Pharmacology, Biochemistry and Behavior 221 (2022) 173490

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

The α_{2C}-adrenoceptor antagonist JP-1302 controls behavioral parameters, tyrosine hydroxylase activity and receptor expression in a rat model of ketamine-induced schizophrenia-like deficits

Nurdan Tekin [a,b], Tuğba Eryiğit Karamahmutoğlu [a], Aslı Aykaç [c], Dilek Akakın [d], Mehmet Zafer Gören [a,*]

[a] Department of Medical Pharmacology, Marmara University Faculty of Medicine, Istanbul, Turkey
[b] Department of Medical Pharmacology, University of Health Sciences, Hamidiye Faculty of Medicine, Istanbul, Turkey
[c] Department of Biophysics, Near East University, Faculty of Medicine, Nicosia, Cyprus
[d] Department of Histology & Embryology, Marmara University Faculty of Medicine, Istanbul, Turkiye

ARTICLE INFO

Keywords:
Schizophrenia
Novel object recognition
HPLC
Western-blotting

ABSTRACT

Schizophrenia is a chronic disabling disease affecting 1 % of the population. Current antipsychotics have limited efficacy in mitigating the severity of the symptoms of the disease. Therefore, searching for new therapeutic targets is essential. Previous studies have shown that α_{2C}-adrenoceptor antagonists may have antipsychotic and pro-cognitive effects. Therefore, the current study evaluates the behavioral and neurochemical effects of JP-1302, a selective α_{2C}-adrenoceptor antagonist, in a model of schizophrenia-like deficits induced by sub-chronic ketamine (KET) administration.

Here, we administered ketamine (25 mg/kg, i.p.) to male and female Wistar rats for eight consecutive days. On the last two days of ketamine administration, rats were pretreated with either JP-1302 (1-3-10 μmol/kg, i.p.), chlorpromazine (0.1 mg/kg, i.p.), or saline, and the behavioral tests were performed. Behaviors related to positive (locomotor activity), negative (social interaction), and cognitive (novel object recognition) symptoms of schizophrenia were assessed. Glutamate, glutamine, GABA levels, and α_{2C}-adrenoceptor expression were measured in the frontal cortex and the hippocampus. Tyrosine hydroxylase immunocytochemical reactivity was also shown in the midbrain regions.

Sub-chronic ketamine administration increased locomotor activity and produced robust social interaction and object recognition deficits, and JP-1302 significantly ameliorated ketamine-induced cognitive deficits. Ketamine induced a hyperdopaminergic activity in the striatum, which was reversed by the treatment with JP-1302. Also, the α_{2C}-adrenoceptor expression was higher in the frontal cortex and hippocampus in the ketamine-treated rats.

Our findings confirm that α_{2C}-adrenoceptor antagonism may be a potential drug target for treating cognitive disorders related to schizophrenia.

1. Introduction

Schizophrenia is a chronic and devastating disorder (James et al., 2018). The etiology of the disease remains elusive; therefore, the current antipsychotic treatment is far from perfect. Currently available antipsychotic treatment is predominantly effective on positive symptoms (hallucinations, delusions, disorganized speech). Even more, it has been shown that certain antipsychotic drugs may exacerbate negative (deficits in social interaction, lack of motivation, anhedonia) and cognitive symptoms (Keefe et al., 2007). Another critical problem in treating schizophrenia is that almost 30 % of patients are refractory to the available treatments.

Dopamine D_2 receptor antagonism is the main target for typical antipsychotics, with their efficacy has been associated with dopamine D_2 receptor occupancy rates (Seeman et al., 1976). In contrast, atypical antipsychotics possess lower D_2 affinity and are more effective on negative and cognitive symptoms of the disease (Keefe et al., 2004; Stroup et al., 2003). Therefore, the additional receptor binding profiles of atypical antipsychotics have driven new antipsychotic drug development. Clozapine is the only drug with proven efficacy in treating

* Corresponding author at: Marmara University, School of Medicine, Department of Medical Pharmacology, 34854 Istanbul, Turkiye.
E-mail address: mgoren@marmara.edu.tr (M.Z. Gören).

https://doi.org/10.1016/j.pbb.2022.173490
Received 20 September 2022; Received in revised form 8 November 2022; Accepted 8 November 2022
Available online 12 November 2022

refractory schizophrenia, with high α_2-adrenoceptors over D_2 receptor selectivity ratios (Uys et al., 2017a). Hence, α_2-adrenoceptors have become an important target.

The α_2-adrenoceptor is a G-protein coupled receptor and regulates the release of several neurotransmitters primarily via the presynaptic inhibition (Bücheler et al., 2002; Hein et al., 1999). The α_{2A}- and α_{2C}-adrenoceptors are widely distributed in the central nervous system, and α_{2C}-adrenoceptors are the most densely present receptor subtype in the brain areas related to schizophrenia, like the striatum and the hippocampus (Uys et al., 2017a). The earliest evidence that the promising role of α_{2C}-adrenoceptor in the treatment of schizophrenia originated from a transgenic mouse model (Scheinin et al., 2001). Furthermore, previous studies have shown that the selective α_{2C}-adrenoceptor antagonists, JP-1302, ORM-10921, and ORM-12741, have antipsychotic-like effects in NMDA-antagonist-induced and neurodevelopmental models of schizophrenia in rats (Dreyer et al., 2016; Sallinen et al., 2007; Sallinen et al., 2013a, 2013b).

Using validated animal models and accurate screening methods is critical in the preclinical development of antipsychotic drugs. There are many pharmacological, neurodevelopmental animal models for schizophrenia (Jones et al., 2011). *N*-methyl-D-aspartate (NMDA) receptor antagonists, phencyclidine, and ketamine can induce schizophrenia-like symptoms in healthy individuals and exacerbate the symptoms of schizophrenia (Cohen et al., 1962; Itil et al., 1967; Krystal et al., 1994). Ketamine is widely used for the induction of schizophrenia-like symptoms in rodents.

The present study aimed to evaluate the behavioral and neurochemical effects of JP-1302, a selective α_{2C}-adrenoceptor antagonist, in a model of schizophrenia-like deficits induced by sub-chronic ketamine (KET) administration. Furthermore, we also compared the effects of the ketamine administration and the treatment with JP-1302 or chlorpromazine on dopamine immunochemistry and the levels of glutamate and GABA in the homogenates of the hippocampus and the frontal cortex. Adrenergic α_{2C} densities were also evaluated by using immunoblotting.

2. Methods

2.1. Animals

Male and female adult Wistar rats aged 10–12 weeks (males 250–300 g, females 200–250 g) were used in the experiment. The animals were obtained from DEHAMER. The rats were housed in transparent shelter cages (20 × 26 × 48 cm) until the beginning of their injection in groups of three to four rats. In a constant temperature (22 ± 4 °C) room, suitable accommodation conditions were provided for 12 h of the light-dark cycle and had ad libitum access to water and food. All behavioral experiments were performed during the light phase (between 07:30 am and 11:30 am). The rats were housed in pairs throughout the experiment to avoid the possible effects of social isolation. The rats were handled and weighed each day during the entire experiment. All efforts were made to minimize animal suffering and to reduce the number of animals used in these experiments. The experiment protocol was approved by Marmara University Animal Experiments Local Ethics Committee (14.2017.mar). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

2.2. Drugs

The drugs used in the study were dissolved in saline at a volume of 1 mL/100 g. Ketamine (KET – CU Chemie Uetikon, Germany) was injected intraperitoneally (i.p.; 25 mg/kg) once daily for eight days as an animal model of schizophrenia. The dosage and duration of administration were selected based on the literature and our pilot experiment to mimic schizophrenia-like symptoms (Calixto et al., 2016; De Oliveira et al., 2011; Zugno et al., 2012). JP-1302 HCl (N-[4-(4-methyl-1-piperazinyl) phenyl]-9-acridinamine dihydrochloride) was purchased from Tocris Bioscience. JP-1302 is defined as a highly selective α_{2C}-adrenoceptor antagonist that displays ~50-fold selectivity over other α_2-adrenoceptor subtypes (K_i values are 28, 1470, and 3150 nM for α_{2C}, α_{2B}, and α_{2A} subtypes, respectively; Tricklebank, 2007). The dosage of JP-1302 (1-3-10 μmol/kg, i.p.) was selected based on previous research (Sallinen et al., 2007). Chlorpromazine (CPZ – Largactil®, Eczacibasi, Turkiye) was administered intraperitoneally at the dose of 0.1 mg/kg. The dosage required to produce a therapeutic effect without inducing catalepsy was determined in our pilot experiments.

