BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 30(4), 320-327 (2022)

Behavioral Deficits in Adolescent Mice after Sub-Chronic Administration of NMDA during Early Stage of Postnatal Development

Keremkleroo Jym Adil[1,†], Chilly Gay Remonde[1,†], Edson Luck Gonzales[1], Kyung-Jun Boo[1], Kyong Ja Kwon[1], Dong Hyun Kim[1], Hee Jin Kim[2], Jae Hoon Cheong[3], Chan Young Shin[1,*] and Se Jin Jeon[1,*]

[1]Department of Pharmacology, School of Medicine, Konkuk University, Seoul 05029,
[2]Uimyung Research Institute for Neuroscience, Department of Pharmacy, Sahmyook University, Seoul 01795,
[3]School of Pharmacy, Jeonbuk National University, Jeonju 54896, Republic of Korea

Abstract

Neurodevelopmental disorders are complex conditions that pose difficulty in the modulation of proper motor, sensory and cognitive function due to dysregulated neuronal development. Previous studies have reported that an imbalance in the excitation/inhibition (E/I) in the brain regulated by glutamatergic and/or GABAergic neurotransmission can cause neurodevelopmental and neuropsychiatric behavioral deficits such as autism spectrum disorder (ASD). NMDA acts as an agonist at the NMDA receptor and imitates the action of the glutamate on that receptor. NMDA however, unlike glutamate, only binds to and regulates the NMDA receptor subtypes and not the other glutamate receptors. This study seeks to determine whether NMDA administration in mice i.e., over-activation of the NMDA system would result in long-lasting behavioral deficits in the adolescent mice. Both gender mice were treated with NMDA or saline at early postnatal developmental period with significant synaptogenesis and synaptic maturation. On postnatal day 28, various behavioral experiments were conducted to assess and identify behavioral characteristics. NMDA-treated mice show social deficits, and repetitive behavior in both gender mice at adolescent periods. However, only the male mice but not female mice showed increased locomotor activity. This study implies that neonatal exposure to NMDA may illicit behavioral features similar to ASD. This study also confirms the validity of the E/I imbalance theory of ASD and that NMDA injection can be used as a pharmacologic model for ASD. Future studies may explore the mechanism behind the gender difference in locomotor activity as well as the human relevance and therapeutic significance of the present findings.

Key Words: Autism spectrum disorder, NMDA, Excitation/inhibition imbalance, Mouse model, Social deficit

INTRODUCTION

Neurodevelopmental disorders are compound conditions that pose difficulty in conceptualizing. One of which is the autism spectrum disorder (ASD), a heterogeneous disorder characterized by impairments in sociability and communication as well as presenting repetitive and patterned behavior (American Psychiatric Association, 2013). Even with the unceasing efforts during the past decades, its pathogenesis is yet to be understood completely. Several studies have identified genetics as an undeniable risk factor for ASD. Studies on twins revealed increased concordance rates especially on monozygotic twins (Rosenberg *et al*., 2009). Broader ASD phenotypes also suggest strong heritability associated with ASD (Kim and Leventhal, 2015). Mutations in genes such as *SHANK3*, *NLGN3*, and *NLGN4* among several others have likewise been implicated in being involved in ASD (Ey *et al*., 2011; Leblond *et al*., 2014). In addition, pre- or early postnatal environmental exposures can significantly increase the risk for ASD (LaSalle, 2013; Kim and Leventhal, 2015; Zerbo *et al*., 2015; Sealey *et al*., 2016).

One of the most recognized theories in ASD is the excitation/inhibition (E/I) imbalance theory. The E/I imbalance theory is conceived as a disturbance in the equilibrium between the

Open Access **https://doi.org/10.4062/biomolther.2021.194**

Received Dec 26, 2021 **Revised** Jan 7, 2022 **Accepted** Jan 11, 2022
Published Online Feb 7, 2022

***Corresponding Authors**
E-mail: chanyshin@kku.ac.kr (Shin CY), sjinjeon@hanmail.net (Jeon SJ)
Tel: +82-2-454-5630 (Shin CY), +82-2-540-5630 (Jeon SJ)
Fax: +82-2-548-5630 (Shin CY), +82-2-548-5630 (Jeon SJ)
[†]The first two authors contributed equally to this work.

glutamatergic and γ-aminobutyric acidergic (GABAergic) inputs (Uzunova *et al*., 2016; Gonçalves *et al*., 2017). It was postulated that either increase or decrease in the ratio of E/I in sensory, mnemonic, social, and emotional systems caused by a combination of variables such as genetic and environmental factors that affect the neural system, can cause some forms of cognitive and behavioral changes such as in autism (Rubenstein and Merzenich, 2003; Gonçalves *et al*., 2017). Previous reports found that the excitatory neurotransmitter glutamate's neurotransmission system is increased in pregenual anterior cingulate cortex of ASD patients (Bejjani *et al*., 2012) and an elevated cellular E/I balance in the mouse medial prefrontal cortex was found to elicit an extensive behavioral impairment, along with social deficits (Yizhar *et al*., 2011).

