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Ketamine administration in early *postnatal* life as a tool for mimicking Autism Spectrum Disorders core symptoms

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ABSTRACT

Autism Spectrum Disorders (ASD) core symptoms include deficits of social interaction, stereotyped behaviours, dysfunction in language and communication. Beyond them, several additional symptoms, such as cognitive impairment, anxiety-like states and hyperactivity are often occurring, mainly overlapping with other neuropsychiatric diseases. To untangle mechanisms underlying ASD etiology, and to identify possible pharmacological approaches, different factors, such as environmental, immunological and genetic ones, need to be considered. In this context, ASD animal models, aiming to reproduce the wide range of behavioural phenotypes of this uniquely human disorder, represent a very useful tool. Ketamine administration in early *postnatal* life of mice has already been studied as a suitable animal model resembling psychotic-like symptoms.

Here, we investigated whether ketamine administration, at *postnatal* days 7, 9 and 11, might induce behavioural features able to mimic ASD typical symptoms in adult mice. To this aim, we developed a 4-days behavioural tests battery, including Marble Burying, Hole Board, Olfactory and Social tests, to assess repetitive and stereotyped behaviour, social deficits and anxiety-like symptoms. Moreover, by using this mouse model, we performed neurochemical and biomolecular analyses, quantifying neurotransmitters belonging to excitatory-inhibitory pathways, such as glutamate, glutamine and gamma-aminobutyric acid (GABA), as well as immune activation biomarkers related to ASD, such as CD11b and glial fibrillary acidic protein (GFAP), in the hippocampus and amygdala. Possible alterations in levels of brain-derived neurotrophic factor (BDNF) expression in the hippocampus and amygdala were also evaluated.

Our results showed an increase in stereotyped behaviours, together with social impairments and anxiety-like behaviour in adult mice, receiving ketamine administration in early *postnatal* life. In addition, we found decreased BDNF and enhanced GFAP hippocampal expression levels, accompanied by elevations in glutamate amount, as well as reduction in GABA content in amygdala and hippocampus.

In conclusion, early ketamine administration may represent a suitable animal model of ASD, exhibiting face validity to mimic specific ASD symptoms, such as social deficits, repetitive repertoire and anxiety-like behaviour.

1. Introduction

Recent lines of evidence have highlighted that positive, negative and cognitive symptoms occurring in schizophrenic patients overlap with those typical of different other neuropsychiatric and neurodevelopmental diseases, such as bipolar disorders, obsessive-compulsive disorder and Autism Spectrum Disorders (ASD) (Carroll and Owen, 2009; Khanzada et al., 2017; Qin et al., 2019). In particular, ASD, although it is different from a diagnostic point of view from schizophrenia, exhibits a number of shared clinical symptoms with this pathology (Stone and Iguchi, 2011). Indeed, its core symptoms, such as deficits of social interaction, stereotyped behaviours and dysfunction in language and communication (Bove et al., 2018a), reproduce important clinical features of schizophrenia (Bristot Silvestrin et al., 2013). Moreover, although not considered among ASD core features, anxiety disorders are the most common comorbidities in both ASD and schizophrenic patients.

ASD etiology is still controversial, mainly due to the implication of

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several factors, such as environmental, immunological and genetic ones (Savino et al., 2020). Different mechanisms underlying ASD pathophysiology have been proposed, mainly involving excitation-inhibition unbalance, immune activation and neuroinflammation (Bristot Silvestrin et al., 2013). Regarding the excitatory-inhibitory system, glutamate, the major excitatory neurotransmitter, has been hypothesized to be crucially implicated in ASD (Rojas, 2014). Indeed, elevated brain and serum levels of glutamate have been found in autistic patients (Rojas, 2014), and disruption of the excitatory-inhibitory balance has been linked to ASD symptoms (Bristot Silvestrin et al., 2013). Accordingly, alterations in inhibitory gamma-aminobutyric acid (GABA)-ergic signaling in ASD animal models have been frequently reported (Lee et al., 2017). Moreover, different clinical studies have shown that both isoforms of glutamate decarboxylase (GAD65 and GAD67), the enzymes that synthesize GABA from glutamate, were reduced in post-mortem ASD brain tissues (Fatemi et al., 2002b; Yip et al., 2007; Rojas, 2014). Concerning central immune functions and neuroinflammation, microglia and astrocytes activation, as well as increased pro-inflammatory cytokines levels, have been found in ASD patients (Pardo et al., 2005; Vargas et al., 2005; Morgan et al., 2010). Astrocytes, synthesizing glutamate from glutamine through the so-called "glutamate-glutamine" cycle (Rojas, 2014), have also been described to be crucially implicated in keeping glutamate homeostasis. Thus, astrocytes activation could contribute to glutamate alteration, endorsing the central role of altered glutamate metabolism in ASD etiopathogenesis (Rojas, 2014).

Different lines of evidence have reported an involvement of brain-derived neurotrophic factor (BDNF) alterations in ASD pathophysiology, probably due to the critical role of this neurotrophin in neurodevelopment (Hellings et al., 2017; Reim and Schmeisser, 2017; Crespi, 2019).

Several data demonstrated that the hippocampus is a brain region particularly involved in ASD (Bristot Silvestrin et al., 2013; Chaddad et al., 2017a; Chaddad et al., 2017b; Xu et al., 2020). In this regard, magnetic resonance imaging scans of ASD subjects reported significant texture differences, occurring predominantly in the hippocampus (Chaddad et al., 2017a). In addition, it has been reported that ASD cognitive dysfunctions importantly involve hippocampal neurotransmission (Guo et al., 2021). Together with the hippocampus, socio-emotional impairments associated to ASD strongly implicate amygdalar circuits (Barnea-Goraly et al., 2014). Indeed, among the brain regions that constitute the neural network called the "social brain", amygdala and hippocampus play a primary role (Baron-Cohen et al., 2000; Felix-Ortiz et al., 2016). Since the "social brain" network is known to be altered in ASD (Volkmar, 2011; Misra, 2014; Elsabbagh and Johnson, 2016), the abnormal development of hippocampus and amygdala could importantly contribute to the onset of ASD symptomatology (Xu et al., 2020).

A strong limitation to the development of clinical studies focused on neuropsychiatric disorders, such as schizophrenia and ASD, is the recruitment of patients, especially those unmedicated, due to the complex burden of symptoms and, mainly for ASD, to the vulnerability of the affected population. Thus, the use of animal models, mimicking schizophrenic and ASD-like symptoms, is crucial to study the underlying neurobiology of these diseases, to untangle their complex etiopathology and to assess the effect of pharmacological interventions in preventing and/or treating these pathologies. In this regard, the administration of subanesthetic doses of ketamine, a non-competitive *N*-methyl-D-aspartate glutamate receptor (NMDAr) antagonist, also acting on different other receptors, such as dopamine, serotonin and opioids ones (Frohlich and Van Horn, 2014), could be considered as a reliable pharmacological tool to mimic schizophrenia-related behavioural and neuropathological dysfunctions in rodents (Cadinu et al., 2018). Moreover, ketamine administration in the early life period represents a neurotoxic insult interfering with physiological neurodevelopmental processes and possibly leading to schizophrenic-like alterations in adult life. Indeed, it has been reported that brain maturation, such as synapse formation, axon elongation, and neurotransmission development, initiates during pregnancy and continue during the early *postnatal* days (PNDs), in both rodents and humans (Kolb and Whishaw, 1989; Viberg, 2009; Teffer and Semendeferi, 2012), specially from PNDs 7 until PNDs 14 for rodents (Plataki et al., 2021). In this regard, we have previously demonstrated that subanesthetic ketamine administration at PNDs 7, 9 and 11 was able to induce behavioural dysfunctions resembling psychotic-like symptoms in adult mice (Schiavone and Trabace, 2016; Bove et al., 2020; Schiavone et al., 2020), accompanied by cortical redox imbalance and increase in pro-inflammatory cytokines.

Concerning ASD, there are several animal models showing strong face validity for the core symptoms (Bossu and Roux, 2019; Iwata, 2019) (Schneider and Przewlocki, 2005; Kazdoba et al., 2016; Meyza and Blanchard, 2017). Since ASD is a uniquely human disorder, with several heterogeneous symptoms, rodent models of ASD aim to reproduce the wide range of behavioural phenotypes, by using behavioural assays able to resemble the most common diagnostic symptoms (Crawley, 2004; Kazdoba et al., 2016).

Here, we investigated whether ketamine administration at PNDs 7, 9 and 11 may induce behavioural features translatable to typical ASD symptoms. To this aim, we developed a 4-days behavioural tests battery (Marble Burying, Hole Board, Olfactory and Social tests) to assess repetitive and stereotyped behaviour, social deficits and anxiety-like symptoms. Moreover, we performed neurochemical and biomolecular analyses, quantifying amino acids belonging to excitatory-inhibitory pathways, such as glutamate, glutamine and GABA in the hippocampus and amygdala, as well as hippocampal and amygdalar immune activation biomarkers related to ASD, such as CD11b and glial fibrillary acidic protein (GFAP). Possible alterations in levels of BDNF expression in the hippocampus and amygdala were also evaluated.

2. Materials and methods

2.1. Animals

Mice were housed at constant room temperature (22 ± 1 °C) and relative humidity (55 ± 5%), under a 12 h light/dark cycle (lights on from 7:00 AM to 7:00 PM) with free access to food and water. Experimental procedures involving animals and their care were performed in conformity with the institutional guidelines of the Italian Ministry of Health (D.Lgs. n. 26/2014), the Guide for the Care and Use of Laboratory Animals: Eight Edition, the Guide for the Care and Use of Mammals in Neuroscience and Behavioural Research, the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, as well as the ARRIVE guidelines. The experimental protocol was approved by the Italian Ministry of Health (approval number 679/2017-PR, protocol n. B2EF8.17) Animal welfare was monitored every day throughout the experimental phase. All the experimental procedures were made to reduce the number of animals used and in order to minimize their suffering.

