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Early treatment with JNJ-46356479, a mGluR2 modulator, improves behavioral and neuropathological deficits in a postnatal ketamine mouse model of schizophrenia

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ABSTRACT

Positive allosteric modulators of the metabotropic glutamate receptor 2 (mGluR2), such as JNJ-46356479 (JNJ), may mitigate the glutamate storm during the early stages of schizophrenia (SZ), which could be especially useful in the treatment of cognitive and negative symptoms. We evaluated the efficacy of early treatment with JNJ or clozapine (CLZ) in reversing behavioral and neuropathological deficits induced in a postnatal ketamine (KET) mouse model of SZ. Mice exposed to KET (30 mg/kg) on postnatal days (PND) 7, 9, and 11 received JNJ or CLZ (10 mg/kg) daily in the adolescent period (PND 35–60). Mice exposed to KET did not show the expected preference for a novel object or for social novelty, but they recovered this preference with JNJ treatment. Similarly, KET group did not show the expected dishabituation in the fifth trial, but mice treated with JNJ or CLZ recovered an interest in the novel animal. Neuronal immunoreactivity also differed between treatment groups with mice exposed to KET showing a reduction in parvalbumin positive cells in the prefrontal cortex and decreased c-Fos expression in the hippocampus, which was normalized with the pharmacological treatment. JNJ-46356479 treatment in early stages may help improve the cognitive and negative symptoms, as well as certain neuropathological deficits, and may even obtain a better response than CLZ treatment. This may have relevant clinical translational applications since early treatment with mGluR2 modulators that inhibit glutamate release at the onset of critical phases of SZ may prevent or slow down the clinical deterioration of the disease.

1. Introduction

Schizophrenia (SZ) is a common and heterogeneous psychiatric disorder that affects approximately 1% of the population worldwide and whose etiopathogenesis remains poorly understood [1,2]. Pharmacological treatment for SZ relies on the use of antipsychotics, which have proven effectiveness for the control of positive symptoms through complex mechanisms that include the blockade of dopamine and serotonin receptors, among other effects. However, these antipsychotics are not able to treat the associated negative symptoms and cognitive deficits, a limitation that complicates and impairs patients' quality of life [3]. Only clozapine (CLZ) has demonstrated superior antipsychotic efficacy; however, despite showing an apparently greater improvement for negative symptoms, it has no clear benefits for cognitive performance [4,5]. Moreover, the ability of CLZ to induce agranulocytosis, a potentially fatal side effect, limits its use in many cases. Therefore, it is necessary to investigate other molecules with different mechanisms of action so as to improve the pharmacological treatment of this disorder.

Glutamatergic dysregulation is one of the main pathophysiological theories of SZ. This theory arose from observations that N-methyl-D-

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aspartate (NMDA) glutamate receptor antagonists, such as ketamine (KET), phencyclidine (PCP), or dizocilpine (MK-801), not only mimic the positive, negative, and cognitive symptoms of SZ in healthy individuals but also exacerbate the psychotic symptoms and cognitive decline in patients with the disease [6,7]. The blockade of NMDA receptors in gamma-aminobutyric (GABA)ergic interneurons, which express parvalbumin (PV), may generate a hyperglutamatergic condition because of the decreased inhibitory control of excitatory pyramidal neurons, leading to neurotoxicity, apoptosis, and synaptic pruning in critical brain areas [8,9]. Consequently, the administration of NMDA antagonists has been proposed to generate animal experimental models of SZ, which produce positive, negative and cognitive schizophrenia symptoms [10]. Interestingly, exposure to NMDA receptor antagonism during the early postnatal period (PND 7–11), seems to affect the function of both the prefrontal cortex (PFC) and hippocampus (HPC) during adulthood, resulting in altered cognitive and social performance across a range of behavioral tasks [11]. Specifically, mice exposed to KET on PND 7, 9, and 11 [12,13] show persistent changes in parvalbumin GABAergic interneurons and c-Fos activity patterns, and induce SZ-like behavioral symptoms in adulthood, as we reported recently [14].

In this scenario, the use of glutamate inhibitors could offer a pharmacological strategy for treating negative and cognitive symptoms. Positive allosteric modulators (PAMs) of metabotropic glutamate receptor 2 (mGluR2) inhibit the presynaptic release of glutamate. We are particularly interested in assessing 8-trifluoromethyl-3-cyclopropylmethyl-7-[(4–(2,4-difluorophenyl)1-piperazinyl)methyl] – 1,2,4-triazolo [4,3-α]pyridine (JNJ-46356479; henceforth JNJ) (Supplementary Figure 1), a new selective and orally bioavailable PAM of mGluR2 [15,16]. *In vitro*, JNJ has a neuroprotective effect in human neuroblastoma cultures [17], while i*n vivo*, JNJ pharmacological treatment in adult postnatal KET mice partially improves neuropathological deficits and SZ-like behavior [14]. However, other mGluR2 PAMs, such as LY2140023 [18] or JNJ40411813 [16,19–21] have failed to treat cases of established SZ [16]. In view of these results, it would be helpful to investigate the possible benefits of these PAMs when used during early critical periods, such as the prodromal stage or during the transition to psychosis. Indeed, dealing with the hypothetical glutamate storm during early stages of SZ may help to prevent disease onset or may slow the progression of psychosis and clinical deterioration of patients [22,23].

We aimed to evaluate the efficacy of early treatment with JNJ-46356479 in reversing the behavioral and neuropathological deficits induced in a postnatal KET mouse model of SZ. Animals were exposed to KET on PND 7, 9, and 11 [12–14] in order to transiently disrupt NMDA receptor function, and were subsequently treated with JNJ or CLZ in the adolescent period (PND 35–60). A wide range of behavioral tests were performed in adult animals (PND >90) to evaluate the cognitive and negative symptoms related to SZ and locomotor activity and anxiety-related behavior. Moreover, PV and c-Fos immunoreactivity were assessed in the PFC and HPC of these animals.

2. Material and methods

2.1. Animals and drug treatment

All experimental procedures involving animals were performed according to European Union guidelines on the care and use of laboratory animals and were approved by the University of Barcelona Animal Care Committee (License number 386/18 P1) and by the Department of the Environment of the Generalitat de Catalunya (Order number 10410).

Mice from the C57BL/6J strain (Charles River Laboratories, Wilmington, MA, USA) were acclimatized in the animal house of the Faculty of Medicine, University of Barcelona. Later, at the age of about 60 days, male and female mice of approximately 22.5 g and 18 g in weight respectively were put to breed in pairs. Mice were housed at 22ºC with ad libitum access to food and drink in an alternating 12-hour light and dark cycle. A total of 73 male and female pups were exposed on PND 7, 9, and 11 with subcutaneous injections of saline vehicle (Veh: approximately 0.1 mL, 25 gauge needle) (N = 37) or ketamine (30 mg/kg, Ketamidor 100 mg/mL, Karizoo, Barcelona, Spain) (N = 36), as previously described [14]. Given that early treatment during critical windows for brain development, such as adolescence, may be more effective than pharmacological treatment in adulthood [22,24], in the present study adolescent mice (PND 35–60) received daily subcutaneous injections (approximately 0.25 mL, 25 gage needle) of vehicle (10% hydroxypropyl-beta- cyclodextrin, HPβCD, and 5% DMSO; N = 24), JNJ (10 mg/kg; N = 24), or CLZ (10 mg/kg; N = 25). The drug doses and administration route selected were based on scientific advice from Janssen Pharmaceuticals and are frequently used in murine models to test CLZ and other mGluR2 positive allosteric modulators [15,21,25,26].

2.2. Behavioral experiments

In adulthood (PND >90), mice in each experimental group underwent a wide range of tests to evaluate cognitive and negative behaviors related to SZ and to assess locomotor activity and anxiety-related behavior. Mice were sex-matched in each of the following groups: Control (Veh+Veh, N = 12); JNJ (Veh+JNJ, N = 12); CLZ (Veh+CLZ, N = 13); KET (KET+Veh, N = 12); KET+JNJ (N = 12) and KET+CLZ (N = 12).

Adult mice underwent behavioral tests between PND 90 and 120 in the following order: motor test battery, rotarod test, open field test, Novel Object Recognition test, Y-maze, Three-chamber sociability and novelty test, and Five-trial social memory test. To minimize potential altered behavioral responses due to prior test history, the most invasive procedures were performed last. The order of animals within one testing session was randomized by cage. All procedures were carried out by the same technician in order to reduce the stress caused to the animals. Each experiment was assessed on the successive mornings, excluding weekends. Most experimental details were described in a previous study by our group [14].

2.2.1. Motor assessment

Motor function, motor coordination, and general activity and anxiety were evaluated using a motor test battery, a rotarod test, and the open field test, respectively.

The motor test battery included the following: (1) visual placing reflex to evaluate cerebellar and vestibular functions by evaluating forepaw extension response after gently lowering mice by the tail toward a flat surface; (2) startle response to a sudden auditory stimulus to evaluate auditory sensitivity; (3) righting reflex after dropping mice from a height and assessing how they land; (4) grip strength by quantifying resistance to separation from a lid of aluminum bars when pulled by the tail; (5) equilibrium by quantifying the capacity of the animal placed on the center of a flat wooden and cylindrical aluminum rod to reach one of its ends, as well as by measuring the latency to fall from these bars; (6) prehensile reflex and traction capacity by assessing their ability to remain suspended by the forepaws when grasping an elevated horizontal wire and assessing the number of hind limbs that the animal raised to reach the wire; and (7) motor coordination with the coat hanger test, placing mice in the middle of a wire in an upside-down position and measuring their activity on the wire and the latency to reach the end of the coat hanger.

