Effects of antipsychotic drugs on the expression of synapse-associated proteins in the frontal cortex of rats subjected to immobilization stress

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A B S T R A C T
The present study examined the effects of antipsychotic drugs on the expression of synapse-associated proteins in the frontal cortex of rats with and without immobilization stress. Rats were subjected to immobilization stress 6 h/day for 3 weeks. The effects of atypical antipsychotic drugs, olanzapine and aripiprazole, on expression of serine9-phosphorylated GSK-3β, β-catenin, BDNF, PSD-95, and synaptophysin were determined by Western blotting. A typical antipsychotic drug, haloperidol, was used for comparison. Immobilization stress significantly decreased the expression of these proteins in the frontal cortex. Chronic administration of olanzapine and aripiprazole significantly attenuated the immobilization stress-induced decrease in the levels of these proteins, whereas haloperidol had no such effect. Additionally, olanzapine and aripiprazole significantly increased levels of phosphorylated GSK-3β under normal conditions without stress, and aripiprazole also increased BDNF levels under this condition. These results indicate that olanzapine and aripiprazole, and, haloperidol, differentially regulate the levels of synapse-associated proteins in the rat frontal cortex. These findings may contribute to explain the neurobiological basis of how olanzapine and aripiprazole up-regulate synapse-associated proteins.

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1. Introduction

Schizophrenia is a severe psychiatric disorder characterized by positive (hallucinations and delusions), negative (affective and social dysfunction), and cognitive (impairments in learning and memory, information processing, and executive function) symptoms (Stahl, 2008). Although the underlying cause of the disorder remains largely unknown, converging lines of evidence propose that disrupted cortical synaptic circuitry, including reduced gray matter volume, synaptic markers, and neuropil, is a main deficit in schizophrenia (Lewis and Lieberman, 2000; Glantz et al., 2006). These neuronal alterations may underpin the cognitive deficits observed in patients with schizophrenia.

Two classes of drugs used in the treatment of schizophrenia, typical and atypical antipsychotic medications, differ in their efficacy for treating the symptoms of this disorder. Although the underlying mechanisms of these actions are not fully understood, it has recently been suggested that atypical antipsychotic drugs up-regulate dendritic spine formation, dendritic outgrowth, and synaptic protein levels in rat hippocampal neurons, whereas the typical antipsychotic drug, haloperidol, down-regulates them or has no effect (Critchlow et al. 2006; Park et al., 2013). Therefore, it can be suggested that the positive effects of atypical antipsychotic drugs on synaptic plasticity reflect a significant difference between typical and atypical antipsychotic medications.

The canonical Wnt signaling pathway regulates various aspects of neural circuit formation, including neural polarity, axon guidance, synapse formation, and synaptic plasticity, in vertebrate and invertebrate nervous systems (Park and Shen, 2012). Glycogen synthase kinase-3β (GSK-3β) is normally inhibited in Wnt signaling, where its primary target is β-catenin. As a result of the inactivation of GSK-3β, intracellular levels of β-catenin increase, allowing its nuclear translocation to activate the expression of Wnt target genes in concert with the TCF/LEF family of transcription factors, whereas active GSK-3β phosphorylates β-catenin, leading to its ubiquitin-dependent degradation (Logan and Nusse, 2004). Additionally, stable β-catenin is a critical regulator of synaptogenesis and synaptic plasticity, acting as a link between cadherins in the plasma membrane (Arikath and Reichardt, 2008).