2.2. Experimental protocol

A total of five C57/Bl6 male mice of 8–10 weeks of age (25–30 g), and ten age and weight-matched adult females (Envigo, San Pietro al Natisone, Italy) were mated (one male and two females per cage). Male and female pups from different litters were divided into the following experimental groups, according to the different treatments they received at PNDs 7, 9, and 11: (1) Saline (10 ml/kg i.p.); (2) Ketamine (Sigma-Aldrich Corporation, Saint Louis, MO, United States; 30 mg/kg i.p., dissolved in saline) (Schiavone et al., 2020). All pups were grown until adulthood (10 weeks of age). At this time point, behavioural tests were performed. Since there were no gender differences (data not shown), experiments were performed by including both males and females in the two experimental groups.

2.3. Behavioural tests battery

2.3.1. Marble burying test

The test was performed according to Agoa-Perez et al. (Angoa-Perez et al., 2013). Briefly, each mouse was placed for 30 min into a corner of an unfamiliar standard polycarbonate rat cage (26 cm × 48 cm × 20 cm) filled with fresh bedding material of 5 cm of thickness and containing 20 marbles, without food and water, paying attention to put the mouse on the bedding far from marbles. . At the end of the test, the number of marbles buried was counted by scoring a marble as buried if two-thirds of its surface area were covered by bedding.

2.3.2. Hole board test

The test was performed according to Souza et al. (Souza et al., 2016). Briefly, the experiment was conducted in a wooden box (40 × 40 × 35 cm) with 16 holes (3 cm of diameter) on the ground. The board was suspended 5 cm to the floor. For 5 min, the number of times the animals poking the hole for a duration of at least 1 s, was recorded as poking holes by using an automatic counter. In addition, the number of hole pokes in the same hole was scored by an experimenter blinded to the experimental groups as a measure of repetitive behaviour, expressed as repetitive hole pokes, according to Lugo et al. (Lugo et al., 2014).

2.3.3. Olfactory "hidden cookie" test

The test was performed according to Moy et al. (Moy et al., 2007). Briefly, 14 days before the test, an unfamiliar food (ChocoKrave, Kellogg's) was placed overnight in the home cages of each mouse, in order to avoid food neophobia and make the food more palatable. On the day of the test, the same food was placed in a large box (40 × 40 × 35 cm), bedding approximately 1 cm below the surface of the litter. The animals were tested for olfactory ability following 16–20 h of food deprivation. The latency to find the buried food, expressed in seconds, was measured. This test was also used as habituation for the social interaction test, in order to minimize the effects of sensory adaptation (Rankin et al., 2009).

2.3.4. Social interaction test

The test was adapted from Kreuters et al. (Kraeuter et al., 2019) and from Laviola et al. (Laviola et al., 2004). Briefly, in the social test, the stimulus mice were of the same strain, age and sex, with no previous encounter, with the subject mice. Five days before the test, the subject mice were individually housed and, on the test day, were placed in the same large box used for the olfactory test (40 × 40 × 35 cm) simultaneously with the stimulus mouse for 5 min. The box was provided with three objects (one ball, one plastic object and one paper cylinder). The test session was video-recorded and, then, scored by a trained observer blind to the treatment. The frequency and duration of the following behaviours was scored:

- Social behaviours (investigative and affiliative) such as allo-grooming, sniffing, exploring the stimulus animal, engaging social contact;
- Non-social behaviours (object-directed) such as exploring the objects, sniffing the objects, playing the objects;
- Self-grooming.

2.4. Post-mortem tissues analyses

Anesthetized animals were euthanized by cervical dislocation. Brains were immediately removed and kept on ice for dissection of hippocampus and amygdala, according to the mouse brain atlas of Paxinos and Franklin (Paxinos and Franklin, 2019). Tissues were immediately frozen and stored at −80 °C until analyses were carried out. Samples were diluted 1:10 *w/v* at 4 °C using PBS buffer, containing 1:100 protease and phosphatase inhibitor cocktail (HALT inhibitors, Thermo Fisher Scientific, Cleveland, OH, USA) for biomolecular analyses, or perchloric acid 0.1 M for neurochemical analyses, as previously described (Bove et al., 2018b). Homogenates were centrifuged at 10.000 ×*g* at 4 °C for 10 min and supernatants were used for the analyses.

2.4.1. Neurochemical quantifications

GABA, glutamate and glutamine levels were measured in the hippocampus and amygdala of mice by High Performance Liquid Chromatography (HPLC) coupled with fluorescence detection (emission length 460 nm; excitation length 340 nm), as previously published (Mele et al., 2019). Analyses were carried out using LC18 reverse phase column (Kinetex, 150 mm × 3.0 mm, ODS 5 μm; Phenomenex, Castel Maggiore- Bologna, Italy) and detection was accomplished by pre-column derivatization with o-phthalaldehyde/mercaptopropionic acid. The mobile phase consisted of 50 mM sodium acetate buffer, at pH 6.95, with gradient methanol increasing linearly from 2 to 30% (*v*/v) over 40 min run. The gradient flow rate was maintained by a pump (JASCO, Tokyo, Japan) at 0.5 ml/min. Results were analyzed by Borwin software (version 1.50; Jasco) and aminoacid concentration was expressed as μM. All data were normalized for total area weight and were expressed as concentration/mg of tissue.

2.4.2. Western blotting quantification

Total amount of proteins was measured in hippocampus and amygdala homogenates by using Pierce BCA Assay (Thermo Fisher Scientific, Cleveland, OH, USA). Forty μg of the total lysate of proteins were separated by SDS-PAGE precast gels (Bio-Rad Laboratories Inc., Segrate (MI), Italy), transferred onto nitrocellulose membranes (Bio-Rad Laboratories Inc., Segrate (MI), Italy) and, then, blocked for 1 h in blocking buffer at room temperature (SigmaAldrich, Milan, Italy), as previously reported (Pieretti et al., 2013; Schiavone et al., 2017a). Mouse monoclonal antibody against GFAP (Ab279290; 1:1000, Abcam, Cambridge, UK), rabbit monoclonal antibody against CD11b (Ab133357, 1:1000, Abcam, Cambridge, UK), rabbit polyclonal antibody against BDNF (Ab226843, 1:1000, Abcam, Cambridge, UK) and mouse monoclonal antibody against β-actin (Ab8226, 1:3000, Abcam, Cambridge, UK) were used to incubate the membranes overnight at 4 °C. After HRP-conjugated specific secondary antibody incubation (Goat Anti-Mouse, Ab205719, and Goat Anti-Rabbit, Ab6721, 1:5000, Abcam, Cambridge, UK), the ECL reagent (Bio-Rad Laboratories Inc., Segrate (MI), Italy) was added to the immune complex and chemiluminescence was detected by ChemiDoc MP system (Bio-Rad Laboratories Inc., Segrate (MI), Italy). Optical densities of the bands were measured using ImageJ software (http://rsb.info.nih.gov/ij/) and normalized against bands relative to β-actin. All original blots are showed in Supplementary Material section.

2.5. Statistical analyses

Data were expressed as mean ± standard error of the mean (SEM). Experiments were analyzed using Unpaired Student's *t*-test, with Welch's correction when variances were unequal. All analyses were performed by using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Differences among groups were considered significant at values of $P < 0.05$.

3. Results

3.1. Effects of postnatal ketamine exposure on stereotyped behaviour in the marble burying test

On day 1, we performed the marble burying test to assess stereotyped and repetitive behaviours. Our results showed an increase in marbles buried in ketamine-treated animals compared to vehicles (Fig. 1, Unpaired Student's *t*-test, $P < 0.05$ ketamine vs. saline).

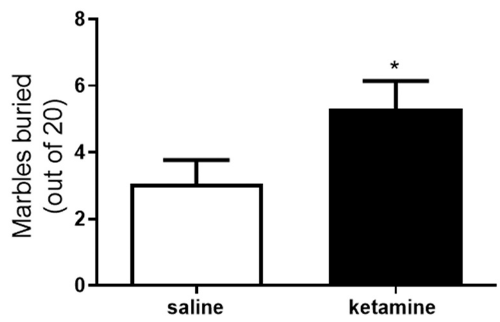

Fig. 1. *Postnatal* ketamine exposure increased stereotyped behaviour in the Marble Burying test.
Marbles buried (out of 20) in saline ($n = 16$) and ketamine-treated ($n = 13$) animals. Unpaired Student's *t*-test, $*P < 0.05$ ketamine vs saline.

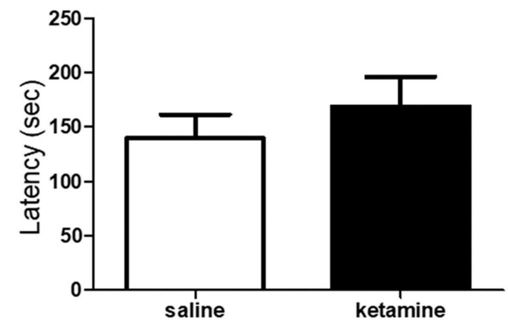

Fig. 3. *Postnatal* Ketamine exposure did not affect olfactory ability in the Olfactory "Hidden Cookie" test.
Latency (*sec*) in saline (n = 16) and ketamine-treated (n = 13) animals. Unpaired Student's t-test, $P = 0.40$.