Motor coordination was evaluated using a rotarod device (Ugo Basile, Comerio, Italy). In the first three tests, the rod was rotated at constant speeds of 10, 25, and 40 revolutions per minute (rpm), respectively. The last trial consisted of an acceleration cycle in which the rod rotated progressively faster, requiring the animal to adapt to the growing demands of the test.

Finally, the open field test was performed to assess locomotor activity, exploratory behavior, and anxiety using a square-shaped open field. Animals were placed in the center of the field and allowed to

explore it freely during a single 5 min trial. We measured (1) total distance traveled, (2) time spent at the periphery and center of the apparatus, (3) mean walking speed, and (4) the number of rearings (vertical activity), using Smart 3.0 video tracking software (Panlab, Barcelona, Spain) to record each parameter automatically.

2.2.2. Y-maze

The spatial working memory task was conducted in a Y-shaped maze. First, mice were allowed 10 min exploration with one arm blocked, and after an interval of 15 s, they were allowed to explore all three arms for 3 min without a plexiglass divider. The times spent inside the novel and familiar arms were recorded.

To evaluate spontaneous alternation, mice were first placed at the start site for 20 s and then freely allowed to choose one arm that was blocked allowing exploration for 25 s. They were then placed at the start site again, the blocked arm was opened, and selection of the new arm was recorded. This procedure was repeated three times with an inter-trial time of 5 min

2.2.3. Novel object recognition test

The novel object recognition test was also used to assess the working memory. We used modifications of the procedures described by Korotkova et al. [27]. Briefly, mice were individually habituated to an open field box (45 × 45× 50 cm) with white walls for 2 days (5 min every day). During the training session on the third day, a couple of identical objects were placed in the open field and the mouse was allowed to explore them for two trials of 5 min each and the time spent exploring each object was recorded. After a 5 min delay, the mouse was returned to the same box where one of the familiar objects was replaced by a novel object, and was allowed to explore freely for 5 min. To avoid spontaneous object preference, objects were presented in a counterbalanced order and cleaned with ethanol between every trial to prevent odor cues. All objects presented were of similar surface structure and size (approximately 10 cm in length) but had distinctive shapes and colors. All sessions were video recorded and interaction times (defined as sniffing and investigating at close proximity) were scored offline.

2.2.4. Three-chamber sociability and social novelty test

The social recognition paradigm was conducted in a three-chambered box with a door connecting each compartment. The animals were habituated for 10 min by allowing them to move freely in all empty areas for two sessions (one per day) before starting the experiment.

In the sociability test phase, after a habituation period, animals were exposed for 10 min to one compartment containing a familiar littermate inside a cage (the social side) and to another compartment with a toy mouse placed inside a cage (the non-social side). Social investigation was assessed by measuring how long the animal sniffed each cage. Then, after placing the mice in their home cage for 10 min, preference for social novelty was tested. Next, a stimulus animal from a different litter was placed in one compartment and a littermate was placed in the other compartment, and again, we measured how long the mice spent sniffing each cage. In both the sociability and the novelty recognition tests, the stimulus animal was placed in alternating compartments, counterbalancing left and right placement to avoid spontaneous preference. The apparatus was cleaned with 70% ethanol after each test and all sessions were video recorded for offline scoring of interaction times (defined as sniffing and investigating at close proximity).

2.2.5. Five-trial social memory test

Experimental mice and two stimulus mice per group (naïve, aged about 2–3 weeks old, to avoid sexual preferences) were housed individually in home cages for at least 24 h prior to experimentation. On the day of testing, a stimulus mouse (intruder) was placed inside a cage and introduced into the experimental animal's home cage four consecutive times for 1 min each. Between exposures, the intruder was returned to its home cage for 10 min. A novel stimulus mouse was then introduced for 1 min into the experimental animal's home cage 10 min after the fourth exposure. All sessions were video recorded and interaction times (defined as sniffing and investigating at close proximity) were scored offline.

2.3. Brain tissue preparation and immunochemistry

Half of the animals in the four main experimental groups: Control (Veh+Veh, N = 6); KET (KET+Veh, N = 6); KET+JNJ (N = 6) and KET+CLZ (N = 6) were perfused one day after completion of behavioral experiments. Mice were deeply anesthetized with pentobarbital (50 mg/kg) and transcardially perfused with phosphate buffer saline 1X (PBS) followed by 4% paraformaldehyde (PFA) in PBS 1X. Brains were removed and submerged in 4% PFA overnight at 4ºC, and then transferred into 30% sucrose and stored for 3 days at 4ºC. Next, the brains were frozen in dry ice and coronal sections were obtained using a cryostat at a thickness of 30 μm. From each animal, 5 series containing 7–9 PFC sections and 8 series containing 8–10 HPC sections were obtained. Sections were stored at − 20ºC in a solution containing 25% (v/v) ethylene glycol, 25% glycerol, and 50% (v/v) sodium phosphate buffer, until processing for histological procedures.

Free-floating sections from the PFC and the HPC were prepared for immunofluorescent double labeling as described previously [14]. Briefly, one series of PFC and HPC from animals was mounted on SuperFrost Plus slides and coverslipped with Fluoro Gel. Following subsequent washes in PBS, sections were blocked in PBS 1X containing 0.3% Triton X-100% and 1% bovine serum albumin for 1 h followed by overnight incubation with primary antibody for PV and c-Fos (1:1000, Abcam and 1:500, Synaptic Systems, respectively) at 4ºC. On the day after washing, the sections were incubated overnight with anti-rabbit Alexa Fluor-594-conjugated and anti-guinea pig Alexa Fluor-488-conjugated secondary antibodies (1:1000, Life Technologies) at 4ºC. Finally, after washing with PBS, total cellular nuclei were stained with 4',96-diamidino-2phenylindole (DAPI) for 12 min in 0.1 N phosphate buffer.

DAPI was used to define the anatomical structures which were identified according to the Franklin and Paxinos atlas [28]. Six independent fields on the prelimbic and infralimbic medial PFC (mPFC) were randomly selected and a dissector counting frame of (403.51 ×403.51 μm) was imaged in each one. Slices from the mPFC were imaged using Zeiss LSM880 with a 40X objective. For the hippocampal area, four random dissectors were photographed obtaining a mosaic image encompassing the entire area; then the specific zones of Dentate Gyrus (DG), CA1, CA2 and CA3 were defined manually, delimiting the granule cell layer in the DG and the pyramidal cell layer in CA1, CA2 and CA3, analyzing average areas of 0.721 mm^2, 0.599 mm^2, 0.081 mm^2 and 0.596 mm^2, respectively. Images from the HPC were obtained using a Leica AF6000 with a 20X objective. Image analysis was performed with the aid of NIH ImageJ software and was conducted blind with regard to treatment groups. PV + cells were counted after applying the ImageJ filter subtract background with a rolling ball radius of 50 pixels. C-Fos marked cells in the dissectors were determined using the thresholding method to estimate the average of mean gray values after manually subtracting the fluorescence intensity of the background for each one of the acquired images.

2.4. Statistical analysis

The Shapiro-Wilk test was used to analyze the normality of data. Differences between groups were analyzed using one or two-way ANOVA, with or without repeated measures (RM), or the Kruskal-Wallis test according to the data distribution. The mean values for each experimental group were compared using Bonferroni post-hoc tests. We also performed intragroup comparisons for the novel object recognition, three-chamber, and five-trial tests using paired Student *t*-

test. Mice were sex-matched in each group and no significant sex effect was detected in any evaluation even when sex was included as a covariable in the analysis. All analyses were performed using IBM SPSS Version 25.0 for Windows (IBM Corp., Armonk, NY, USA) with a significance level of $p < 0.05$.

3. Results

3.1. Behavioral experiments

3.1.1. Motor assessment

Regarding the motor test battery, no significant differences were observed between groups for most trials (Supplementary Table 1). Differences were observed in grip strength ($F_{(5,67)} = 5.20$, $p = 0.00043$), with the CLZ group showing less resistance to separation from the lid of bars when pulled by the tail (Bonferroni post-hoc: $p = 0.002$ vs control group). Differences were also observed for prehensile reflex ($F_{(5,67)} = 4.39$, $p = 0.0016$) and traction capacity ($F_{(5,67)} = 3.81$, $p = 0.0042$) with the KET group being less able to remain suspended on the wire (Bonferroni post-hoc: $p = 0.025$ vs control group) and raising fewer hind limbs to reach the wire after the three trials (Bonferroni post-hoc: $p = 0.006$) (Supplementary Table 1). Animals exposed to KET and treated with JNJ or CLZ recovered their prehensile reflex and traction capacity, reaching the same levels as the control group.

In the rotarod, the two-way RM ANOVA revealed significant differences in latency to fall between the three different speeds ($F_{(2,70)} = 533.06$; $p = 5.1 \times 10^{-50}$) but no effect of drug treatment, as the animals treated with different drugs showed similar latencies to fall from the rotarod at the different constant speeds (Fig. 1A). During the acceleration cycle, groups presented significant differences ($H_{(5,67)} = 14.19$, $p = 0.014$). In particular, mice treated with JNJ showed a lower latency to fall (post-hoc comparison: $p = 0.004$ vs control group) (Fig. 1B).

