Psychopharmacology (2020) 237:199–208
https://doi.org/10.1007/s00213-019-05357-2

ORIGINAL INVESTIGATION

Improvement of autistic-like behaviors in adult rats prenatally exposed to valproic acid through early suppression of NMDA receptor function

Somayeh Mohammadi[1] · Majid Asadi-Shekaari[2] · Mohsen Basiri[2] · Mahdieh Parvan[3] · Mohammad Shabani[2] · Masoumeh Nozari[3]

Received: 8 February 2019 / Accepted: 2 September 2019 / Published online: 8 October 2019

Abstract

Rationale Autism spectrum disorder (ASD), the fastest growing neurodevelopmental disorder, is characterized by social deficits, repetitive/stereotypic activity, and impaired verbal and nonverbal communication and is commonly diagnosed at early stages of life. Based on the excitatory-inhibitory imbalance theory of autism, some recent animal experiments have reported amelioration in autistic-like phenotypes in adult animals following acute treatment of NMDA antagonists. However, we suggested the neonatal period as a critical period for NMDA antagonist intervention.

Objectives This experiment was designed to determine the role of postnatal MK-801, an NMDA receptor blocker, in the prenatal valproic acid (VPA) rat model of ASD.

Methods The model of autism was induced by subcutaneous administration of valproic acid (600 mg/kg) to pregnant rats at gestational day 12.5. The effects of MK-801 (0.03 mg/kg, from postnatal day 6–10) in correcting ASD-associated behaviors in male offspring were assessed by open-field, three-chambered social interaction tests. Moreover, the nociceptive threshold was measured by tail flick and hot plate. Behavioral tests were performed on PND 55–60. Nissl staining was performed to confirm the safety of 0.03 mg/kg MK-801 for the brain.

Results We reported that MK-801 rescued social deficits, repetitive behaviors (self-grooming), anxiety-related behavior, and the low nociceptive threshold in the VPA-treated rats. Further, histological examination showed that there were no significant differences among all the groups in terms of the neuronal survival rate.

Conclusions Our results showed that postnatal low-dose MK-801 improved ASD-associated behaviors in the VPA-treated rats and that early exposure to NMDA antagonist resulted in permanent changes in adult behavior.

Keywords Autism · Valproic acid · MK-801 · NMDA antagonist · Postnatal period

Introduction

Autism spectrum disorder (ASD) is one of the fastest growing neurodevelopmental disorders in the world with a prevalence rate of roughly 1 in every 59 (1.7%) children and generally develops in the first 3 years of life (Benger et al. 2018). ASD is diagnosed by behavioral symptoms including decreased social interaction, increased repetitive, or stereotypic movements, as well as some non-core features such as self-injury, hyperactivity, and unusual sensory sensitivity to stimulation (Schneider and Przewłocki 2005). The neurodevelopmental hypothesis of autism associates the etiology of autism with early prenatal development and pathological processes that begin before the brain fully matures (Zwaigenbaum et al. 2005). Prenatal valproic acid (VPA) exposure is a risk factor for autism in

Majid Asadi-Shekaari and Masoumeh Nozari contributed equally to this work.

✉ Majid Asadi-Shekaari
majidasadi@kmu.ac.ir

✉ Masoumeh Nozari
Masoumeh.Nozari@gmail.com

[1] Department of Anatomical Sciences, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

[2] Neuroscience Research Center, Neuropharmacology Institute, Kerman University of Medical Sciences, Kerman, Iran

[3] Neuroscience Research Center, Institute of Neuropharmacology, Department of Physiology and Pharmacology, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

humans and a well-known animal model of the disease. Similar to human autistic phenotypes, utero VPA-exposed rats showed defects in communication and had stereotypic behavior (Schneider and Przewłocki 2005).

Imbalance of excitatory and inhibitory (E/I) circuits is considered as a possible etiology of neurodevelopmental disorders like autism (Won et al. 2012; W. S, L. L, A. G, L. S 2012; Kang and Kim 2015). Furthermore, recent researches have suggested that acute pharmacological blockade of *N*-methyl-D-aspartate (NMDA) receptor rescues behavioral deficits in adult rodents prenatally exposed to valproic acid (Kang and Kim 2015; Kumar and Sharma 2016; Kim et al. 2017). Surprisingly, no attempt has been made to find the effect of the neonatal block of the NMDA receptor on behavioral and histological impairment by prenatal VPA exposure. However, the accumulating evidence suggests that many of the behavioral domains associated with autism are commonly diagnosed in early childhood (~ 15 months), and even can be predicted beforehand, as early as infanthood (Dietz et al. 2006; Samango-Sprouse et al. 2015). In addition to g-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the adult brain is known as an excitatory transmitter in the immature brain, and GABAergic neurons express glutamatergic receptors in early stages of development (Tyzio et al. 2006; Ben-Ari et al. 2012). Based on the excitatory-inhibitory imbalance theory of autism and critical roles of GABA and glutamate during early development of neuronal circuits, we hypothesized that neonatal stage is a critical period for pharmacological interventions such as using NMDA antagonists to prevent or reduce the severity of autism-like behavior.

Materials and method

Animals

Female Wistar rats ($n = 10$) from the Neuroscience Research Center (Kerman, Iran) were maintained under standard laboratory conditions at 22 ± 2 °C, with 25 ± 5% relative humidity and on a 12 h-light/dark cycle, with free access to food and water. The animals, with a controlled fertility cycle, were mated overnight and the morning when spermatozoa were found in vaginal secretion was considered as the gestational day (GD 0.5). On GD 12.5, half of the pregnant rats were randomly exposed to valproic acid (dissolved in saline, 600 mg/kg., subcutaneously), which was a gift from the Raha Pharmaceutical Company (Isfahan, Iran). The remaining half of the pregnant rats were subjected to saline (1 cc/kg) (Kerr et al. 2013). Only the male pups were used in this study, and approximately, three to five male pups were born from each dam. They were randomly assigned to four groups ($n = 8$). Group I was control rats whose mothers were subjected to saline on GD 12.5, and which received saline (1 cc/kg, i.p., once per day on PND 6–10). Group II was MK-801-treated rats prenatally exposed to saline and postnatally subjected to MK-801. The drug was purchased from Tocris and dissolved in saline, 0.03 mg/kg, i.p., repeated for 5 days from PND 6 to PND 10. Group III was VPA-exposed rats which received a single subcutaneously dose of 600 mg/kg valproic acid 12.5 days after conception. Group IV was VPA+ MK-801 rats treated prenatally with VPA and postnatally with MK-801, as described above. Mothers were kept with their litters until weaning on postnatal day 21 (PND 21).