Postsynaptic brain-derived neurotrophic factor (BDNF), the
most abundant neurotrophin in the brain, contributes to axonal branching, dendritic differentiation, and connectivity among neurons (Poo, 2001; Lessmann et al., 2003; Ji et al., 2005). Increased levels of BDNF are associated with improved learning and memory, and a reduction in BDNF plays a role in age-related memory deficits (Bimonte et al., 2003). Postsynaptic density protein PSD-95 is a scaffolding protein that anchors receptors, including glutamate receptors (Han and Kim, 2008). It is located preferentially in dendritic spines and plays a critical role in regulating dendritic spine size and shape (Ehrlich et al., 2007; Han and Kim, 2008). Thus, PSD-95 is widely used as a postsynaptic marker (Okabe et al., 1999). Synaptophysin is the major integral membrane protein of presynaptic vesicles required for vesicle formation and exocytosis (Valtorta et al., 2004). It is widely used as a presynaptic marker for synapse activity, and an increase in synaptophysin is generally correlated with synaptogenesis (Eastwood and Harrison, 2001). In this context, increased BDNF, PSD-95, or synaptophysin levels may reflect increased synaptic density, activity, and vesicles, indicating improved functioning of synapses. Decreased levels of GSK-3β phosphorylation, β-catenin, BDNF, PSD-95, or synaptophysin have been reported in the postmortem brains of patients with schizophrenia (Cotter et al., 1998; Karson et al., 1999; Vawter et al., 1999; Durany and Thome, 2004; Kozlovsky et al., 2004; Nadri et al., 2004; Funk et al., 2012). Changes in GSK-3β phosphorylation, β-catenin, or PSD-95 levels have been identified in rat brains following chronic administration of antipsychotic drugs (Bai et al., 2003; Alimohamad et al., 2005a, 2005b; Park et al., 2006, 2009a, 2011). However, the effects of antipsychotic drugs on the regulation of presynaptic and postsynaptic proteins, PSD-95, and synaptophysin have not been explored in any detail under in vivo conditions. Atypical antipsychotic drugs may exert beneficial effects by reversing deficits in the levels of these synapse-associated proteins. To test this hypothesis, we used an established immobilization stress model in which levels of GSK-3β phosphorylation, β-catenin, BDNF expression, and synaptic proteins are decreased (Park et al., 2006, 2009a, 2011; Fang et al., 2013). Moreover, immobilization stress for 21 days decreases the neurogenesis in the dentat gyrus of rat hippocampus (Park et al., 2007). Another common observation is that this stress model causes atrophy of the apical dendrite and dendritic spine loss in rat prefrontal cortex, as well as working memory impairment (Hains et al., 2009). These cellular and molecular changes in response to stress have been also observed in the studies for the investigation of postsynaptic mechanisms of schizophrenia (Cotter et al., 1998; Durany and Thome, 2004; Kozlovsky et al., 2004; Nadri et al., 2004; Glantz et al., 2006; Benarroch, 2013; Eich et al., 2014).

The present study investigated the effects of haloperidol and the atypical antipsychotic drugs olanzapine and aripiprazole on phosphorylated-serine9-phosphorylated GSK-3β, β-catenin, BDNF, PSD-95, and synaptophysin expression in the frontal cortex, a brain region known to be involved in the pathophysiology of schizophrenia and in key cognitive functions.

2. Materials and methods
2.1. Drugs and reagents
Olanzapine was supplied by Eli Lilly Research Laboratories (Indianapolis, IN, USA), aripiprazole was supplied by Otsuka Pharmaceuticals (Tokushima, Japan), and haloperidol was purchased from Sigma (St. Louis, MO, USA). Antibodies used for Western blot analysis were obtained from the following sources: anti-BDNF (sc-546) and anti-GSK-3 (sc-7291) from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-phosphorylated-GSK-3β (Ser 9; 9336) from Cell Signaling Technology (Beverly, MA, USA); anti-PSD-95 (AB9634) from Millipore (Temecula, CA, USA); anti-synaptophysin (ab52636) from Abcam (Cambridge, UK); and anti-β-catenin (C2206) and anti-α-tubulin (T9026) from Sigma. Anti-mouse IgG peroxidase conjugates were obtained from Sigma, and goat-anti-rabbit IgG horseradish-peroxide conjugates were from Santa Cruz Biotechnology.

2.2. Animals and drug administration
The procedures used in the present study complied with the animal care guidelines in the “Principles of Laboratory Animal Care” (NIH publication no. 23-85, 1996). All experiments involving animals were approved by the Committee for Animal Experimentation and the Institutional Animal Laboratory Review Board of Inje Medical College (Approval no. 2012-0110). Male Sprague–Dawley rats ( Orient Bio, GyeongGi-Do, Korea) weighing 200–250 g were housed two or three per cage with food and water available ad libitum; they were maintained at 21 °C on a 12:12 h light/dark cycle. After 7 days of acclimation, the rats were randomly divided into eight groups of five rats each. All drugs were dissolved in vehicle (0.8% glacial acetic acid in 0.9% saline) and injected intraperitoneally (i.p.) into the animals. The first group (vehicle) received vehicle (1 mL/kg, i.p.) without immobilization stress. The second (olanzapine), third (aripiprazole), and fourth (haloperidol) groups received olanzapine (2 mg/kg, i.p.), aripiprazole (1.5 mg/kg, i.p.), and haloperidol (1 mg/kg, i.p.), respectively, and were then immobilized in the same way as were the rats in the fifth group. These procedures were repeated once daily for 3 weeks.