All groups displayed similar general activity and anxiety levels in the open field. Mice traveled similar total distances (Fig. 2A), spending more time at the periphery of the apparatus (236.2 ± 5.0 s) than the center (63.8 ± 5.0 s) ($F_{(1,144)} = 622.14$; $p = 3.4 \times 10^{-52}$) without differences between treatment groups (Fig. 2B). Although differences appeared in the average speed ($F_{(5,67)} = 3.21$, $p = 0.012$) and rearing activity ($F_{(5,67)} = 2.41$, $p = 0.046$), they were no longer significant after Bonferroni correction (Fig. 2C, D).

3.1.2. Spatial working memory Y-maze test

The results of the two-way ANOVA showed that animals spent almost three times longer in the novel arm (72.0 ± 2.4 s) than in the familiar arm (26.8 ± 1.5 s) during the 3 min trial after initial exploration ($F_{(1,144)} = 256.76$; $p = 6.1 \times 10^{-33}$), but no differences were found between treatment groups (Fig. 3A). Animals under different drug treatments also showed similar spontaneous alternations of around 80% (Fig. 3B).

3.1.3. Novel object recognition (NOR) memory test

The results of the two-way ANOVA showed that animals spent twice as long exploring the novel object compared to the familiar object (16.3 ± 1.5 s vs 8.3 ± 0.6 s, respectively) ($F_{(1,144)} = 25.16$; $p = 2.0 \times 10^{-6}$) and, although the differences did not reach statistical significance, a certain effect of drug treatment was also observed ($F_{(5,140)} = 1.97$; $p = 0.08$). While, as expected, controls and mice treated with JNJ or CLZ spent significantly more time exploring the novel object ($t = -2.31$, $p = 0.042$; $t = -4.69$, $p = 0.00066$, $t = -4.80$, $p = 0.00043$ respectively), mice exposed to KET did not show a preference for the novel object; this preference was recovered by treatment with JNJ ($t = -2.97$, $p = 0.013$) but not with CLZ (Fig. 4).

3.1.4. Three-chamber sociability and social novelty test

In the three-chamber test, the results of the two-way ANOVA showed that animals demonstrated a significant preference for spending time sniffing a familiar littermate rather than a toy mouse (62.0 ± 2.6 s vs 34.6 ± 1.5 s; $F_{(1,144)} = 83.73$; $p = 8.3 \times 10^{-16}$). Drug treatment did not show any effect, as similar preferences were observed in all treatment groups (controls: $t = 4.06$, $p = 0.002$; JNJ: $t = 3.18$, $p = 0.009$; CLZ: $t = 5.45$, $p = 0.0001$; KET: $t = 4.62$, $p = 0.0007$; KET+JNJ: $t = 5.18$, $p = 0.0003$; KET+CLZ: $t = 5.89$, $p = 0.0001$; Fig. 5A). When the choice was between the familiar littermate and an unfamiliar conspecific mouse (Fig. 5B), the results of the two-way ANOVA also showed that mice demonstrated a significant preference for spending time sniffing the novel animal (61.7 ± 3.5 s vs 35.9 ± 1.6 s; $F_{(1,144)} = 51.0$; $p = 5.3 \times 10^{-11}$), but this time a significant effect of drug treatment was observed ($F_{(1,140)} = 2.88$; $p = 0.017$). Interestingly, the KET group did not demonstrate a preference for social novelty, while animals exposed to KET and treated with JNJ recovered this preference ($t = -4.71$, $p = 0.0006$), as did controls ($t = -3.89$, $p = 0.002$) and mice treated with JNJ ($t = -5.75$, $p = 0.00013$) or CLZ ($t = -3.79$, $p = 0.0025$). Mice exposed to KET and treated with CLZ did not show this recovery.

3.1.5. Five-trial social memory test

Mice were evaluated for their social recognition abilities in the five-trial test (Fig. 6). The two-way RM ANOVA revealed significant differences between trials ($F_{(4,360)} = 32.81$; $p = 1.8 \times 10^{-17}$) and drug treatment ($F_{(5,359)} = 4.94$; $p = 0.00066$). After Bonferroni post-hoc correction, mice treated with CLZ or KET+CLZ spent significantly less time investigating the mouse than controls ($p = 0.0023$ and $p = 0.0075$ respectively).

Animals from the same treatment group spent similar or shorter times in the four first trials when exposed to the same mouse. Even the

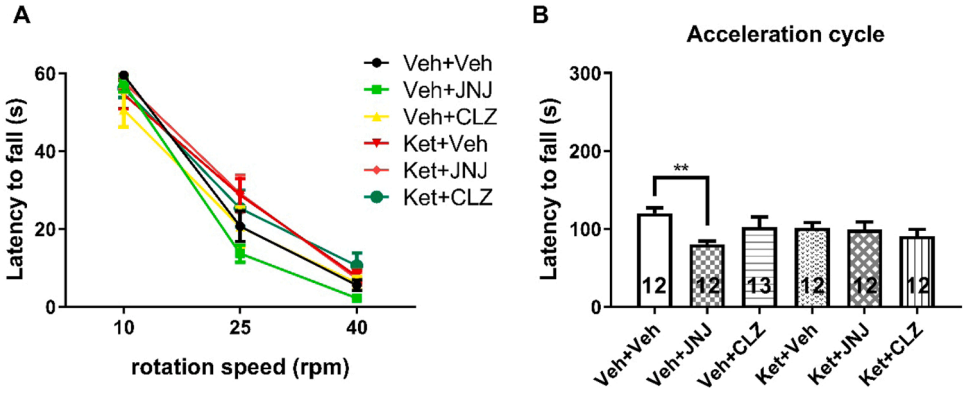

Fig. 1. Motor coordination in the rotarod test. Mean values ± SEM of latency to fall from the rotarod at different constant speeds (A) and during the acceleration cycle (B). The number of animals in each experimental group is displayed within the bars. * *$p < 0.01$. Abbreviations: CLZ = clozapine; JNJ = JNJ-46356479; Ket = ketamine; SEM = standard error of the mean; Veh = vehicle.

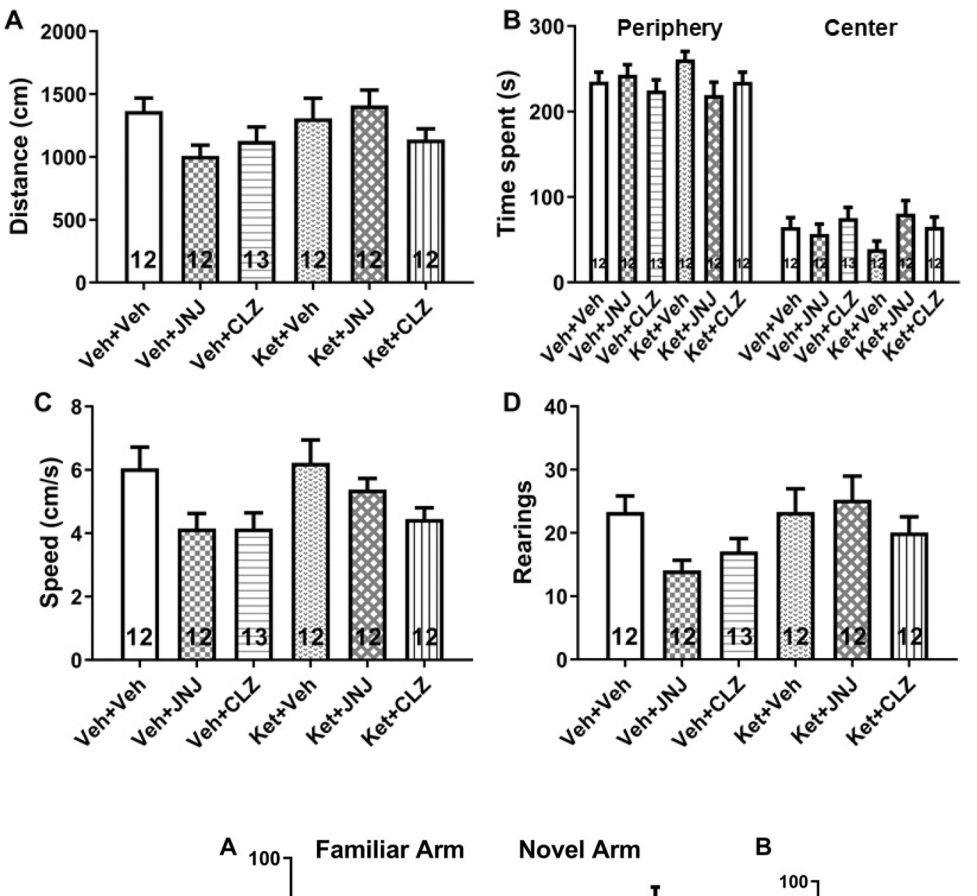

Fig. 2. Activity in the open field test. Figure shows total distance traveled (A), time spent at the periphery and center of the apparatus (B), walking speed (C), and number of rearings (vertical activity) (D) performed during the test. Results are shown as the means ± SEM of the total activity. The number of animals in each experimental group is displayed within the bars. Abbreviations: CLZ = clozapine; JNJ = JNJ-46356479; Ket = ketamine; SEM = standard error of the mean; Veh = vehicle.