Behavioral procedures were performed on PND 55–60, and all the tested animals were at a range of 100–110 g. The experiments were carried out between 08:00 a.m. and 2:00 p.m. The schedules of the animal experiments are summarized in Fig.1.

Ethics statement

All the following experiments and animal care protocols were approved by the Animal Experiment Committee at the Kerman Medical University (Ethics code: IR.KMU.REC.1396.183) and performed in line with the "NIH Guide for the Care and Use of Laboratory Animals."

Experimental procedure

The open-field test

Locomotor and exploratory activity as well as stereotypic behaviors in a novel environment were assessed by an open-field test. Each rat was individually placed in a plexiglass-made box (90 × 90 × 30 cm); subsequently, the activities of the animal were recorded automatically and then analyzed using a video tracking system (the Borj Sanat Iran Company). The floor of the field was divided into 16 equal-sized squares, with the four central squares being defined as a central area and the square of the residual as a peripheral area. Increased locomotor activity in the peripheral area (distance and time spent in the peripheral area) was considered as anxiety-like behavior (Blume et al. 2018).

Vertical locomotor activities (rearing and climbing) and stereotypic behavior (grooming: rubbing the body with paws or mouth and rubbing the head with paws) were scored by experimenters blind to the animal status. The apparatus was located in a dim-light and sound-attenuated room, and all male animals were exposed to open field for 5 min. After each trial, the chamber was cleaned (Nozari et al. 2015).

The three-chambered social interaction test

We performed the three-chambered social interaction test to identify the preventive and protective effect of MK-801 on the reduced social behavior of the VPA-exposed rats. This

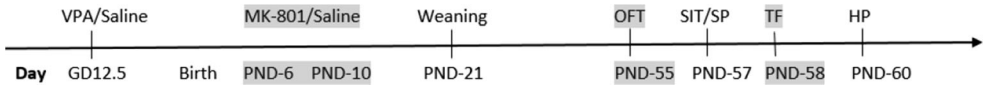


Fig. 1 The diagram for the study schedule. GD, gestational day; PND, postnatal day; OFT, open-field test; SIT, social interaction test; SP, social preference; TF, tail flick; HP, hot plate

behavioral task is based on rodent instinct to explore a novel context. The experimental apparatus consisted of a center chamber (40 × 40 × 50 cm) and two side chambers (40 × 40 × 50 cm), under a light intensity of ~ 120 lx. The utilized test session consisted of three 10-min sessions. The first session was an acclimation period, during which each rat was habituated to the social box. In the second session, known as the sociability phase, a small empty wire cage (14 cm in diameter and 20 cm in length) was placed on one side of the chamber (the empty cage chamber), and an age-matched male rat that had no previous contact with the subject was placed in another wire cage on the other side (the stranger zone 1). Both the left and right chambers were chosen randomly to avoid the side preference chamber. The subject animal was placed in the center chamber and allowed to explore the entire apparatus freely.

Directly after the termination of the second session, the third session was started and lasted for 10 min. This third session was called the social preference phase. In this phase, a novel age- and sex-matched rat (the stranger 2) was added to the empty cage, after which the subject rat was allowed to explore all the three chambers. In this phase, the stranger zone 1 in the second phase was called the familiar chamber while the empty chamber was called the stranger zone 2. The stranger rats were habituated to the wire cages in the three-chamber apparatus for 30 min and 24 h before the test, as described previously (Moy et al. 2004). Wire cage sniffing time, frequency of entry into each compartment, and stay duration in each chamber (chamber time) were measured using a video tracking system (the Borj Sanat Iran Company) during the sociability and social preference phases.

The sociability index (SI) was calculated as the ratio between the time spent by the test animal in the stranger zone 1 to the empty zone; and the social preference index (SPI) was defined as the ratio between the time spent by the test animal in the stranger zone 2 to the familiar side (the stranger zone 1). SI and SPI were also calculated based on sniffing time (time spent actively sniffing the empty cage, familiar animal or unfamiliar animal) and frequency of entry into each compartment (Crawley 2004; Zhang-James et al. 2014; Kang and Kim 2015).

The nociceptive threshold (tail flick and hot plate)

Reduced pain sensitivity appears to be a feature of autism (Nader et al. 2004). The latency of spinal tail flick reflex was evaluated in response to acute thermal noxious stimuli. The procedure was carried out using a tail flick analgesia meter (the Borj Sanat Iran Company). An animal was gently restrained by a restrainer cage and the light emitted from a heat source was directed onto its tail (3 cm from the distal end of the tail). In the meantime, the tail flick latency was recorded. Tail flick measurements were taken three times at 30-s intervals, and the mean value of them was calculated to represent the thermal nociception threshold. The cutoff time was fixed at 9 s to avoid tail injury (Schneider and Przewocki 2001; Schneider and Przewłocki 2005).

The hot plate test was also used to assess the sensitivity to pain. The animal was placed directly on a 52 °C hot plate (the Borj Sanat Iran Company), and the latency to the first reaction to the thermal stimulus was recorded (licking, moving the paws, little leaps or a jump to escape the heat). The cutoff time was 30 s to avoid tissue damage (Schneider and Przewłocki 2005; MMed et al. 2012).

Histology: Nissl staining

The rats were sacrificed on PND 62, and the brains were collected for histological analysis. Briefly, sections were deparaffinized through xylene and alcohols into tap water, stained in a 0.1% cresyl violet solution for 8 min, dehydrated in 95 and 100% alcohols, and then cleared in xylene. For assessment of neurodegeneration, Nissl-stained sections of the frontal association area, a subsection of the medial prefrontal cortex, were evaluated. The area was designated in sagittal sections based on the Paxinos and Watson atlas (Paxinos and Watson 2013). Neurons were counted in four microscopic fields (0.107 mm2; 89.82 × 120.70 μm), and neurons with visible round nucleus, prominent nucleolus, and intact cytoplasm having discernable and rich Nissl staining were counted as viable neurons. However, neurons with shrunken cell bodies and condensed cytoplasm were considered as damaged neurons (Gao et al. 2012; Huang et al. 2018). The neuronal survival rate was estimated as the percentage of intact neurons.