3.2. Effects of postnatal ketamine exposure on anxiety-like and repetitive behaviour in the hole board test

On day 2, we carried out a behavioural test to analyze anxiety-like behaviour, known as the Hole Board test. We found a significant decrease in the number of poking holes in mice exposed to *postnatal* ketamine compared to saline (Fig. 2A, Unpaired Student's *t*-test, $P < 0.01$ ketamine vs. saline). Moreover, this test is also used to analyze repetitive behaviour, by scoring the number of total hole pokes repetitions in the same hole. Our results showed an increase in the number of repetitive hole pokes in mice treated with ketamine compared to vehicles (Fig. 2B, Unpaired Student's *t*-test, $P < 0.05$ ketamine vs. saline).

3.3. Effects of postnatal ketamine exposure on olfactory ability in the olfactory "hidden cookie" test

On day 3, we performed the olfactory "Hidden Cookie" test, in order to evaluate olfactory capacity in our experimental groups. There was no difference in the latency time to reach the buried food between ketamine- and saline- treated animals (Fig. 3, Unpaired Student's *t*-test, n.s.).

3.4. Effects of postnatal ketamine exposure on social behaviour and self-grooming in the social interaction test

On day 4, we performed the social interaction test. We found no differences in non social frequency between the two groups (Fig. 4A, Unpaired *t*-test, n.s.), while the social frequency was significantly decreased in ketamine-treated animals compared to vehicles (Fig. 4B, Unpaired Student's *t*-test, $P < 0.01$ ketamine vs. saline). Regarding self-grooming frequency, ketamine administration significantly increased this repetitive and anxiety-like behaviour compared to the control group (Fig. 4C, Unpaired Student's *t*-test, $P < 0.01$ ketamine vs. saline). Concerning duration measurements, the time spent in performing social and non-social behaviours was not significantly changed between saline and ketamine-treated mice (Fig. 4D-E, Unpaired *t*-test, n.s.), while there was a significant increase in self-grooming duration in ketamine-treated mice over the saline administrated animals (Fig. 4F, Unpaired Student's t-test, $P < 0.01$ ketamine vs. saline).

3.5. Effects of postnatal ketamine exposure on hippocampal BDNF, CD11b and GFAP expression levels

Concerning hippocampal BDNF expression, we found a significant decrease in ketamine-treated animals compared to vehicles (Figs. 5A and D, Unpaired Student's *t*-test, $P < 0.05$ ketamine vs. saline). As shown in Figs. 5B and E, no difference in hippocampal CD11b expression was retrieved between the two experimental groups (Figs. 5B and E, Unpaired Student's *t*-test, n.s.). Furthermore, in Figs. 5C and F, a significant increase of GFAP expression in ketamine-treated mice compared to saline ones is reported (Figs. 5C and F, Unpaired Student's *t*-test, $P < 0.05$ ketamine vs. saline).

3.6. Effects of postnatal ketamine exposure on amygdalar BDNF, CD11b and GFAP expression levels

In order to evaluate the impact of ketamine administration on amygdala, we evaluated BDNF, CD11b and GFAPB expression. According to our results, there was no significant difference between BDNF, CD11b and GFAP levels in the amygdalar tissues of the saline and ketamine-treated mice (Figs. 6A-C and D-F, Unpaired *t*-test, n.s.).

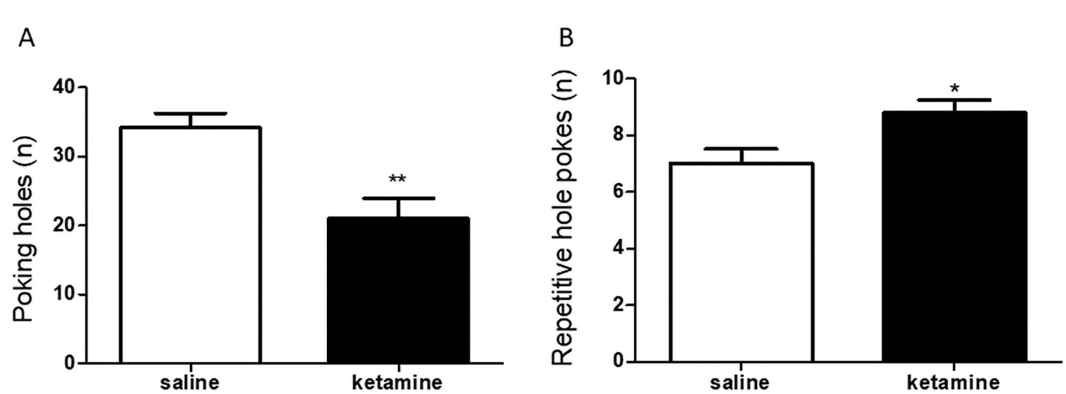

Fig. 2. *Postnatal* ketamine exposure induced anxiety-like and repetitive behaviours in the Hole Board test.
A) Poking holes (n) in saline ($n = 11$) and ketamine-treated ($n = 10$) animals. Unpaired Student's t-test, $**P < 0.01$ ketamine vs saline.
B) Repetitive poking holes (n) in saline (n = 11) and ketamine-treated (n = 10) animals. Unpaired Student's t-test, $*P < 0.05$ ketamine vs saline.

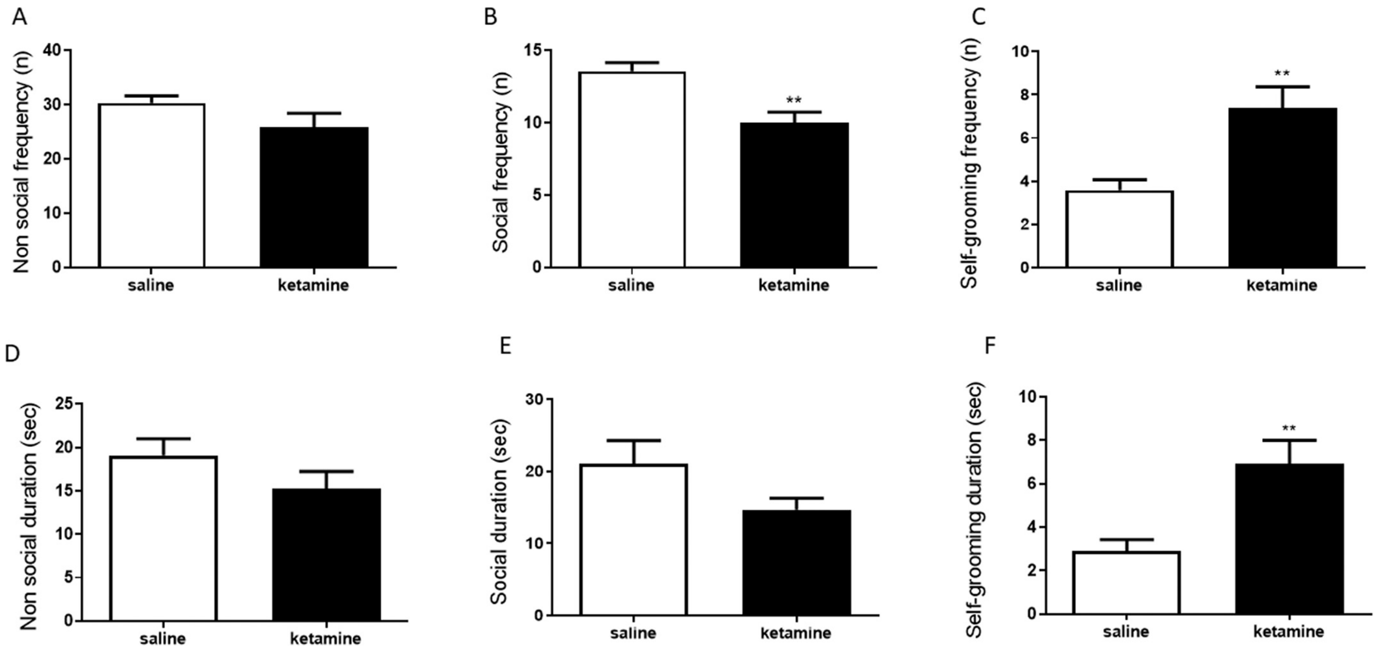

Fig. 4. *Postnatal* Ketamine exposure decreased social frequency, did not affect social duration, non social frequency and non social duration, and increased self-grooming frequency and self-grooming duration in the Social Interaction test.
A) Non social frequency (n) in saline ($n = 12$) and ketamine-treated (n = 10) animals. Unpaired Student's t-test, $P = 0.12$.
B) Social frequency (n) in saline (n = 12) and ketamine-treated (n = 10) animals. Unpaired Student's t-test, $^{**}P < 0.01$ ketamine vs saline.
C) Self-grooming frequency (n) in saline (n = 12) and ketamine-treated (n = 10) animals. Unpaired Student's t-test, $^{**}P < 0.01$ ketamine vs saline.
D) Non social duration (sec) in saline (n = 12) and ketamine-treated (n = 10) animals. Unpaired Student's t-test, $P = 0.18$.
E) Social duration (sec) in saline (n = 12) and ketamine-treated (n = 10) animals. Unpaired Student's t-test, P = 0.12.
F) Self-grooming duration (sec) in saline (n = 12) and ketamine-treated (n = 10) animals. Unpaired Student's t-test, $^{**}P < 0.01$ ketamine vs saline.