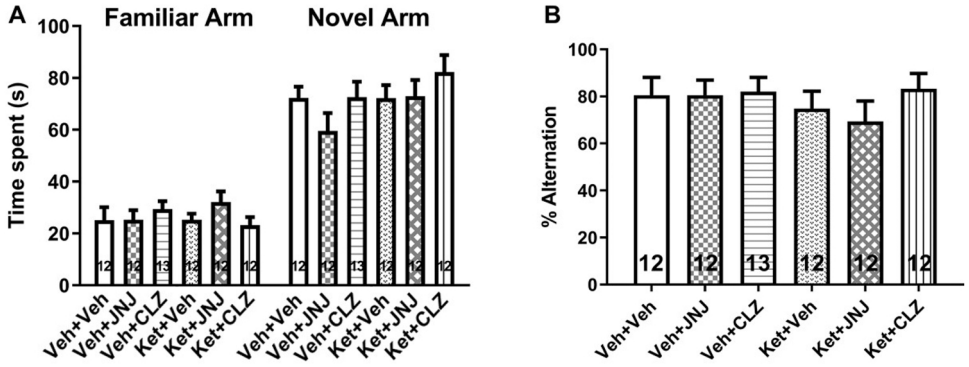

Fig. 3. Spatial working memory in the Y-maze test. Figure shows time spent in the familiar or novel arms in the Y-maze for the working memory task (A) and the spontaneous alternation test with 3 trials (B). Results are shown as the means ± SEM. The number of animals in each experimental group is displayed within the bars. Abbreviations: CLZ = clozapine; JNJ = JNJ-46356479; Ket = ketamine; SEM = standard error of the mean; Veh = vehicle.

JNJ group showed a proportional reduction ($r^2 = 0.99$) in time exploring the animal (18.0 ± 1.2 s, 13.8 ± 1.6 s, 10.2 ± 1.3 s and 7.5 ± 1.3 s in the trials 1, 2, 3, and 4 respectively).

After four trials of exposure to the same mouse, the control group and the animals treated with JNJ or CLZ showed increased interaction times when presented with the novel mouse in the fifth trial ($t = -3.65$, $p = 0.001$; $t = -5.86$, $p = 7 \times 10^{-7}$; $t = -2.47$, $p = 0.023$ respectively). This expected dishabituation was not shown in the KET group. Interestingly, when mice were exposed to KET and treated with JNJ or CLZ, they recovered an interest in the novel animal ($t = -2.66$, $p = 0.014$; $t = -2.48$, $p = 0.021$ respectively).

3.2. Immunochemistry

3.2.1. Parvalbumin and c-Fos immunoreactivity in the medial prefrontal cortex

The number of PV+ cells and mean gray values of c-Fos in mPFC are shown in Fig. 7. Significant differences in PV immunoreactivity were detected between the main four treatment groups ($F_{(3,21)} = 4.32$, $p = 0.017$; Fig. 7A). While a reduction of almost 25% in PV+ interneuron density was observed in the KET group, mice exposed to KET and treated with CLZ recovered control PV values, the difference reaching significance after Bonferroni post-hoc comparisons ($p = 0.036$, KET vs KET+CLZ). We also quantified c-Fos immunoreactivity in the mPFC as a marker of neural activity. Although mice exposed to KET also seemed to show a reduction in c-Fos expression, the differences between the groups were not significant (Fig. 7B). Representative confocal images of slices from the mPFC stained for DAPI, PV and c-Fos are shown in Fig. 7C.

3.2.2. Parvalbumin and c-Fos immunoreactivity in the hippocampus

The number of PV+ cells and mean gray values of c-Fos in the four sections of the HPC DG, CA1, CA2 and CA3 are shown in Fig. 8. No significant differences in the number of PV+ cells were detected between groups. However, significant differences in c-Fos immunoreactivity were observed in all four HPC sections (GD: $F_{(3,21)} = 3.68$,

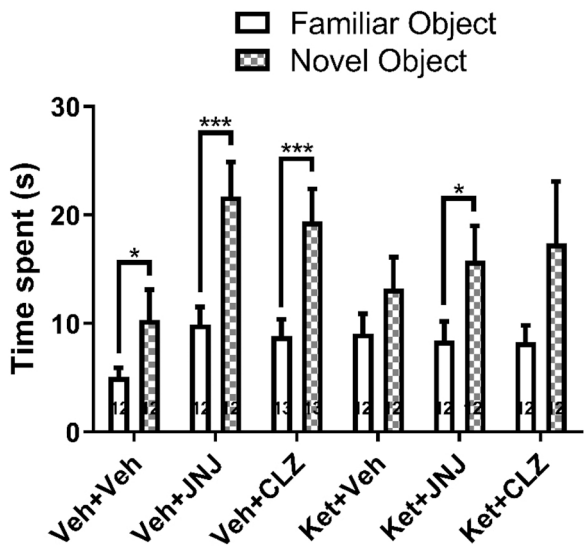

Fig. 4. Novel object recognition (NOR) memory test. Results are shown as the means ± SEM of the time mice spent exploring the familiar and novel objects. The number of animals in each experimental group is displayed within the bars. *p < 0.05, * **p < 0.001. Abbreviations: CLZ = clozapine; JNJ = JNJ-46356479; Ket = ketamine; SEM = standard error of the mean; Veh = vehicle.

p = 0.029; CA1: $F_{(3,21)}$ = 4.18, p = 0.019; CA2: $F_{(3,21)}$ = 6.44, p = 0.003; CA3: $F_{(3,21)}$ = 4.00, p = 0.022; Fig. 8B). The same distribution was observed in the four HPC sections, with mice exposed to KET showing a reduction of up to 40% in c-Fos immunoreactivity that was partially or completely normalized after CLZ or JNJ treatment respectively. Bonferroni post-hoc correction showed significant differences between the KET and KET+JNJ groups (GD: p = 0.027; CA1: p = 0.016; CA2: p = 0.002; CA3: p = 0.016; Fig. 8B). Representative hippocampal mosaic images stained for DAPI, PV and c-Fos are shown in Fig. 8C.

4. Discussion

In this study, we explored the effect of pharmacological treatment with JNJ or CLZ during adolescence in reversing the behavioral and neuropathological deficits induced in a postnatal KET mouse model of SZ. The results showed that both cognitive and social impairments and the alterations in neuronal markers experienced by the adult animals can be mitigated by JNJ treatment, even more so than CLZ treatment, administered during early stages of development.

Animal exposure to NMDA receptor antagonists, such as KET, PCP, and MK-801 has been used to induce different models of SZ in rodents [13,29]. Exposure of neonatal mice to NMDA receptor antagonism can induce differential cognitive impairments during adulthood depending on the temporal window of drug exposure. Disturbing brain development during the early postnatal period (PND 7–11) seems to affect the function of both the PFC and HPC during adulthood, resulting in altered cognitive and social performance across a range of behavioral tasks [11]. The postnatal mouse model used in the present study exposes animals to KET on PND 7, 9, and 11 [12,13] so as to transiently disrupt the NMDA receptor function, which shows persistent changes in parvalbumin GABAergic interneurons and induces SZ-like behavioral symptoms in adulthood, as we have recently reported [14]. In the present study, animals exposed to postnatal KET also showed impaired memory and social performance in the NOR, three-chamber social novelty, and the five-trial social memory tests, during adulthood. Regarding the spatial working memory assessed with the Y-maze, this time no significant differences were observed. However, in our previous study [14], mice exposed postnatally to KET spent more time in the familiar arm and showed impaired spontaneous alternation, indicating that spatial working memory may also be impaired in this animal model,

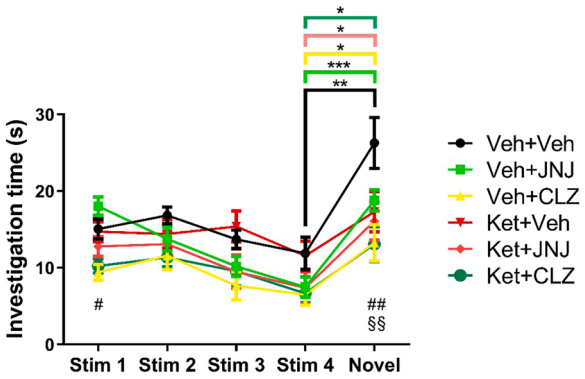

Fig. 6. Social memory in the five-trial test. After four exposures to the same stimulus mouse, experimental mice were exposed to a novel stimulus mouse. Results are shown as means ± SEM. #p < 0.05 Veh+CLZ vs Veh+Veh; ##p < 0.01 Veh+CLZ vs Veh+Veh; §§p < 0.01 KET+CLZ vs Veh+Veh; *p < 0.05; * *p < 0.01; * **p < 0.001. Abbreviations: CLZ = clozapine; JNJ = JNJ-46356479; Ket = ketamine; SEM = standard error of the mean; Veh = vehicle.

