 5% skim milk powder, and incubated in primary antibodies and secondary antibodies, respectively. The immunoreactive bands were visualised using Amersham Hyperfilm ECL (GE Healthcare, #28-9068-36) and Luminata Classico

Western HRP substrate (Millipore, #WBLUC0500). All Western blot experiments were performed in duplicate to ensure consistency.

The following antibodies were used to detected corresponding proteins: anti-PKA-Cα (1:1000; Santa Cruz Biotechnology, #SC-903), anti-phosphor-PKA-C (Thr197) (1:1000; Cell Signaling Technology, #5661), anti-Akt (1:2000; Cell Signaling Technology, #4691), anti-phosphor-Akt (Thr308) (1:1000; Cell Signaling Technology, #13038), anti-GSK3β (1:2000; Cell Signaling Technology, #5676), anti-phospho-GSK3β (Ser9) (1:1000; Cell Signaling Technology, #9322), anti-Dvl-3 (1:1000; Santa Cruz Biotechnology, #SC-8027), anti-β-catenin (1:1000; Santa Cruz Biotechnology, #SC-7963), anti-$GABA_A$ β-1 (1:1000; Abcam, #ab154822), anti-CREB1 (1:2000, Abcam, #ab32515), and anti-phospho-CREB1 (1:2000, Abcam, #ab32096). Mouse anti-actin primary polyclonal antibody (1:10000; Millipore, #MAB1501) was used to determine the actin levels. The secondary antibodies were HRP-conjugated anti-rabbit IgG antibody (1:3000; Cell Signaling Technology, #7074) and HRP-conjugated anti-mouse IgG antibody (1:3000; Cell Signaling Technology, #7076).

Statistics

The immunoreactive signals were quantified using Bio-Rad Image Lab (version 6.0). All data were analysed by using SPSS Statistics (version 24.0). The data of each targeted protein were then corrected based on their corresponding actin levels. Data normal distribution was tested using histograms and a Kolmogorov–Smirnov Z-test. One-way analysis of variance (ANOVA) was performed if the data were normally distributed, the post-hoc Dunnett *t* test was used to compare each drug treatment group with the control

group (using raw data). If the data were not normally distributed, the protein expression in each brain region was analysed by a Kruskal-Wallis H-test, followed by the post-hoc Mann–Whiney U-test. The results of each protein expression were expressed by taking the value of the control group as 100%. The ratios of each phosphorylated proteins were analysed by a Kruskal–Wallis H-test and the post-hoc Mann–Whiney U-test. Pearson's correlation tests were employed to analyse the relationships among certain measurements. Statistical significance was accepted when $p \leq 0.05$.

Results

The effect of antipsychotics on Akt and GSK3β

In the NAc, antipsychotic administration did not exert any effects on Akt (Akt, $F_{3, 20}$ = 2.486, $p > 0.05$; p-Akt (Thr308), $F_{3, 20}$ = 1.182, $p > 0.05$), whereas GSK3β was significantly affected by antipsychotic treatment (GSK3β, $F_{3, 20}$ = 4.003, $p < 0.05$; p-GSK3β, $F_{3, 20}$ = 4.696, $p > 0.05$). Post-hoc tests have shown that risperidone down-regulated the expression of GSK3β in the NAc by 64.0% ($p < 0.05$); in addition, both aripiprazole ($p < 0.05$) and olanzapine ($p < 0.01$) increased the expression of p-GSK3β (Ser9) by 59.4% and 90.0%, respectively (Figure 1(b), 1(d)). Furthermore, all three antipsychotics significantly elevated the ratio of p-GSK3β/GSK3β (aripiprazole, $p < 0.01$; olanzapine, $p < 0.05$; risperidone, $p < 0.01$) (Figure 1(b)).

In the CPu, on the other hand, the protein levels of Akt, p-Akt, GSK3β, and p-GSK3β were not significantly affected by any antipsychotic administration (Akt, $F_{3, 20} = 0.910$, $p > 0.05$; p-Akt (Thr308), $F_{3, 20} = 2.159$, $p > 0.05$; GSK3β, $F_{3, 20} = 1.671$, $p > 0.05$; p-GSK3β (Ser9), $F_{3, 20}$ = 0.091, $p > 0.05$) (Figure 2), nor the ratio of p-Akt/Akt and p-

GSK3β/GSK3β (both $p > 0.05$).

The effect of antipsychotics on Dvl-3 and β-catenin

No antipsychotic treatment showed significant effect on the protein expression of Dvl-3 in either the NAc or CPu (NAc, $F_{3, 20} = 1.170$, $p > 0.05$; CPu, $F_{3, 20} = 0.647$, $p > 0.05$). However, β-catenin was significantly altered in both regions. In the NAc, antipsychotics significantly affected the expression of β-catenin ($F_{3, 20} = 4.430$, $p < 0.05$); while in the CPu, the levels of p-β-catenin were changed (NAc, $F_{3, 20} = 5.698$, $p < 0.01$). Post-hoc tests have indicated that in the NAc, both aripiprazole and risperidone significantly reduced the expression of β-catenin by 34.9% ($p < 0.01$) and 24.5% ($p < 0.05$), respectively (Figure 1(c), 1(d)); they also significantly elevated the ratio of p-β-catenin/β-catenin (both $p < 0.05$) (Figure 1(c)). Olanzapine also tended to decrease the expression of β-catenin ($p = 0.63$, -20.6%) and increase the ratio of p-β-catenin/β-catenin in the NAc ($p = 0.092$). In the CPu, only risperidone was able to exert significant effects on the protein levels of p-β-catenin (+139.7%, $p < 0.01$) (Figure 2(c), 2(d)) as well as the ratio of p-β-catenin/β-catenin ($p < 0.01$) (Figure 2(c)).

The effects of antipsychotics on PKA

The protein levels of PKA-C (NAc, $F_{3, 20} = 1.196$, $p > 0.05$; CPu, $F_{3, 20} = 0.158$, $p > 0.05$) and p-PKA-C (Thr197) ($F_{3, 20} = 0.409$, $p > 0.05$; CPu, $F_{3, 20} = 0.644$, $p > 0.05$) were not significantly affected by any antipsychotic treatment in the two brain regions (Figure 3(a), 3(d), 4(a), 4(d)).

The effects of antipsychotics on CREB1

Significant changes in the protein levels of phosphorylated CREB1 were found in the

NAc (CREB1, $F_{3, 20} = 0.449$, $p > 0.05$; p-CREB1, $F_{3, 20} = 4.451$, $p < 0.05$), but no change was found in the CPu (CREB1, $F_{3, 20} = 0.088$, $p > 0.05$; p-CREB1, $F_{3, 20} = 1.439$, $p > 0.05$). Individual comparisons have shown that in the NAc, aripiprazole and risperidone significantly elevated the levels of p-CREB1 by 34.9% ($p < 0.05$) and 59.1% ($p < 0.01$), as well as the ratios of p-CREB1/CREB1 (both $p < 0.01$) (Figure 3(b), 3(d)). Moreover, the ratio of p-CREB1/CREB1 was shown to be positively correlated with the ratio of p-GSK3β/GSK3β ($p < 0.01$, $r = 0.493$) (Figure 5(a)).

The effects of antipsychotics on NMDA receptor subunits

In the NAc, both NMDA NR1 and NR2A expression were significantly altered by antipsychotic treatment (NR1, $F_{3, 20} = 5.099$, $p < 0.01$; NR2A, $F_{3, 20} = 10.903$, $p < 0.01$). Post-hoc comparisons have indicated that all three antipsychotics up-regulated the protein expression of the NDMA NR1 subunit (aripiprazole, +35.0%, $p < 0.05$; olanzapine, +47.2%, $p < 0.01$; risperidone, +53.1%, $p < 0.01$); additionally, both aripiprazole and olanzapine significantly elevated the expression of the NMDA NR2A subunit (aripiprazole, +67.1%, $p < 0.01$; olanzapine, +106.2%, $p < 0.01$) (Figure 2(c), 2(d)). In the CPu, no drug was able to alter the expression of NMDA receptor subunits (NR1, $F_{3, 20} = 1.127$, $p > 0.05$; NR2A, $F_{3, 20} = 0.404$, $p > 0.05$) (Figure 4(c), 4(d)). Furthermore, correlation tests have demonstrated that the expression of NMDA NR1 subunit was positively correlated with the phosphorylation ratio of β-catenin ($p < 0.01$, $r = 0.705$) and CREB1 ($p < 0.01$, $r = 0.475$) (Figure 5(b), 5(c)).