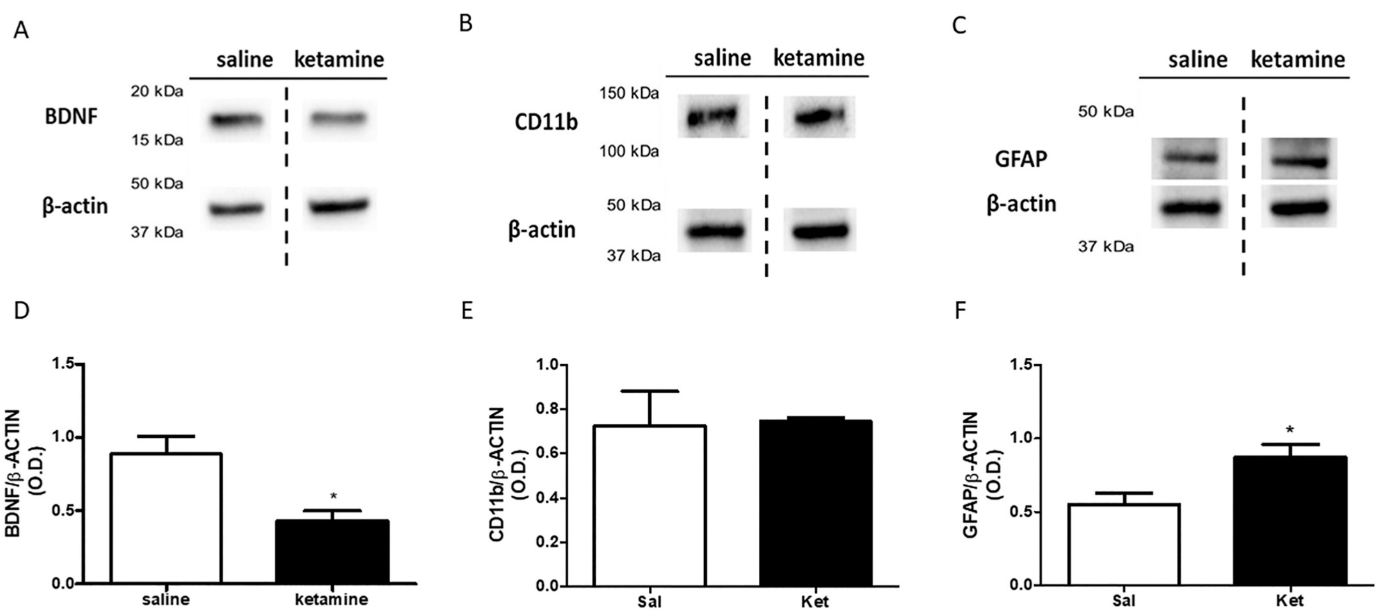

Fig. 5. *Postnatal* Ketamine exposure decreased BDNF, did not affect CD11b and increased GFAP expression levels in the hippocampus.
A) Representative images of Western Blotting bands of BDNF and β-actin housekeeping gene in the hippocampus of saline and ketamine-treated animals.
B) Representative images of Western Blotting bands of CD11b and β-actin housekeeping gene in the hippocampus of saline and ketamine-treated animals.
C) Representative images of Western Blotting bands of GFAP and β-actin housekeeping gene in the hippocampus of saline and ketamine-treated animals.
D) Quantification of the optical band density of BDNF normalized for optical band density of β-actin housekeeping gene in the hippocampus of saline ($n = 4$) and ketamine-treated (n = 4) animals. Unpaired Student's t-test, $^{*}P < 0.05$ ketamine vs saline.
E) Quantification of the optical band density of CD11b normalized for optical band density of β-actin housekeeping gene in the hippocampus of saline (n = 4) and ketamine-treated (n = 4) animals. Unpaired Student's t-test, $P = 0.92$.
F) Quantification of the optical band density of GFAP normalized for optical band density of β-actin housekeeping gene in the hippocampus of saline (n = 4) and ketamine-treated (n = 4) animals. Unpaired Student's t-test, $^{*}P < 0.05$ ketamine vs saline.

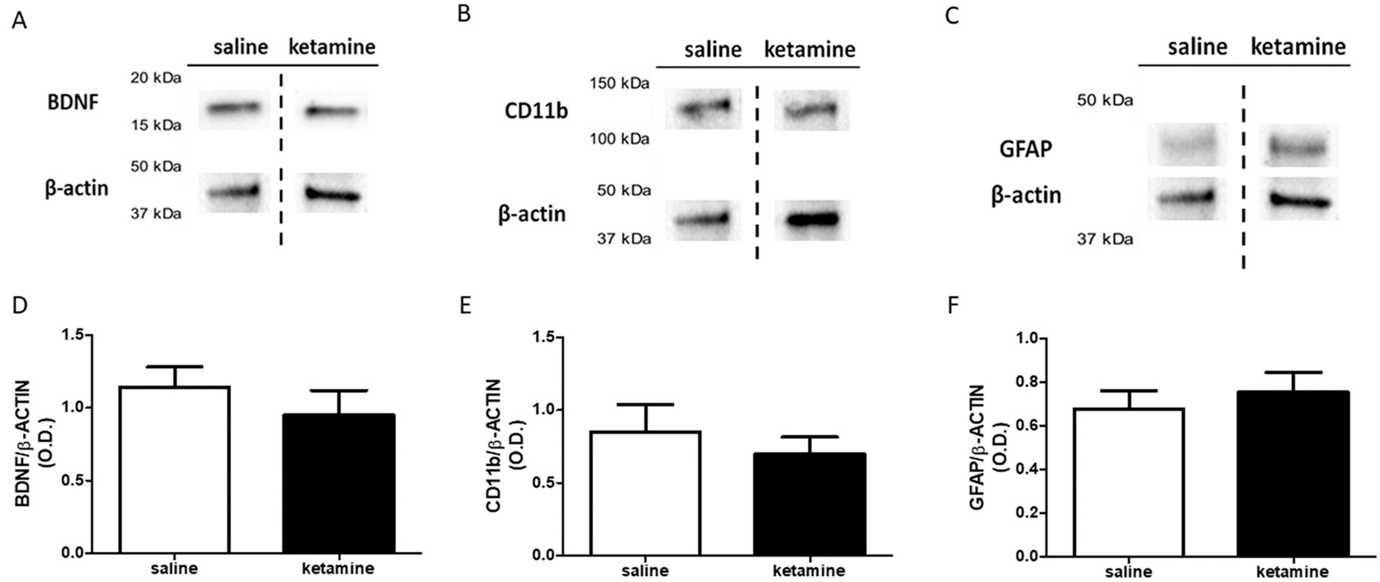

Fig. 6. *Postnatal* Ketamine exposure did not affect BDNF, CD11b and GFAP expression levels in the amygdala.
A) Representative images of Western Blotting bands of BDNF and β-actin housekeeping gene in the amygdala of saline and ketamine-treated animals.
B) Representative images of Western Blotting bands of CD11b and β-actin housekeeping gene in the amygdala of saline and ketamine-treated animals.
C) Representative images of Western Blotting bands of GFAP and β-actin housekeeping gene in the amygdala of saline and ketamine-treated animals.
D) Quantification of the optical band density of BDNF normalized for optical band density of β-actin housekeeping gene in the amygdala of saline (n = 4) and ketamine-treated (n = 4) animals. Unpaired Student's t-test, $P = 0.42$.
E) Quantification of the optical band density of CD11b normalized for optical band density of β-actin housekeeping gene in the amygdala of saline (n = 4) and ketamine-treated (n = 4) animals. Unpaired Student's t-test, $P = 0.52$.
F) Quantification of the optical band density of GFAP normalized for optical band density of β-actin housekeeping gene in the amygdala of saline (n = 4) and ketamine-treated (n = 4) animals. Unpaired Student's t-test, $P = 0.55$.