2.3. The experimental design

The rats were randomly assigned into seven groups (male/female:1/1). A total of 88 animals were used in the study. An outline of the experimental design is illustrated briefly in Fig. 1. Briefly, all rats received either KET or vehicle (0.9 % physiological saline) for eight consecutive days. On the experimental days (days 7 and 8), the rats were treated with either vehicle (SAL), CPZ (0.1 mg/kg), or three different doses of JP-1302 (1–3-10 μmol/kg) 30 min before KET injection. On the seventh day, (i) the open field test (to model the positive symptoms of SCZ) and (ii) the social interaction test (to model the negative symptoms); on the eighth day, (iii) the novel object recognition test (to model the cognitive symptoms) was performed on each animal. Behavioral tests were conducted 45 min after the ketamine. The animals were sacrificed immediately after the object recognition test (See Supplementary Methods and **Fig. S1**).

2.4. Behavioral experiments

2.4.1. Open-field test for locomotor activity

The spontaneous locomotor activity was measured for 15 min using a computerized system (Locomotor Activity Cage ACT 508, Commat, Turkiye). At the beginning of each session, the rats were placed in the center of the open field. The data on the percentage of resting time and stereotypic behavior was evaluated (See Supplementary Methods).

2.4.2. Social interaction test

The general design of the test was adapted from (Becker and Grecksch, 2004). Two rats from the same group that had not previously been exposed to each other were placed in opposite corners of the test arena and were recorded for 15 mins. The rats were matched for sex- and weight-matched (± 10 g difference in body weight) to control for the potential effect of dominance. The interaction times were scored based on the records (See Supplementary Methods).

2.4.3. Novel Object Recognition (NORT)

Novel object recognition is defined as spending more time exploring the novel object than the familiar object. The general design of the test was adapted from (Osborne et al., 2017). In the acquisition phase, the rat was placed into the arena and was allowed to explore two identical objects for 5 min. In the testing phase, one of the familiar objects was replaced by a new object. Following the 1-h inter-trial interval, the rat was returned to explore the objects for 5 min. The exploration times of each object were scored by viewing the digital video. The recognition index was calculated for each rat (Antunes and Biala, 2012; See Supplementary Methods).

2.5. Western blotting analysis

Frozen tissue was homogenized with the buffer using a centrifuge. After incubation with protease inhibitor solution, the amount of protein in each brain tissue was determined by the Lowry method (Lowry et al., 1951). Samples were prepared for 12 % SDS-PAGE separation. The transferred samples were incubated with primary antibodies [α-2c (sc-1480, 1:200) and β-actin (sc-130,657, 1:200), Santa Cruz Biotechnology,

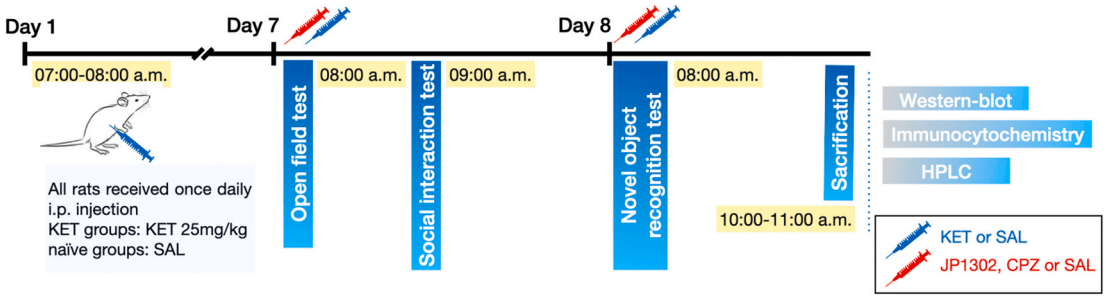

Fig. 1. Timeline of the experiment. KET: ketamine, CPZ: chlorpromazine.

Inc. (Santa Cruz, CA, USA)] then were incubated with rabbit monoclonal anti-goat IgG secondary antibodies. NBT/BCIP (nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl- phosphate; Promega, Wisconsin, USA) was used for the visibility of specific bands. The publicly available software was used for the densitometric analysis of the resulting membranes (Bio-Rad Molecular Analyst, www.totallab.com; (See Supplementary Methods).

2.6. High-performance liquid chromatography analysis of neurotransmitters

The HPLC analysis of glutamate, glutamine, and GABA was assayed according to described the previously published studies (Gören et al., 2007; Küçükibrahimoğlu et al., 2009; Özakman et al., 2021).

For method details see Supplementary Methods.

2.7. Immunocytochemistry

The sections, encompassing the striatum, ventral tegmental area (VTA), substantia nigra pars compacta (SNpc), and nucleus accumbens (NAc), were processed for floating-section immunohistochemistry to detect tyrosine hydroxylase. Briefly, after quenching, the sections were incubated with the primer antibody solution (Mouse AntiTyrosine Hydroxylase Antibody, Milliporefor). The next day, the sections were incubated in the seconder antibody solution (Biotinylated Horse Anti-Mouse IgG Antibody, Vector Laboratorie) and an avidin-biotin complex (Vectastain ABC kit from Vector). The colour reaction was developed with the DAB solution (Sigma-Aldrich). The sections were mounted on slides and coverslipped after dehydration with ethanol (See Supplementary Material). The JP-1302 treated naïve group was excluded from immunocytochemistry studies due to technical difficulties.

The optical density of TH-positive fibers and neurons was examined from digitized images using ImageJ software (Version 1.50i, NIH, USA). After eliminating background staining, the total density of TH-positive dopaminergic neurons was expressed as a percentage change from basal levels, with 100 % defined as the average count for vehicle-treated control rats (See Supplementary Methods).

2.8. Statistical analysis

All statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, SanDiego, USA, trial version). The Shapiro-Wilk test was used to assess the normality of the data. Data with a normal distribution ($p > 0.05$ = normal distribution) were analyzed by one-way ANOVA followed, when appropriate, by the Tukey or Bonferroni *post-hoc* tests. In cases where one or more data sets in a comparison failed normality testing, data were analyzed by the Kruskal-Wallis ANOVA with *post-hoc* Dunn's test. All parametric data are presented as the mean ± standard error of the mean (SEM), and differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Behavioral tests

3.1.1. Open field test

In the open field test, the percentage of resting and stereotyped behavior was compared between groups. The statistical analysis showed that the percentage of resting time differed among groups ($F_{(6, 76)} = 4223$, $p = 0.001$). The post-hoc test showed that KET decreased the percentage of resting time ($p < 0.05$), and CPZ prevented the decrease in resting time ($p < 0.01$). The percentage of stereotyped activity also differed among the group (Kruskal-Wallis statistic $H = 16.65$, $p < 0.05$). KET administration significantly increased the stereotyped activity compared to the control group ($p < 0.05$). The JP-1302 treatment at three different concentrations did not produce a significant change in resting time and stereotyped behavior. While 1 μmol/kg and 3 μmol/kg dose of JP-1302 tended to increase the resting time and decrease the stereotyped activity compared to the KET group, 10 μmol/kg dose of JP-1302 had opposite effects on stereotyped activity and the resting time (Fig. 2A-2B).

3.1.2. Social interaction test

One-way ANOVA revealed a significant difference in the social interaction time among groups ($F_{(6,31)} = 16.69$; $p < 0.0001$). The post-hoc analysis with Bonferroni showed that KET administration significantly reduced the social interaction time compared to the control group ($p < 0.001$), and CPZ did not attenuate the deficit. JP-1302 slightly but not significantly increased the social interaction time dose-dependently (Fig. 2C).

3.1.3. Novel object recognition test

Here, we evaluate if the KET administration impairs cognitive performance in the novel object recognition test and if the JP-1302 treatment at various doses effects the possible impairment in cognitive performance. There were no significant differences in the total exploration times during both phases among the different treatment groups, indicating that the treatments did not influence sensorimotor parameters such as motivation (data not shown). The statistical analysis showed that the recognition index (RI) significantly differed among groups ($F_{(6,68)} = 7.5$; $p < 0.0001$). KET administration significantly reduced the RI compared to the control group ($p < 0.005$), and a 10 μmol/kg dose of JP-1302 produced significant improvement ($p < 0.05$). The CPZ treatment did not improve the KET-induced deficit (Fig. 2D).