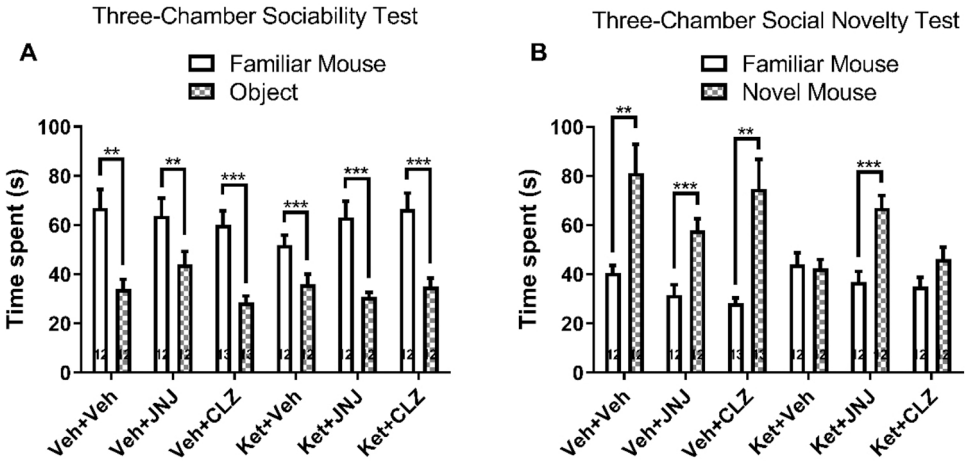

Fig. 5. Social behavior in the three-chamber test. Time was measured for sociability with (A) and preference for a novel mouse (B). Results are shown as means ± SEM. The number of animals in each experimental group is shown within the bars. * *p < 0.01, * **p < 0.001. Abbreviations: CLZ = clozapine; JNJ = JNJ-46356479; Ket = ketamine; SEM = standard error of the mean; Veh = vehicle.

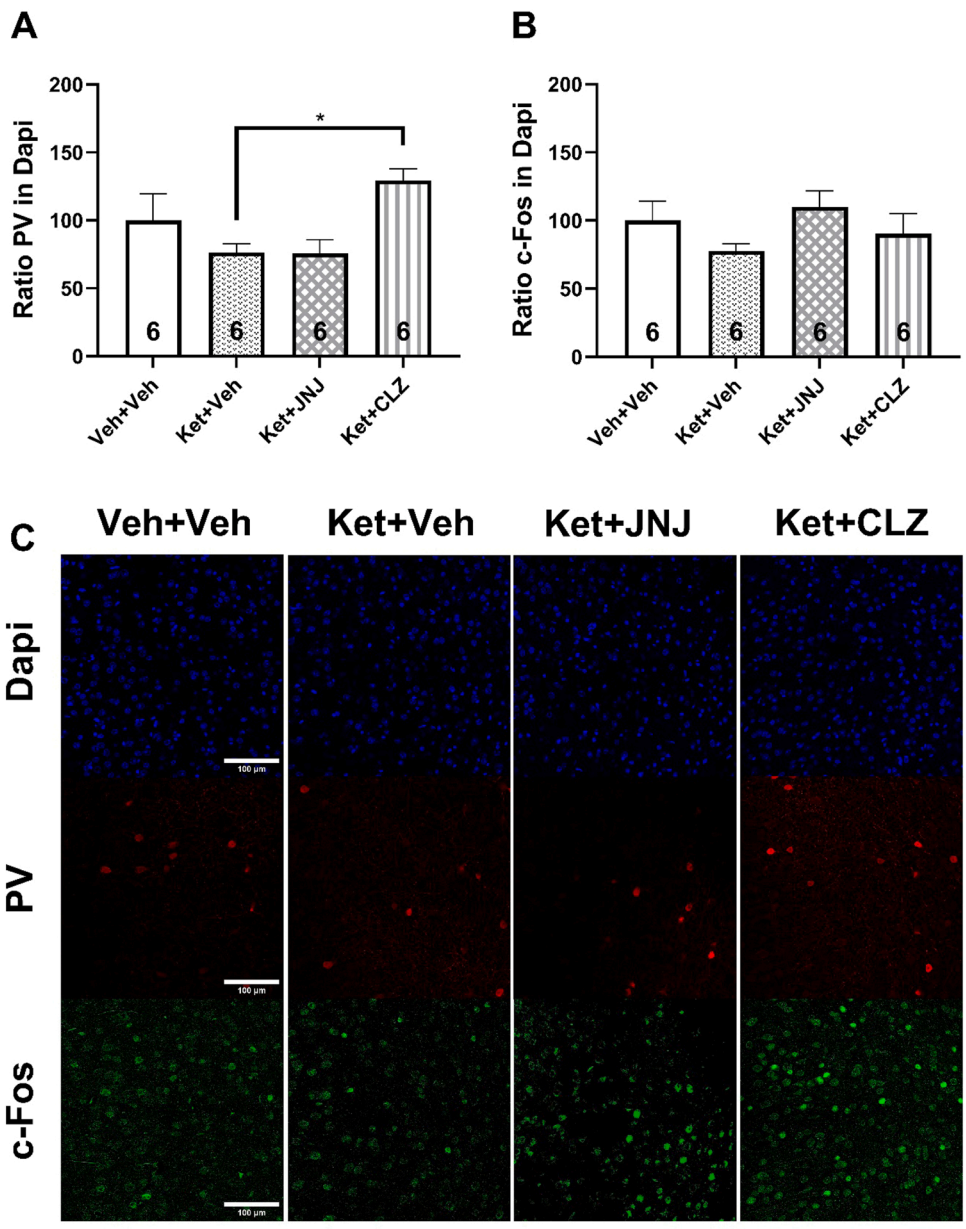

Fig. 7. Quantification of parvalbumin (PV) positive neurons (A) and mean gray values for c-Fos (B) in the medial prefrontal cortex (mPFC). PV and c-Fos values were corrected by Dapi levels. Results are expressed as percentages of the control group. Bars represent means ± SEM, with the number of each condition displayed within the bars. *$p < 0.05$. (C) Representative confocal images slices from the mPFC stained for Dapi (blue), PV (red) and c-Fos (green) from all groups. Scale bars are 100 µm. Abbreviations: CLZ = clozapine; JNJ = JNJ-46356479; Ket = ketamine; SEM = standard error of the mean; Veh = vehicle.

in accordance with other reports [30,31]. Memory and social deficits observed in these mice may indicate impaired functioning of important brain regions involved, such as PFC and HPC. Interestingly, significant differences between treatment groups were observed in PV immunoreactivity in the PFC, with mice exposed to KET showing reduced numbers of PV+ cells, in accordance with previous results in the literature showing PV reductions in this and other rodent models of SZ [12–14]. Moreover, significant differences between treatment groups were also observed in c-Fos immunoreactivity across all four sections of the HPC, with animals exposed to KET showing reductions in this neuronal activity marker of up to 40%. Although conflicting results may be found in the literature showing c-Fos increases in murine models exposed to NMDA antagonists [14,32,33], the present results are in line with those obtained by Hauser et al. [34] in a KET mouse model which also showed reduced c-Fos expression in the HPC, together with reductions in PV expression in the PFC and impairments in novelty discrimination. The expression of c-Fos is involved in neuronal activity, memory and learning-related plasticity in the HPC and other neuronal regions associated with these processes [35,36], with c-Fos knockout resulting in deficits in HPC-dependent spatial and associative learning tasks in adult mice [37]. The observation of all these behavioral and neuronal impairments in the postnatal KET mouse model increases the body of evidence favoring this animal model for preclinical investigation in SZ.

The main objective of this study was to evaluate whether pharmacological treatment during a critical period of neurodevelopment, such as adolescence, could reverse the impairments induced in the postnatal KET mouse model in adult animals. Notably, JNJ treatment mitigated these deficits, with treated mice recovering the preference for exploring the novel object in the NOR test, for sniffing a familiar littermate rather than a toy in the three-chamber social novelty test, and for exploring an unfamiliar conspecific mouse rather than a familiar littermate in the five-trial test. Moreover, JNJ treatment also normalized c-Fos expression in GD, CA1, CA2 and CA3 sections of the HPC, as JNJ-treated animals showed significant increases in this neuronal marker compared to mice exposed to postnatal KET. Interestingly, it has been reported that antipsychotic treatment with olanzapine also increased c-Fos expression in different brain structures [35]. These results are consistent with previous results reported by group showing that JNJ treatment during