The clinical effect of many antipsychotic drugs are reflected in dopamine D2 receptor occupancy of 60–70% (Farde et al., 1988; Kapur et al., 2001). Drug doses were calculated based on rat studies that investigated D2 receptor occupancy (Barth et al., 2006; Natesan et al., 2006) and had plasma levels well within the therapeutic range of the doses used for treatment of patients with schizophrenia (Anderson et al., 2000).

2.3. Protein extraction and Western blot
Rats were sacrificed 24 h after the last immobilization session. The brain was removed, and the frontal cortex was dissected out. Whole frontal cortex samples were homogenized in ice-cold lysis buffer containing 20 mM Tris–HCl, 137 mM NaCl, 10% glycerol, 1% Nonidet P-40, 0.1% sodium deoxycholate, 2 mM EDTA, one tablet of complete protease inhibitor (Roche, Canada), 20 mM NaF, and 1 mM Na3VO4. The tissue homogenates were centrifuged (13,000 rpm, 30 min, 4 °C), and the supernatants were collected and used to quantitate the total protein. Equal amounts of protein (30 μg) from tissue extracts under each treatment condition were separated by SDS-polyacrylamide gel electrophoresis and transferred electrophoretically onto polyvinylidene fluoride (PVDF) membranes. The PVDF membranes were blocked by incubation in 5% (w/v) nonfat milk in Tris-buffered saline (TBS) with 0.15% Tween 20 (TBS-T) for 1 h. After incubation with a primary antibody (anti-phospho-ser9-GSK-3β, 1:1000; anti-GSK-3, 1:1000; anti-β-catenin, 1:1000; anti-BDNF, 1:1000; anti-PSD-95, 1:1000; anti-synaptophysin, 1:1000; anti-α-tubulin, 1:2000) in TBS-T at 4 °C overnight, the membranes were washed three times in TBS-T for 10 min. The membranes were then incubated in 1 h in TBS-T containing horseradish peroxidase-conjugated secondary antibody (goat-anti-rabbit IgG for anti-phospho-ser9-GSK-3β, 1:2000; anti-β-catenin, 1:10,000; anti-BDNF, 1:2000; anti-PSD-95, 1:2000; anti-synaptophysin, 1:2000; anti-α-tubulin, 1:10,000). Immunoreactive bands were visualized and quantified using ECL Western blotting reagents (Bio-Rad, Hercules, CA), and chemiluminescence was detected with the Las-3000 Image Reader software (Fuji Film, Tokyo, Japan). Protein levels were normalized to the housekeeping protein α-tubulin to adjust for variability in protein loading. Data are expressed as percentages of the vehicle control (deemed to be 100%).

2.4. Statistical analysis
To determine the individual and interactive effects of drug administration and immobilization stress on protein levels, two-way ANOVA was performed. Scheffe’s test was used for the post hoc comparison. A p-value < 0.05 was considered to indicate statistical significance.

3. Results
3.1. Effects of olanzapine, aripiprazole, and haloperidol on levels of serine2-phosphorylated GSK-3β and β-catenin in the frontal cortex
Immobilization stress significantly reduced the levels of GSK-3β phosphorylation and β-catenin in the frontal cortex of

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experimental subjects, to about 30% and 40%, respectively, compared with those of vehicle-treated controls (all $p < 0.01$; Fig. 1). Chronic treatment with olanzapine and aripiprazole prevented the reduction in phosphorylated GSK-3β (stress + olanzapine = 61% of control, $p < 0.01$; stress + aripiprazole = 93% of control, $p < 0.01$; Fig. 1A) and β-catenin (stress + olanzapine = 71% of control, $p < 0.05$; stress + aripiprazole = 94% of control, $p < 0.01$; Fig. 1B). Moreover, chronic treatment with olanzapine and aripiprazole significantly increased the levels of phosphorylated GSK-3β under the no-stress condition (olanzapine = 126% of control, $p < 0.05$; aripiprazole = 133% of control, $p < 0.01$; Fig. 1A). In contrast, chronic treatment with haloperidol had no effect on the levels of these proteins in the prefrontal cortex of rats, irrespective of whether they were subjected to immobilization stress.