3.2. The level of glutamate, glutamine, and GABA in the frontal cortex and hippocampus

See Supplementary Results for more details.

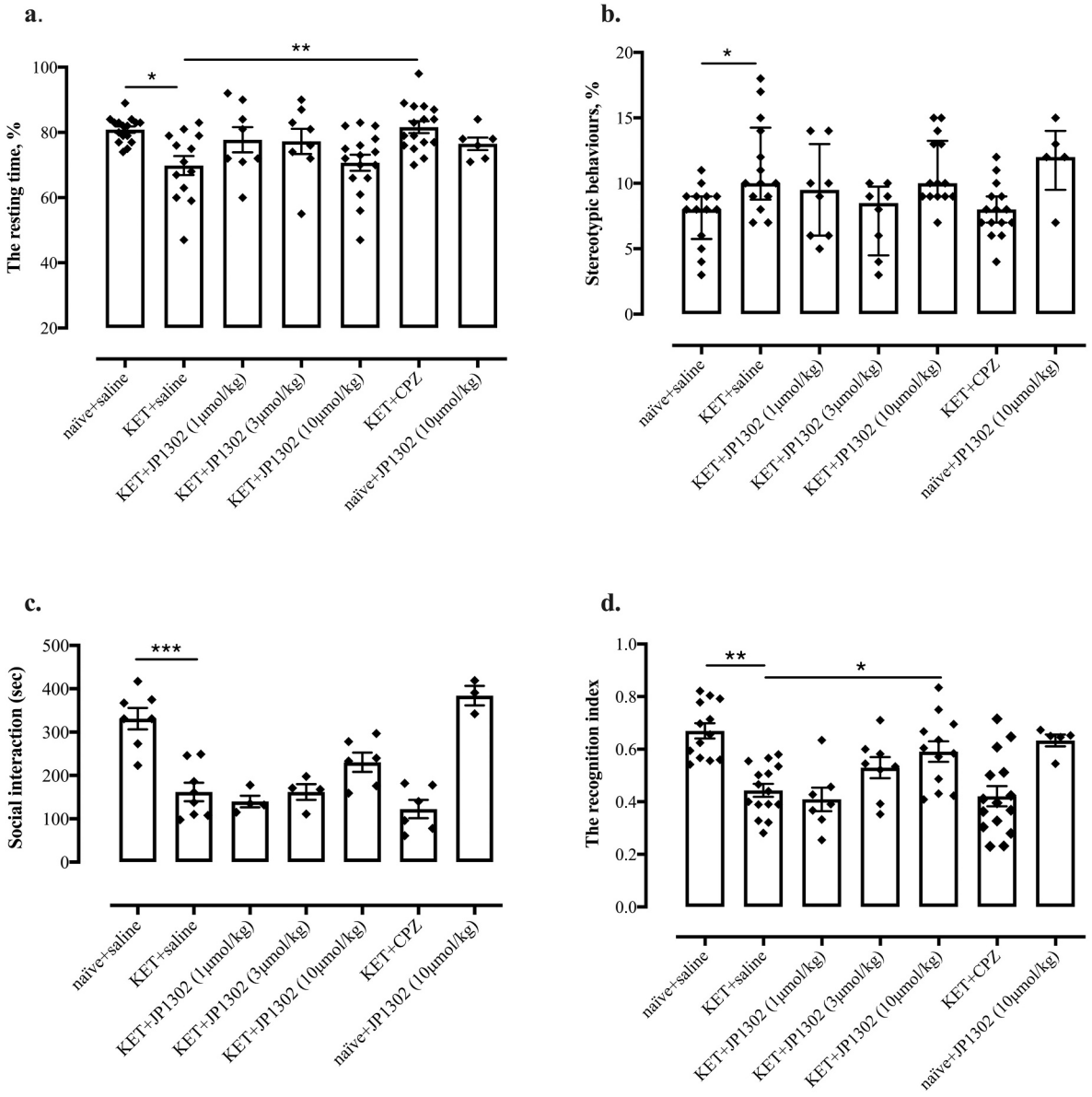

Fig. 2. The effect of ketamine and different treatments on behavioral parameters. (A-B) Locomotor activity was assessed for 15 min using a computerized system (Activity Monitor) 45 min after the last ketamine administration ($n = 8$–16). (A) The percentage of resting time. Bars represent means ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ (ANOVA, Bonferroni *post-hoc* test). (B) The percentage of stereotyped behaviors. Bars represent median and interquartile ranges. $^{*}p < 0.05$ (Kruskal Wallis ANOVA, Dunn's *post-hoc* test). (C) Social interaction test. The duration of sniffing, tracking, grooming, etc. social behaviors of the two rats was evaluated for 15 min. ($n = 8$). Bars represent means ± SEM. $^{***}p < 0.001$ (ANOVA, Bonferroni *post-hoc* test). (D) Novel object recognition test. The recognition index (RI) was calculated by evaluating the time rats spent examining the familiar and the novel objects. ($RI = t_{novel} / (t_{novel} + t_{familiar})$; t_{novel}: time spent with novel object, $t_{familiar}$: time spent with familiar object; n = 8). Bars represent means ± SEM. $^{*}p < 0.05$, $^{**}p < 0.005$ (ANOVA, Bonferroni *post-hoc* test). KET: ketamine, CPZ: chlorpromazine.

3.3. Immunocytochemical analysis of dopamine

3.3.1. Tyrosine hydroxylase (TH) immunoreactivity in striatum/optical density of tyrosine hydroxylase

Significant differences in TH immunoreactivity were detected between groups ($F_{(5,18)} = 3.94$; $p < 0.05$). The post-hoc analysis revealed that KET administration significantly increased TH immunoreactivity in dopamine terminals in the striatum compared to the control group ($p < 0.05$), and this effect was attenuated by treatment with 10 μmol/kg JP-1302 ($p < 0.01$). Moreover, JP-1302 tended to reduce the increased TH immunoreactivity in KET-treated rats in a dose-dependent manner (Fig. 3**)**.

3.3.2. Tyrosine hydroxylase immunoreactivity in NAc/optical density of tyrosine hydroxylase

The one-way ANOVA revealed a significant difference in the TH immunoreactivity in NAc among groups ($F_{(5,30)} = 4.17$; $p < 0.01$). There was no significant difference between KET and the control group. Administration of 3 and 10 μmol/kg JP-1302 significantly reduced TH immunoreactivity compared to the KET group ($p < 0.05$; Fig. 4).

3.3.3. Tyrosine hydroxylase immunoreactivity in VTA/optical density of tyrosine hydroxylase

We evaluated TH immunoreactivity in VTA, and the statistical analysis showed that there were significant differences among groups ($F_{(5,20)} = 3.942$; $p < 0.05$). Again, there was no significant difference between KET and the control group. Administration of 3 μmol/kg JP-1302 significantly reduced TH immunoreactivity compared to the KET