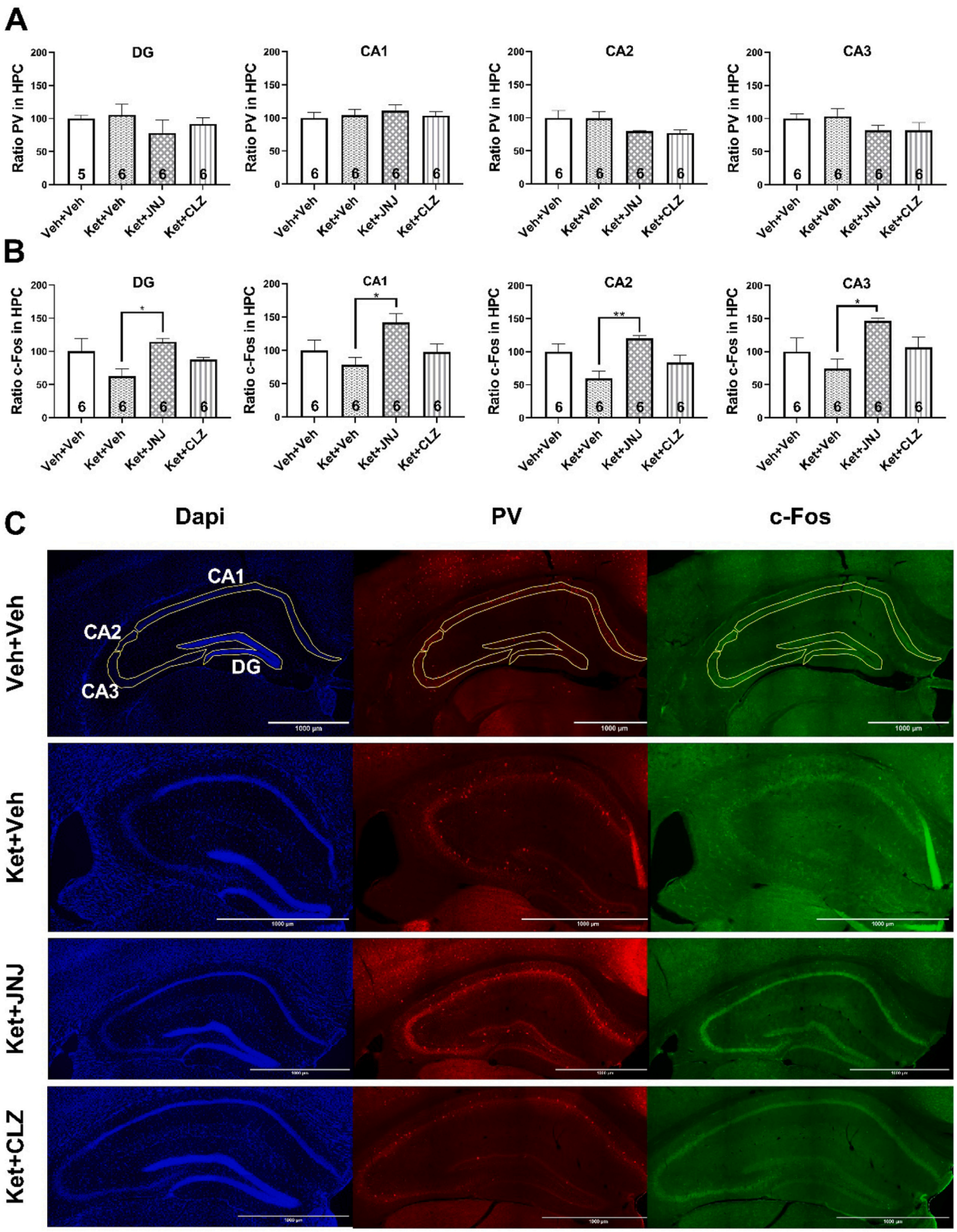

Fig. 8. Quantification of parvalbumin (PV) positive neurons (A) and mean gray values for c-Fos (B) in the dentate gyrus (DG), CA1, CA2 and CA3 subfields of the hippocampus (HPC). PV and c-Fos values were corrected by analyzed area in μm^2. Results are expressed as percentages of normalized PV and c-Fos measured in Veh + Veh group. Bars represent means ± SEM, with the number of each condition displayed within the bars. *$p < 0.05$; * *$p < 0.01$. (C) Distribution pattern of PV and c-Fos positive neurons in the mouse HPC. Representative hippocampal mosaic images acquired with LAS AF Power mosaic software from Leica, stained for Dapi (blue), PV (red) and c-Fos (green) from all groups. Scale bars are 1000 µm. Abbreviations: CLZ = clozapine; JNJ = JNJ-46356479; Ket = ketamine; SEM = standard error of the mean; Veh = vehicle.

adulthood can also reverse SZ-like behaviors and neuropathological impairments [14], thereby reinforcing the notion that mGluR2 PAMs may be effective in the treatment of cognitive deficits and negative symptoms in patients with SZ.

Extensive evidence has identified all mGluRs as viable therapeutic targets for SZ, since their agonists and subtype-selective PAMs show efficacy in dopaminergic, serotonergic, and glutamatergic models of positive, negative and cognitive symptoms [38]. Regarding mGluR2, there are several PAMs in development [39] that have produced promising preclinical findings, and recently some of them have even been tested in humans. Although the results of clinical trials were negative, this may have been due to patient selection issues. Future trials with patients selected by disease stage and prior antipsychotic usage may yield different outcomes [16,38]. The use of these molecules in critical periods of the disease, such as the prodrome or transition to psychosis, rather than when the disease is already established, may increase their effectiveness in preventing or slowing down the progression of clinical and cognitive deterioration [22]. Our present results support this idea, showing that JNJ treatment during adolescence reversed memory, social, and neuropathological deficits which otherwise occur in adulthood, perhaps by limiting the neurotoxic glutamate storm at this critical juncture [23]. An excess of glutamate would trigger neurotoxicity and synaptic apoptosis-pruning by local activation of caspase-3, leading to loss of dendritic spine density in critical brain areas [8,9]. Our group previously demonstrated the importance of apoptosis in antipsychotic-naïve patients with a first-psychotic episode [40–42]. JNJ treatment during early stages may reduce the excessive apoptosis in this critical neurodevelopmental period, thus helping deleterious synaptic pruning and therefore mitigating SZ-related symptoms. In fact, we have recently observed that JNJ-46356479 seems to attenuate the apoptosis in vitro, particularly the caspase-3 activation induced by dopamine and glutamate [17].

Although, this is the first time that pharmacological treatment in adolescence has been assessed in a postnatal KET mouse model, juvenile treatment with a mGluR2/3 agonist has previously been shown to restore dendritic spine loss and learning and memory deficits generated in a neurodevelopmental animal model of SZ [43]. Interestingly, early treatment with a mGluR5 PAM drug in a neonatal NMDAR antagonist animal model also reversed the neurodevelopmental progression of certain SZ-like behaviors, an effect that was not observed when the drug was administered in adults [44,45]. Moreover, other experimental treatments administered during development in animal models of SZ have reported beneficial outcomes, including improved cognitive function [24]. The presentation of psychosis does not represent the onset of the disorder, but is rather a prominent consequence of developmental alterations that could be prevented by early intervention before they become irreversible [46]. It is clear that core features of SZ, such as disruption of PV GABAergic interneurons [47], are already present in individuals at high risk for psychosis. Early pharmacological treatments that can effectively reverse these initial alterations could be the key to preventing or at least reducing the effects of the disorder.

Motor assessment was also performed to evaluate the impact of drug treatment. Exceptionally, animals treated with JNJ showed lower latency to fall in the rotarod. Notably, however, this effect was not observed when JNJ treatment was given in adult animals [14]. Nor did JNJ induced any other impairments in motor function, coordination, general activity, anxiety, memory, or sociability, which is consistent with its observed lack of neurotoxicity in neuroblastoma cell culture [17]. Nevertheless, further studies are required to assess the safety of this mGluR2 PAM.

The present study also carried out the first evaluation of the efficacy of early CLZ treatment in the postnatal KET mouse model. CLZ reverted certain social deficits, particularly those observed in the five-trial test, but had no effect on the memory deficits observed in these animals. This is consistent with the results from a dual-hit SZ model in which CLZ treatment normalized the resulting social impairments but not the cognitive deficits [48]. Damage in the PFC is frequently associated with behavioral and social impairments. Given that in our study early CLZ treatment significantly increased the number of PV+ cells in the PFC in mice exposed to postnatal KET, this may be involved, at least in part, in the improvement in social behavior also showed by animals under CLZ treatment. In patients, although CLZ has no clear beneficial effects on cognitive performance [4], it is the only medication indicated for refractory SZ due to its superior efficacy compared with all antipsychotic agents, resulting in a greater improvement in negative symptoms [5]. However, CLZ treatment has also been shown to improve PCP-induced emotional memory impairments in mice, suggesting a capacity to modulate glutamatergic memory dysfunction [49]. The putative effect of CLZ on both negative and cognitive symptoms of SZ could be due to its slight activation of the NMDA receptor. Interestingly, combining CLZ and a powerful neuroprotectant that specifically binds NMDA receptors, has been shown to improve MK801-induced SZ behavior, including cognitive impairment [50].

This study has certain limitations that should be noted. First, we used a very specific pharmacological mouse model based on just one pathological hypothesis of SZ. As such, the model cannot be assumed to mimic such a complex disease with complete accuracy. Second, it is unclear whether the doses tested would represent the doses used therapeutically in humans. Despite these concerns, our results are interesting and call for more research at neuropathological level to clarify the biological effects of these pharmacological treatments.

Our results support the efficacy of JNJ treatment for counteracting the deleterious cognitive and social effects, as well as the neuropathological deficits, induced by postnatal NMDA receptor blockade not only by treating adult animals, as we showed in a previous work [14], but also by treating animals during critical neurodevelopmental periods. The results are particularly relevant with regard to the possible prevention or deceleration of the deteriorating course of SZ. Adolescent JNJ treatment also produced better outcomes than CLZ in our animal model, indicating that the glutamatergic pathway may be an important therapeutic target for both the negative symptoms and cognitive deficits in SZ.