The effect of antipsychotics on $GABA_A$ receptor

In the NAc, the expression of $GABA_A$ (β-1) receptor was significantly altered by

antipsychotic administration ($F_{3, 20} = 3.363$, $p < 0.05$). Elevated expression of $GABA_A$ (β-1) receptor induced by aripiprazole (+52.5%, $p < 0.05$) has been observed; additionally, risperidone tended to increase $GABA_A$ (β-1) receptor expression ($p = 0.1$, +37.4%) (Figure 3(c), 3(d)). In the CPu, $GABA_A$ (β-1) receptor was also significantly influenced by antipsychotic administration ($F_{3, 20} = 9.732$, $p < 0.01$), and its expression was promoted by the administration with both aripiprazole and olanzapine (aripiprazole, +90.0%, $p < 0.01$; olanzapine, +85.6%, $p < 0.01$) (Figure 4(c), 4(d)).

Discussion

The present study has examined the antipsychotic modulations on PKA- and GSK3β-dependent signalling pathways, as well as NMDA and $GABA_A$ receptors, in the NAc and CPu of juvenile male rats. Our results indicated that aripiprazole, olanzapine, and risperidone differentially affected these signalling pathways and receptors; and their effects are also brain-regionally dependent (Table 1).

Modulations of antipsychotics on the GSK3β-associated signalling pathways

Abnormal GSK3β signalling has been reported in a number of mental disorders, including schizophrenia, autism, bipolar disorders, and depression (Hur and Zhou, 2010). The present study has revealed that all three antipsychotics were able to significantly increase the ratio of phosphorylated GSK3β in the NAc of the juvenile rats, indicating that the function of GSK3β in the juvenile rats was inhibited by these antipsychotic drugs. These findings are generally consistent with those of various previous studies in adult rats (Emamian et al., 2004; Alimohamad et al., 2005a; Alimohamad et al., 2005b; Beaulieu et al., 2009; Li et al., 2007). Furthermore, our previous studies that examined

294 the effects of antipsychotics on adult rats have also shown that acute, short-term, and
295 chronic administration with aripiprazole elevated the phosphorylation levels of GSK3β in
296 the NAc (Pan et al., 2015; Pan et al., 2016a; Pan et al., 2016b). Taken together, it is very
297 likely that antipsychotics (at least aripiprazole) modulate GSK3β activity in juvenile rats
298 in a similar manner as in adults.

299 A number of previous studies, including two studies from our group, have demonstrated
300 that various classes of antipsychotics (e.g. aripiprazole, olanzapine, and risperidone) can
301 increase the signalling of β-catenin in the striatum of adult animals (Alimohamad et al.,
302 2005b; Alimohamad et al., 2005a; Sutton and Rushlow, 2011; Seo et al., 2015; Pan et al.,
303 2016a; Pan et al., 2016b; Park et al., 2011). Consistent with these previous studies, the
304 current study has also found up-regulation of the phosphorylation levels of β-catenin in
305 the NAc of juvenile rats by all three antipsychotics (although the effect of olanzapine did
306 not reach significance). Therefore, it could be concluded that β-catenin-mediated
307 signalling in the NAc is very likely to be one of the major targets of antipsychotics in
308 both youths and adults.

309 *Modulations of antipsychotics on CREB1*

310 Novel variants in the CREB1 gene have been identified in schizophrenic subjects
311 (Kawanishi et al., 1999), and a number of *in vivo* studies reported that antipsychotics can
312 increase the phosphorylation levels of CREB1 in adult animals (Pozzi et al., 2003;
313 Konradi and Heckers, 1995; Mavrikaki et al., 2014; Pan et al., 2016c; Pan et al., 2016b;
314 Rogoz et al., 2017; Einoch et al., 2017). CREB1 has also been found to be associated
315 with neurodevelopment (Sakamoto et al., 2011) and involved in childhood-onset mood

316 disorders (Burcescu et al., 2010). In the present study in juvenile rats, both aripiprazole
317 and risperidone, but not olanzapine, significantly elevated the phosphorylation levels of
318 CREB1 in the NAc. These data were consistent with those findings from adult animals or
319 the neurons from adult animals, suggesting that in both juveniles and adults,
320 antipsychotics react with CREB1 in similar patterns.

321 It is interesting that similar to the influences of aripiprazole, risperidone, and olanzapine
322 on the phosphorylation levels of GSK3β and β-catenin, aripiprazole and risperidone also
323 induced larger alterations in the phosphorylation of CREB1 than that induced by
324 olanzapine in the NAc. It has been reported that the affinity of aripiprazole and
325 risperidone for D_2Rs is higher than that of olanzapine (Correll, 2010). Therefore, the
326 stronger influences induced by aripiprazole and risperidone on CREB1 is very likely to
327 be caused by their higher affinity for D_2Rs. However, the signalling pathway(s) through
328 which D_2Rs regulate CREB1 requires further exploration.

329 It has been revealed that extensive communication occurs between CREB1 and GSK3β
330 (Lonze and Ginty, 2002). Consistent with the findings of our previous study in adult rats
331 (Pan et al., 2016b), the data of the present study revealed a positive correlation between
332 the phosphorylation level of GSK3β and that of CREB1 in the NAc. The phenomenon
333 that CREB1 activity can be enhanced by inhibition of GSK3β was observed in both *in*
334 *vitro* and *in vivo* studies (Liang and Chuang, 2006; Park et al., 2011). Moreover, it has
335 been revealed that patients with novel variants in the CREB1 gene experienced positive
336 symptoms of schizophrenia (Kawanishi et al., 1999). Therefore, taken together, it is
337 suggested that activation of CREB1 via inhibition of the function of GSK3β in the NAc is
338 very likely to be associated with the actions of antipsychotics in both juveniles and

339 adults. Considering the extent of the alterations in GSK3β and CREB1 caused by these
340 antipsychotics, it is also suggested that the activation of CREB1 via inhibiting GSK3β
341 functions is likely to be associated with the levels of the binding affinity for D_2Rs of
342 antipsychotics.

343 *Antipsychotics might modulate NMDA receptor subunits via GSK3β-β-*
344 *catenin and/or CREB1 signalling*

345 It has been widely accepted that abnormal NMDAR neurotransmission is associated with
346 many types of mental disorders, including schizophrenia, depression, bipolar disorder,
347 and autism (Yamamoto et al., 2015). Previous studies showed that antipsychotic drug
348 administration (e.g. clozapine and aripiprazole) elevated the NMDAR binding density
349 and expression of protein and mRNA of NMDAR subunits in various brain regions of
350 adult rats, including the NAc, hippocampus, and cortex (Pan et al., 2016b; Schmitt et al.,
351 2003). The present study demonstrated that both aripiprazole and olanzapine
352 administration for 20 days were able to raise the expression levels of NMDA NR1 and
353 NR2A subunits in the NAc of juvenile rats. Therefore, elevating NDMAR expression is
354 very likely to be a shared action of antipsychotics in both juvenile and adult rats.