Statistical analysis

We ensured normality using the Shapiro-Wilk test. Data were expressed as the mean ± standard error of the mean (SEM) and analyzed for statistical significance using two-way analysis of variance (ANOVA). Independent variables (fixed factors) were status (saline vs. VPA) and treatment (saline or MK-801). If the interaction between the status and treatment was

significant, then one-way ANOVA was carried out using the Tukey post hoc test for multiple comparisons. When one or both main effects were statistically significant (without the interaction effect), unpaired *t* test was carried out to determine the differences between the means (i.e., the effect of prenatal VPA or postnatal MK801). Differences were considered statistically significant when the *p* value was less than 0.05 ($p < 0.05$). All statistical analyses were conducted using the SPSS software package (23.0; SPSS Inc., Chicago, IL, USA).

Results

The postnatal MK-801 treatment alleviating repetitive/stereotypic-like activities and anxiety-like behaviors in the VPA-exposed rats

We studied the therapeutic effect of MK-801 in the stereotypical self-grooming behavior, which are the core symptoms of ASD. The two-way ANOVA revealed significant effects of the both treatments (saline or MK-801) [$F(1,28) = 24.37$, $p < 0.001$] and treatment status interaction [$F(1,28) = 14.12$, $p = 0.001$] without a significant effect of status (saline vs. VPA) [$F(1,28) = 0.26$, $p = 0.61$] on grooming. Moreover, a one-way ANOVA conducted on the main effect of the prenatal VPA and postnatal MK-801 treatment indicated that the VPA-exposed animals exhibited significantly increased the number of grooming compared to the control group ($p = 0.026$). The excessive grooming behavior in the VPA-treated group was significantly corrected by MK-801 ($p < 0.001$; the VPA+MK-801 rats compared to the VPA-treated rats, Fig. 2a).

Two-way ANOVA showed a significant effect of status [$F(1,28) = 17.35$, $p < 0.001$] on rearing while the treatment and the interaction effect between the treatment and status were not significant [treatment: $F(1,28) = 0.71$, $p = 0.40$; treatment status interaction: $F(1,28) = 0.34$, $p = 0.56$]. Analysis with two-tailed unpaired *t* test revealed that prenatal VPA treatment significantly increased the rearing number ($p < 0.001$, Fig. 2b).

In the open-field test, less entry into the center of the arena is indicative of enhanced anxiety-related responses (Blume et al. 2018). The two-way ANOVA of the time spent at the center in the open-field test showed significant effects of both the status and treatment [status $F(1,28) = 26.94$, $p < 0.001$, treatment $F(1,28 = 55.49$, $p < 0.05$; status treatment interaction $F(1,28) = 9.59$, $p < 0.01$]. A one-way ANOVA demonstrated that the VPA-treated rats spent significantly less time ($p < 0.001$) at the center of the arena compared to the control groups. The MK-801 treatment rescued anxiety behaviors in the VPA-exposed group [(the VPA group compared to the VPA+MK-801 rats ($p = 0.003$) and VPA+MK-801 compared to the control animals; ($p > 0.05$, Fig. 2c)]. Two-way ANOVA for total distance traveled in the center of the open field showed a significant main effects of prenatal VPA administration [$F(1,28) = 42.14$, $p < .0.001$] and postnatal MK-801 treatment [$F(1,28) = 12.48$, $p > 0.001$], whereas there was no significant interaction between these factors [$F(1,28) = 0.01$, $p > 0.05$]. Prenatal VPA exposure significantly decreased the traveled distance in the center of the open field (*t* test, $p < 0.001$, Fig. 2d) compared to prenatal saline exposure. Postnatal treatment with low-dose of MK-801 increased this variable (*t* test, $p < 0.05$, Fig. 2e).

There was no significant difference for total distance traveled between the groups ($p > 0.05$; data not shown).

The postnatal MK-801 treatment rescuing the social impairment in the VPA-exposed rats

In the first session, SI was measured as the ratio of duration in the stranger zone to duration in the empty side. Two-way ANOVA revealed a significant status difference, [$F(1, 28) = 8.00$, $p = 0.009$], and interaction between status × treatment $F(1,28) = 26.86$, $p < 0.001$, but no significant effect for the treatment $F(1,28) = 0.12$, $p = 0.72$). Overall, SI decreased by VPA exposure (Fig. 3a; $p < 0.001$), representing autism-like behaviors. The VPA+MK-801 group spent more time in encountering a strange rat than an empty cage (Fig. 3a; $p < 0.01$; the VPA+MK-801 group compared with the VPA-treated rats).

After adding a new rat in the empty wire cage, SPI was also calculated, which showed whether the experiment rats socially preferred an unfamiliar rat or a familiar one. In the SPI analysis, there was a main effect for status on SPI, [$F(1,28) = 22.83$, $p < 0.001$], but not for treatment [$F(1,28) = 1.18$, $p = 0.28$)]. Moreover, there was a significant interaction between the status and treatment ($F(1,28) = 15.18$, $p < 0.001$), and post hoc comparisons demonstrated that the VPA-treated animals showed a low significant curiosity to the unfamiliar rats compared to the control group (Fig. 3b; $p < 0.001$). However, this aberrant behavior was relatively improved by the postnatal MK-801 treatment (Fig. 3b; $p < 0.001$ VPA+MK-801 compared to the control group, and $p < 0.05$ VPA+MK-801 compared to the VPA group). SI and SPI significantly decreased in the MK-801-treated group (Fig. 3 a and b; $p < 0.01$ MK-801 compared to the control group), but the effect of MK-801 in the VPA group was completely different, leading to increased SPI value in the VPA-treated animals.