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CRediT authorship contribution statement

Albert Martínez-Pinteño: Methodology, Formal analysis, Writing – review & editing. **Natalia Rodríguez**: Methodology, Formal analysis, Writing – review & editing. **David Olivares:** Methodology, Formal analysis, Writing – review & editing**. Santiago, Madero**: Investigation. **Marta Gómez**: Investigation. **Llucia Prohens**: Data curation, Methodology. **Clemente García-Rizo**: Investigation. **Sergi Mas**: Supervision. **Constanza Moren**: Data curation, Writing – review & editing. **Eduard Parellada**: Conceptualization, Funding acquisition, Writing – review & editing. **Patricia Gassó**: Conceptualization, Funding acquisition, Writing – original draft preparation.

Conflict of interest

The authors declared no potential conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.114079.

References

[1] S.R. Marder, T.D. Cannon, Schizophrenia, N. Engl. J. Med. 381 (2019) 1753–1761, https://doi.org/10.1056/NEJMra1808803.
[2] M.J. Owen, A. Sawa, P.B. Mortensen, Schizophrenia, Lancet 388 (2016) 86–97, https://doi.org/10.1016/S0140-6736(15)01121-6.
[3] D.C. Goff, The pharmacologic treatment of Schizophrenia-2021, JAMA 325 (2021) 175–176, https://doi.org/10.1001/jama.2020.19048.
[4] R.S. Keefe, R.M. Bilder, S.M. Davis, P.D. Harvey, B.W. Palmer, J.M. Gold, H. Y. Meltzer, M.F. Green, G. Capuano, T.S. Stroup, J.P. McEvoy, M.S. Swartz, R. A. Rosenheck, D.O. Perkins, C.E. Davis, J.K. Hsiao, J.A. Lieberman, Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE trial, Arch. Gen. Psychiatry 64 (2007) 633–647, https://doi.org/10.1001/archpsyc.64.6.633.
[5] Nucifora F.C.Jr, Mihaljevic M., Lee B.J., Sawa A. Clozapine as a model for antipsychotic development neurotherapeutics. 2017; 14: 750–761. doi: 10.1007/s13311–017-0552–9.
[6] D.A. Lewis, B. Moghaddam, Cognitive dysfunction in schizophrenia: convergence of gamma-aminobutyric acid and glutamate alterations, Arch. Neurol. 63 (2006) 1372–1376, https://doi.org/10.1001/archneur.63.10.1372.
[7] Y. Uno, J.T. Coyle, Glutamate hypothesis in schizophrenia, Psychiatry Clin. Neurosci. 73 (2019) 204–215, https://doi.org/10.1111/pcn.12823.
[8] E. Parellada, P. Gassó, Glutamate and microglia activation as a driver of dendritic apoptosis: a core pathophysiological mechanism to understand schizophrenia, Transl. Psychiatry 11 (2021) 271, https://doi.org/10.1038/s41398-021-01385-9.
[9] C.M. Yuede, D.F. Wozniak, C.E. Creeley, G.T. Taylor, J.W. Olney, N.B. Farber, Behavioral consequences of NMDA antagonist-induced neuroapoptosis in the infant mouse brain, PLoS One 5 (2010), e11374, https://doi.org/10.1371/journal.pone.0011374.
[10] M. Białoń, A. Wąsik, Advantages and limitations of animal schizophrenia models, Int. J. Mol. Sci. 23 (2022) 5968, https://doi.org/10.3390/ijms23115968.
[11] M.E. Plataki, K. Diskos, C. Sougklakos, M. Velissariou, A. Georgilis, V. Stavroulaki, K. Sidiropoulou, Effect of neonatal treatment with the NMDA receptor antagonist, MK-801, during different temporal windows of postnatal period in adult prefrontal cortical and hippocampal function, Front Behav. Neurosci. 15 (2021), 689193, https://doi.org/10.3389/fnbeh.2021.689193.
[12] V. Jeevakumar, C. Driskill, A. Paine, M. Sobhanian, H. Vakil, B. Morris, J. Ramos, S. Kroener, Ketamine administration during the second postnatal week induces enduring schizophrenia-like behavioral symptoms and reduces parvalbumin expression in the medial prefrontal cortex of adult mice, Behav. Brain Res 282 (2015) 165–175, https://doi.org/10.1016/j.bbr.2015.01.010.
[13] S.B. Powell, T.J. Sejnowski, M.M. Behrens, Behavioral and neurochemical consequences of cortical oxidative stress on parvalbumin-interneuron maturation in rodent models of schizophrenia, Neuropharmacology 62 (2012) 1322–1331, https://doi.org/10.1016/j.neuropharm.2011.01.049.
[14] A. Martínez-Pinteño, S. García-Cerro, S. Mas, T. Torres, D. Boloc, N. Rodríguez, A. Lafuente, P. Gassó, J.A. Arnaiz, E. Parellada, The positive allosteric modulator of the mGlu2 receptor JNJ-46356479 partially improves neuropathological deficits and schizophrenia-like behaviors in a postnatal ketamine mice model, J. Psychiatr. Res. 126 (2020) 8–18, https://doi.org/10.1016/j.jpsychires.2020.04.005.
[15] J.M. Cid, G. Tresadern, J.A. Vega, A.I. de Lucas, A. Del Cerro, E. Matesanz, M. L. Linares, A. García, L. Iturrino, L. Pérez-Benito, G.J. Macdonald, D. Oehlrich, H. Lavreysen, L. Peeters, M. Ceusters, A. Ahnaou, W. Drinkenburg, C. Mackie, M. Somers, A.A. Trabanco, Discovery of 8-trifluoromethyl-3-cyclopropylmethyl-7-[(4-(2,4-difluorophenyl)-1-piperazinyl)methyl]-1,2,4-triazolo[4,3-a]pyridine (JNJ-46356479), a selective and orally bioavailable mGlu2 receptor positive allosteric modulator (PAM, J. Med Chem. 59 (2016) 8495–8507, https://doi.org/10.1021/acs.jmedchem.6b00913.
[16] M.L. Li, X.Q. Hu, F. Li, W.J. Gao, Perspectives on the mGluR2/3 agonists as a therapeutic target for schizophrenia: still promising or a dead end, Prog. Neuropsychopharmacol. Biol. Psychiatry 60 (2015) 66–76, https://doi.org/10.1016/j.pnpbp.2015.02.012.
[17] Gassó P., Martínez-Pinteño A., Rodríguez N., Madero S., Gómez M., Segura A.G., García-Rizo C., Morén C., Mas S., Parellada E. Neuroprotective effect of the positive allosteric modulator of the mGluR2 JNJ-46356479 in human neuroblastoma cell cultures. Int J Mol Sci 2022 (under review).
[18] O. Gruber, A. Chadha Santuccione, H. Aach, Magnetic resonance imaging in studying schizophrenia, negative symptoms, and the glutamate system, Front Psychiatr. 5 (2014) 32, https://doi.org/10.3389/fpsyt.2014.00032.
[19] J.M. Cid, G. Tresadern, G. Duvey, R. Lütjens, T. Finn, J.P. Rocher, S. Poli, J.A. Vega, A.I. de Lucas, E. Matesanz, M.L. Linares, J.I. Andrés, J. Alcazar, J.M. Alonso, G. J. Macdonald, D. Oehlrich, H. Lavreysen, A. Ahnaou, W. Drinkenburg, C. Mackie, S. Pype, D. Gallacher, A.A. Trabanco, Discovery of 1-butyl-3-chloro-4-(4-phenyl-1-piperidinyl)-(1H)-pyridone (JNJ-40411813): a novel positive allosteric modulator of the metabotropic glutamate 2 receptor, J. Med Chem. 57 (2014) 6495–6512, https://doi.org/10.1021/jm500496m.
[20] H. Lavreysen, A. Ahnaou, W. Drinkenburg, X. Langlois, C. Mackie, S. Pype, R. Lütjens, E. Le Poul, A.A. Trabanco, J.M. Nuñez, Pharmacological and pharmacokinetic properties of JNJ-40411813, a positive allosteric modulator of the mGlu2 receptor, Pharm. Res. Perspect. 3 (2015), e00096, https://doi.org/10.1002/prp2.96.
[21] H. Lavreysen, X. Langlois, L.V. Donck, J.M. Nuñez, S. Pype, R. Lütjens, A. Megens, Preclinical evaluation of the antipsychotic potential of the mGlu2-positive allosteric modulator JNJ-40411813, Pharm. Res. Perspect. 3 (2015), e00097, https://doi.org/10.1002/prp2.97.
[22] M.J. Millan, A. Andrieux, G. Bartzokis, K. Cadenhead, P. Dazzan, P. Fusar-Poli, J. Gallinat, J. Giedd, D.R. Grayson, M. Heinrichs, R. Kahn, M.O. Krebs, M. Leboyer, D. Lewis, O. Marin, P. Marin, A. Meyer-Lindenberg, P. McGorry, P. McGuire, M. J. Owen, P. Patterson, A. Sawa, M. Spedding, P. Uhlhaas, F. Vaccarino, C. Wahlestedt, D. Weinberger, Altering the course of schizophrenia: progress and perspectives, Nat. Rev. Drug Disco 15 (2016) 485–515, https://doi.org/10.1038/nrd.2016.
[23] S.A. Schobel, N.H. Chaudhury, U.A. Khan, B. Paniagua, M.A. Styner, I. Asllani, B. P. Inbar, C.M. Corcoran, J.A. Lieberman, H. Moore, S.A. Small, Imaging patients with psychosis and a mouse model establishes a spreading pattern of hippocampal dysfunction and implicates glutamate as a driver, Neuron 78 (2013) 81–93, https://doi.org/10.1016/j.neuron.2013.02.011.
[24] O. Marín, Developmental timing and critical windows for the treatment of psychiatric disorders, Nat. Med. 22 (2016) 1229–1238, https://doi.org/10.1038/nm.4225.
[25] S. Dedeurwaerdere, C. Wintmolders, R. Straetemans, D. Pemberton, X. Langlois, Memantine-induced brain activation as a model for the rapid screening of potential novel antipsychotic compounds: exemplified by activity of an mGlu2/3 receptor agonist, Psychopharmacology 214 (2011) 505–514, https://doi.org/10.1007/s00213-010-2052-z.
[26] C.S. Metcalf, B.D. Klein, M.D. Smith, M. Ceusters, H. Lavreysen, S. Pype, N. Van Osselaer, R. Twyman, H.S. White, Potent and selective pharmacodynamic synergy between the metabotropic glutamate receptor subtype 2-positive allosteric modulator JNJ-46356479 and levetiracetam in the mouse 6-Hz (44-mA) model, Epilepsia 59 (2018) 724–735, https://doi.org/10.1111/epi.14005.
[27] T. Korotkova, E.C. Fuchs, A. Ponomarenko, J. von Engelhardt, H. Monyer, NMDA receptor ablation on parvalbumin-positive inter, Neurons Impairs Hippocampal Synchrony Spat. Represent. Work. Mem. Neuron 68 (2010) 557–569, https://doi.org/10.1016/j.neuron.2010.09.017.
[28] Franklin K.B., Paxinos G. Paxinos and Franklin's the mouse brain in stereotaxic coordinates. Academic Press 2013, fourth ed. An imprint of Elsevier, Amsterdam.
[29] D. Cadinu, B. Grayson, G. Podda, M.K. Harte, N. Doostdar, J.C. Neill, NMDA receptor antagonist rodent models for cognition in schizophrenia and identification of novel drug treatments, an update, Neuropharmacology 142 (2018) 41–62, https://doi.org/10.1016/j.neuropharm.2017.11.045.
[30] B. Ben-Azu, A.O. Aderibigbe, A.O. Eneni, A.M. Ajayi, S. Umukoro, E.O. Iwalewa, Morin attenuates neurochemical changes and increased oxidative/nitrergic stress in brains of mice exposed to ketamine: prevention and reversal of schizophrenia-like symptoms, Neurochem. Res. 43 (9) (2018) 1745–1755, https://doi.org/10.1007/s11064-018-2590-z.
[31] Y. Hou, H. Zhang, G. Xie, X. Cao, Y. Zhao, Y. Liu, Z. Mao, J. Yang, C. Wu, Neuronal injury, but not microglia activation, is associated with ketamine-induced experimental schizophrenic model in mice, Progr. Neuropsychopharmacol. Biol. Psychiatry 45 (2013) 107–116, https://doi.org/10.1016/j.pnpbp.2013.04.006.
[32] A. Castañé, N. Santana, F. Artigas, PCP-based mice models of schizophrenia: differential behavioral, neurochemical and cellular effects of acute and subchronic treatments, Psychopharmacology 232 (21–22) (2015) 4085–4097, https://doi.org/10.1007/s00213-015-3946-6.
[33] M.E. Hervig, M.S. Thomsen, I. Kalló, J.D. Mikkelsen, Acute phencyclidine administration induces c-Fos-immunoreactivity in interneurons in cortical and subcortical regions, Neuroscience 334 (2016) 13–25, https://doi.org/10.1016/j.neuroscience.2016.07.028.
[34] M.J. Hauser, D. Isbrandt, J. Roeper, Disturbances of novel object exploration and recognition in a chronic ketamine mouse model of schizophrenia, Behav. Brain Res. 332 (2017) 316–326, https://doi.org/10.1016/j.bbr.2017.06.013.
[35] F.T. Gallo, C. Katche, J.F. Morici, J.H. Medina, N.V. Weisstaub, Immediate early genes, memory and psychiatric disorders: focus on c-Fos, Egr1 and Arc, Front Behav. Neurosci. 12 (2018) 79, https://doi.org/10.3389/fnbeh.2018.00079.
[36] K. Minatohara, M. Akiyoshi, H. Okuno, Role of immediate-early genes in synaptic plasticity and neuronal ensembles underlying the memory trace, Front. Mol. Neurosci. 8 (2015) 78, https://doi.org/10.3389/fnmol.2015.00078.
[37] A. Fleischmann, O. Hvalby, V. Jensen, T. Strekalova, C. Zacher, L.E. Layer, A. Kvello, M. Reschke, R. Spanagel, R. Sprengel, E.F. Wagner, P. Gass, Impaired long-term memory and NR2A-type NMDA receptor-dependent synaptic plasticity in mice lacking c-Fos in the CNS, J. Neurosci. 23 (2003) 9116–9122, https://doi.org/10.1523/JNEUROSCI.23-27-09116.2003.