355 It should be noted that antipsychotics do not directly bind with NMDARs. Thus, it is
356 possible that antipsychotics modulate NMDARs via D_2R-mediated signalling pathways.
357 Previous evidence has revealed the association between GSK3β-β-catenin signalling and
358 the activity of NMDARs (Saiepour et al., 2017; Singh et al., 2017; Wu et al., 2016; Wan
359 et al., 2012; Mills et al., 2014; Sanges et al., 2013). In the current study, we found that the
360 expression of NMDA NR1 subunit was positively correlated with the phosphorylation

level of β-catenin in the NAc after antipsychotic treatment (Figure 5(c)). Thus, taken together with previous studies, our finding further proposes a potential regulation by antipsychotics of NMDARs via GSK3β-β-catenin signalling through the D_2R (Figure 6).

It is also worth noting that antipsychotics might regulate NMDARs via CREB1, as has been reported by several previous studies (Mavrikaki et al., 2014; Yuan et al., 2010; Snyder and Gao, 2013). The present study has shown that the NMDA NR1 expression was positively correlated with the CREB1 phosphorylation in the NAc, further confirming the relationship between CREB1 and NMDARs. Taken together with previous evidence (Lonze and Ginty, 2002), it has been suggested that antipsychotics might modulation NMDARs via PKA-CREB1 signalling (Figure 6). However, exact evidence is still required.

Modulations of antipsychotics on the $GABA_A$ (β-1) receptor

The $GABA_A$ receptor has also been widely reported to be involved in various mental disorders in children/adolescents, such as schizophrenia, depression, bipolar disorder, and autism (Rudolph and Mohler, 2014; Chiapponi et al., 2016), while antipsychotics can regulate $GABA_A$ receptors. For example, 1-week treatment with both haloperidol and olanzapine increased the binding density of $GABA_A$ receptors in the prefrontal cortex of adult rats (Skilbeck et al., 2007). A 6-month clozapine administration reduced the bindings of $GABA_A$ receptors in the anterior cingulate and infralimbic cortex of adult rats (Zink et al., 2004). Our previous studies have found that the expression of $GABA_A$ receptors was elevated by both 1-week and 10-week aripiprazole administration in the NAc of adult rats (Pan et al., 2016c; Pan et al., 2016b). In this study on juvenile rats, both

aripiprazole and olanzapine administration were able to elevate $GABA_A$ receptor expression, which were generally consistent with the results of previous studies (Skilbeck et al., 2007; Zink et al., 2004; Pan et al., 2016c; Pan et al., 2016b), suggesting that the modulation of antipsychotics (at least aripiprazole) on $GABA_A$ (β-1) receptors are similar in both youths and adults.

Like NMDARs, although antipsychotics can impact $GABA_A$ receptors, they do not directly interact with these receptors. Previous studies pointed out that $GABA_A$ receptors can be regulated by the D_2R-downstream PKA signalling pathway (Poisbeau et al., 1999; Connelly et al., 2013). Our previous studies revealed that 1-week antipsychotic treatment modulated both the PKA phosphorylation levels and the expression of $GABA_A$ receptors in the NAc (Pan et al., 2016c), whereas 10-week antipsychotic treatment affected $GABA_A$ receptor expression only (Pan et al., 2016b). The results of the current study in which animals were treated for 3 weeks were similar as those of the 10-week *in vivo* study in adult rats (Pan et al., 2016b). It seems that antipsychotics alter PKA in a time-dependent manner (Figure 6), probably due to adaptive changes in dopamine D_2 receptors after a relatively long period (more than 1 week) of treatment, which however needs further validation.

The brain-regional differences of the modulations of antipsychotics

In the present study, the antipsychotics had very limited affections in the CPu in comparison with those in the NAc, indicating brain-regional differences of the modulations of antipsychotics in juvenile rats. This phenomenon is generally consistent with that in adult animals in our previous studies (Pan et al., 2016a; Pan et al., 2016b).

405 The exact reason for these differences in these two brain regions (NAc and CPu) remains
406 unclear. It is possible that these differences might be caused by the heterogeneous
407 structures of these brain regions that possess different neural inputs and outputs
408 connected with various brain regions (Yager et al., 2015). For example, the NAc and CPu
409 receive dopaminergic inputs from different brain areas – the ventral tegmental area and
410 substantia nigra pars, respectively; in addition, outputs of NAc connect with the limbic
411 areas and prefrontal cortex, while neurons in the CPu project to neocortical areas (Yager
412 et al., 2015).

413 *Notes of intrinsic activity of the three antipsychotics for D_2Rs*

414 In the present study, the three agents have different intrinsic activities for D_2Rs. As a D_2R
415 partial agonist, the intrinsic activity of aripiprazole for D_2R is lower than that of
416 endogenous dopamine. Thus, when aripiprazole competes with endogenous dopamine to
417 bind with D_2Rs in normal animals, the overall activation of D_2Rs could be weaker than
418 that caused by endogenous dopamine solely, thereby showing antagonistic effects on
419 D_2Rs. Therefore, in the present study, aripiprazole displayed antagonistic effects on D_2Rs
420 as haloperidol.

421 In our previous studies (Pan et al., 2016; Pan et al., 2016b; Pan et al., 2016c), we found
422 that bifeprunox, a potent D_2R partial agonist, also exerted certain antagonistic effects on
423 D_2Rs instead of agonistic effects in healthy animals. However, the intrinsic activity of
424 bifeprunox is higher than that of aripiprazole, thereby, the observed antagonistic effects
425 of bifeprunox were relatively weaker.

426

Conclusion

In conclusion, the present study investigated the modulations of aripiprazole, olanzapine, and risperidone on various signalling pathways in the NAc and CPu of juvenile rats, revealing that these antipsychotics share some common effects on these signalling pathways, but differential modulations of these antipsychotics also existed. Furthermore, this study found that NMDA and $GABA_A$ receptors can be modulated by these antipsychotics and revealed possible involvement of GSK3β-β-catenin and/or CREB1 pathways in these modulations. Overall, in view of the involvement of NDMA and $GABA_A$ receptors in the pathophysiology of various mental disorders, this study suggests that antipsychotics might exert their therapeutic effects in treating mental disorders by modulating NMDA and $GABA_A$ receptors via PKA- and GSK3β-dependent signalling pathways in childhood-adolescence. The current study has provided *in vivo* evidence at the molecular level that could be a reference for clinical prescription of childhood schizophrenia, further studies, however, are still necessary by using juvenile animal disease models (such as bipolar disorder, autism, schizophrenia, etc.) to investigate how antipsychotics impact behaviours and reverse deficits of animals via these signalling pathways, as well as to examine direct regulations of antipsychotics on genes and protein expression of downstream targets.

Funding

This work was supported by the National Health and Medical Research Council (APP1104184), Australia to Chao Deng and Jiamei Lian. Bo Pan was supported by the Natural Science Foundation of the Higher Education Institutions of Jiangsu Province, China (17KJB310018), the China Postdoctoral Science Foundation (2018M632401), and the Natural Science Foundation of Jiangsu Province of China (BK20171290). Jiamei Lian was also supported by a National Health and Medical Research Council Early Career Fellowship (APP1125937). The funding organisation did not play a role in the design and conduct of the study, in data interpretation or paper writing.

Declaration of conflicting interests

None of the authors has a conflict of interest.

References

Alimohamad H, Rajakumar N, Seah YH, et al. (2005a) Antipsychotics alter the protein expression levels of beta-catenin and GSK-3 in the rat medial prefrontal cortex and striatum. *Biol Psychiatry* 57: 533-542.

Alimohamad H, Sutton L, Mouyal J, et al. (2005b) The effects of antipsychotics on beta-catenin, glycogen synthase kinase-3 and dishevelled in the ventral midbrain of rats. *J Neurochem* 95: 513-525.

Beaulieu JM, Gainetdinov RR and Caron MG. (2009) Akt/GSK3 signaling in the action of psychotropic drugs. *Annu Rev Pharmacol Toxicol* 49: 327-347.

Brenhouse HC and Andersen SL. (2011) Developmental trajectories during adolescence in males and females: a cross-species understanding of underlying brain changes. *Neurosci Biobehav Rev* 35: 1687-1703.