N-methyl-D-aspartic acid is an amino acid derivative that acts as a prototype agonist specifically at the ionotropic glutamate receptor's NMDA subtype. NMDA imitates the action of glutamate but only binds to and regulates the NMDA receptor subtypes expressed in certain regions of the brain such as the dentate gyrus, striatum, and hippocampal CA1 (Coppola and Moshé, 2012). Interruptions of brain processes in any stage of the brain growth spurt period will determine the pattern of neuronal developments and alterations of the brain circuitry as well as the manifestation of neurobehavioral disorders (Du Bois and Huang, 2007). Since E/I imbalance has been associated with ASD, we seek to determine whether inducing the over-activation of the NMDA system through NMDA injection during early life where substantial developmental synaptogenesis in the brain occurs in rodents. This study aims to determine whether intraperitoneal injection of NMDA during early postnatal developmental stages of mice, which corresponds to neonatal periods in human, induces autistic-like behavior and if it can be a possible pharmacological model for autism.

MATERIALS AND METHODS

Study design

Postnatal day 7 male and female ICR mice from Orient Bio (Seoul, Korea) were used for the NMDA injection and behavioral experiments. These same mice were housed in groups of six and kept under a standard condition (12 h light/dark cycle, 24 ± 2°C, 55 ± 5% humidity). N-methyl-D-aspartate in varying doses was administered via intraperitoneal administration on postnatal days 7-11. Behavioral experiments were performed starting from postnatal day 28 to 42 (Fig. 1). The behavioral experiments were performed under weak light and were recorded using the EthoVision XT 24 software (Noldus, Wageningen, the Netherlands). Guidelines set by the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Institutional Animal Care and Use Committee of Konkuk University, Seoul, Korea (KU18054) were followed for the proper handling and care of the animals.

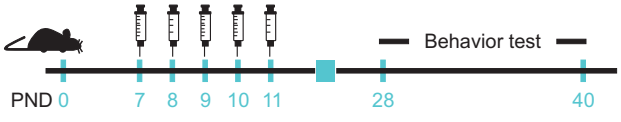

Fig. 1. NMDA Injection was performed at PND 7, 8, 9, 10 and 11 and behavioral tests were performed from P 28-P 40.

Drug preparation and administration

N-methyl-D-aspartate (NMDA), acquired from Sigma-Aldrich Co. (St. Louis, MO, USA), was diluted in saline (0.9% w/v NaCl) in varying doses (2.5 mg/kg and 5 mg/kg). On postnatal day 7, 8, 9, 10, and 11, mice were randomly divided into three groups and were administered with saline, 2.5 mg/kg NMDA, and 5 mg/kg NMDA, respectively, via the intraperitoneal route.

Three-chamber sociability and social novelty test

The three-chamber sociability test was conducted to determine the sociability and social novelty preference of the mice (Kim *et al*., 2019a). Sociability (Phase 1) is evaluated by tracking the rodent's exploration on a stranger rodent in a wire cage over an empty cage, and social preference (Phase 2) compares a rodent's preference to a novel stranger rodent to an already familiar one (Moy *et al*., 2004). A three-chamber apparatus (23×40×22 cm in each compartment) was used for the test with opening access connected to the center area. Three phases completed one trial. First was the 5-min habituation period where the mouse was allowed to freely move in all three chambers. The sociability test follows, marking the second phase, which encouraged the subject mouse to explore between a stranger mouse contained in a wire cage in either of the side compartments and an empty cage on the opposite side. The accumulated times spent within the stranger and empty compartments were automatically tracked by a camera connected to the EthoVision XT 24 software (Noldus). The third phase immediately follows the second by introducing a novel stranger mouse in the previously empty cage to assess the preference for the social novelty of the subject mouse by exploring the three chambers for another 10 min. The stranger mice used were conspecific ICR mice of younger age and same-sex to the test mouse. In this test, the accumulated times spent in each compartment were also measured using the EthoVision tracking software (Noldus).

Self-grooming test

The self-grooming test is a tool used to assess the sequential organization and repetitive behavior in rodents (Kalueff *et al*., 2016; Adil *et al*., 2021). Subject mice were placed in a covered cage measuring 27 cm×22 cm×13 cm and were allowed to habituate for 10 min. The next 10 min were then recorded using a video recorder and the spontaneous self-grooming time was manually measured by a blind observer after.