3.2. Effects of olanzapine, aripiprazole, and haloperidol on levels of postsynaptic proteins BDNF and PSD-95 and presynaptic synaptophysin in the frontal cortex

Chronic immobilization stress significantly decreased the levels of BDNF, PSD-95, and synaptophysin in the frontal cortex of the experimental subjects, to about 31%, 32%, and 50% of the values for vehicle-treated controls, respectively ($p < 0.01$; Fig. 2). Chronic treatment with olanzapine and aripiprazole markedly reversed the immobilization stress-induced decrease in BDNF expression levels (stress + olanzapine = 77% of control, $p < 0.01$; stress + aripiprazole = 86% of control, $p < 0.01$; Fig. 2A), PSD-95 (stress + olanzapine = 56% of control, $p < 0.01$; stress + aripiprazole = 70% of control, $p < 0.01$; Fig. 2B), and synaptophysin (stress + olanzapine = 84% of control, $p < 0.05$; stress + aripiprazole = 90% of control, $p < 0.01$; Fig. 2C). Furthermore, chronic aripiprazole increased BDNF expression levels in experimental animals compared with vehicle-treated controls under the stress-free condition (138%, $p < 0.01$; Fig. 2A). However, chronic haloperidol treatment did not affect the levels of these proteins, irrespective of whether the animals were subjected to immobilization stress.

The results of the two-way ANOVA are summarized in Table 1. Significant individual effects of olanzapine and aripiprazole, but not of haloperidol, were found on phosphorylated GSK-3β, β-catenin, BDNF, PSD-95, and synaptophysin levels in the frontal cortex ($p < 0.05$ or $p < 0.01$). The individual effect of stress was also significant ($p < 0.05$ or $p < 0.01$). The analysis revealed a significant drug × stress interaction reflecting the significant effects of olanzapine and aripiprazole, but not haloperidol, on β-catenin and PSD-95 ($p < 0.05$ or $p < 0.01$) under the stress condition. No drug was found to significantly affect phosphorylated GSK-3β levels under the stress condition. Only olanzapine was found to significantly affect BDNF levels under the stress condition ($p < 0.05$). Only aripiprazole was found to significantly affect synaptophysin levels under the stress condition ($p < 0.05$).

4. Discussion

The main finding of the present study is that immobilization stress decreased the expression of synapse-associated proteins, suggesting an essential role of the frontal cortex in responses to stress and that the atypical antipsychotic drugs olanzapine and aripiprazole and the typical antipsychotic drug haloperidol differentially affect these protein levels in the frontal cortex of rats subjected to immobilization stress.

In rodents, exposure to chronic stress induces profound behavioral changes, including depressive and anxiogenic behavior, decreased social interaction, and learning and memory deficits (Pittenger and Duman, 2008). These changes seem to be related to structural and functional alterations in specific brain regions and disturbed synaptic plasticity (Pittenger and Duman, 2008). In the present study, chronic immobilization stress markedly decreased the levels of GSK-3β phosphorylation, β-catenin, BDNF, PSD-95, and synaptophysin in the frontal cortex. Some of these findings are consistent with our previous study, which found that reduced levels of BDNF, GSK-3β phosphorylation, and β-catenin in the rat hippocampus were associated with immobilization stress (Park et al., 2011). Moreover, several previous studies using various animal stress models support the findings presented here. Chronic immobilization stress (3 weeks) reduced BDNF expression in the hippocampus and neocortex (Park et al., 2006, 2009a). Prenatal stress (2 weeks) decreased GSK-3β phosphorylation in the rat frontal cortex (Szyszmańska et al., 2009). Chronic swim stress (2 weeks) induced significant behavior changes and was associated with decreased levels of phosphorylated GSK-3β and β-catenin in the rat medial prefrontal cortex (Chen et al., 2012). Chronic unpredictable mild stress (5 weeks) also decreased BDNF, PSD-95, and synaptophysin expression in the rat amygdala (Luo et al., 2013). Immobilization stress (5 days) reduced synaptophysin expression in the rat hippocampus (Thome et al., 2001). However, a study conducted by Kožlovsky et al. (2002) reported that GSK-3β
Effects of antipsychotic drugs on the levels of brain-derived neurotrophic factor (BDNF), postsynaptic density protein-95 (PSD-95), and synaptophysin expression in the frontal cortex of rats subjected to immobilization stress. Psychiatry Research (2015), http://dx.doi.org/10.1016/j.psychres.2015.05.098