SI and SPI were also calculated based on sniffing time (time spent actively sniffing the empty cage, familiar animal or unfamiliar animal) and frequency of entry into each compartment. Two-way ANOVA revealed the effect of the status [sniffing SI: $F(1,28) = 8.86$, $p < 0.01$; sniffing SPI: $F(1,28) = 29.66$, $p < 0.001$] and the status × treatment interaction [sniffing SI: $F(1,28) = 7.79$, $p < 0.01$; sniffing SPI: $F(1,28) = 40.64$, $p < 0.001$]. Analysis of the data collected from the frequency of entry into each compartment revealed that the effect

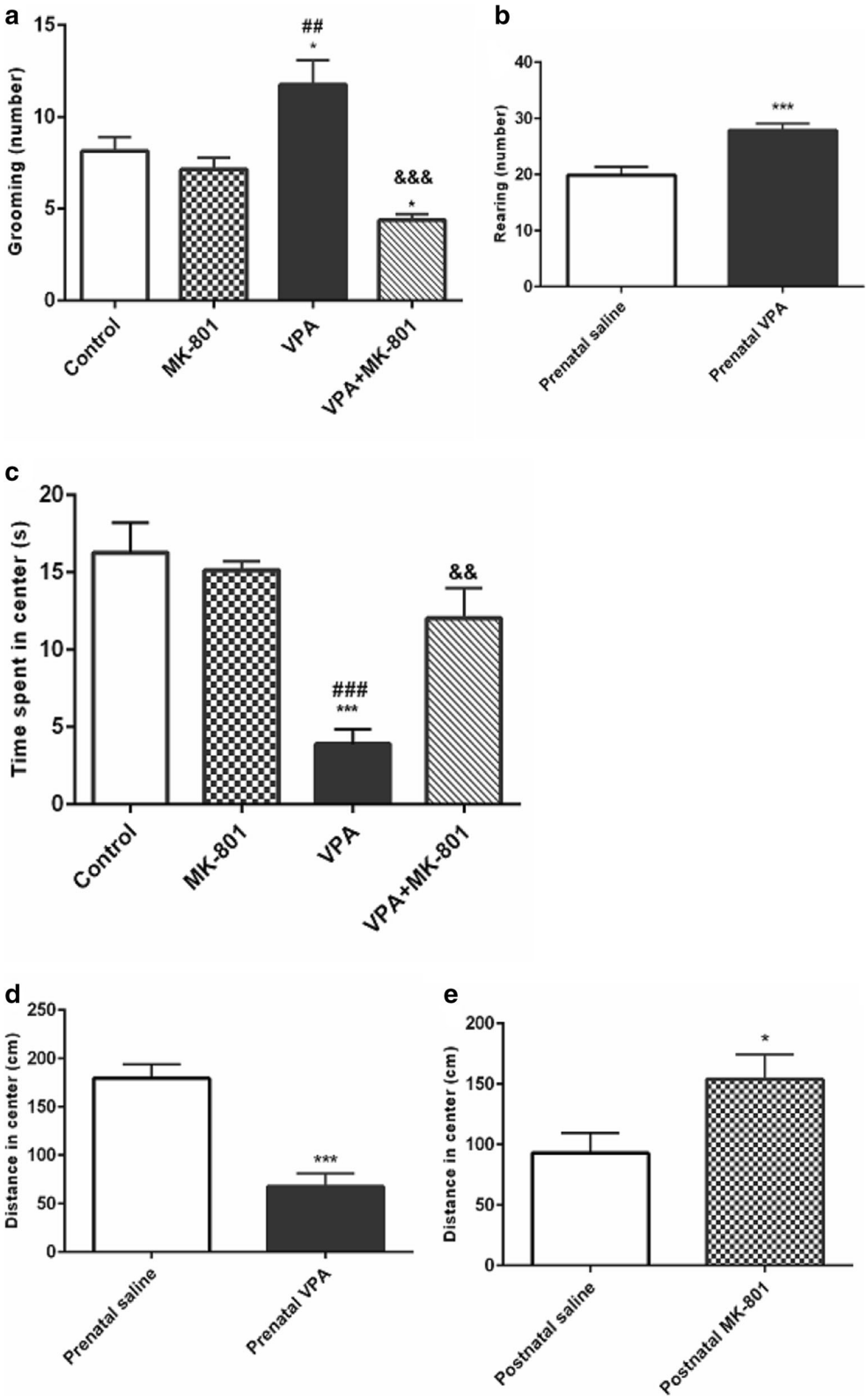
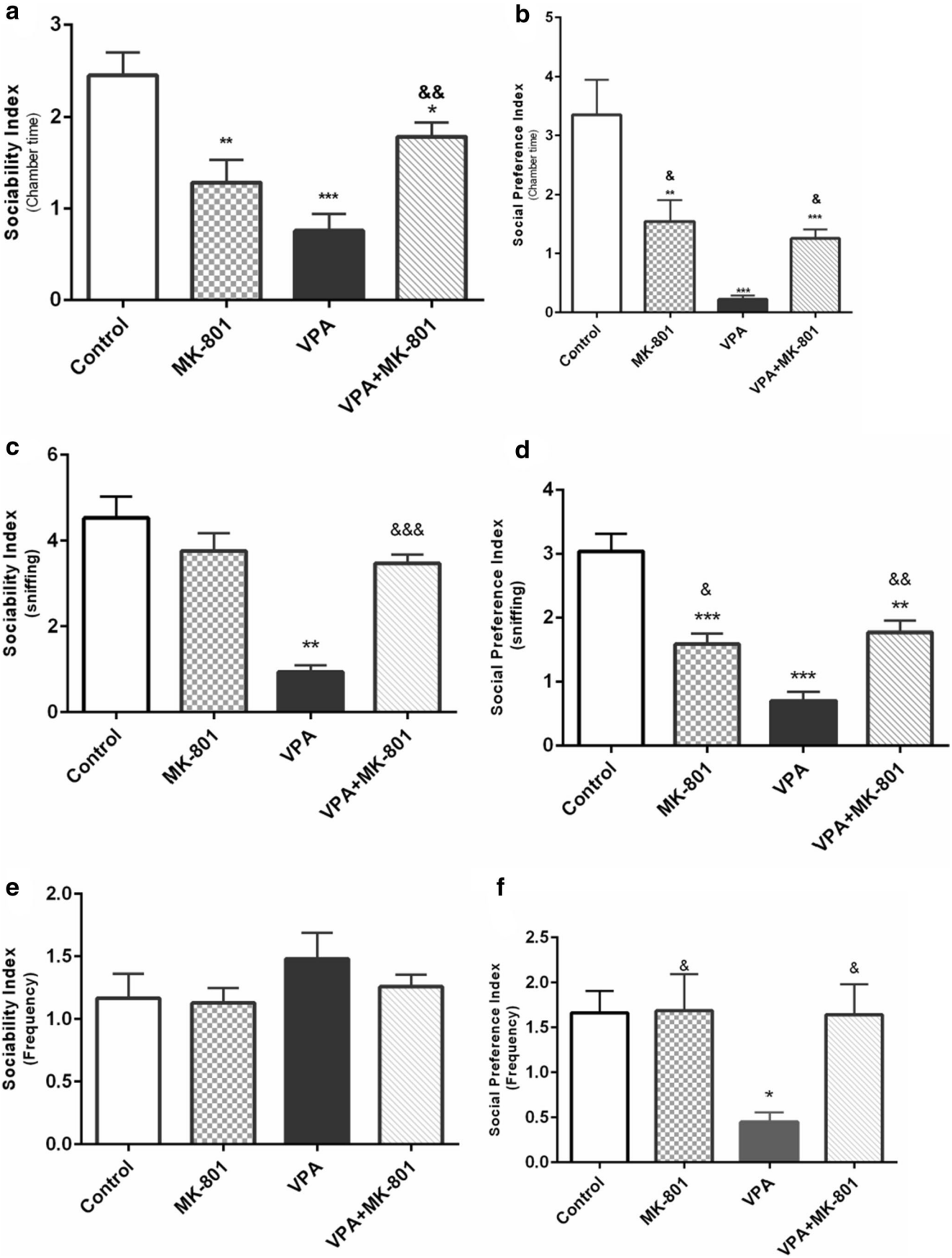