Burcescu I, Wigg K, King N, et al. (2010) Association study of CREB1 and childhood - onset mood disorders. *American Journal of Medical Genetics Part B Neuropsychiatric Genetics* 137B: 45-50.

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

Chiapponi C, Piras F, Piras F, et al. (2016) GABA System in Schizophrenia and Mood Disorders: A Mini Review on Third-Generation Imaging Studies. *Front Psychiatry* 7: 61.

Connelly WM, Errington AC, Di Giovanni G, et al. (2013) Metabotropic regulation of extrasynaptic GABAA receptors. *Front Neural Circuits* 7: 171.

Correll CU. (2010) From receptor pharmacology to improved outcomes: individualising the selection, dosing, and switching of antipsychotics. *European Psychiatry* 25, Supplement 2: S12-S21.

DelBello MP, Adler CM and Strakowski SM. (2006) The neurophysiology of childhood and adolescent bipolar disorder. *CNS Spectr* 11: 298-311.

Einoch R, Weinreb O, Mandiuk N, et al. (2017) The involvement of BDNF-CREB signaling pathways in the pharmacological mechanism of combined SSRI-antipsychotic treatment in schizophrenia. *Eur Neuropsychopharmacol* 27: 470-483.

Emamian ES, Hall D, Birnbaum MJ, et al. (2004) Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat Genet* 36: 131-137.

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

Fraguas D, Correll CU, Merchan-Naranjo J, et al. (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: 621-645.

Fritschy JM, Paysan J, Enna A, et al. (1994) Switch in the expression of rat GABAA-receptor subtypes during postnatal development: an immunohistochemical study. *J Neurosci* 14: 5302-5324.

Gabbay V, Ely BA, Li Q, et al. (2013) Striatum-based circuitry of adolescent depression and anhedonia. *J Am Acad Child Adolesc Psychiatry* 52: 628-641 e613.

506 Ginovart N and Kapur S. (2012) Role of dopamine D(2) receptors for antipsychotic
507 activity. *Handb Exp Pharmacol*: 27-52.
508 Greenaway Mi and Elbe D. (2009) Focus on Aripiprazole: A Review of its use in Child
509 and Adolescent Psychiatry. *Journal of the Canadian Academy of Child and*
510 *Adolescent Psychiatry* 18: 250-260.
511 Hur EM and Zhou FQ. (2010) GSK3 signalling in neural development. *Nat Rev Neurosci*
512 11: 539-551.
513 James A, Joyce E, Lunn D, et al. (2016) Corrigendum to "Abnormal frontostriatal
514 connectivity in adolescent-onset schizophrenia and its relationship to cognitive
515 functioning" [Eur. Psychiatry 35C (2016) 32–38]. *European Psychiatry* 35: 32.
516 Kapur S, VanderSpek SC, Brownlee BA, et al. (2003) Antipsychotic dosing in preclinical
517 models is often unrepresentative of the clinical condition: a suggested solution
518 based on in vivo occupancy. *J Pharmacol Exp Ther* 305: 625-631.
519 Karanges EA, Stephenson CP and McGregor IS. (2014) Longitudinal trends in the
520 dispensing of psychotropic medications in Australia from 2009-2012: focus on
521 children, adolescents and prescriber specialty. *Aust N Z J Psychiatry* 48: 917-931.
522 Kawanishi Y, Harada S, Tachikawa H, et al. (1999) Novel variants in the promoter region
523 of the CREB gene in schizophrenic patients. *J Hum Genet* 44: 428-430.
524 Konradi C and Heckers S. (1995) Haloperidol-induced Fos expression in striatum is
525 dependent upon transcription factor cyclic AMP response element binding
526 protein. *Neuroscience* 65: 1051-1061.
527 Langen M, Schnack HG, Nederveen H, et al. (2009) Changes in the developmental
528 trajectories of striatum in autism. *Biol Psychiatry* 66: 327-333.
529 Li X, Rosborough KM, Friedman AB, et al. (2007) Regulation of mouse brain glycogen
530 synthase kinase-3 by atypical antipsychotics. *Int J Neuropsychopharmacol* 10: 7-
531 19.
532 Lian J, Pan B and Deng C. (2016) Early antipsychotic exposure affects serotonin and
533 dopamine receptor binding density differently in selected brain loci of male and
534 female juvenile rats. *Pharmacol Rep* 68: 1028-1035.
535 Liang MH and Chuang DM. (2006) Differential roles of glycogen synthase kinase-3
536 isoforms in the regulation of transcriptional activation. *J Biol Chem* 281: 30479-
537 30484.
538 Lonze BE and Ginty DD. (2002) Function and regulation of CREB family transcription
539 factors in the nervous system. *Neuron* 35: 605-623.
540 Lopez-Tellez JF, Vela J, del Rio JC, et al. (2004) Postnatal development of the alpha1
541 containing GABAA receptor subunit in rat hippocampus. *Brain Res Dev Brain*
542 *Res* 148: 129-141.
543 Mavrikaki M, Schintu N, Kastellakis A, et al. (2014) Effects of lithium and aripiprazole
544 on brain stimulation reward and neuroplasticity markers in the limbic forebrain.
545 *Eur Neuropsychopharmacol* 24: 630-638.
546 Mills F, Bartlett TE, Dissing-Olesen L, et al. (2014) Cognitive flexibility and long-term
547 depression (LTD) are impaired following beta-catenin stabilization in vivo. *Proc*
548 *Natl Acad Sci U S A* 111: 8631-8636.
549 Mouri A, Noda Y, Enomoto T, et al. (2007) Phencyclidine animal models of
550 schizophrenia: approaches from abnormality of glutamatergic neurotransmission
551 and neurodevelopment. *Neurochem Int* 51: 173-184.

552 Natesan S, Reckless GE, Nobrega JN, et al. (2006) Dissociation between in vivo
553 occupancy and functional antagonism of dopamine D2 receptors: comparing
554 aripiprazole to other antipsychotics in animal models. *Neuropsychopharmacology*
555 31: 1854-1863.
556 Nutt DJ and Blier P. (2016) Neuroscience-based Nomenclature (NbN) for Journal of
557 Psychopharmacology. *J Psychopharmacol* 30: 413-415.
558 Olfson M, Crystal S, Huang C, et al. (2010) Trends in Antipsychotic Drug Use by Very
559 Young, Privately Insured Children. *J Am Acad Child Adolesc Psychiatry* 49: 13-
560 23.
561 Pan B, Chen J, Lian J, et al. (2015) Unique Effects of Acute Aripiprazole Treatment on
562 the Dopamine D2 Receptor Downstream cAMP-PKA and Akt-GSK3beta
563 Signalling Pathways in Rats. *PLoS One* 10: e0132722.
564 Pan B, Huang XF and Deng C. (2016a) Aripiprazole and Haloperidol Activate
565 GSK3beta-Dependent Signalling Pathway Differentially in Various Brain Regions
566 of Rats. *Int J Mol Sci* 17: 459.
567 Pan B, Huang XF and Deng C. (2016b) Chronic administration of aripiprazole activates
568 GSK3beta-dependent signalling pathways, and up-regulates GABAA receptor
569 expression and CREB1 activity in rats. *Sci Rep* 6: 30040.
570 Pan B, Lian J, Huang XF, et al. (2016c) Aripiprazole Increases the PKA Signalling and
571 Expression of the GABAA Receptor and CREB1 in the Nucleus Accumbens of
572 Rats. *J Mol Neurosci* 59: 36-47.
573 Panaccione I, Napoletano F, Forte AM, et al. (2013) Neurodevelopment in schizophrenia:
574 the role of the wnt pathways. *Curr Neuropharmacol* 11: 535-558.
575 Park SW, Seo MK, Cho HY, et al. (2011) Differential effects of amisulpride and
576 haloperidol on dopamine D2 receptor-mediated signaling in SH-SY5Y cells.
577 *Neuropharmacology* 61: 761-769.
578 Paxinos G and Watson C. (2005) *The rat brain in stereotaxic coordinates,* San Diego,
579 CA: Elsevier Academic Press.
580 Poisbeau P, Cheney MC, Browning MD, et al. (1999) Modulation of synaptic GABAA
581 receptor function by PKA and PKC in adult hippocampal neurons. *J Neurosci* 19:
582 674-683.
583 Pozzi L, Hakansson K, Usiello A, et al. (2003) Opposite regulation by typical and
584 atypical anti-psychotics of ERK1/2, CREB and Elk-1 phosphorylation in mouse
585 dorsal striatum. *J Neurochem* 86: 451-459.
586 Reagan-Shaw S, Nihal M and Ahmad N. (2008) Dose translation from animal to human
587 studies revisited. *Faseb j* 22: 659-661.
588 Rogoz Z, Kaminska K, Panczyszyn-Trzewik P, et al. (2017) Repeated co-treatment with
589 antidepressants and risperidone increases BDNF mRNA and protein levels in rats.
590 *Pharmacol Rep* 69: 885-893.
591 Ronsley R, Scott D, Warburton WP, et al. (2013) A population-based study of
592 antipsychotic prescription trends in children and adolescents in British Columbia,
593 from 1996 to 2011. *Canadian Journal of Psychiatry Revue Canadienne De*
594 *Psychiatrie* 58: 361-369.
595 Roth BL and Driscol J. (2018) PDSP Ki database. *Psychoactive Drug Screening Program*
596 *(PDSP).* University of North Carolina at Chapel Hill and the United States
597 National Institute of Mental Health.