Fig. 2. Effects of antipsychotic drugs on the levels of brain-derived neurotrophic factor (BDNF), postsynaptic density protein-95 (PSD-95), and synaptophysin expression in the frontal cortex of rats subjected to immobilization stress. Rats (n = 5 animals/group) were given a daily injection of vehicle (Veh; 1 ml/kg), olanzapine (OLA; 2 mg/kg), aripiprazole (ARP; 1.5 mg/kg), or haloperidol (HAL; 1 mg/kg) for 21 days with or without immobilization stress (6 h daily for 3 weeks). Levels of BDNF in brain homogenates from the prefrontal cortex were detected by SDS-PAGE and Western blotting with anti-BDNF, anti-PSD-95, and anti-synaptophysin antibody. A representative image from the prefrontal cortex were detected by SDS-PAGE and Western blotting with α-tubulin band, are shown. Results are expressed as the percentage of vehicle control levels and represent the means ± S.E.M. of five animals per group. *p < 0.05 versus vehicle controls; **p < 0.01 versus vehicle; †p < 0.05 versus vehicle + stress; ††p < 0.01 versus vehicle + stress.

phosphorylation did not affect the rat frontal cortex following acute (1 day), subchronic (6 days), or chronic cold-restraint stress (14 days). Taken together, most of the results reported by previous studies indicate that exposure to stress significantly decreases synapse-associated protein levels in various brain regions. However, little is known about changes in postsynaptic protein PSD-95 and presynaptic synaptophysin expression in the frontal cortex during the stress response. Thus, the present study demonstrated, for the first time, that expression of PSD-95 and synaptophysin is down-regulated in the frontal cortex by chronic immobilization stress. Moreover, low levels of BDNF, phosphorylated GSK-3β, β-catenin, BDNF, PSD-95, and synaptophysin were found in postmortem studies of patients with schizophrenia (Cotter et al., 1998; Vawter et al., 1999; Karson et al., 1999; Durany and Thome, 2004; Kozlovsky et al., 2004; Nadri et al., 2004; Funk et al., 2012). In this context, the chronic immobilization stress-induced changes in the expression of these proteins may be associated, at least in part, with the structural and functional changes previously observed after chronic stress and may suggest that a form of disturbed synaptic plasticity underlies the pathophysiology of stress-related disorders.

The present study revealed that the deficits in GSK-3β phosphorylation and β-catenin levels in the frontal cortex induced by this stress model were effectively reversed by olanzapine and aripiprazole but not by haloperidol. Our previous study identified similar alterations in the hippocampus of rats exposed to chronic immobilization (Park et al., 2011). These results are also supported by our in vitro studies showing that olanzapine prevented serum deprivation-induced decreases in the levels of GSK-3β phosphorylation and β-catenin in SH-SYSY cells, whereas haloperidol reduced the levels of these proteins (Kim et al., 2008). The differential effects of aripiprazole and haloperidol on GSK-3β phosphorylation levels have been reported in SH-SYSY cells (Park et al., 2009b). Moreover, in vivo studies have shown that acute administration of atypical antipsychotic drugs, including clozapine, olanzapine, quetiapine, and ziprasidone, rapidly increased phosphorylated GSK-3β levels in the cortex, hippocampus, striatum, and cerebellum of mice (Li et al., 2007), whereas acute haloperidol treatment did not increase GSK-3β phosphorylation in the frontal cortex of mice (Li et al., 2004). However, several differences between the present study and other studies with respect to the effects of haloperidol on β-catenin levels should be noted. Specifically, several studies demonstrated an increase in β-catenin levels in various brain regions (the striatum and prefrontal cortex; the ventral midbrain, consisting of the substantia nigra and ventral tegmental area; and the prefrontal cortex) following haloperidol (1 mg/kg for 7 days, 1 mg/kg for 28 days, and 0.5 mg/kg for 14 days, respectively) (Alimohamad et al., 2005a, 2005b; Sutton et al., 2007). Although the reasons for these differences are difficult to identify, one possible explanation may involve differences in doses, durations of administration, participants’ sex, and brain regions across studies.