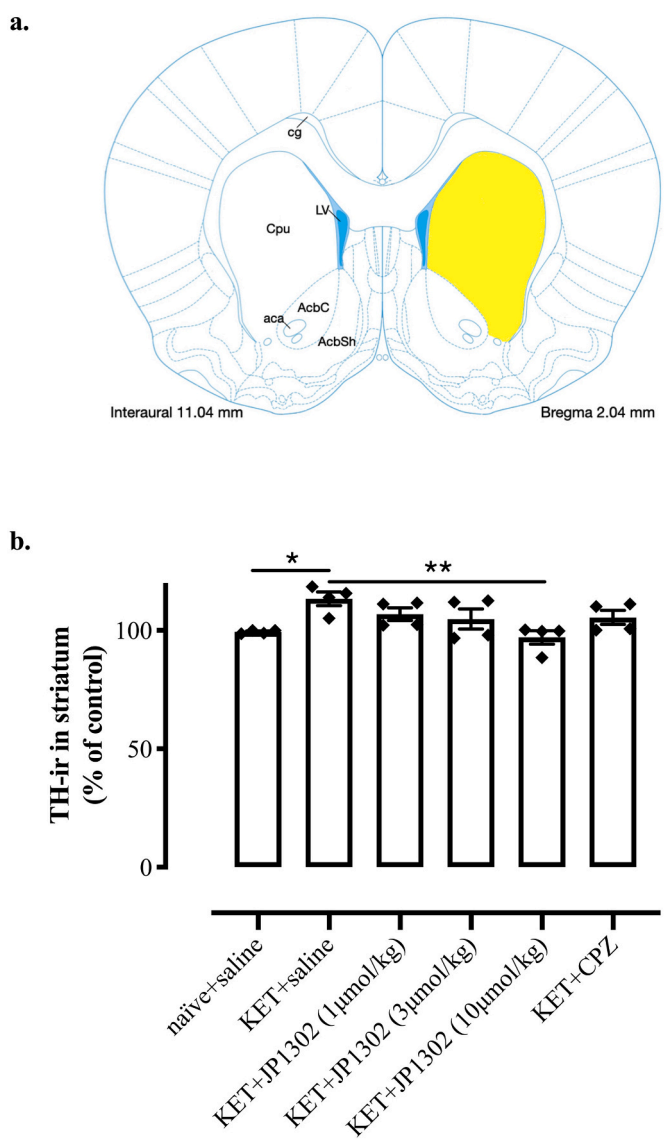

Fig. 3. The effect of ketamine and different treatment on TH-ir in the striatum ($n = 4$). (A) Schematic of the region studied: striatum (yellow area), adapted from Paxinos and Watson (2007), (B) The percentage of TH-ir positive fibers in the striatum compared to naïve. Bars represent means ± SEM. *p < 0.05, **$p <$ 0.01 (ANOVA, Bonferroni *post-hoc* test). KET: ketamine, CPZ: chlorpromazine, AcbC: nucleus accumbens core, AcbSh: nucleus accumbens, shell, aca: anterior commissure, anterior, cg: cingulum, Cpu: caudate putamen (striatum), LV: lateral ventricle, LAcbSn: lateral accumbens shell.

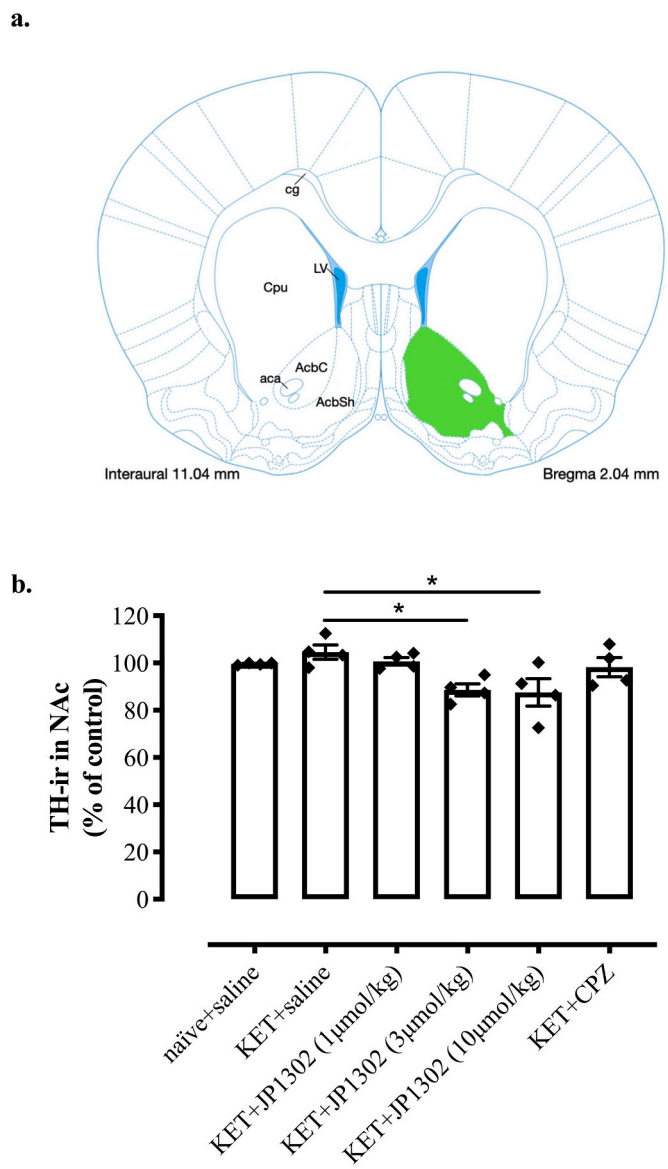

Fig. 4. The effect of ketamine and different treatment on TH-ir in NAc ($n = 4$). (A) Schematic of the region studied: NAc (green area), adapted from Paxinos and Watson (2007), (B) The percentage of TH-ir positive cell bodies in NAc compared to naïve. Bars represent means ± SEM. *$p <$ 0.05, (ANOVA, Bonferroni *post-hoc* test). KET: ketamine, CPZ: chlorpromazine, AcbC: nucleus accumbens core, AcbSh: nucleus accumbens, shell, aca: anterior commissure, anterior, cg: cingulum, Cpu: caudate putamen (striatum), LV: lateral ventricle, LAcbSn: lateral accumbens shell.

group ($p < 0.05$; Fig. 5).

Our data showed no significant difference between all groups in the TH immunoreactivity in SNpc ($F_{(5,11)} = 2.69$; $p = 0.079$; data not shown).

3.4. Effect of the sub-chronic administration of KET and different treatments on the expression of α_{2C}-adrenoceptor

We measured the expression levels of α_{2C}-adrenoceptor in the frontal cortex (FC) and hippocampus (HPC) via Western blotting **(**Fig. 6**)**. There were significant differences in the expression of α_{2C}-adrenoceptor in the FC and HPC among groups ($F_{(6,21)} = 9.881$; $p < 0.01$; $F_{(6,21)} = 30.12$; $p <$ 0.0001, respectively). The post-hoc analysis showed that KET administration significantly increased the total α_{2C}-adrenoceptor level in the FC compared to the control. Also, 10 μmol/kg JP-1302 treatment reduced the α_{2C}-adrenoceptor level ($p < 0.01$). Same as the FC, 10 μmol/kg JP-1302 treatment reduced the α_{2C}-adrenoceptor level ($p < 0.0001$) in the HPC, and this effect was also significantly observed in CPZ treatment ($p < 0.001$; Fig. 7).

4. Discussion

Our results demonstrate that KET administration increased locomotor activity, increased stereotype behavior, social withdrawal, and memory impairment, and JP-1302 reversed the memory impairment. Notably, KET increased the tyrosine hydroxylase activity in the striatum, indicating hyperdopaminergic activity, and a high dose of JP-1302 alleviated this effect. Also, KET administration caused a significant increase in α_{2C}-adrenoceptor density in the hippocampus and frontal cortex.