[38] J. Maksymetz, S.P. Moran, P.J. Conn, Targeting metabotropic glutamate receptors for novel treatments of schizophrenia, Mol. Brain 10 (2017) 15, https://doi.org/10.1186/s13041-017-0293-z.
[39] A.A. Trabanco, J.M. Bartolomé, J.M. Cid, mGluR2 positive allosteric modulators: an updated patent review (2013-2018), Expert Opin. Ther. Pat. 29 (2019) 497–507, https://doi.org/10.1080/13543776.2019.1637421.
[40] A. Batalla, N. Bargalló, P. Gassó, O. Molina, D. Pareto, S. Mas, J.M. Roca, M. Bernardo, A. Lafuente, E. Parellada, Apoptotic markers in cultured fibroblasts correlate with brain metabolites and regional brain volume in antipsychotic-naive first-episode schizophrenia and healthy controls, Transl. Psychiatry 5 (2015), e626, https://doi.org/10.1038/tp.2015.122.
[41] P. Gassó, S. Mas, O. Molina, A. Lafuente, M. Bernardo, E. Parellada, Increased susceptibility to apoptosis in cultured fibroblasts from antipsychotic-naïve first-episode schizophrenia patients, J. Psychiatr. Res 48 (2014) 94–101, https://doi.org/10.1016/j.jpsychires.2013.09.017.
[42] P. Gassó, S. Mas, N. Rodríguez, D. Boloc, S. García-Cerro, M. Bernardo, A. Lafuente, E. Parellada, Microarray gene-expression study in fibroblast and lymphoblastoid cell lines from antipsychotic-naïve first-episode schizophrenia patients, J. Psychiatr. Res. 95 (2017) 91–101, https://doi.org/10.1016/j.jpsychires.2017.08.003.
[43] B. Xing, G. Han, M.J. Wang, M.A. Snyder, W.J. Gao, Juvenile treatment with mGluR2/3 agonist prevents schizophrenia-like phenotypes in adult by acting through GSK3β, Neuropharmacology 137 (2018) 359–371, https://doi.org/10.1016/j.neuropharm.2018.05.019.
[44] N.E. Clifton, N. Morisot, S. Girardon, M.J. Millan, F. Loiseau, Enhancement of social novelty discrimination by positive allosteric modulators at metabotropic glutamate 5 receptors: adolescent administration prevents adult-onset deficits induced by neonatal treatment with phencyclidine, Psychopharmacology 225 (2013) 579–594, https://doi.org/10.1007/s00213-012-2845-3.
[45] P. Miller-Rhodes, N. Piazza, A. Mattle, E. Teboul, M. Ehmann, K. Morris-Schaffer, V.P. Markowski, Sex-specific behavioral impairments produced by neonatal exposure to MK-801 are partially reversed by adolescent CDPPB treatment, Neurotoxicol. Teratol. 89 (2022), 107053, https://doi.org/10.1016/j.ntt.2021.107053.
[46] T.R. Insel, Rethinking schizophrenia, Nature 468 (2010) 187–193, https://doi.org/10.1038/nature09552.
[47] D.A. Lewis, A.A. Curley, J.R. Glausier, D.W. Volk, Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia, Trends Neurosci. 35 (2012) 57–67, https://doi.org/10.1016/j.tins.2011.10.004.
[48] A.M. Hamieh, D. Babin, E. Sablé, A.M. Hernier, V. Castagné, Neonatal phencyclidine and social isolation in the rat: effects of clozapine on locomotor activity, social recognition, prepulse inhibition, and executive functions deficits, Psychopharmacology 238 (2021) 517–528, https://doi.org/10.1007/s00213-020-05700-y.
[49] A. Adem, N. Madjid, O. Stiedl, A. Bonito-Oliva, Å. Konradsson-Geuken, S. Holst, G. Fisone, S.O. Ögren, Atypical but not typical antipsychotic drugs ameliorate phencyclidine-induced emotional memory impairments in mice, Eur. Neuropsychopharmacol. 29 (2019) 616–628, https://doi.org/10.1016/j.euroneuro.2019.03.007.
[50] X. Zhou, G. Cai, S. Mao, D. Xu, X. Xu, R. Zhang, Z. Yao, Modulating NMDA receptors to treat MK-801-induced schizophrenic cognition deficit: effects of clozapine combining with PQQ treatment and possible mechanisms of action, BMC Psychiatry 20 (2020) 106, https://doi.org/10.1186/s12888-020-02509-z.