The involvement of GSK-3β and β-catenin in neurite outgrowth has been reported in previous studies. GSK-3β inhibition-mediated neurite outgrowth is accompanied by cytosolic β-catenin accumulation (García-Perez et al., 1999; Orme et al., 2003). Particularly, overexpression of β-catenin is associated with up-regulation of axonal length, dendritic processes, and the density of dendritic spines in primary hippocampal neurons (Yu and Malenka, 2003, 2004). Our recent study showed that dendritic outgrowth in rat hippocampal neurons was induced by olanzapine and aripiprazole, whereas haloperidol had no effect in this regard (Park et al., 2013). Thus, the effects of olanzapine on dendritic outgrowth may be accompanied by the up-regulation of GSK-3β phosphorylation and β-catenin levels.

Specifically, recent studies have found an association between Wnt signaling and BDNF expression, suggesting that BDNF is a direct target of Wnt signaling (David et al., 2008; Yi et al., 2012). In the present study, changes in BDNF levels in the frontal cortex were correlated with chronic stress, whereas chronic olanzapine and aripiprazole, but not haloperidol, treatment reversed these effects. In several studies, chronic treatment with atypical antipsychotic drugs, but not haloperidol, also prevented BDNF down-regulation in the frontal cortex induced by chronic immobilization stress. Psychiatry Research (2015), http://dx.doi.org/10.1016/j.psychres.2015.05.098
regulation in response to stress (Park et al., 2006, 2009b, 2011). Moreover, atypical antipsychotic drugs enhanced dendritic outgrowth and spine formation in hippocampal neurons, whereas haloperidol had no effect or was associated with decreases in this regard (Park et al., 2013). Combined with these findings, the present study further suggests that GSK-3β/β-catenin signaling, mediated by BDNF, may stimulate synapse formation, which enhances synaptic plasticity and connectivity in the brain regions associated with chronic immobilization stress.

This study is an extension of our previously published study on the effects of olanzapine, aripiprazole, and haloperidol on BDNF, GSK-3β, and β-catenin in hippocampus of rats subjected to immobilization stress (Park et al., 2011). To the best our knowledge, the present study is the first to demonstrate that administration of olanzapine and aripiprazole increases the expression of PSD-95 and synaptophysin, which are associated with synapse structure and activity in the frontal cortex, in immobilized but not in control rats, whereas haloperidol treatment has no effect in this regard. This is consistent with a recent study that found that olanzapine and aripiprazole, but not haloperidol, increased these proteins levels in response to cytotoxicity in hippocampal cultures (Park et al., 2013). Although synapse density was not directly assessed in the present study, the increased PSD-95 levels associated with olanzapine and aripiprazole may represent increased spine synapse density in the frontal cortex. Furthermore, the increased expression of synaptophysin induced by these drugs may reflect an increase in the synaptic vesicles, with a consequent increase in neurotransmitter release. Few studies have investigated the effects of antipsychotic drugs on PSD-95 and synaptophysin in non-stressed animals. One study demonstrated that acute administration of haloperidol or olanzapine did not modulate PSD-95 expression in rat forebrains (de Bartolomeis et al., 2002). Another study reported that chronic treatment with haloperidol significantly increased PSD-95 mRNA expression in the rat cortex but did not affect the hippocampus (Lasevoli et al., 2010). Other studies reported that haloperidol decreased synaptophysin levels in the hippocampus of rats (Eastwood et al., 1997), but no change were observed in the primate cerebral cortex (Lidow et al., 2001). Thus, olanzapine- and haloperidol-induced changes in PSD-95 or synaptophysin are not always consistent. The effects of these medications on these proteins appear to vary depending on brain region, duration of administration, and presence or absence of stress.