Hyperlocomotion is considered a putative correlate of positive

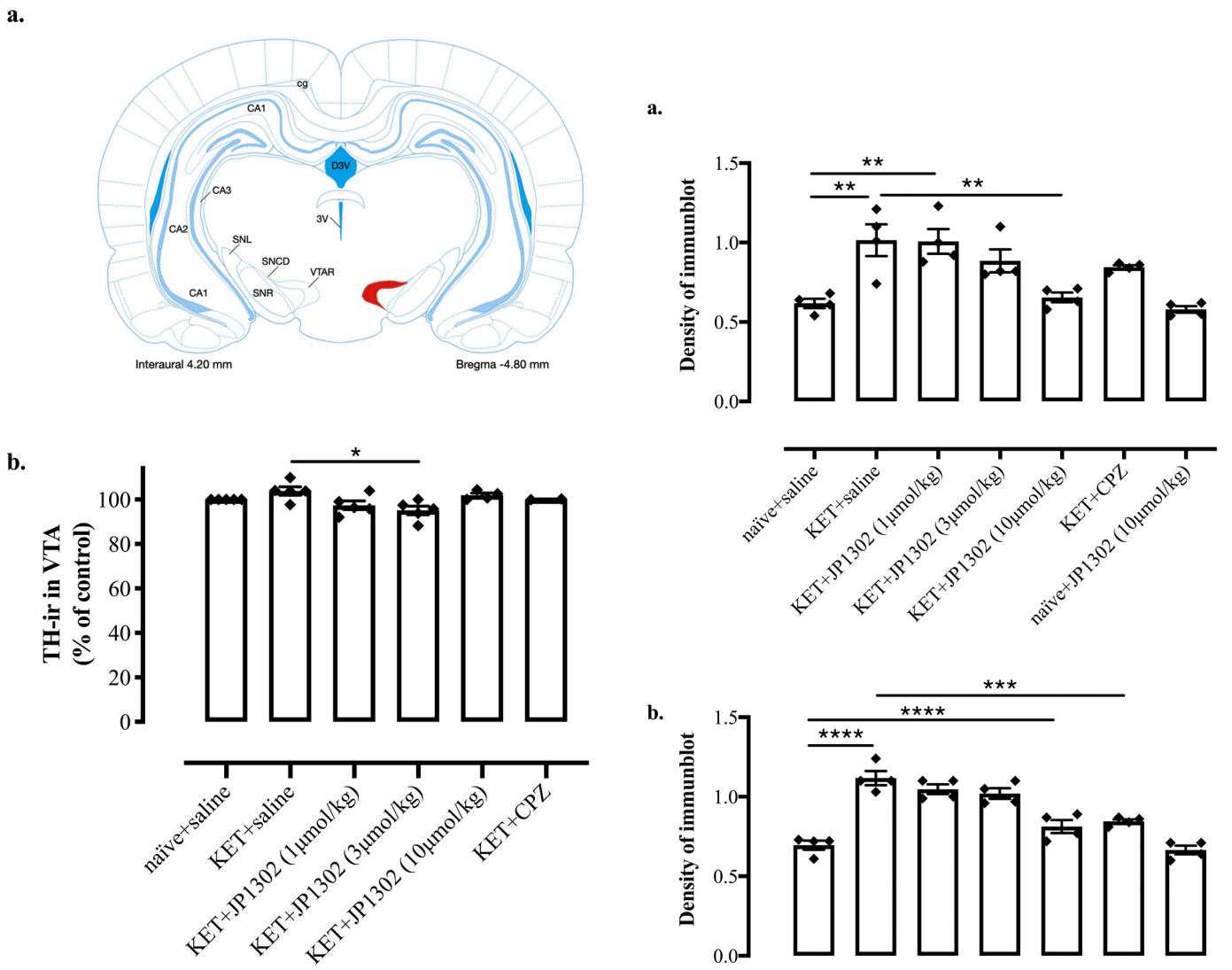

Fig. 5. The effect of ketamine and different treatment on TH-ir in VTA (n = 4). (A) Schematic of the region studied: VTA (red area), adapted from Paxinos and Watson (2007), (B) The percentage of TH-ir positive cell bodies in VTA compared to naïve. Bars represent means ± SEM. *p < 0.05, (ANOVA, Bonferroni *post-hoc* test). KET: ketamine, CPZ: chlorpromazine, 3 V: 3rd ventricle, CA1: field CA1 of the hippocampus, CA2: field CA2 of the hippocampus, CA3: field CA3 of the hippocampus, cg: cingulum, D3V: dorsal 3rd ventricle, LV: lateral ventricle, pc: posterior commissure, SNCD: substantia nigra, compacta, dorsal tier, SNL: substantia nigra, lateral part, SNR: substantia nigra, reticular part, VTAR: ventral tegmental area, rostral.

Fig. 7. The levels of α_{2C}-adrenoceptor expression in the (A) frontal cortex and (B) hippocampus were obtained from the western blotting assay ($n = 4$). **$p < 0.01$, ****$p < 0.0001$. Bars represent means ± SEM. (ANOVA, Bonferroni *post-hoc* test). KET: ketamine, CPZ: chlorpromazine.

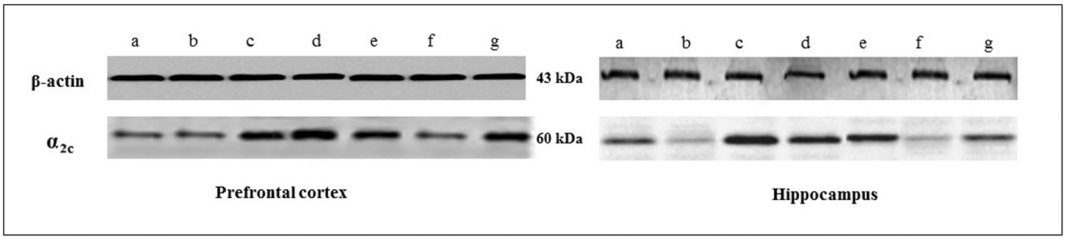

Fig. 6. The representative membrane images were obtained from the frontal cortex and hippocampus brain regions by western blotting assays. The blots were corrected according to corresponding beta-actin values. The experimental groups consisted of (a) naïve + saline, (b) naïve + JP1302 (10 μmol/kg) (c) KET + saline, (d) KET + JP1302 (1 μmol/kg), (e) KET + JP1302 (3 μmol/kg), (f) KET + JP1302 (10 μmol/kg), (g) KET + CPZ. KET: ketamine, CPZ: chlorpromazine.

symptoms of schizophrenia in rodents (Wilson and Terry, 2010; Wolff et al., 2018). In this study, KET administration increased locomotor activity, but JP-1302 did not reduce the KET-induced activity. In fact, the high dose of JP-1302 appeared to have the opposite effect, decreasing the resting time and increasing stereotyped behavior in naïve rats, albeit not statistically significant. There are conflicting results in the literature; Sallinen and coworkers reported that high dose JP-1302 (100 μmol/kg, 300 μmol/kg, subcutaneous) inhibited locomotor activity in naïve mice (Sallinen et al., 2007). Another study revealed that ORM-10921 produced a more prominent effect at lower doses as it might have dual engagement with α_{2A}- and α_{2C}-adrenoceptor at higher doses. (Uys et al., 2017b). Therefore, the increased stereotyped behavior in the present study may be relevant to the α_{2A}-adrenoceptor or non-α_2-adrenoceptor activity of JP-1302. Further studies are needed to address this issue.

Social withdrawal can be considered one of the classical negative signs of schizophrenia. Preclinical studies have shown that selective α_{2C}-adrenoceptor antagonism reverses social interaction deficits (Sallinen et al., 2013a). We show that repetitive KET administration decreased social interaction, in line with the literature (Adell et al., 2012; Becker et al., 2003). However, JP-1302 did not produce a statistically significant improvement.

Cognitive impairments are one of the main symptoms of schizophrenia (Green et al., 2004). Our study is consistent with the literature that has shown the recognition index was significantly lower in the KET-group (Goulart et al., 2010; Nikiforuk et al., 2013a; Pitsikas et al., 2008). The pro-cognitive properties of α_{2C}-adrenoceptor antagonism have been shown in preclinical and clinical studies. Age-related memory and learning decline was attenuated by ORM-12741 (Sallinen et al., 2013a), and this effect was confirmed in a clinical trial (Rinne et al., 2016). In the present study, we demonstrated that JP-1302 at a dose of 10 μmol/kg ameliorated the KET-induced cognitive deficits.

The present findings indicate that CPZ failed to attenuate the effects of KET on social interaction or cognition. One of the major limitations of our study is drug choice for positive control since, as a typical antipsychotic, it was not expected that CPZ would improve these deficits (Corbett et al., 1995). There are contradictory results about the efficacy of atypical antipsychotics in animal studies (Corbett et al., 1995; Sams-Dodd, 1996, 1997; Boulay et al., 2004). It might be speculated that no true positive control compound exists because there is no drug of choice for treating negative and cognitive symptoms in patients (Savolainen et al., 2019). Furthermore, most antipsychotics have an affinity for multiple receptor systems (e.g., serotonergic, adrenergic, and cholinergic). CPZ has a lower α_{2C}/D_2 selectivity than clozapine and risperidone but not olanzapine and haloperidol (Kalkman and Loetscher, 2003). Also, the lack of significant effects of treatments on behavioral parameters might relate to the acute effect of ketamine.

Most animal models of neuropsychiatric diseases include only male rats, and schizophrenia is no exception; studies including both sexes in animal models of schizophrenia are limited (Hill, 2016). There was no significant difference between sexes in the behavioral experiments (**Fig. S3**); these data were analyzed cumulatively. Although, we observed some differences between sexes in the open field test. While the percentage of the resting time was similar between sexes, the female rats had a higher total distance traveled than male rats, and sub-chronic KET had a more pronounced effect on this parameter in female rats. Previous studies have shown that female rodents exhibited more pronounced hyperlocomotion after the administration of NMDA antagonists (Damazio Pacheco et al., 2019; Frantz and Van Hartesveldt, 1999; McDougall et al., 2019; Wilson et al., 2007). The observed difference in KET effects on locomotion may be related to the pharmacokinetics of the KET (Saland and Kabbaj, 2018).