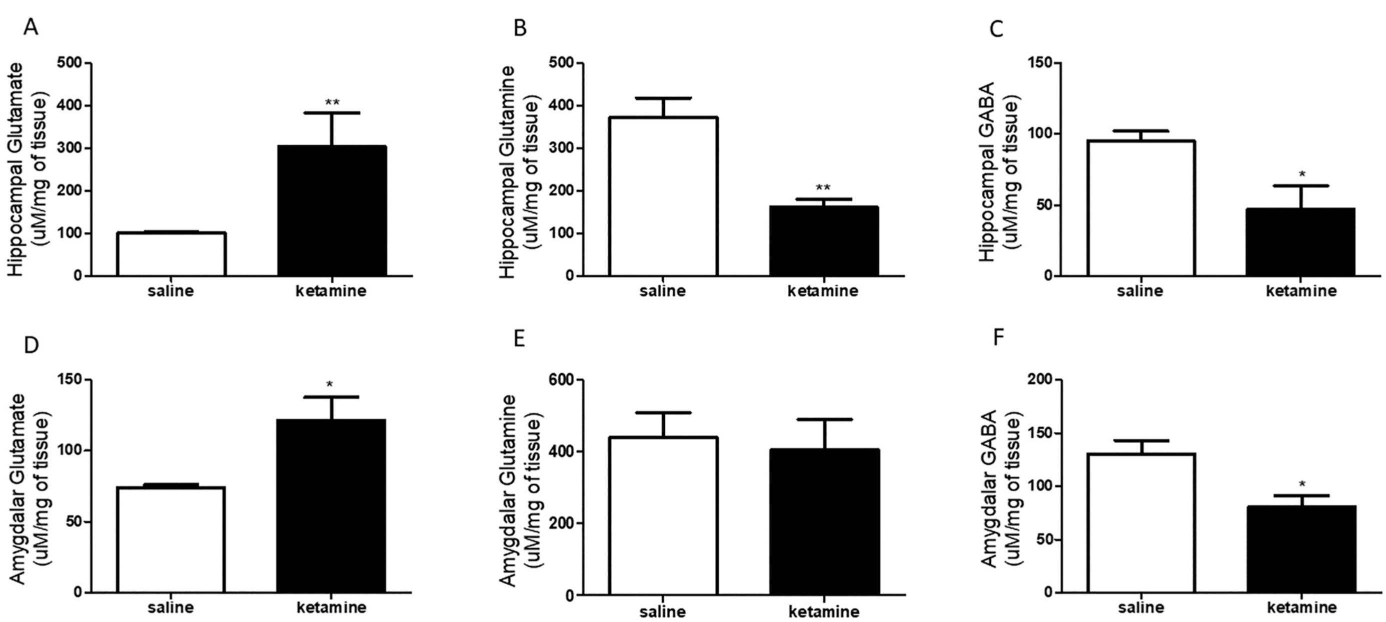

Fig. 7. *Postnatal* Ketamine exposure altered hippocampal and amygdalar glutamate, glutamine and GABA contents.
A) Hippocampal Glutamate (μM/mg of tissue) levels in saline (*n* = 5) and ketamine-treated (n = 5) animals. Unpaired Student's t-test, $^{**}P < 0.01$ ketamine vs saline.
B) Hippocampal Glutamine (μM/mg of tissue) levels in saline (n = 5) and ketamine-treated (n = 5) animals. Unpaired Student's t-test, $^{**}P < 0.01$ ketamine vs saline.
C) Hippocampal GABA (μM/mg of tissue) levels in saline (n = 5) and ketamine-treated (n = 5) animals. Unpaired Student's t-test, $^{*}P < 0.05$ ketamine vs saline.
D) Amygdalar Glutamate (μM/mg of tissue) levels in saline (n = 5) and ketamine-treated (n = 5) animals. Unpaired Student's t-test, $^{*}P < 0.05$ ketamine vs saline.
E) Amygdalar Glutamine (μM/mg of tissue) levels in saline (n = 5) and ketamine-treated (n = 5) animals. Unpaired Student's t-test, $P = 0.75$.
F) Amygdalar GABA (μM/mg of tissue) levels in saline (n = 5) and ketamine-treated (n = 5) animals. Unpaired Student's t-test, $^{*}P < 0.05$ ketamine vs saline.

3.7. Effects of postnatal ketamine exposure on glutamate, glutamine and GABA levels in hippocampus and amygdala

As shown in Fig. 7A, hippocampal glutamate levels were significantly increased in ketamine-treated animals compared to controls (Fig. 7A, Unpaired Student's *t*-test, $P < 0.05$ ketamine vs. saline), whereas *postnatal* ketamine administration induced a reduction in glutamine and GABA contents (Figs. 7B and C, Unpaired Student's *t*-test, $P < 0.05$ ketamine vs. saline). Concerning amygdalar quantifications, we found a significant increase in glutamate levels in adult mice after *postnatal* ketamine administration (Fig. 7D, Unpaired Student's *t*-test, $P < 0.05$ ketamine vs. saline), while no difference was retrieved in glutamine content between the two groups (Fig. 7E, Unpaired *t*-test, n.s.). Lastly, ketamine injections during *postnatal* period were able to significantly reduce amygdalar GABA levels in adult mice (Fig. 7F, Unpaired Student's *t*-test, $P < 0.05$ ketamine vs. saline).

4. Discussion

In the present study, we proposed an animal model of subanesthetic *postnatal* ketamine administration as a suitable tool to study ASD typical dysfunctions. Indeed, we performed a 4-days behavioural tests battery focusing on ASD core symptoms, such as stereotyped and repetitive behaviour and social impairments, together with anxiety-like behaviour, the major ASD comorbidity, accompanied by molecular and neurochemical quantifications related to ASD dysfunctions, including BDNF, CD11b and GFAP expressions, as well as glutamate, glutamine and GABA contents in hippocampus and amygdala.

An important finding of our study relates to the increase in Marble Buried observed in ketamine-treated animals compared to controls. This behavioural test has been widely used to assess repetitive and persistent behaviour in neuropsychiatric disorders, mainly due to its reliability and scoring celerity (Angoa-Perez et al., 2013; Chang et al., 2017). In line with our results, several mouse models of ASD reported an increase in Marble Buried (Angoa-Perez et al., 2013; Nadeem et al., 2019; Wiebe et al., 2019; Witt et al., 2019), confirming the pivotal aspect of the repetitive domain in the ASD phenotype. As regarding the social sphere, we performed a Social Interaction test by introducing in the arena not only the stimulus animal, but also three different non socially related stimuli, scoring social, non social and self-grooming durations and frequencies. Our results showed no difference in non social approaches frequency and duration between the two experimental groups, while ketamine-administrated animals reported a decrease in social interactions frequency, but not duration. Since social withdrawal is one of the pivotal diagnostic symptoms occurring in ASD patients (Bove et al., 2018a), social dysfunctions are a fundamental aspect to provide face validity in a developing model of ASD phenotype. Moreover, *postnatal* ketamine administration increased self-grooming frequency and duration, a behaviour related to anxiety and repetitive domains (Mcfarlane et al., 2008; Estanislau et al., 2019; Wei et al., 2020). Supporting our results, several evidence reported that BTBR T+ tf/J mice, the inbred strain most widely used to mimic ASD symptoms, showed high levels of repetitive self-grooming and low levels of social behaviours (Mcfarlane et al., 2008; Pobbe et al., 2011; Meyza and Blanchard, 2017; Cai et al., 2019; Queen et al., 2020).

Here, we also reported an anxiety-like state in ketamine-treated animals, as shown by the decrease in the nose poking counts in the Hole Board test. Interestingly, the Hole Board test has also been used to evaluate repetitive behaviours, expressed as going repetitively in the same hole, or preference for a restricted number of holes (Moy et al., 2008; Patterson, 2011; Lugo et al., 2014). Here, stereotyped behaviour has been extrapolated from the Hole Board test, by scoring the frequency of the repetitive nose pokes in the same hole. Our results showed an increase in repetitive hole pokes in ketamine-treated animals compared to saline ones. Accordingly, the repeated nose pokes into one hole, alternatively called back-to-back head dipping, have been considered as a sign of stereotypy long ago (Makanjuola et al., 1977a; Makanjuola et al., 1977b; Meeking et al., 2020). Hence, *postnatal* ketamine administration was able to reproduce core ASD symptoms in adulthood, such as stereotyped behaviours and social dysfunctions, providing an important face validity to our proposed animal model. However, from a translational point of view, ASD is mainly diagnosed during childhood and adolescence, thus further researches will be needed in order to investigate the effects of early ketamine administration also during juvenile phase. Moreover, we did not explore another ASD key symptom regarding communication impairments. The investigation of communication deficits, by the means of ultrasonic vocalizations, scent-marking or social transmission of food preference (Mcfarlane et al., 2008; Wohr et al., 2011), could represent a useful tool in order to make the model more robust and exhibit stronger face validity.

In this study, by using the Olfactory "Hidden Cookie" test, we also showed no difference in the latency to reach the palatable food between the two experimental groups, attesting that the olfactory ability of ketamine-administrated mice is similar to control one. Since olfactory system plays a critical role in social interactions, this test is commonly used to exclude olfactory impairments when social tests are performed (Yamada et al., 2001; Yang and Crawley, 2009) Indeed, our results, showing intact olfactory abilities in ketamine-treated animals, strongly endorsed that this animal model is able to induce alterations in social behaviour.

In order to corroborate behavioural results with neurochemical and biomolecular analyses, we evaluated the effects of *postnatal* ketamine administration on immune activation markers and on the excitatory-inhibitory balance in hippocampus and amygdala. As regarding immune activation, we found no difference in themicroglial marker CD11b expression levels, in both amygdala and hippocampus, while there was an increase in GFAP, a specific marker for astrocytes (Fatemi et al., 2002a), in the hippocampus, of ketamine-treated animals compared to controls. This increase in GFAP expression levels was not retrieved in the amygdala, thus revealing possible area-specific changes related to the hippocampal brain region. In this regard, recent clinical and preclinical studies showed that neuroglial pathological development might exacerbate or even cause ASD symptoms (Bronzuoli et al., 2018). Accordingly, neurogliopathology, reflected by elevated GFAP levels, has been found in adult autistic brains (Fatemi et al., 2002b; Laurence and Fatemi, 2005; Vargas et al., 2005). Moreover, ASD animal models and post-mortem brains reported astrogliosis, microgliosis and neuroinflammation (Codagnone et al., 2015). In keeping with this hypothesis, we have previously demonstrated a cortical increase in pro-inflammatory cytokines expressions in ketamine-treated animals, thus denoting a significant neuroinflammation in this animal model (Bove et al., 2020).

Furthermore, our results showed a decrease of hippocampal, but not amygdalar, BDNF expression levels in ketamine-administrated mice. The same trend was also found in a clinical study performing peripheral measurements, in particular, lower serum BDNF levels have been reported in adult autistic patients compared to age-matched controls (Hashimoto et al., 2006). Accordingly enough, newborn children, lately developing ASD, also showed a decrease in blood BDNF levels (Skogstrand et al., 2019). In addition, different preclinical studies reported that ketamine treatment was able to alter neurotrophin expression levels in hippocampus and prefrontal cortex (Schiavone et al., 2017b; Sedky and Magdy, 2021). Conversely, a number of studies reported BDNF gene and protein expression increase in ASD animal models (Almeida et al., 2014; Bryn et al., 2015; Meng et al., 2017), in particular at birth, with a subsequent decrease during the juvenile period (Fuentealba et al., 2019). Although further studies need to be warranted, in particular related to the age of the experimental groups and the putative hippocampal brain region-specificity, BDNF and its related pathways could be an important aspect of the unrevealed ASD etiology. Indeed, age of testing represent a limitation of our study, since it has been shown that also social interaction deficits can be stronger revealed during juvenile phase (Panksepp et al., 2007).