Intriguingly, the present and previous (Park et al., 2011) studies found that aripiprazole had slightly superior effects compared with olanzapine on the expression of GSK-3β, β-catenin, and BDNF in the frontal cortex, whereas its effect on hippocampal changes were less robust compared with that of olanzapine. Our Western blot data revealed a discrepancy between the frontal cortex and hippocampus with respect to the effect of these two drugs on changes in these proteins. Indeed, the effects of medications may depend on the pharmacological characteristics of the drugs. Aripiprazole is an antipsychotic drug with a pharmacological profile that differs from all other atypical and typical antipsychotic drugs. It shows partial agonist activity at 5-HT1A, antagonism at 5-HT2A receptors, and partial agonism at D2 receptors, unlike other antipsychotic drugs which are D2 receptor antagonists. Aripiprazole, increases dopamine release in the medial prefrontal cortex of rats, an effect that depends on the activation of 5-HT1A receptors localized in the medial prefrontal cortex (Bortolozzi et al., 2007). Given the crucial role of prefrontal dopamine in cognitive functioning (Castner et al., 2004), the increase in dopamine release in the medial prefrontal cortex may underlie the slightly superior effects of aripiprazole on the synapse-associated proteins levels in the frontal cortex.

The results of the present study show that immobilization stress affects the expression of synapse-associated proteins in the frontal cortex of rats and that the atypical antipsychotics olanzapine and aripiprazole and the typical antipsychotic haloperidol differentially affect stress-induced changes in the expression of synapse-associated proteins. It is also proposed that olanzapine and aripiprazole, but not haloperidol, may ameliorate the compromised plasticity, which was induced by immobilization stress, by up-regulation synapse-associated protein levels.

**Conflict of interest**

None to declare.

**Acknowledgments**

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**References**


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**Table 1**

Summary of the two-way analysis of variance for changes in phosphorylated GSK-3β, β-catenin, BDNF, PSD-95, and synaptophysin under the drug, stress, and interaction of drug and stress.

<table>
<thead>
<tr>
<th></th>
<th>Olanzapine F</th>
<th>Aripiprazole F</th>
<th>Haloperidol F</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-GSK-3β</td>
<td>20.926 &lt; 0.001</td>
<td>41.099 &lt; 0.001</td>
<td>0.552 0.465</td>
</tr>
<tr>
<td>Stress</td>
<td>117.851 &lt; 0.001</td>
<td>54.312 &lt; 0.001</td>
<td>217.083 &lt; 0.001</td>
</tr>
<tr>
<td>Drug × stress</td>
<td>6.813 0.064</td>
<td>4.090 0.054</td>
<td>0.046 0.831</td>
</tr>
<tr>
<td>β-catenin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>10.082 0.003</td>
<td>14.602 0.001</td>
<td>0.494 0.478</td>
</tr>
<tr>
<td>Stress</td>
<td>88.689 &lt; 0.001</td>
<td>20.804 &lt; 0.001</td>
<td>61.635 &lt; 0.001</td>
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<tr>
<td>Drug × stress</td>
<td>12.291 0.001</td>
<td>10.463 0.003</td>
<td>1.746 0.195</td>
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<tr>
<td>BDNF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>16.963 &lt; 0.001</td>
<td>22.887 &lt; 0.001</td>
<td>0.135 0.717</td>
</tr>
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<td>Stress</td>
<td>58.052 &lt; 0.001</td>
<td>38.795 &lt; 0.001</td>
<td>205.627 &lt; 0.001</td>
</tr>
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<td>Drug × stress</td>
<td>7.812 0.010</td>
<td>0.820 0.374</td>
<td>0.082 0.777</td>
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<td>PSD-95</td>
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<td>29.283 &lt; 0.001</td>
<td>0.741 0.398</td>
</tr>
<tr>
<td>Stress</td>
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<td>143.580 &lt; 0.001</td>
<td>102.780 &lt; 0.001</td>
</tr>
<tr>
<td>Drug × stress</td>
<td>8.701 0.007</td>
<td>7.863 0.010</td>
<td>0.124 0.728</td>
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<tr>
<td>Synaptophysin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>19.401 &lt; 0.001</td>
<td>25.566 &lt; 0.001</td>
<td>0.008 0.929</td>
</tr>
<tr>
<td>Stress</td>
<td>48.998 &lt; 0.001</td>
<td>44.343 &lt; 0.001</td>
<td>112.859 &lt; 0.001</td>
</tr>
<tr>
<td>Drug × stress</td>
<td>2.167 0.148</td>
<td>2.778 0.010</td>
<td>0.002 0.962</td>
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</tbody>
</table>

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