In the present study, we conducted tyrosine hydroxylase (TH) immunostaining in the subcortical brain areas. TH is the rate-limiting enzyme that catalyzes catecholamine biosynthesis (Hökfelt et al., 1984) and is used in immunocytochemistry studies to reveal dopaminergic neurons (Nair-Roberts et al., 2008). Postmortem and animal studies suggest that alteration of TH expression in subcortical brain areas may reflect the dopaminergic dysfunction in schizophrenia. The present study demonstrated that KET administration leads to a significant increase in TH expression in the striatum.

It has been shown that KET exerts this psychosis-like effect mainly by inhibiting the NMDA receptor on GABAergic interneuron, leading to disinhibition of dopamine in the striatal regions (Moghaddam et al., 1997; Lorrain et al., 2003; Balla et al., 2009). Earlier studies revealed increased dopamine levels in the dorsal striatum following the administration of NMDA antagonists (Carboni et al., 1989; Irifune et al., 1991). These studies also show the correlation between locomotor activity and striatal dopamine levels. As reported in the literature, we found that KET caused hyperdopaminergic activity in the striatum. JP-1302 alleviated this activity, but CPZ failed. It is difficult to interpret these results since JP-1302 did not decrease locomotor activity; indeed, a dose of 10 μmol/kg of JP-1302 tended to increase locomotion in naïve rats. There is growing evidence that the striatum may be as crucial as the prefrontal cortex in the formation of cognitive symptoms in schizophrenia (Simpson et al., 2010). One can assume that reducing the striatal dopaminergic activity may contribute to the pro-cognitive action of JP-1302 in the NORT.

The neurochemical analysis in the present study was conducted on frontal cortices and hippocampi. It has been shown that functional connectivity between these areas is disrupted in schizophrenia (Sigurdsson, 2016). In the present study, the α_{2C}-adrenoceptor level was higher in the KET group. To our knowledge, there is no study in the literature evaluating the α_{2C}-adrenoceptor expression in the schizophrenia model. In transgenic studies, it has been shown that α_{2C}-OE mice had impaired performance on water maze navigation tests (Björklund et al., 2000). We also reported a decreased number of α_{2C}-adrenoceptor as a response to high dose JP-1302. Antagonist-induced receptor downregulation is an unexpected phenomenon and might be a consequence of complex signal transduction cascade systems. Further molecular studies are required to elucidate this downregulation.

One possible mechanism for the role of α_{2C}-adrenoceptor in antipsychotic treatment has been obtained from transgenic animal studies that revealed an important relationship between α_{2C}-adrenoceptor and dopamine levels in the striatum and the frontal cortex (Sallinen et al., 1997). The results indicate that the deactivation of α_{2C}-adrenoceptor may decrease dopamine turnover in the striatum but not in the frontal cortex. Taken together with the idea of the dopamine hypothesis, which suggests that mesocortical hypodopaminergic and mesolimbic hyperdopaminergic state in schizophrenia, α_{2C}-adrenoceptor may be a potential therapeutic target in disease which has mesolimbic-cortical dopamine imbalance (Uys et al., 2017a).

4.1. Strengths and limitations

To date, this is the first study to assess the effects of JP-1302 treatment on behavioral and molecular alteration in a KET-induced schizophrenia model.

Some limitations of present studies are 1) the estral cycle phases were not determined in female rats; 2) the washout period was not applied; thus, the acute effect of ketamine cannot be ruled out; 3) the neurotransmitter levels were analyzed in the brain homogenates, so the levels are the final amount whether they could be intra or extracellular molecules. A microdialysis study may be more important to delineate neurotransmitter changes; 4) a typical antipsychotic was chosen as treatment in the positive control group, but it is also known that typical antipsychotics have limited effects on negative and cognitive symptoms.

In conclusion, sub-chronic sub-anesthetic KET induces schizophrenia-like behavioral and biochemical alteration in Wistar rats. The selective α_{2C}-adrenoceptor antagonist, JP-1302, could ameliorate the KET-induced cognitive impairment and attenuate the hyperdopaminergic activity, especially in the striatum. These results may

support the hypothesis that drugs targeting α_{2C}-adrenoceptors may be a promising approach for developing antipsychotic drugs.

Declaration of competing interest

None of the authors has any conflict of interest to disclose.

Part of the data was presented as a poster at the British Pharmacology Society Meeting 2019 in Edinburg, Scotland.

Data availability

Data will be made available on request.

Acknowledgments

This project was supported by Marmara University Scientific Research Committee (SAG-A-111017-0576).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbb.2022.173490.

References

Adell, A., Jimenez-Sanchez, L., Lopez-Gil, X., Romon, T., 2012. Is the acute NMDA receptor hypofunction a valid model of schizophrenia? Schizophr. Bull. 9, 14. https://doi.org/10.1093/schbul/sbr133.

Antunes, M., Biala, G., 2012. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn. Process. 13 (2), 93–110. https://doi.org/10.1007/s10339-011-0430-z.

Balla, A., Nattini, M.E., Sershen, H., Lajtha, A., Dunlop, D.S., Javitt, D.C., 2009. GABAB/NMDA receptor interaction in the regulation of extracellular dopamine levels in rodent prefrontal cortex and striatum. Neuropharmacology 56 (5), 915–921. https://doi.org/10.1016/j.neuropharm.2009.01.021.

Becker, A., Grecksch, G., 2004. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Test of predictive validity. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 28 (8), 1267–1277. https://doi.org/10.1016/j.pnpbp.2004.06.019.

Becker, A., Peters, B., Schroeder, H., Mann, T., Huether, G., Grecksch, G., 2003. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 27 (4), 687–700. https://doi.org/10.1016/S0278-5846(03)00080-0.

Björklund, M., Sirviö, J., Riekkinen, M., Sallinen, J., Scheinin, M., Riekkinen, P., 2000. Overexpression of alpha2C-adrenoceptors impairs water maze navigation. Neuroscience 95 (2), 481–487. https://doi.org/10.1016/S0306-4522(99)00428-5.

Boulay, D., Depoortère, R., Louis, C., Perrault, G., Griebel, G., Soubrié, P., 2004. SSR181507, a putative atypical antipsychotic with dopamine D2 antagonist and 5-HT1A agonist activities: improvement of social interaction deficits induced by phencyclidine in rats. Neuropharmacology 46 (8), 1121–1129. https://doi.org/10.1016/j.neuropharm.2004.02.008.

Bücheler, M.M., Hadamek, K., Hein, L., 2002. Two α2-adrenergic receptor subtypes, α2A and α2C, inhibit transmitter release in the brain of gene-targeted mice. Neuroscience 109 (4), 819–826. https://doi.org/10.1016/S0306-4522(01)00531-0.

Calixto, O.P., de Oliveira, M.B., Damázio, L.S., Zugno, A.I., Heylmann, A.S., Canever, L., Pedro, T.H., Budni, J., Wessler, P.G., Pacheco, F.D., Pereira, F.P., Macan, T.P., Schuck, P.F., Steckert, A., Quevedo, J., Mastella, G.A., 2016. Effect of folic acid on oxidative stress and behavioral changes in the animal model of schizophrenia induced by ketamine. J. Psychiatr. Res. 81, 23–35. https://doi.org/10.1016/j.jpsychires.2016.06.013.

Carboni, E., Imperato, A., Perezzani, L., Di Chiara, G., 1989. Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28 (3), 653–661. https://doi.org/10.1016/0306-4522(89)90012-2.

Cohen, B.D., Rosenbaum, G., Luby, E.D., Gottlieb, J.S., 1962. Comparison of phencyclidine hydrochloride (Sernyl) with other drugs. Simulation of schizophrenic performance with phencyclidine hydrochloride (Sernyl), lysergic acid diethylamide (LSD-25), and amobarbital (Amytal) sodium; II. Symbolic and sequential thinking. Arch. Gen. Psychiatry 6, 395–401. https://doi.org/10.1001/archpsyc.1962.01710230063007.

Corbett, R., Camacho, F., Woods, A.T., Kerman, L.L., Fishkin, R.J., Brooks, K., Dunn, R. W., 1995. Antipsychotic agents antagonize non-competitive N-methyl-D-aspartate antagonist-induced behaviors. Psychopharmacology 120 (1), 67–74. https://doi.org/10.1007/BF02246146.