598 Rudolph U and Mohler H. (2014) GABAA receptor subtypes: Therapeutic potential in
599 Down syndrome, affective disorders, schizophrenia, and autism. *Annu Rev*
600 *Pharmacol Toxicol* 54: 483-507.
601 Saiepour MH, Min R, Kamphuis W, et al. (2017) beta-Catenin in the Adult Visual Cortex
602 Regulates NMDA-Receptor Function and Visual Responses. *Cereb Cortex*: 1-12.
603 Sakamoto K, Karelina K and Obrietan K. (2011) CREB: a multifaceted regulator of
604 neuronal plasticity and protection. *J Neurochem* 116: 1-9.
605 Sanges D, Romo N, Simonte G, et al. (2013) Wnt/beta-catenin signaling triggers neuron
606 reprogramming and regeneration in the mouse retina. *Cell Rep* 4: 271-286.
607 Schmidt MJ and Mirnics K. (2015) Neurodevelopment, GABA system dysfunction, and
608 schizophrenia. *Neuropsychopharmacology* 40: 190-206.
609 Schmitt A, Zink M, Muller B, et al. (2003) Effects of long-term antipsychotic treatment
610 on NMDA receptor binding and gene expression of subunits. *Neurochem Res* 28:
611 235-241.
612 Schneider C, Taylor D, Zalsman G, et al. (2014) Antipsychotics use in children and
613 adolescents: An on-going challenge in clinical practice. *J Psychopharmacol* 28:
614 615-623.
615 Segnitz N, Ferbert T, Schmitt A, et al. (2011) Effects of chronic oral treatment with
616 aripiprazole on the expression of NMDA receptor subunits and binding sites in rat
617 brain. *Psychopharmacology (Berl)* 217: 127-142.
618 Seo MK, Lee CH, Cho HY, et al. (2015) Effects of antipsychotic drugs on the expression
619 of synapse-associated proteins in the frontal cortex of rats subjected to
620 immobilization stress. *Psychiatry Res* 229: 968-974.
621 Sheng M, Cummings J, Roldan LA, et al. (1994) Changing subunit composition of
622 heteromeric NMDA receptors during development of rat cortex. *Nature* 368: 144-
623 147.
624 Singh S, Mishra A, Srivastava N, et al. (2017) MK-801 (Dizocilpine) Regulates Multiple
625 Steps of Adult Hippocampal Neurogenesis and Alters Psychological Symptoms
626 via Wnt/beta-Catenin Signaling in Parkinsonian Rats. *ACS Chem Neurosci* 8: 592-
627 605.
628 Skilbeck KJ, O'Reilly JN, Johnston GA, et al. (2007) The effects of antipsychotic drugs
629 on GABAA receptor binding depend on period of drug treatment and binding site
630 examined. *Schizophr Res* 90: 76-80.
631 Snyder MA and Gao WJ. (2013) NMDA hypofunction as a convergence point for
632 progression and symptoms of schizophrenia. *Front Cell Neurosci* 7: 31.
633 Sutton LP and Rushlow WJ. (2011) The effects of neuropsychiatric drugs on glycogen
634 synthase kinase-3 signaling. *Neuroscience* 199: 116-124.
635 Vitiello B, Correll C, van Zwieten-Boot B, et al. (2009) Antipsychotics in children and
636 adolescents: increasing use, evidence for efficacy and safety concerns. *Eur*
637 *Neuropsychopharmacol* 19: 629-635.
638 Wan XZ, Li B, Li YC, et al. (2012) Activation of NMDA receptors upregulates a
639 disintegrin and metalloproteinase 10 via a Wnt/MAPK signaling pathway. *J*
640 *Neurosci* 32: 3910-3916.
641 Wu HF, Chen PS, Chen YJ, et al. (2016) Alleviation of N-Methyl-D-Aspartate Receptor-
642 Dependent Long-Term Depression via Regulation of the Glycogen Synthase
643 Kinase-3beta Pathway in the Amygdala of a Valproic Acid-Induced Animal Model

644 of Autism. *Mol Neurobiol*.
645 Yager LM, Garcia AF, Wunsch AM, et al. (2015) The ins and outs of the striatum: role in
646 drug addiction. *Neuroscience* 301: 529-541.
647 Yamamoto H, Hagino Y, Kasai S, et al. (2015) Specific Roles of NMDA Receptor
648 Subunits in Mental Disorders. *Curr Mol Med* 15: 193-205.
649 Yuan P, Zhou R, Wang Y, et al. (2010) Altered levels of extracellular signal-regulated
650 kinase signaling proteins in postmortem frontal cortex of individuals with mood
651 disorders and schizophrenia. *J Affect Disord* 124: 164-169.
652 Zink M, Schmitt A, May B, et al. (2004) Differential effects of long-term treatment with
653 clozapine or haloperidol on GABAA receptor binding and GAD67 expression.
654 *Schizophr Res* 66: 151-157.
655

656

Table 1. Summary of the effects of three antipsychotics in the nucleus accumbens (NAc) and caudate putamen (CPu) of juvenile rats.

Effects of antipsychotics in NAc	
Aripiprazole	p-GSK3β↑, Ratio of GSK3β↑; β-catenin↓, Ratio of β-catenin↑; p-CREB↑, Ratio of CREB↑; NMDA NR1↑, NMDA NR2A↑; $GABA_A$ β-1↑
Olanzapine	p-GSK3β↑, Ratio of GSK3β↑; NMDA NR1↑, NMDA NR2A↑
Risperidone	GSK3β↓, Ratio of GSK3β↑; β-catenin↓, Ratio of β-catenin↑; p-CREB↑, Ratio of CREB↑; NMDA NR1↑
Effects of antipsychotics in CPu	
Aripiprazole	$GABA_A$ β-1↑
Olanzapine	$GABA_A$ β-1↑
Risperidone	β-catenin↑, Ratio of β-catenin↑

CREB: cAMP-responsive element-binding protein; GABA: γ-aminobutyric acid; GSK3β: glycogen synthase kinase 3 beta; NMDA: N-methyl-D-aspartate.