Concerning excitatory-inhibitory neurotransmitters, we found an increase of glutamate content in hippocampus and amygdala, accompanied by a decrease of GABA levels in both brain regions, while glutamine content was decreased in hippocampus, but not in amygdala. However not fully understood yet, different clinical studies reported an increase in glutamate levels in ASD patients (Rojas, 2014). This may be due to the neuroglial activation, in particular to the astrocytic involvement. Indeed, in the glutamate-glutamine cycle, astrocytes reabsorb extracellular glutamate and reconvert it in glutamine (Rojas, 2014). In this regard, alterations of glutamate astrocytic clearance have been reported in ASD (Bristot Silvestrin et al., 2013), supporting our hypothesis that astrocytes activation might contribute to the increase in glutamate availability reported in our animal model. Moreover, the glutamate-to-GABA converting enzymes, GAD65 and GAD67, could also be crucially implicated, since their reduction is commonly reported in several clinical ASD studies (Fatemi et al., 2002b; Yip et al., 2007; Blatt and Fatemi, 2011; Rojas, 2014). In particular, GAD67 reduction might explain the increase in glutamate levels and the decrease in GABA levels commonly reported in different brain regions of ASD patients and animal models (Chao et al., 2010; Puts et al., 2017; Horder et al., 2018). We have previously demonstrated that BTBR T + tf/J mice exhibited cortical and amygdalar increase in glutamate and decrease in GABA (Bove et al., 2018a), supporting the central role of excitation-inhibition unbalance in ASD etiopathogenesis. Interestingly, the BTBR T + tf/J inbred strain showed, among others, a single nucleotide polymorphism of the KMO gene encoding for an enzyme involved in the metabolism of kynurenic acid, a well-known glutamate antagonist (Mcfarlane et al., 2008). Thus, this polymorphism might lead to an abnormal glutamatergic activity and consequently participate in the development of the ASD-like behavioural phenotype (Mcfarlane et al., 2008; Lim et al., 2016).

To summarize, behavioural dysfunctions induced by sub-chronic *postnatal* ketamine administration well resembled ASD core symptoms and its comorbidities, such as anxiety-like behaviour, further endorsing strong face validity of our proposed animal model. In line with our results, it has been reported that animal models of NMDAr hypofunction showed similarity with schizophrenia-like symptoms, but also social dysfunctions and communication impairments related to the autistic phenotype (Carlson, 2012). Moreover, impaired NMDAr-mediated neurotransmission has been correlated to different ASD risk alleles (Deutsch et al., 2022). However, ketamine interferes in a non-competitive and non-specific way with NMDAr, thus future studies are warranted in order to investigate changes in expression and distribution of specific NMDAr subtypes in our model. Conventional NMDAr is a heterotetrameric complex, with two GluN1 subunits, binding the co-agonist glycine, and two GluN2 subunits, binding the glutamate (Sengar et al., 2019). Interestingly, it has been reported the GluN1 subunits downregulation was able to prompt social impairments and repetitive behaviour (Lee et al., 2017). In particular, GluN1 Knock-Out mice showed different abnormal behaviours, resembling negative symptom domains and commonly occurring across several neuropsychiatric and neurodevelopmental diseases (Gandal et al., 2012). In line with these studies, our results support the hypothesis that early disruption of NMDAr activation might lead to the development of behavioural abnormalities resembling different phenotypic ASD-like traits and to pathological consequences that might affect neuroinflammatory processes.

Unfortunately, we could not evaluate predictive validity in our proposed animal model since no therapeutic interventions are currently available for concomitantly treating the heterogeneous ASD symptoms. Nonetheless, our study might shed new light on novel biological targets as possible therapeutic candidates aiming to prevent and/or treat ASD core symptoms.

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Ethical statement

Experimental procedures involving animals and their care were performed in conformity with the institutional guidelines of the Italian Ministry of Health (D.Lgs. n. 26/2014), the Guide for the Care and Use of Laboratory Animals: Eight Edition, the Guide for the Care and Use of Mammals in Neuroscience and Behavioural Research (National Research Council, 2004), the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, as well as the ARRIVE guidelines. The experimental protocol was approved by the Italian Ministry of Health (approval number 679/2017-PR, protocol n. B2EF8.17) Animal welfare was monitored every day throughout the experimental phase. All the experimental procedures were made to reduce the number of animals used and in order to minimize their suffering.

CRediT authorship contribution statement

Maria Bove: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Writing – original draft, Writing – review & editing. **Stefania Schiavone:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Software, Supervision, Validation, Visualization, Writing – review & editing. **Paolo Tucci:** Investigation, Methodology, Writing – review & editing. **Vladyslav Sikora:** Investigation, Methodology, Writing – review & editing. **Stefania Dimonte:** Investigation, Methodology, Writing – review & editing. **Anna Laura Colia:** Investigation, Methodology, Writing – review & editing. **Maria Grazia Morgese:** Data curation, Formal analysis, Software, Validation, Visualization, Writing – review & editing. **Luigia Trabace:** Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2022.110560.

References

Almeida, L.E., Roby, C.D., Krueger, B.K., 2014. Increased BDNF expression in fetal brain in the valproic acid model of autism. Mol. Cell. Neurosci. 59, 57–62.
Angoa-Perez, M., Kane, M.J., Briggs, D.I., Francescutti, D.M., Kuhn, D.M., 2013. Marble burying and nestlet shredding as tests of repetitive, compulsive-like behaviors in mice. J. Vis. Exp. 50978.
Barnea-Goraly, N., Frazier, T.W., Piacenza, L., Minshew, N.J., Keshavan, M.S., Reiss, A. L., Hardan, A.Y., 2014. A preliminary longitudinal volumetric MRI study of amygdala and hippocampal volumes in autism. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 48, 124–128.
Baron-Cohen, S., Ring, H.A., Bullmore, E.T., Wheelwright, S., Ashwin, C., Williams, S.C., 2000. The amygdala theory of autism. Neurosci. Biobehav. Rev. 24, 355–364.
Blatt, G.J., Fatemi, S.H., 2011. Alterations in GABAergic biomarkers in the autism brain: research findings and clinical implications. Anat Rec (Hoboken) 294, 1646–1652.
Bossu, J.L., Roux, S., 2019. The valproate model of autism. Med. Sci. (Paris) 35, 236–243.
Bove, M., Ike, K., Eldering, A., Buwalda, B., De Boer, S.F., Morgese, M.G., Schiavone, S., Cuomo, V., Trabace, L., Kas, M.J.H., 2018a. The visible burrow system: A behavioral