Fig. 2 MK-801 corrected the stereotypic and anxiety behaviors in the VPA-exposed rats. Self-grooming test (**a**, $n = 8$), rearing (**b**, $n = 16$), time spent in the center (**c**, n = 8), and traveled distance in the center (**d**, **e**, $n = 16$). *, **, ***, $p < 0.05$, 0.01, 0.001 vs. control group, $^{\#\#, \#\#\#}$, 0.01, 0.001 vs. MK-801 group, $^{\&\&, \&\&\&}$, 0.01, 0.001 vs. VPA group. The data were expressed as mean ± SEM

of the status [SI: $F(1,28) = 2.85$, $p = 0.10$; SPI: $F(1,28) = 3.59$, $p = 0.06$], treatment [SI: $F(1,28) = 1.72$, $p = 0.20$; SPI: $F(1,28) = 3.29$, $p = 0.08$], and status × treatment interaction [SI: $F(1,28) = 1.33$, $p = 0.25$; SPI: $F(1,28) = 12.31$, $p < 0.01$]. Post hoc analysis indicated that VPA decreased SI derived from the sniffing parameters (Fig. 3c; $p < 0.01$) and did not have any effect on SI derived from the frequency of entry into each compartment (Fig. 3e; $p > 0.05$).


a
Sociability Index
(Chamber time)
3
2
1
0
**
***
&&
*
Control
MK-801
VPA
VPA+MK-801
b
Social Preference Index
(Chamber time)
5
4
3
2
1
0
&
**
***
&
***
Control
MK-801
VPA
VPA+MK-801
c
Sociability Index
(sniffing)
6
4
2
0
**
&&&
Control
MK-801
VPA
VPA+MK-801
d
Social Preference Index
(sniffing)
4
3
2
1
0
&
***
***
&&
**
Control
MK-801
VPA
VPA+MK-801
e
Sociability Index
(Frequency)
2.0
1.5
1.0
0.5
0.0
Control
MK-801
VPA
VPA+MK-801
f
Social Preference Index
(Frequency)
2.5
2.0
1.5
1.0
0.5
0.0
&
*
&
Control
MK-801
VPA
VPA+MK-801

◀ **Fig. 3** The MK-801 restored social impairment in the VPA-exposed rats. The sociability and social preference tests were performed based on the total duration in each chamber (**a**, **b**), sniffing time (**c**, **d**), and number of entrance to each compartment (**e**, **f**). All the data were expressed as mean ± SEM ($n = 8$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control group; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ vs. VPA-treated group

Sniffing SPI decreased both by VPA exposure and MK-801 [Fig. 3d; $F(3,31) = 23.73$, $p < 0.001$]. However, VPA+MK-801 and MK-801 rats had more SPI than the VPA-treated animals [Fig. 3d; $p < 0.05$ in VPA compared to MK-801 and $p < 0.01$ in VPA compared to VPA+MK-801]. VPA-exposed rats had the lowest frequency of entry into the familiar zone [Fig. 3f; $F(3,31) = 4.22$, $p < 0.05$].

The postnatal MK-801 treatment improving nociceptive response (hypoalgesia) in the VPA-exposed rats

Two-way ANOVA was conducted separately for the nociceptive threshold in tail flick (latency to the first reaction) and the hot plate test (the thermal stimulus). The analysis indicated the effect of the status [tail flick: $F(1,28) = 62.57$, $p < 0.001$; hot plate: $F(1,28) = 34.46$, $p < 0.001$], treatment [tail flick: $F(1,28) = 29.93$, $p < 0.001$; hot plate: $F(1,28) = 14.88$, $p = 0.001$], and status × treatment interaction [tail flick: $F(1,28) = 73.18$, $p < 0.001$; hot plate: $F(1,28) = 40.37$, $p < 0.001$].

In the tail flick test, the VPA-treated rats presented a higher nociceptive threshold than the control group (Fig. 4a; $p < 0.001$). Treatment with MK-801 decreased the nociceptive threshold of the VPA-treated animals (Fig. 4a; $p < 0.001$).

In the hot plate test, we found a significant increase in latency to the first reaction to the thermal stimulus in the prenatal VPA-exposed rats in comparison to the control (Fig. 4b; $p < 0.001$). Reduced latency was found following the treatment with MK-801 as compared with the VPA group (VPA+MK-801 compared to VPA; $p < 0.001$; Fig. 2).