Damazio Pacheco, F., Canever, L., Antunes Mastella, G., Gomes Wessler, P., Kunz Godoi, A., Hubbe, I., da Costa Afonso, A., Celso, D., Quevedo, J., Ioppi Zugno, A., 2019. Effects of ketamine on prepubertal Wistar rats: implications on behavioral parameters for childhood-onset schizophrenia. Int. J. Dev. Neurosci. 79, 49–53. https://doi.org/10.1016/J.IJDEVNEU.2019.10.006.

De Oliveira, L., Fraga, D.B., De Luca, R.D., Canever, L., Ghedim, F.V., Matos, M.P.P., Streck, E.L., Quevedo, J., Zugno, A.I., 2011. Behavioral changes and mitochondrial dysfunction in a rat model of schizophrenia induced by ketamine. Metab. Brain Dis. 26 (1), 69–77. https://doi.org/10.1007/s11011-011-9234-1.

Dreyer, W., Sallinen, J., Shahid, M., Uys, M., Harvey, B.H., Cockeran, M., 2016. The α2C-adrenoceptor antagonist, ORM-10921, has antipsychotic-like effects in social isolation reared rats and bolsters the response to haloperidol. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 71, 108–116. https://doi.org/10.1016/j.pnpbp.2016.07.002.

Frantz, K., Van Hartesveldt, C., 1999. Locomotion elicited by MK801 in developing and adult rats: temporal, environmental, and gender effects. Eur. J. Pharmacol. 369 (2), 145–157. https://doi.org/10.1016/S0014-2999(99)00070-9.

Gören, M.Z., Küçükibrahimoglu, E., Berkman, K., Terzioglu, B., 2007. Fluoxetine partly exerts its actions through GABA: a neurochemical evidence. Neurochem. Res. 32, 1559–1565. https://doi.org/10.1007/s11064-007-9357-2.

Goulart, B.K., de Lima, M.N., de Farias, C.B., Reolon, G.K., Almeida, V.R., Quevedo, J., Kapczinski, F., Schröder, N., Roesler, R., 2010. Ketamine impairs recognition memory consolidation and prevents learning-induced increase in hippocampal brain-derived neurotrophic factor levels. Neuroscience 167 (4), 969–973. https://doi.org/10.1016/j.neuroscience.2010.03.032.

Green, M.F., Nuechterlein, K.H., Gold, J.M., Barch, D.M., Cohen, J., Essock, S., Fenton, W.S., Frese, F., Goldberg, T.E., Heaton, R.K., Keefe, R.S.E., Kern, R.S., Kraemer, H., Stover, E., Weinberger, D.R., Zalcman, S., Marder, S.R., 2004. Approaching a consensus cognitive battery for clinical trials in schizophrenia: the NIMH-MATRICS conference to select cognitive domains and test criteria. Biol. Psychiatry 56 (5), 301–307. https://doi.org/10.1016/J.BIOPSYCH.2004.06.023.

Hein, L., Altman, J.D., Kobilka, B.K., 1999. Two functionally distinct α2-adrenergic receptors regulate sympathetic neurotransmission. Nature. 402, 181–184. https://doi.org/10.1038/46040.

Hill, R.A., 2016. Sex differences in animal models of schizophrenia shed light on the underlying pathophysiology. Neurosci. Biobehav. Rev. 67, 41–56. https://doi.org/10.1016/j.neubiorev.2015.10.014.

Hökfelt, T., Martensson, R., Bjorklund, A., Kleinau, S., Goldstein, M., 1984. Handbook of Chemical Neuroanatomy,Vol. 2, Part 1. Elsevier.

Irifune, M., Shimizu, T., Nomoto, M., 1991. Ketamine-induced hyperlocomotion associated with alteration of presynaptic components of dopamine neurons in the nucleus accumbens of mice. Pharmacol. Biochem. Behav. 40 (2), 399–407. https://doi.org/10.1016/0091-3057(91)90571-i.

Itil, T., Keskiner, A., Kiremitci, N., Holden, J.M., 1967. Effect of phencyclidine in chronic schizophrenics. Can. Psychiatr. Assoc. J. 12 (2), 209–212. https://doi.org/10.1177/070674376701200217.

James, S.L., Abate, D., Abate, K.H., Abay, S.M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R.S., Abebe, Z., Abera, S.F., Abil, O.Z., Abraha, H.N., Abu-Raddad, L.J., Abu-Rmeileh, N. M.E., Accrombessi, M.M.K., Murray, C.J.L., 2018. Global, regional, and national incidence, prevalence, and years lived with disability for 354 Diseases and Injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392 (10159), 1789–1858. https://doi.org/10.1016/S0140-6736(18)32279-7.

Jones, C., Watson, D., Fone, K., 2011. Animal models of schizophrenia. Br. J. Pharmacol. 164 (4), 1162–1194. https://doi.org/10.1111/j.1476-5381.2011.01386.x.

Kalkman, H.O., Loetscher, E., 2003. alpha2C-Adrenoceptor blockade by clozapine and other antipsychotic drugs. Eur. J. Pharmacol. 462 (1–3), 33–40. https://doi.org/10.1016/s0014-2999(03)01308-6.

Keefe, R.S.E., Goldberg, T.E., Harvey, P.D., Gold, J.M., Poe, M.P., Coughenour, L., 2004. The brief assessment of cognition in schizophrenia: reliability, sensitivity, and comparison with a standard neurocognitive battery. Schizophr. Res. 68 (2-3), 283–297. https://doi.org/10.1016/j.schres.2003.09.011.

Keefe, R.S.E., Bilder, R.M., Davis, S.M., Harvey, P.D., Palmer, B.W., Gold, J.M., Meltzer, H.Y., Green, M.F., Capuano, G., Stroup, T.S., McEvoy, J.P., Swartz, M.S., Rosenheck, R.A., Perkins, D.O., Davis, C.E., Hsiao, J.K., Lieberman, J.A., 2007. Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE trial. Arch. Gen. Psychiatry 64 (6), 633–647. https://doi.org/10.1001/archpsyc.64.6.633.

Krystal, J.H., Karper, L.P., Seibyl, J.P., Freeman, G.K., Delaney, R., Bremner, J.D., Heninger, G.R., Bowers Jr., M.B., Charney, D.S., 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch. Gen. Psychiatry 51 (3), 199–214. https://doi.org/10.1001/archpsyc.1994.03950030035004.

Küçükibrahimoğlu, E., Saygın, M.Z., Çalışkan, M., Kaplan, O.K., Ünsal, C., Gören, M.Z., 2009. The change in plasma GABA, glutamine and glutamate levels in fluoxetine- Or S-citalopram-treated female patients with major depression. Eur. J. Clin. Pharmacol. 65, 571–577. https://doi.org/10.1007/s00228-009-0650-7.

Lorrain, D.S., Baccei, C.S., Bristow, L.J., Anderson, J.J., Varney, M.A., 2003. Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: modulation by a group II selective metabotropic glutamate receptor agonist LY379268. Neuroscience 117 (3), 697–706. https://doi.org/10.1016/s0306-4522(02)00652-8.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 (1), 265–275. https://doi.org/10.1016/s0021-9258(19)52451-6.

McDougall, S.A., Park, G.I., Ramirez, G.I., Gomez, V., Adame, B.C., Crawford, C.A., 2019. Sex-dependent changes in ketamine-induced locomotor activity and ketamine pharmacokinetics in preweanling, adolescent, and adult rats. Eur.