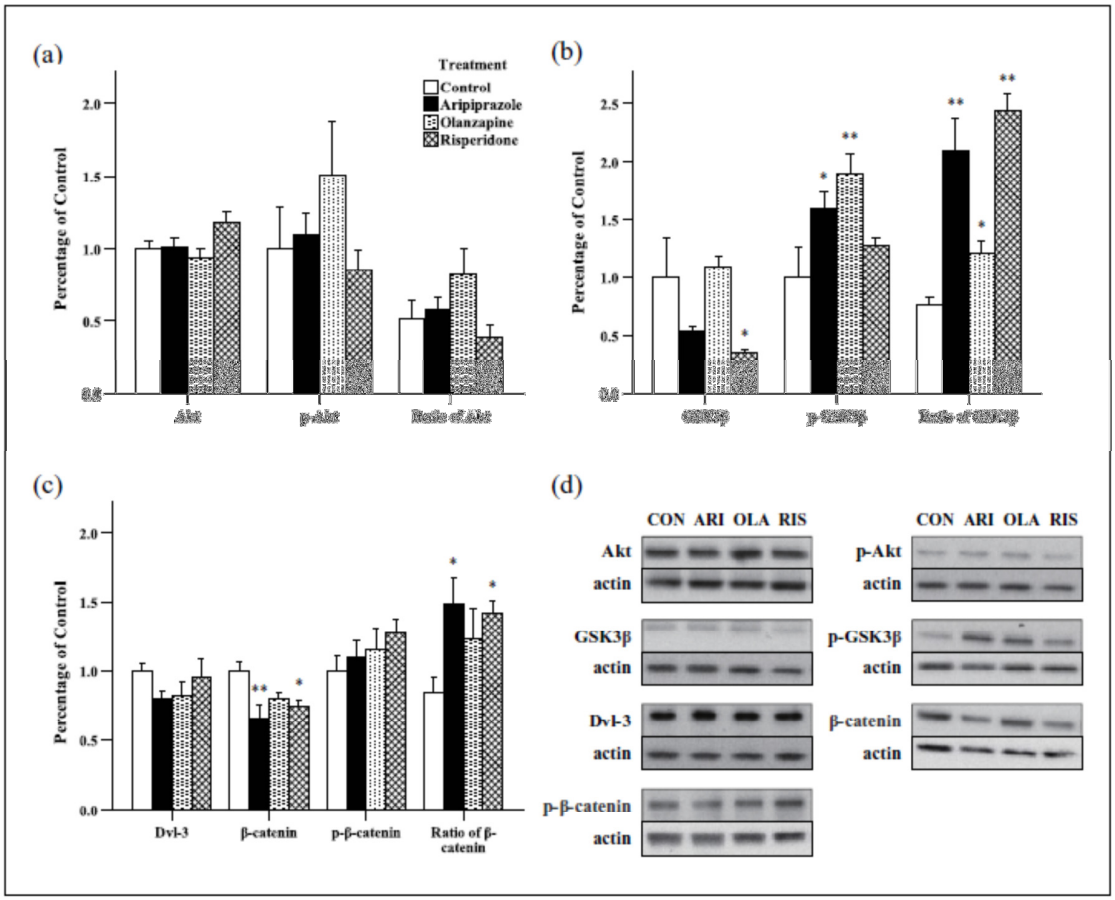


Figure 1. Effects of three antipsychotics on Akt, GSK3β, Dvl-3 and β-catenin in the nucleus accumbens. The effects of aripiprazole (ARI), olanzapine (OLA) and risperidone (RIS) on Akt (a), GSK3β (b), Dvl-3 and β-catenin (c) were measured in the nucleus accumbens. The representative bands of Western blot are shown in (d). Akt was quantified at 60kDa; p-Akt (Thr308) was quantified at 60kDa; GSK3β was quantified at 46kDa; p-GSK3β (Ser9) was quantified at 46kDa; Dvl-3 was quantified at 85kDa; β-catenin was quantified at 92kDa; p-β-catenin was quantified at 92kDa. The data were normalised by taking the average value of the control group as 100% and expressed as mean ± S.E.M. (* $p \leq 0.05$, ** $p < 0.01$ versus the control)