paradigm to assess sociability and social withdrawal in BTBR and C57BL/6J mice strains. Behav. Brain Res. 344, 9–19.
Bove, M., Mhillaj, E., Tucci, P., Giardino, I., Schiavone, S., Morgese, M.G., Trabace, L., 2018b. Effects of n-3 PUFA enriched and n-3 PUFA deficient diets in naive and Abeta-treated female rats. Biochem. Pharmacol. 155, 326–335.
Bove, M., Tucci, P., Dimonte, S., Trabace, L., Schiavone, S., Morgese, M.G., 2020. Postnatal antioxidant and anti-inflammatory treatments prevent early ketamine-induced cortical dysfunctions in adult mice. Front. Neurosci. 14, 590088.
Bristot Silvestrin, R., Bambini-Junior, V., Galland, F., Daniele Bobermim, L., Quincozes-Santos, A., Torres Abib, R., Zanotto, C., Batassini, C., Brolese, G., Goncalves, C.A., Riesgo, R., Gottfried, C., 2013. Animal model of autism induced by prenatal exposure to valproate: altered glutamate metabolism in the hippocampus. Brain Res. 1495, 52–60.
Bronzuoli, M.R., Facchinetti, R., Ingrassia, D., Sarvadio, M., Schiavi, S., Steardo, L., Verkhratsky, A., Trezza, V., Scuderi, C., 2018. Neuroglia in the autistic brain: evidence from a preclinical model. Mol Autism 9, 66.
Bryn, V., Halvorsen, B., Ueland, T., Isaksen, J., Kolkova, K., Ravn, K., Skjeldal, O.H., 2015. Brain derived neurotrophic factor (BDNF) and autism spectrum disorders (ASD) in childhood. Eur. J. Paediatr. Neurol. 19, 411–414.
Cadinu, D., Grayson, B., Podda, G., Harte, M.K., Doostdar, N., Neill, J.C., 2018. NMDA receptor antagonist rodent models for cognition in schizophrenia and identification of novel drug treatments, an update. Neuropharmacology 142, 41–62.
Cai, Y., Wang, L., Nalvarte, I., Xiao, R., Li, X., Fan, X., 2019. Citalopram attenuates social behavior deficits in the BTBR T(+)Itpr3(tf)/J mouse model of autism. Brain Res. Bull. 150, 75–85.
Carlson, G.C., 2012. Glutamate receptor dysfunction and drug targets across models of autism spectrum disorders. Pharmacol. Biochem. Behav. 100, 850–854.
Carroll, L.S., Owen, M.J., 2009. Genetic overlap between autism, schizophrenia and bipolar disorder. Genome Med 1, 102.
Chaddad, A., Desrosiers, C., Hassan, L., Tanougast, C., 2017a. Hippocampus and amygdala radiomic biomarkers for the study of autism spectrum disorder. BMC Neurosci. 18, 52.
Chaddad, A., Desrosiers, C., Toews, M., 2017b. Multi-scale radiomic analysis of sub-cortical regions in MRI related to autism, gender and age. Sci. Rep. 7, 45639.
Chang, Y.C., Cole, T.B., Costa, L.G., 2017. Behavioral phenotyping for autism Spectrum disorders in mice. Curr Protoc Toxicol 72 (11 22 11-11 22 21).
Chao, H.T., Chen, H., Samaco, R.C., Xue, M., Chahrour, M., Yoo, J., Neul, J.L., Gong, S., Lu, H.C., Heintz, N., Ekker, M., Rubenstein, J.L., Noebels, J.L., Rosenmund, C., Zoghbi, H.Y., 2010. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. Nature 468, 263–269.
Codagnone, M.G., Podesta, M.F., Uccelli, N.A., Reines, A., 2015. Differential local connectivity and Neuroinflammation profiles in the medial prefrontal cortex and Hippocampus in the Valproic acid rat model of autism. Dev. Neurosci. 37, 215–231.
Crawley, J.N., 2004. Designing mouse behavioral tasks relevant to autistic-like behaviors. Ment. Retard. Dev. Disabil. Res. Rev. 10, 248–258.
Crespi, B.J., 2019. Comparative psychopharmacology of autism and psychotic-affective disorders suggests new targets for treatment. Evol Med Public Health 2019, 149–168.
Deutsch, S.I., Luyo, Z.N.M., Burket, J.A., 2022. Targeted NMDA receptor interventions for autism: developmentally determined expression of GluN2B and GluN2A-containing receptors and balanced allosteric modulatory approaches. Biomolecules 12.
Elsabbagh, M., Johnson, M.H., 2016. Autism and the social brain: the first-year puzzle. Biol. Psychiatry 80, 94–99.
Estanislau, C., Veloso, A.W.N., Filgueiras, G.B., Maio, T.P., Dal-Col, M.L.C., Cunha, D.C., Klein, R., Carmona, L.F., Fernandez-Teruel, A., 2019. Rat self-grooming and its relationships with anxiety, dearousal and perseveration: evidence for a self-grooming trait. Physiol. Behav. 209, 112585.
Fatemi, S.H., Halt, A.R., Realmuto, G., Earle, J., Kist, D.A., Thuras, P., Merz, A., 2002a. Purkinje cell size is reduced in cerebellum of patients with autism. Cell. Mol. Neurobiol. 22, 171–175.
Fatemi, S.H., Halt, A.R., Stary, J.M., Kanodia, R., Schulz, S.C., Realmuto, G.R., 2002b. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. Biol. Psychiatry 52, 805–810.
Felix-Ortiz, A.C., Burgos-Robles, A., Bhagat, N.D., Leppla, C.A., Tye, K.M., 2016. Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex. Neuroscience 321, 197–209.
Frohlich, J., Van Horn, J.D., 2014. Reviewing the ketamine model for schizophrenia. J. Psychopharmacol. 28, 287–302.
Fuentealba, C.R., Fiedler, J.L., Peralta, F.A., Avalos, A.M., Aguayo, F.I., Morgado-Gallardo, K.P., Aliaga, E.E., 2019. Region-specific reduction of BDNF protein and transcripts in the Hippocampus of juvenile rats prenatally treated with sodium valproate. Front. Mol. Neurosci. 12, 261.
Gandal, M.J., Anderson, R.L., Billingslea, E.N., Carlson, G.C., Roberts, T.P., Siegel, S.J., 2012. Mice with reduced NMDA receptor expression: more consistent with autism than schizophrenia? Genes Brain Behav 11, 740–750.
Guo, D., Peng, Y., Wang, L., Sun, X., Wang, X., Liang, C., Yang, X., Li, S., Xu, J., Ye, W.C., Jiang, B., Shi, L., 2021. Autism-like social deficit generated by Dock4 deficiency is rescued by restoration of Rac1 activity and NMDA receptor function. Mol. Psychiatry 26 (5), 1505–1519. https://doi.org/10.1038/s41380-019-0472-7.
Hashimoto, K., Iwata, Y., Nakamura, K., Tsujii, M., Tsuchiya, K.J., Sekine, Y., Suzuki, K., Minabe, Y., Takei, N., Iyo, M., Mori, N., 2006. Reduced serum levels of brain-derived neurotrophic factor in adult male patients with autism. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 30, 1529–1531.
Hellings, J.A., Arnold, L.E., Han, J.C., 2017. Dopamine antagonists for treatment resistance in autism spectrum disorders: review and focus on BDNF stimulators loxapine and amitriptyline. Expert. Opin. Pharmacother. 18, 581–588.
Horder, J., Petrinovic, M.M., Mendez, M.A., Bruns, A., Takumi, T., Spooren, W., Barker, G.J., Kunnecke, B., Murphy, D.G., 2018. Glutamate and GABA in autism spectrum disorder-a translational magnetic resonance spectroscopy study in man and rodent models. Transl. Psychiatry 8, 106.
Iwata, K., 2019. Characterization of an animal model of autism and social interaction. Methods Mol. Biol. 1916, 149–155.
Kazdoba, T.M., Leach, P.T., Yang, M., Silverman, J.L., Solomon, M., Crawley, J.N., 2016. Translational mouse models of autism: advancing toward pharmacological therapeutics. Curr. Top. Behav. Neurosci. 28, 1–52.
Khanzada, N.S., Butler, M.G., Manzardo, A.M., 2017. GeneAnalytics pathway analysis and genetic overlap among autism Spectrum disorder, bipolar disorder and schizophrenia. Int. J. Mol. Sci. 18.
Kolb, B., Whishaw, I.Q., 1989. Plasticity in the neocortex: mechanisms underlying recovery from early brain damage. Prog. Neurobiol. 32, 235–276.
Kraeuter, A.K., Guest, P.C., Sarnyai, Z., 2019. Free dyadic social interaction test in mice. Methods Mol. Biol. 1916, 93–97.
Laurence, J.A., Fatemi, S.H., 2005. Glial fibrillary acidic protein is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects. Cerebellum 4, 206–210.
Laviola, G., Adriani, W., Rea, M., Aloe, L., Alleva, E., 2004. Social withdrawal, neophobia, and stereotyped behavior in developing rats exposed to neonatal asphyxia. Psychopharmacology 175, 196–205.
Lee, E., Lee, J., Kim, E., 2017. Excitation/inhibition imbalance in animal models of autism Spectrum disorders. Biol. Psychiatry 81, 838–847.
Lim, C.K., Essa, M.M., De Paula Martins, R., Lovejoy, D.B., Bilgin, A.A., Waly, M.I., Al-Farsi, Y.M., Al-Sharbati, M., Al-Shaffae, M.A., Guillemin, G.J., 2016. Altered kynurenine pathway metabolism in autism: implication for immune-induced glutamatergic activity. Autism Res. 9, 621–631.
Lugo, J.N., Smith, G.D., Arbuckle, E.P., White, J., Holley, A.J., Floruta, C.M., Ahmed, N., Gomez, M.C., Okonkwo, O., 2014. Deletion of PTEN produces autism-like behavioral deficits and alterations in synaptic proteins. Front. Mol. Neurosci. 7, 27.
Makanjuola, R.O., Hill, G., Dow, R.C., Campbell, G., Ashcroft, G.W., 1977a. The effects of psychotropic drugs on exploratory and stereotyped behaviour of rats studied on a hole-board. Psychopharmacology 55, 67–74.
Makanjuola, R.O., Hill, G., Maben, I., Dow, R.C., Ashcroft, G.W., 1977b. An automated method for studying exploratory and stereotyped behaviour in rats. Psychopharmacology 52, 271–277.
Mcfarlane, H.G., Kusek, G.K., Yang, M., Phoenix, J.L., Bolivar, V.J., Crawley, J.N., 2008. Autism-like behavioral phenotypes in BTBR T+tf/J mice. Genes Brain Behav 7, 152–163.
Meeking, M.M., Macfabe, D.F., Mepham, J.R., Foley, K.A., Tichenoff, L.J., Boon, F.H., Kavaliers, M., Ossenkopp, K.P., 2020. Propionic acid induced behavioural effects of relevance to autism spectrum disorder evaluated in the hole board test with rats. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 97, 109794.