Neuropsychopharm. 29 (6), 740–755. https://doi.org/10.1016/J.EURONEURO.2019.03.013.
Moghaddam, B., Adams, B., Verma, A., Daly, D., 1997. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J. Neurosci. 17 (8), 2921–2927. https://doi.org/10.1523/JNEUROSCI.17-08-02921.1997.
Nair-Roberts, R.G., Chatelain-Badie, S.D., Benson, E., White-Cooper, H., Bolam, J.P., Ungless, M.A., 2008. Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. Neuroscience 152 (4), 1024–1031. https://doi.org/10.1016/J.NEUROSCIENCE.2008.01.046.
Nikiforuk, A., Fija, ł.K., Potasiewicz, A., Popik, P., Kos, T., 2013a. The 5-hydroxytryptamine (serotonin) receptor 6 agonist EMD 386088 ameliorates ketamine-induced deficits in attentional set shifting and novel object recognition, but not in the prepulse inhibition in rats. J. Psychopharmacol. 27, 469–476. https://doi.org/10.1177/0269881113480991.
Osborne, A.L., Solowij, N., Babic, I., Huang, X.F., Weston-Green, K., 2017. Improved social interaction, recognition and working memory with cannabidiol treatment in a prenatal infection (poly I:C) Rat Model. Neuropsychopharmacology 42 (7), 1447–1457. https://doi.org/10.1038/npp.2017.40.
Özakman, S., Gören, M.Z., Nurten, A., Tekin, N., Kalaycı, R., Enginar, N., 2021. Effects of tamoxifen and glutamate and glutamine levels in brain regions in repeated sleep deprivation–induced mania model in mice. Naunyn Schmiedeberg's Arch. Pharmacol. 394, 619–629. https://doi.org/10.1007/s00210-020-02001-1.
Paxinos, G., Watson, C., 2007. The Rat Brain: In Stereotaxis Coordinates, sixth ed. Elsevier Inc.
Pitsikas, N., Boultadakis, A., Sakellaridis, N., 2008. Effects of sub-anesthetic doses of ketamine on rats' spatial and non-spatial recognition memory. Neuroscience 154 (2), 454–460. https://doi.org/10.1016/j.neuroscience.2008.04.001.
Rinne, J.O., Wesnes, K., Cummings, J.L., Hakulinen, P., Hallikainen, M., Hänninen, J., Murphy, M., Riordan, H., Scheinin, M., Soininen, H., Rouru, J., 2016. Tolerability of ORM-12741 and effects on episodic memory in patients with Alzheimer's disease. Alzheimer's Dementia 3(1), 1–9. https://doi.org/10.1016/j.trci.2016.11.004.
Saland, S.K., Kabbaj, M., 2018. Sex differences in the pharmacokinetics of low-dose ketamine in plasma and brain of male and female rats. J. Pharmacol. Exp. Ther. 367 (3), 393. https://doi.org/10.1124/JPET.118.251652.
Sallinen, Link, R.E., Haapalinna, A., Viitamaa, T., Kulatunga, M., Sjöholm, B., Macdonald, E., Pelto-Huikko, M., Leino, T., Barsh, G.S., Kobilka, B.K., Scheinin, M., 1997. Genetic alteration of alpha 2C-adrenoceptor expression in mice: influence on locomotor, hypothermic, and neurochemical effects of dexmedetomidine, a subtype-nonselective alpha 2-adrenoceptor agonist. Mol. Pharmacol. 51 (1), 36–46. https://doi.org/10.1124/MOL.51.1.36.
Sallinen, J., Höglund, I., Engström, M., Lehtimäki, J., Virtanen, R., Sirviö, J., Wurster, S., Savola, J.M., Haapalinna, A., 2007. Pharmacological characterization and CNS effects of a novel highly selective α 2C-adrenoceptor antagonist JP-1302. Br. J. Pharmacol. 150 (4), 391–402. https://doi.org/10.1038/sj.bjp.0707005.
Sallinen, J., Rouru, J., Lehtimaki, J., Ivi Marjamäki, P., Haaparanta-Solin, M., Arponen, E., Helin, S., Solin, O., Tarazi, F., Shahid, M., 2013a. ORM-12741: Receptor pharmacology of a novel alpha2c-adrenergic receptor subtype selective antagonist with multi-therapeutic potential. Poster Session III-Wednesday. Neuropsychopharmacology 38 (Suppl. 2), S435–S593. https://doi.org/10.1038/npp.2013.281.
Sallinen, J., Holappa, J., Koivisto, A., Kuokkanen, K., Chapman, H., Lehtimäki, J., Piepponen, P., Mijatovic, J., Tanila, H., Virtanen, R., Sirviö, J., Haapalinna, A., 2013b. Pharmacological characterisation of a structurally novel α2C -adrenoceptor antagonist ORM-10921 and its effects in neuropsychiatric models. Basic Clin. Pharmacol. Toxicol. 113 (4), 239–249. https://doi.org/10.1111/bcpt.12090.
Sams-Dodd, F., 1996. Phencyclidine-induced stereotyped behaviour and social isolation in rats: a possible animal model of schizophrenia. Behav. Pharmacol. 7, 3–23.
Sams-Dodd, F., 1997. Effect of novel antipsychotic drugs on phencyclidine-induced stereotyped behaviour and social isolation in the rat social interaction test. Behav. Pharmacol. 8, 196–215.
Savolainen, K., Ihalainen, J., Jalkanen, A.J., Forsberg, M.M., 2019. Selective adrenergic alpha2C receptor antagonist ameliorates acute phencyclidine-induced schizophrenia-like social interaction deficits in rats. Psychopharmacology 236 (4), 1245–1253. https://doi.org/10.1007/s00213-018-5130-2.
Scheinin, M., Sallinen, J., Haapalinna, A., 2001. Evaluation of the α 2C -adrenoceptor as a neuropsychiatric drug target: Studies in transgenic mouse models. Life Sci. 68 (19-20), 2277–2285. https://doi.org/10.1016/S0024-3205(01)01016-5.
Seeman, P., Lee, T., Chau-Wong, M., Wong, K., 1976. Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 717-719. https://doi.org/10.1038/261717a0.
Sigurdsson, T., 2016. Neural circuit dysfunction in schizophrenia: insights from animal models. Neuroscience 321, 42–65. https://doi.org/10.1016/j.neuroscience.2015.06.059.
Simpson, E.H., Kellendonk, C., Kandel, E., 2010. A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia. Neuron 65 (5), 585–596. https://doi.org/10.1016/j.neuron.2010.02.014.
Stroup, T.S., McEvoy, J.P., Swartz, M.S., Byerly, M.J., Glick, I.D., Canive, J.M., McGee, M.F., Simpson, G.M., Stevens, M.C., Lieberman, J.A., 2003. The National Institute of Mental Health Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) project: schizophrenia trial design and protocol development. Schizophr. Bull. 29 (1), 15–31. https://doi.org/10.1093/oxfordjournals.schbul.a006986.
Tricklebank, M.D., 2007. JP-1302: a new tool to shed light on the roles of α 2C-adrenoceptors in brain. Br. J. Pharmacol. 150 (4), 381–382. https://doi.org/10.1038/sj.bjp.0707007.
Uys, Madeleine Monique, Shahid, M., Harvey, B.H., 2017a. Therapeutic potential of selectively targeting the α2C -adrenoceptor in cognition, depression, and schizophrenia—New developments and future perspective. Front. Psychiatry 8, 144. https://doi.org/10.3389/fpsyt.2017.00144.
Uys, M.M., Shahid, M., Sallinen, J., Harvey, B.H., 2017b. The α2C-adrenoceptor antagonist, ORM-10921, exerts antidepressant-like effects in the Flinders Sensitive Line rat. Behav. Pharmacol. 28 (1), 9–18. https://doi.org/10.1097/FBP.0000000000000261.
Wilson, C., Terry, A.V., 2010. Neurodevelopmental animal models of schizophrenia: role in novel drug discovery and development. Clin. Schizophrenia Relat. Psychoses 4, 124–137. https://doi.org/10.3371/CSRP.4.2.4.
Wilson, C., Kercher, M., Quinn, B., Murphy, A., Fiegel, C., McLaurin, A., 2007. Effects of age and sex on ketamine-induced hyperactivity in rats. Physiol. Behav. 91 (2–3), 202–207. https://doi.org/10.1016/J.PHYSBEH.2007.02.010.
Wolff, A.R., Bygrave, A.M., Sanderson, D.J., Boyden, E.S., Bannerman, D.M., Kullmann, D.M., 2018. Optogenetic induction of the schizophrenia-related endophenotype of ventral hippocampal hyperactivity causes rodent correlates of positive and cognitive symptoms. Sci. Report. 8-12871 https://doi.org/10.1038/s41598-018-31163-5.
Zugno, A.I., Heylmann, A.S., Luca, R.D., Pedrini, M., Massuda, R., Canever, L., Deroza, P. F., Fraga, D.B., Gama, C.S., Stertz, L., de Lucena, D.F., Gubert, C., Panizzutti, B., 2012. Effects of omega-3 dietary supplement in prevention of positive, negative and cognitive symptoms: a study in adolescent rats with ketamine-induced model of schizophrenia. Schizophr. Res. 141 (2–3), 162–167. https://doi.org/10.1016/j.schres.2012.08.002.