The neuronal survival rate

The percentage of the intact neurons was named the neuronal survival rate. Statistical analysis of this parameter showed that there were no significant differences in terms of interaction between the status and treatment [$F(1,32) = 3.36$, $p = 0.07$]; with significant differences between the status: $F(1,32) = 4.83$, $p = 0.03$ and the treatment: [$F(1,32) = 4.88$, $p = 0.03$]. The survival rate was 81.61 ± 2.79 in the control group; 80.95 ± 0.93 in the MK-801 group; 88.79 ± 0.97 in the VPA group; and 81.60 ± 1.73 in the VPA+MK-801 group (Fig. 5).

Discussion

In this study, we demonstrated transient low-dose exposure to MK-801 during the neonatal period to alleviate the autism-like behavioral alterations in rats prenatally exposed to VPA. Previous studies have shown that prenatal VPA treatment increases repetitive, stereotyped behavior as an ASD core symptom. In our study, the VPA-exposed rats showed increased self-grooming and exploratory behavior (vertical locomotor activity: number of rearing and climbing); MK-801 rescued self-grooming behavior without a change in exploratory rearing and locomotor activity. The VPA-treated animals also showed more anxiety-related behavior and less nociceptive threshold value than control animals, as non-core symptoms of ASD. MK-801 had the therapeutic anxiolytic effects and increased the nociception threshold. Kim et al. (2017) reported that the administration of a single dose of agmatine, 30 min before behavioral experiments, improved the impaired social interaction, hyperactive, and repetitive behaviors in a VPA rat model (Kim et al. 2017). They did not observe any therapeutic effects of the NMDA antagonist on abnormal anxiety behavior in the open-field test. Before that, Kumar and Sharma (2016), Kang (2015), and Kim et al. (2014) reported therapeutic effects of acute NMDA antagonist administration on autism-like behavior in prenatally VPA-exposed rats (Kim et al. 2014; Kang and Kim 2015; Kumar and Sharma 2016).

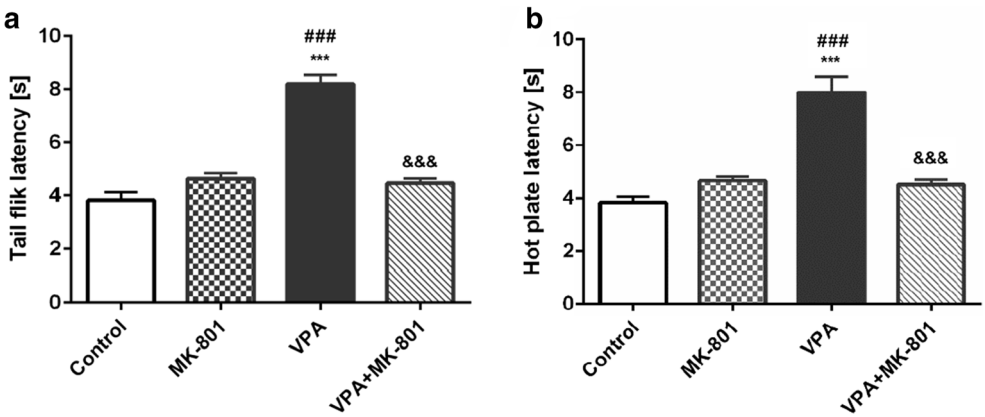


Fig. 4 The effect of MK-801 on the thermal nociceptive threshold in rats prenatally exposed to VPA; tail flick (**a**) and hot-plate test (**b**). Data were expressed as mean ± SEM, $n = 8$ per group. ***$p < 0.001$ vs. control, $^{\&\&\&}p < 0.001$ vs. VPA, $^{\#\#\#}p < 0.001$ vs. MK-801

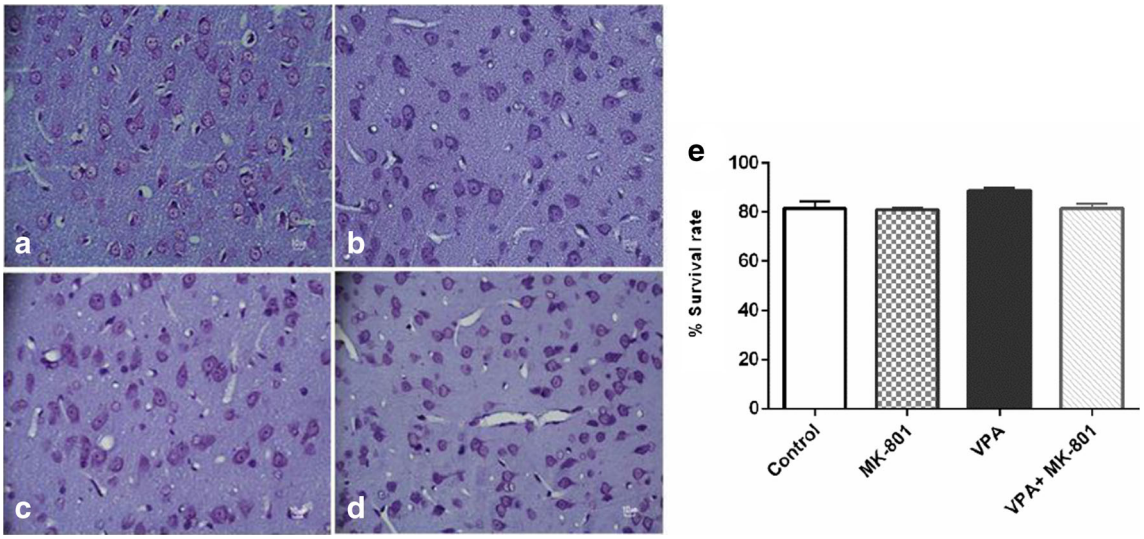


Fig. 5 Representative photomicrographs showing prefrontal cortex neurons of rats in different groups (**a**, **b**, **c**, **d**). The normal morphology of neurons including round nucleus with prominent nucleolus and light cytoplasm is visible in the figure. **a** control, **b** MK-801, **c** VPA, **d** VPA+ MK-801. Scale bar 10 μm. MK-801 did not induce insults to the neurons (**e**)

All of these studies were designed in the same way (acute injection, in adult age) to confirm the excitatory-inhibitory imbalance theory of autism.