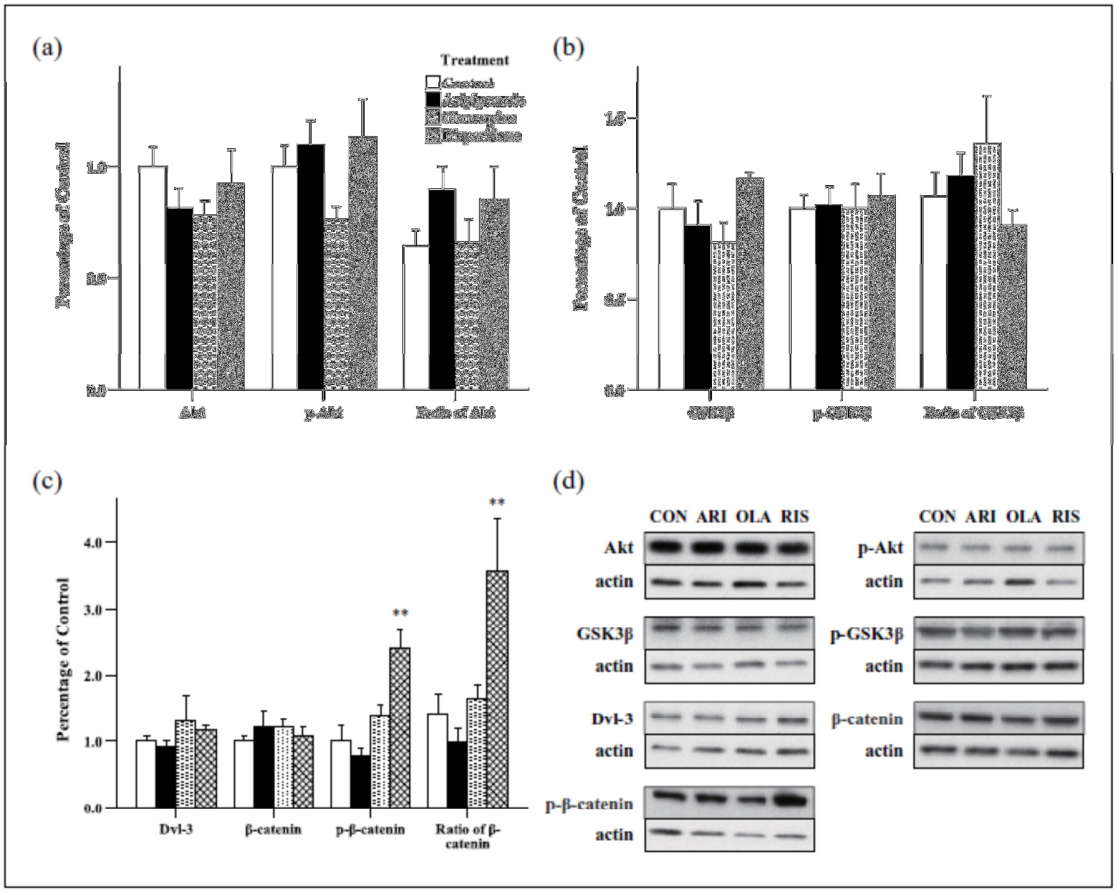
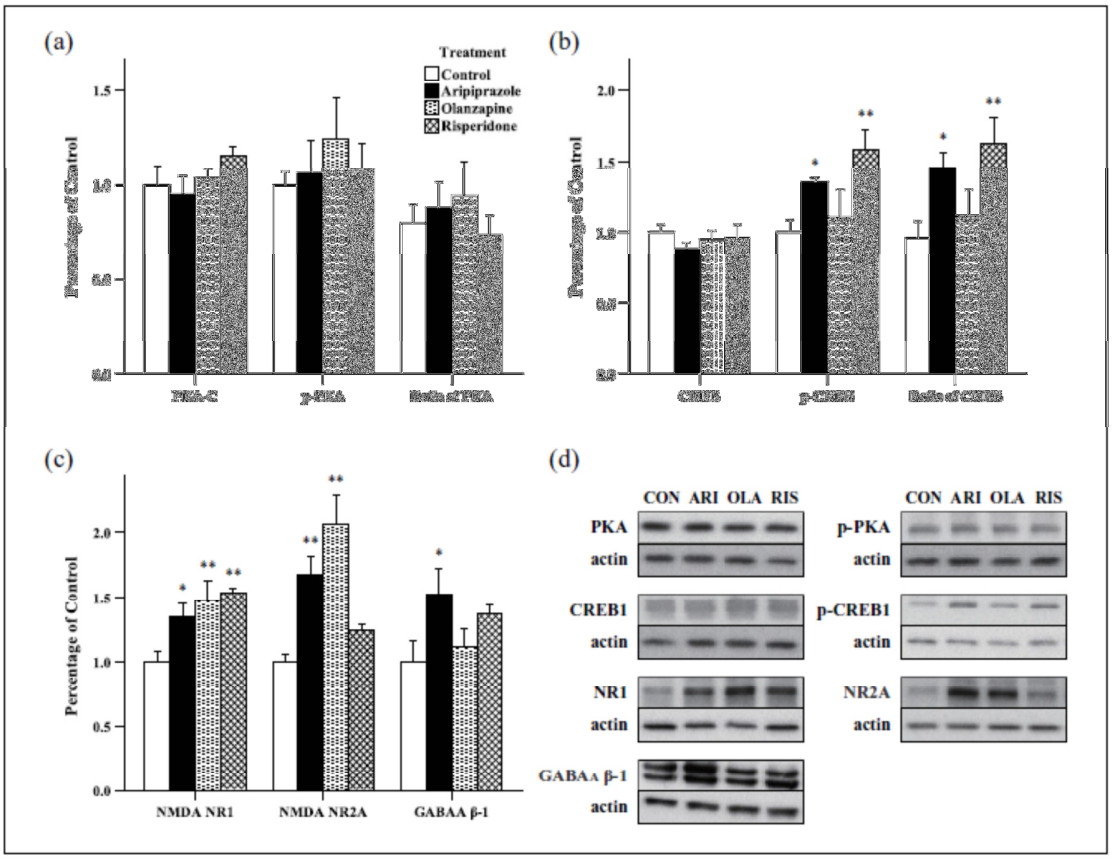
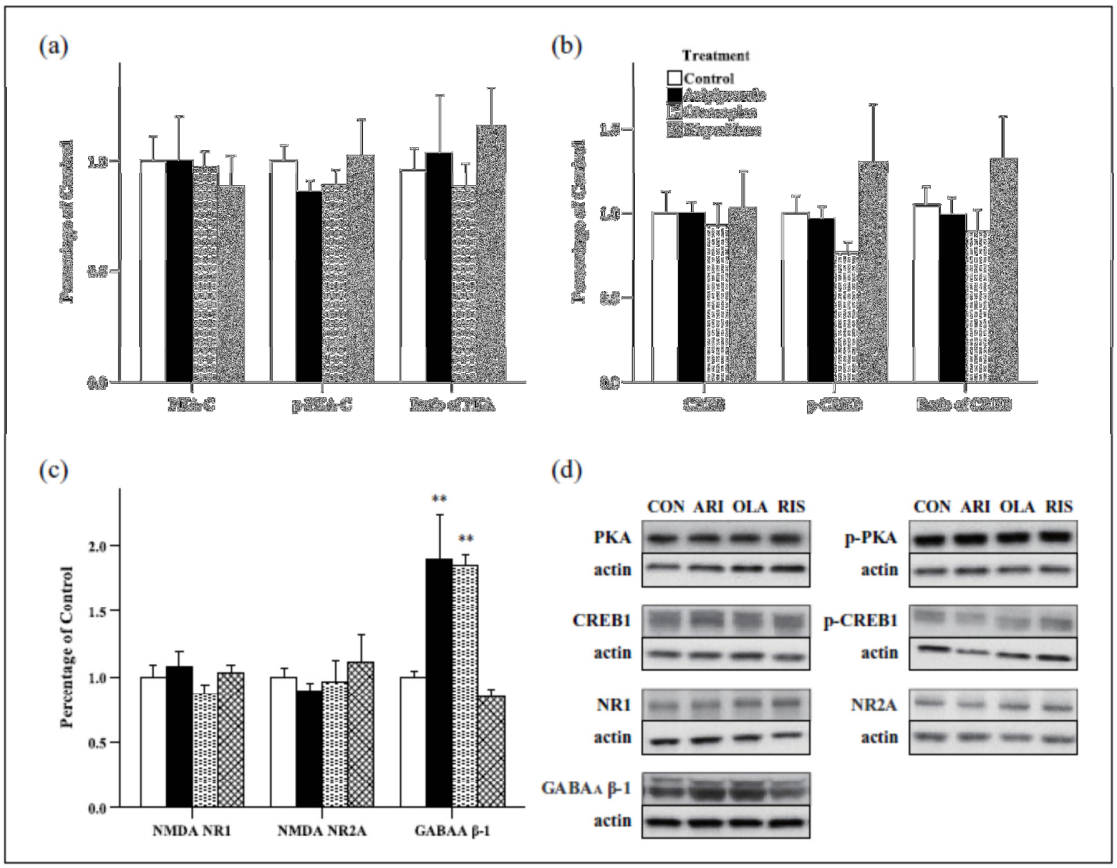


Figure 2. Effects of three antipsychotics on Akt, GSK3β, Dvl-3 and β-catenin in the caudate putamen. The effects of aripiprazole (ARI), olanzapine (OLA) and risperidone (RIS) on Akt (a), GSK3β (b), Dvl-3 and β-catenin (c) were measured in the caudate putamen. The representative bands of Western blot are shown in (d). Akt was quantified at 60kDa; p-Akt (Thr308) was quantified at 60kDa; GSK3β was quantified at 46kDa; p-GSK3β (Ser9) was quantified at 46kDa; Dvl-3 was quantified at 85kDa; β-catenin was quantified at 92kDa; p-β-catenin was quantified at 92kDa. The data were normalised by taking the average value of the control group as 100% and expressed as mean ± S.E.M. (* $p \leq 0.05$, ** $p < 0.01$ versus the control)



680

681 **Figure 3. Effects of three antipsychotics on PKA-C, CREB1, NMDA NR1, NR2A**
682 **and $GABA_A$ β-1 receptors in the nucleus accumbens.** The effects of aripiprazole
683 (ARI), olanzapine (OLA) and risperidone (RIS) on PKA-C (a), CREB1 (b), NMDA NR1
684 and NR2A (c) and $GABA_A$ β-1 receptor (c) were measured in the nucleus accumbens.
685 The representative bands of Western blot are shown in (d). PKA-C was quantified at
686 42kDa; p-PKA-C (Thr197) was quantified at 42kDa; CREB1 was quantified at 40kDa; p-
687 CREB1 was quantified at 37kDa; NMDA NR1 subunit was quantified at 105kDa; NMDA
688 NR2A subunit was quantified at 165kDa; and $GABA_A$ β-1 receptors were quantified at
689 54kDa. The data were normalised by taking the average value of the control group as
690 100% and expressed as mean ± S.E.M. (* $p \leq 0.05$, ** $p < 0.01$ versus the control)



691

692 **Figure 4. Effects of three antipsychotics on PKA-C, CREB1, NMDA NR1, NR2A**
693 **and $GABA_A$ β-1 receptors in the caudate putamen.** The effects of aripiprazole (ARI),
694 olanzapine (OLA) and risperidone (RIS) on PKA-C (a), CREB1 (b), NMDA NR1 and
695 NR2A (c) and $GABA_A$ β-1 receptor (c) were measured in the caudate putamen. The
696 representative bands of Western blot are shown in (d). PKA-C was quantified at 42kDa;
697 p-PKA-C (Thr197) was quantified at 42kDa; CREB1 was quantified at 40kDa; p-CREB1
698 was quantified at 37kDa; NMDA NR1 subunit was quantified at 105kDa; NMDA NR2A
699 subunit was quantified at 165kDa; and $GABA_A$ β-1 receptors were quantified at 54kDa.
700 The data were normalised by taking the average value of the control group as 100% and
701 expressed as mean ± S.E.M. (* $p \leq 0.05$, ** $p < 0.01$ versus the control)