Mele, A., Mantuano, P., De Bellis, M., Rana, F., Sanarica, F., Conte, E., Morgese, M.G., Bove, M., Rolland, J.F., Capogrosso, R.F., Pierno, S., Camerino, G.M., Trabace, L., De Luca, A., 2019. A long-term treatment with taurine prevents cardiac dysfunction in mdx mice. Transl. Res. 204, 82–99.
Meng, W.D., Sun, S.J., Yang, J., Chu, R.X., Tu, W., Liu, Q., 2017. Elevated serum brain-derived neurotrophic factor (BDNF) but not BDNF gene Val66Met polymorphism is associated with autism Spectrum disorders. Mol. Neurobiol. 54, 1167–1172.
Meyza, K.Z., Blanchard, D.C., 2017. The BTBR mouse model of idiopathic autism - current view on mechanisms. Neurosci. Biobehav. Rev. 76, 99–110.
Misra, V., 2014. The social brain network and autism. Ann. Neurosci. 21, 69–73.
Morgan, J.T., Chana, G., Pardo, C.A., Achim, C., Semendeferi, K., Buckwalter, J., Courchesne, E., Everall, I.P., 2010. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol. Psychiatry 68, 368–376.
Moy, S.S., Nadler, J.J., Young, N.B., Perez, A., Holloway, L.P., Barbaro, R.P., Barbaro, J. R., Wilson, L.M., Threadgill, D.W., Lauder, J.M., Magnuson, T.R., Crawley, J.N., 2007. Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. Behav. Brain Res. 176, 4–20.
Moy, S.S., Nadler, J.J., Poe, M.D., Nonneman, R.J., Young, N.B., Koller, B.H., Crawley, J. N., Duncan, G.E., Bodfish, J.W., 2008. Development of a mouse test for repetitive, restricted behaviors: relevance to autism. Behav. Brain Res. 188, 178–194.
Nadeem, A., Ahmad, S.F., Al-Harbi, N.O., Attia, S.M., Bakheet, S.A., Ibrahim, K.E., Alqahtani, F., Alqinyah, M., 2019. Nrf2 activator, sulforaphane ameliorates autism-like symptoms through suppression of Th17 related signaling and rectification of oxidant-antioxidant imbalance in periphery and brain of BTBR T+tf/J mice. Behav. Brain Res. 364, 213–224.
Panksepp, J.B., Jochman, K.A., Kim, J.U., Koy, J.J., Wilson, E.D., Chen, Q., Wilson, C.R., Lahvis, G.P., 2007. Affiliative behavior, ultrasonic communication and social reward are influenced by genetic variation in adolescent mice. PLoS One 2, e351.
Pardo, C.A., Vargas, D.L., Zimmerman, A.W., 2005. Immunity, neuroglia and neuroinflammation in autism. Int Rev Psychiatry 17, 485–495.
Patterson, P.H., 2011. Modeling autistic features in animals. Pediatr. Res. 69, 34R–40R.
Paxinos, G., Franklin, K.B.J., 2019. The Mouse Brain in Stereotaxic Coordinates. Elsevier Academic Press.
Pieretti, S., Mastriota, M., Tucci, P., Battaglia, G., Trabace, L., Nicoletti, F., Scaccianoce, S., 2013. Brain nerve growth factor unbalance induced by anabolic androgenic steroids in rats. Med. Sci. Sports Exerc. 45, 29–35.
Plataki, M.E., Diskos, K., Sougklakos, C., Velissariou, M., Georgilis, A., Stavroulaki, V., Sidiropoulou, K., 2021. 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, 689193.
Pobbe, R.L., Defensor, E.B., Pearson, B.L., Bolivar, V.J., Blanchard, D.C., Blanchard, R.J., 2011. General and social anxiety in the BTBR T+ tf/J mouse strain. Behav. Brain Res. 216, 446–451.
Puts, N.A.J., Wodka, E.L., Harris, A.D., Crocetti, D., Tommerdahl, M., Mostofsky, S.H., Edden, R.A.E., 2017. Reduced GABA and altered somatosensory function in children with autism spectrum disorder. Autism Res. 10, 608–619.
Qin, J., Sui, J., Ni, H., Wang, S., Zhang, F., Zhou, Z., Tian, L., 2019. The shared and distinct White matter networks between drug-naive patients with obsessive-compulsive disorder and schizophrenia. Front. Neurosci. 13, 96.
Queen, N.J., Boardman, A.A., Patel, R.S., Siu, J.J., Mo, X., Cao, L., 2020. Environmental enrichment improves metabolic and behavioral health in the BTBR mouse model of autism. Psychoneuroendocrinology 111, 104476.
Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D.F., Colombo, J., Coppola, G., Geyer, M.A., Glanzman, D.L., Marsland, S., Mcsweeney, F.K., Wilson, D. A., Wu, C.F., Thompson, R.F., 2009. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiol. Learn. Mem. 92, 135–138.
Reim, D., Schmeisser, M.J., 2017. Neurotrophic factors in mouse models of autism Spectrum disorder: focus on BDNF and IGF-1. Adv. Anat. Embryol. Cell Biol. 224, 121–134.
Rojas, D.C., 2014. The role of glutamate and its receptors in autism and the use of glutamate receptor antagonists in treatment. J. Neural Transm. (Vienna) 121, 891–905.
Savino, R., Carotenuto, M., Polito, A.N., Di Noia, S., Albenzio, M., Scarinci, A., Ambrosi, A., Sessa, F., Tartaglia, N., Messina, G., 2020. Analyzing the potential biological determinants of autism Spectrum disorder: from Neuroinflammation to the kynurenine pathway. Brain Sci 10.
Schiavone, S., Trabace, L., 2016. Pharmacological targeting of redox regulation systems as new therapeutic approach for psychiatric disorders: A literature overview. Pharmacol. Res. 107, 195–204.
Schiavone, S., Mhillaj, E., Neri, M., Morgese, M.G., Tucci, P., Bove, M., Valentino, M., Di Giovanni, G., Pomara, C., Turillazzi, E., Trabace, L., Cuomo, V., 2017a. Early loss of blood-brain barrier integrity precedes NOX2 elevation in the prefrontal cortex of an animal model of psychosis. Mol. Neurobiol. 54, 2031–2044.
Schiavone, S., Tucci, P., Mhillaj, E., Bove, M., Trabace, L., Morgese, M.G., 2017b. Antidepressant drugs for beta amyloid-induced depression: A new standpoint? Prog. Neuro-Psychopharmacol. Biol. Psychiatry 78, 114–122.
Schiavone, S., Morgese, M.G., Bove, M., Colia, A.L., Maffione, A.B., Tucci, P., Trabace, L., Cuomo, V., 2020. Ketamine administration induces early and persistent neurochemical imbalance and altered NADPH oxidase in mice. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 96, 109750.
Schneider, T., Przewlocki, R., 2005. Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. Neuropsychopharmacology 30, 80–89.
Sedky, A.A., Magdy, Y., 2021. Reduction in TNF alpha and oxidative stress by liraglutide: impact on ketamine-induced cognitive dysfunction and hyperlocomotion in rats. Life Sci. 278, 119523.
Sengar, A.S., Li, H., Zhang, W., Leung, C., Ramani, A.K., Saw, N.M., Wang, Y., Tu, Y., Ross, P.J., Scherer, S.W., Ellis, J., Brudno, M., Jia, Z., Salter, M.W., 2019. Control of Long-term synaptic potentiation and learning by alternative splicing of the NMDA receptor subunit GluN1. Cell Rep. 29 (4285–4294), e4285.
Skogstrand, K., Hagen, C.M., Borbye-Lorenzen, N., Christiansen, M., Bybjerg-Grauholm, J., Baekvad-Hansen, M., Werge, T., Borglum, A., Mors, O., Nordentoft, M., Mortensen, P.B., Hougaard, D.M., 2019. Reduced neonatal brain-derived neurotrophic factor is associated with autism spectrum disorders. Transl. Psychiatry 9, 252.
Souza, L.S., Silva, E.F., Santos, W.B., Asth, L., Lobao-Soares, B., Soares-Rachetti, V.P., Medeiros, I.U., Gavioli, E.C., 2016. Lithium and valproate prevent methylphenidate-induced mania-like behaviors in the hole board test. Neurosci. Lett. 629, 143–148.
Stone, W.S., Iguchi, L., 2011. Do apparent overlaps between schizophrenia and autistic Spectrum disorders reflect superficial similarities or etiological commonalities? N Am J Med Sci (Boston) 4, 124–133.
Teffer, K., Semendeferi, K., 2012. Human prefrontal cortex: evolution, development, and pathology. Prog. Brain Res. 195, 191–218.
Vargas, D.L., Nascimbene, C., Krishnan, C., Zimmerman, A.W., Pardo, C.A., 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann. Neurol. 57, 67–81.
Viberg, H., 2009. Exposure to polybrominated diphenyl ethers 203 and 206 during the neonatal brain growth spurt affects proteins important for normal neurodevelopment in mice. Toxicol. Sci. 109, 306–311.
Volkmar, F.R., 2011. Understanding the social brain in autism. Dev. Psychobiol. 53, 428–434.
Wei, H., Wang, M., Lv, N., Yang, H., Zhao, M., Huang, B., Li, R., 2020. Increased repetitive self-grooming occurs in Pax2 mutant mice generated using CRISPR/Cas9. Behav. Brain Res. 393, 112803.
Wiebe, S., Nagpal, A., Truong, V.T., Park, J., Skalecka, A., He, A.J., Gamache, K., Khoutorsky, A., Gantois, I., Sonenberg, N., 2019. Inhibitory interneurons mediate autism-associated behaviors via 4E-BP2. Proc. Natl. Acad. Sci. U. S. A. 116, 18060–18067.
Witt, N.A., Lee, B., Ghent, K., Zhang, W.Q., Pehrson, A.L., Sanchez, C., Gould, G.G., 2019. Vortioxetine reduces marble burying but only transiently enhances social interaction preference in adult male BTBR T(+)Itpr3(tf)/J mice. ACS Chem. Neurosci. 10, 4319–4327.
Wohr, M., Roullet, F.I., Hung, A.Y., Sheng, M., Crawley, J.N., 2011. Communication impairments in mice lacking Shank1: reduced levels of ultrasonic vocalizations and scent marking behavior. PLoS One 6, e20631.
Xu, Q., Zuo, C., Liao, S., Long, Y., Wang, Y., 2020. Abnormal development pattern of the amygdala and hippocampus from childhood to adulthood with autism. J. Clin. Neurosci. 78, 327–332.
Yamada, K., Wada, E., Wada, K., 2001. Female gastrin-releasing peptide receptor (GRP-R)-deficient mice exhibit altered social preference for male conspecifics: implications for GRP/GRP-R modulation of GABAergic function. Brain Res. 894, 281–287.
Yang, M., Crawley, J.N., 2009. Simple behavioral assessment of mouse olfaction. Curr Protoc Neurosci. https://doi.org/10.1002/0471142301.ns0824s48 (chapter 8, unit 8.24).
Yip, J., Soghomonian, J.J., Blatt, G.J., 2007. Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. Acta Neuropathol. 113, 559–568.