It is well accepted that the early postnatal period is a critical period for structural, functional, and behavioral cortical circuit development (Hensch 2004). In the immature brain, GABA is the primary excitatory neurotransmitter, and GABAergic neurons express glutamatergic receptors. During fetal and postnatal periods, there is an excitatory-to-inhibitory switch of GABA actions (Herlenius and Lagercrantz 2004; Ben-Ari et al. 2012). Moreover, previous studies reported that early exposure to NMDA antagonist caused many long-lasting changes in behavior and different neurotransmitter systems (Lim et al. 2012). Therefore, we hypothesized that this critical period could be more effective than an acute injection of NMDA antagonist in rescue autism-like behavior through balancing excitatory-inhibitory circuits. However, the precise exploration of the optimal therapeutic time windows and dosage is necessary given that postnatal NMDA receptor blockade induces schizophrenia-like symptoms. In this study, we showed that using low-dose MK-801 (0.03 mg/kg) during P6-P10 improved behavioral deficits in the VPA-treated animals. In the rodent brain, the maximum expression of NMDA receptors occurs in the early weeks after birth, especially P7–P14 (du Bois and Huang 2007). Previous studies showed that low-dose of MK-801 in this time window could induce schizophrenia-like symptoms (Ikonomidou et al. 1999; Latysheva and Rayevsky 2003; Lim et al. 2012). Consistent with our reports, Chung et al. (2018) demonstrated that early correction of NMDA receptor hyperfunction rescued autistic-like behaviors in adult Shank2 mutant mice (Chung et al. 2018). Although our study did not thoroughly examine the underlying mechanism of the effects of MK-801 on repetitive behaviors, there were previous reports indicating that the interruption of cortico-striatal-thalamic loop circuits by MK-801 could selectively reduce repetitive behaviors (Presti et al. 2003; Lewis et al. 2007). Along with repetitive behavior, perturbed social interaction was improved by MK-801, suggesting the possible regulatory role of autism-like behaviors by the NMDA pathway. Moreover, recent research has suggested that the AMPA receptor can also be a potential candidate for the treatment of the social behavior deficits associated with ASD (Kim et al. 2019). Despite the protective effect of MK-801 in the VPA-treated animals, it disrupted SI and preference in the control rats, demonstrating that NMDA blockers had different effects on VPA and intact animals in terms of social interaction. Further investigation into modulating the dosage is necessary in order to eliminate its impact on intact animals.

Lower sensitivity to pain including both spinal (tail flick) and supraspinal (hot plate) levels was observed in this study. Although patients with autism reacted differently to pain (Baronio et al. 2015), the VPA model of autism shows less sensitivity to pain including both spinal and supraspinal levels, similar to what was observed in this study (Padurariu et al. 2017). The mechanism by which postnatal MK-801 treatment induces long-lasting effects on pain sensitivity should be further investigated in the future.

Conclusion

In conclusion, our results provided experimental evidence that early suppression of NMDAR can prevent autistic-like behavioral in the VPA rat model of ASD. Such beneficial effects of MK-801 may suggest consideration of NMDA blockers as the

preferential choice for anesthesia in children with ASD or autism family history.

Funding information This research article is a part of the first author's M.Sc. thesis. Funding for this study was provided by Kerman University of Medical Sciences, Kerman, Iran (Grant ID: 96000183).

Compliance with ethical standards

All the following experiments and animal care protocols were approved by the Animal Experiment Committee at the Kerman Medical University (Ethics code: IR.KMU.REC.1396.183) and performed in line with the "NIH Guide for the Care and Use of Laboratory Animals."

Conflict of interest The authors declare that there are no conflicts of interest.

References

Baronio D, Castro K, Gonchoroski T, de Melo GM, Nunes GDF, Bambini-Junior V, Gottfried C, Riesgo R (2015) Effects of an H3R antagonist on the animal model of autism induced by prenatal exposure to valproic acid. PLoS One 10:e0116363. https://doi.org/10.1371/journal.pone.0116363

Ben-Ari Y, Khalilov I, Kahle KT, Cherubini E (2012) The GABA excitatory/inhibitory shift in brain maturation and neurological disorders. Neurosci 18:467–486. https://doi.org/10.1177/1073858412438697

Benger M, Kinali M, Mazarakis ND (2018) Autism spectrum disorder: prospects for treatment using gene therapy. Mol Autism 9:39. https://doi.org/10.1186/s13229-018-0222-8

Blume SR, Nam H, Luz S, Bangasser DA, Bhatnagar S (2018) Sex- and age-dependent effects of orexin 1 receptor blockade on open-field behavior and neuronal activity. Neuroscience 381:11–21. https://doi.org/10.1016/j.neuroscience.2018.04.005

Chung C, Ha S, Kang H, Lee J, Um SM, Yan H, Yoo YE, Yoo T, Jung H, Lee D, Lee E, Lee S, Kim J, Kim R, Kwon Y, Kim W, Kim H, Duffney L, Kim D, Mah W, Won H, Mo S, Kim JY, Lim CS, Kaang BK, Boeckers TM, Chung Y, Kim H, Jiang YH, Kim E (2018) Early correction of N-methyl-D-aspartate receptor function improves autistic-like social behaviors in adult Shank2-/- mice. Biol Psychiatry 85:534–543. https://doi.org/10.1016/j.biopsych.2018.09.025

Crawley JN (2004) Designing mouse behavioral tasks relevant to autistic-like behaviors. Ment Retard Dev Disabil Res Rev 10:248–258. https://doi.org/10.1002/mrdd.20039

Dietz C, Swinkels S, Van Daalen E et al (2006) Screening for autistic spectrum disorder in children aged 14-15 months. II: population screening with the early screening of autistic traits questionnaire (ESAT). Design and general findings. J Autism Dev Disord 36:713–722. https://doi.org/10.1007/s10803-006-0114-1

du Bois TM, Huang X-F (2007) Early brain development disruption from NMDA receptor hypofunction: relevance to schizophrenia. Brain Res Rev 53:260–270. https://doi.org/10.1016/j.brainresrev.2006.09.001

Gao CJ, Niu L, Ren PC, et al (2012) Hypoxic preconditioning attenuates global cerebral ischemic injury following asphyxial cardiac arrest through regulation of delta opioid receptor system. Pergamon

Hensch TK (2004) Critical period regulation. Annu Rev Neurosci 27:549–579. https://doi.org/10.1146/annurev.neuro.27.070203.144327

Herlenius E, Lagercrantz H (2004) Development of neurotransmitter systems during critical periods. Exp Neurol 190:8–21. https://doi.org/10.1016/J.EXPNEUROL.2004.03.027

Huang K, Wang Z, Gu Y, et al (2018) Glibenclamide prevents water diffusion abnormality in the brain after cardiac arrest in rats. Humana Press Inc.

Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vöckler J, Dikranian K, Tenkova TI, Stefovska V, Turski L, Olney JW (1999) Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 283:70–74

Kang J, Kim E (2015) Suppression of NMDA receptor function in mice prenatally exposed to valproic acid improves social deficits and repetitive behaviors. Front Mol Neurosci 8:17. https://doi.org/10.3389/fnmol.2015.00017

Kerr DM, Downey L, Conboy M, Finn DP, Roche M (2013) Alterations in the endocannabinoid system in the rat valproic acid model of autism. Behav Brain Res 249:124–132. https://doi.org/10.1016/j.bbr.2013.04.043

Kim KC, Lee DK, Go HS, Kim P, Choi CS, Kim JW, Jeon SJ, Song MR, Shin CY (2014) Pax6-dependent cortical glutamatergic neuronal differentiation regulates autism-like behavior in prenatally valproic acid-exposed rat offspring. Mol Neurobiol 49:512–528. https://doi.org/10.1007/s12035-013-8535-2

Kim JW, Seung H, Kim KC, Gonzales ELT, Oh HA, Yang SM, Ko MJ, Han SH, Banerjee S, Shin CY (2017) Agmatine rescues autistic behaviors in the valproic acid-induced animal model of autism. Neuropharmacology 113:71–81. https://doi.org/10.1016/j.neuropharm.2016.09.014

Kim J-W, Park K, Kang RJ, Gonzales ELT, Kim DG, Oh HA, Seung H, Ko MJ, Kwon KJ, Kim KC, Lee SH, Chung CH, Shin CY (2019) Pharmacological modulation of AMPA receptor rescues social impairments in animal models of autism. Neuropsychopharmacology 44:314–323. https://doi.org/10.1038/s41386-018-0098-5

Kumar H, Sharma B (2016) Memantine ameliorates autistic behavior, biochemistry & blood brain barrier impairments in rats. Brain Res Bull 124:27–39. https://doi.org/10.1016/j.brainresbull.2016.03.013

Latysheva NV, Rayevsky KS (2003) Chronic neonatal N-methyl-D-aspartate receptor blockade induces learning deficits and transient hypoactivity in young rats. Prog Neuro-Psychopharmacology Biol Psychiatry 27:787–794. https://doi.org/10.1016/S0278-5846(03)00110-6

Lewis MH, Tanimura Y, Lee LW, Bodfish JW (2007) Animal models of restricted repetitive behavior in autism. Behav Brain Res 176:66–74. https://doi.org/10.1016/j.bbr.2006.08.023

Lim AL, Taylor DA, Malone DT (2012) Consequences of early life MK-801 administration: long-term behavioural effects and relevance to schizophrenia research. Behav Brain Res 227:276–286. https://doi.org/10.1016/j.bbr.2011.10.052

MMed ML, Liu X, Zhang Y, Guo S-W (2012) Valproic acid and progestin inhibit lesion growth and reduce hyperalgesia in experimentally induced endometriosis in rats. Reprod Sci 19:360–373. https://doi.org/10.1177/1933719111424453

Moy SS, Nadler JJ, Perez A, Barbaro RP, Johns JM, Magnuson TR, Piven J, Crawley JN (2004) Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes, Brain Behav 3:287–302. https://doi.org/10.1111/j.1601-1848.2004.00076.x

Nader R, Oberlander TF, Chambers CT, Craig KD (2004) Expression of pain in children with autism. Clin J Pain 20:88–97

Nozari M, Mansouri FAFAFA, Shabani M et al (2015) Postnatal MK-801 treatment of female rats impairs acquisition of working memory, but not reference memory in an eight-arm radial maze; no beneficial effects of enriched environment. Psychopharmacology 232:2541–2550. https://doi.org/10.1007/s00213-015-3890-5

Padurariu M, Antioch I, Ciobica A et al (2017) Intranasal oxytocin in autism: models, pain and oxidative stress. Rev Chim 68

Paxinos G, Watson C (2013) The rat brain in stereotaxic coordinates : hard Cover Edition. Elsevier Science

Presti MF, Mikes HM, Lewis MH (2003) Selective blockade of spontaneous motor stereotypy via intrastriatal pharmacological manipulation. Pharmacol Biochem Behav 74:833–839. https://doi.org/10.1016/S0091-3057(02)01081-X

Samango-Sprouse CA, Stapleton EJ, Aliabadi F, Graw R, Vickers R, Haskell K, Sadeghin T, Jameson R, Parmele CL, Gropman AL (2015) Identification of infants at risk for autism spectrum disorder and developmental language delay prior to 12 months. Autism 19: 327–337. https://doi.org/10.1177/1362361314521329

Schneider T, Przewłocki R (2005) Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. Neuropsychopharmacology 30:80–89. https://doi.org/10.1038/sj.npp.1300518

Schneider T, Przewocki R (2001) Preliminary communication nociceptive changes in rats after prenatal exposure to valproic acid. europepmc.org 531–534

Tyzio R, Cossart R, Khalilov I et al (2006) Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. Science (80-) 314:1788–1792. https://doi.org/10.1126/science.1133212

W. S, L. L, A. G, L. S (2012) Synapse dysfunction in autism: a molecular medicine approach to drug discovery in neurodevelopmental disorders. Trends Pharmacol Sci 33:669–684. https://doi.org/10.1016/j.tips.2012.09.004LK

Won H, Lee H, Gee H, et al (2012) Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. nature.com

Zhang-James Y, Yang L, Middleton FA, Yang L, Patak J, Faraone SV (2014) Autism-related behavioral phenotypes in an inbred rat substrain. Behav Brain Res 269:103–114. https://doi.org/10.1016/j.bbr.2014.04.035

Zwaigenbaum L, Bryson S, Rogers T, Roberts W, Brian J, Szatmari P (2005) Behavioral manifestations of autism in the first year of life. Int J Dev Neurosci 23:143–152. https://doi.org/10.1016/J.IJDEVNEU.2004.05.001