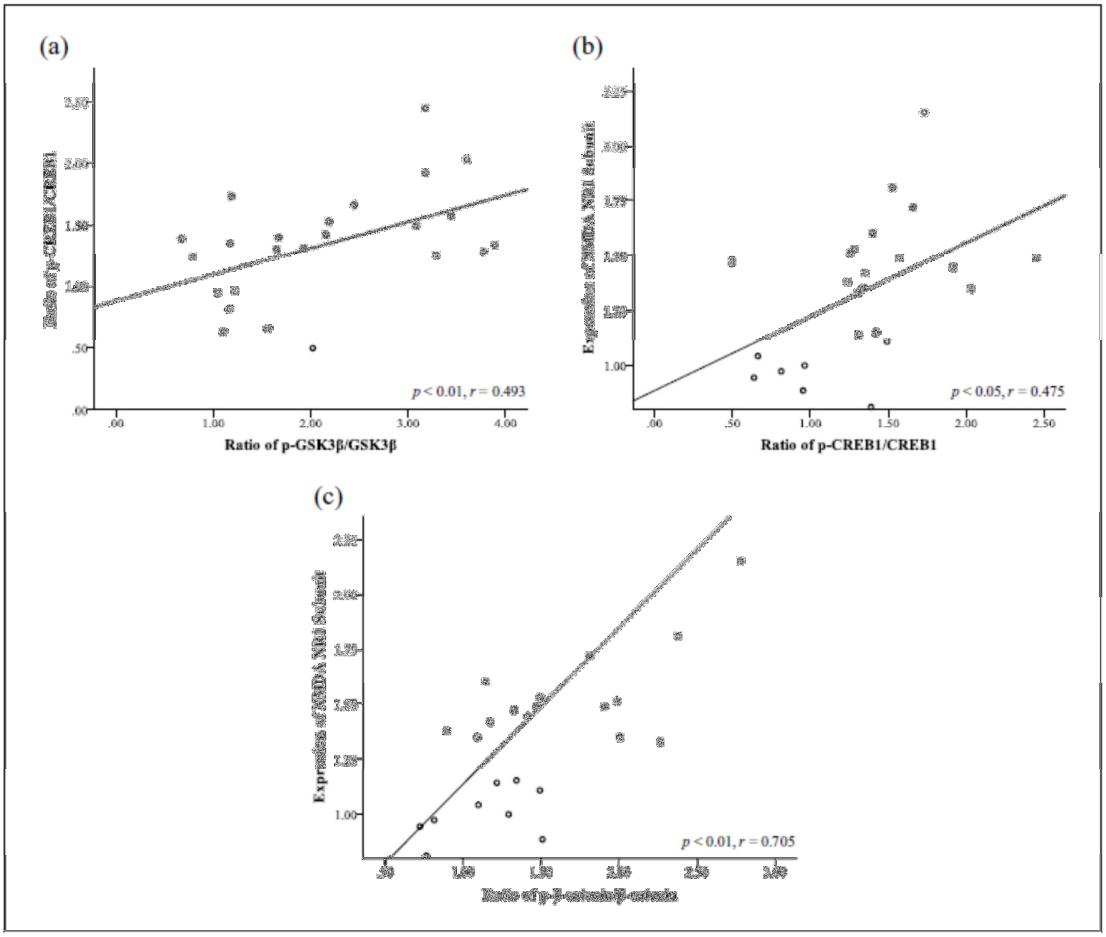
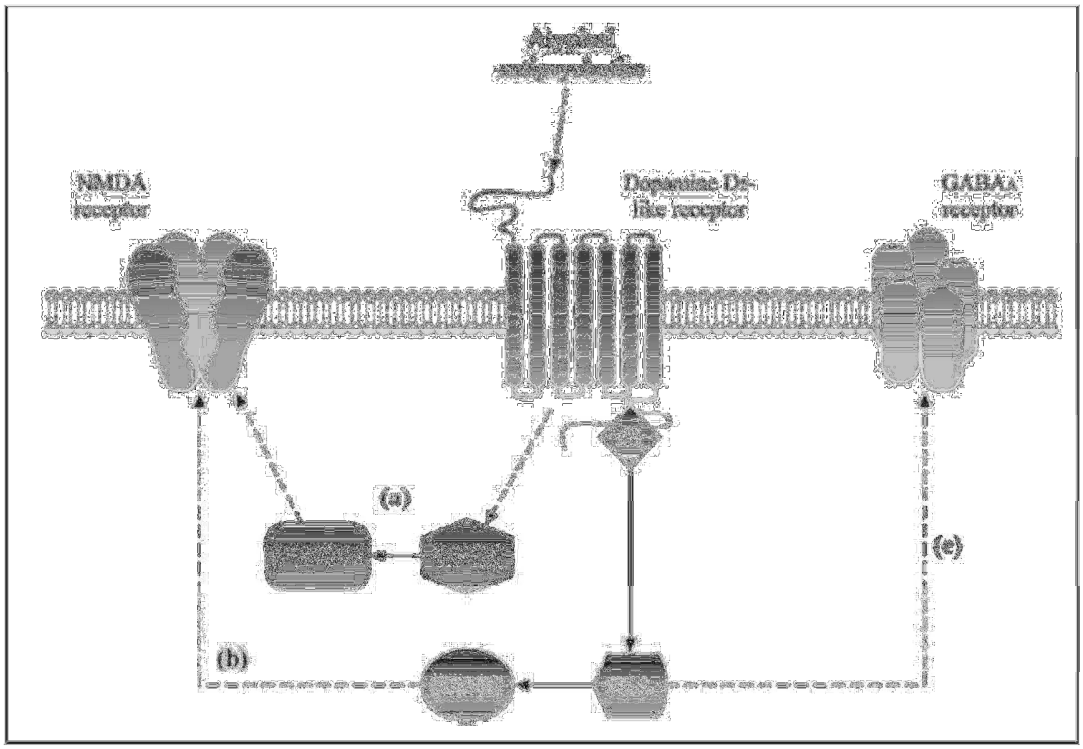


Figure 5. Correlations between the ratio of p-GSK3β/GSK3β and the ratio of p-CREB1/CREB1, the ratio of p-β-catenin/β-catenin and the expression of NMDA NR1 subunit, and the ratio of p-CREB1/CREB1 and the expression of NMDA NR1 subunit in the nucleus accumbens. The ratio of p-CREB1/CREB1 was positively correlated with the ratio of p-GSK3β/GSK3β (a); the expression of NMDA NR1 subunit was positively correlated with the ratio of p-β-catenin/β-catenin (b), as well as the ratio of p-CREB1/CREB1 (c).



712

713 **Figure 6. A proposed schematic diagram illustrating the possible signalling**
714 **pathways through which antipsychotics affect NMDA and $GABA_A$ receptors in the**
715 **nucleus accumbens.** Antipsychotics bind with the dopamine D_2-like receptor, probably
716 resulting in the phosphorylation of GSK3β and β-catenin, which finally induces the
717 elevation in the expression of NMDA receptor subunits (a). Reaction with D_2-like
718 receptors by antipsychotics results in the increase in the expression of NMDA receptors
719 probably via the PKA-CREB1 signalling pathway (b). Antipsychotics might modulate
720 $GABA_A$ receptors via PKA signalling in juveniles in a time-dependent manner (c). (The
721 dashed arrows indicate our speculative ideas.)

722