Open field test

Open field test was conducted to evaluate the exploratory and general locomotor activity of mice. It also provides an initial screen for anxiety-related behavior in rodents. Each subject mouse was placed in an open arena measuring 40 cm×40 cm and allowed to explore for 25 min. Using the EthoVision XT 24 software (Noldus), the total distance moved and the total time spent in the center of the arena (20 cm×20 cm) were measured to assess the anxiety behavior.

Rotarod test

To assess the motor coordination of the subject mice, rotarod test was performed. The mice were placed in a rotating rod with an accelerating speed over a 5-min period. The mouse was assessed in three different trials in 20-min intervals. The latency to fall was recorded.

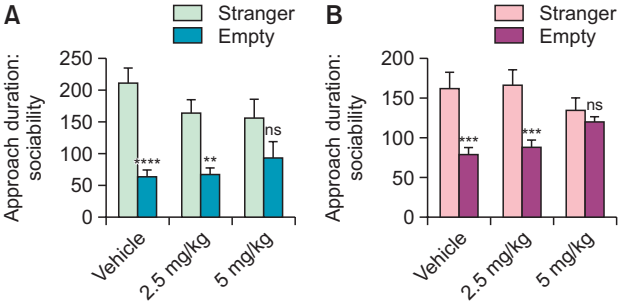

Fig. 2. Three-chamber sociability test of male and female NMDA injected mice. Approach duration in male (A) and female (B) mice injected with saline, 2.5 mg/kg and 5 mg/kg of NMDA at PND 7-P 11. Bars expresses the mean ± SEM. ***p*<0.01, ****p*<0.001, and *****p*<0.0001. N=10 per group.

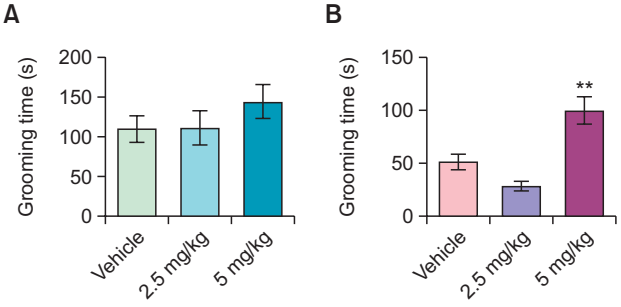

Fig. 3. Self-grooming test of male and female NMDA injected mice. Grooming time of male (A) and female (B) mice injected with saline, 2.5 mg/kg and 5 mg/kg of NMDA at PND 7-P 11. Bars expresses the mean ± SEM. ***p*<0.01. N=10 per group.

Elevated plus maze test

Elevated plus maze test was performed to assess the impulsivity and anxiety-related behavior of rodents. The open arm allows for the assessment of approach or exploration while the close arm asses its avoidance and anxiety (Kishikawa *et al*., 2014). The maze is made of four perpendicular arms having two open or plain arms (30 cm×6 cm) and two walled arms (30 cm×6 cm×20 cm). Each arm meets in a central delimited area of 6 cm×6 cm. The EPM was elevated to 50 cm above the floor. Each subject was placed in the center of the maze facing one of the open arms to explore the maze for 8 min. An arm entry is defined as the entry of all four paws to the lined boundary of each arm. The percentage of open arms exploration against the exploration of all arms was calculated to assess anxiety behaviors.

Data analysis

Data were analyzed using GraphPad Prism software (GraphPad software, San Diego, CA, USA). All data analyzed were expressed as the mean ± of the standard error of the mean (SEM). Two-way ANOVA was used for comparing multiple variables and one-way ANOVA was used for column analysis partnered with Tukey's multiple comparison post hoc analysis.

RESULTS

Altered sociability in NMDA injected mice

Three-chamber sociability test was conducted to determine if sub-chronic NMDA injection during early life can affect the social behavior in later life (Castillo-Gomez *et al*., 2017; Latusz and Mackowiak, 2020; Golitabari *et al*., 2021). The social interaction behavior was measured and analyzed using the EthoVision behavioral assessment system (Noldus). Results show that in both male and female mice, there is a significant difference in the sociability between the control mice and the NMDA injected mice (Fig. 2). At higher dose of NMDA (5 mg/kg) but not at the lower dose (2.5 mg/kg), the sociability of both male [Preference, $F_{1, 52}$=39.65, p<0.0001; Treatment, $F_{2, 52}$=0.6283, p=0.5375; interaction preference×treatment, $F_{2, 52}$=2.216, p=0.1192] (Fig. 2A) and female mice [Preference, $F_{1, 54}$=27.29, p<0.0001; Treatment, $F_{2, 54}$=0.1785, p=0.8370; interaction $_{\text{preference}}\times_{\text{treatment}}$, $F_{2, 54}$=3.882, p=0.0266] (Fig. 2B) was significantly impaired. This result shows that subchronic activation of excitatory neurotransmission by 5 mg/kg of NMDA injection during brain development in early life induces social deficits in both male and female mice.

NMDA injection increased repetitive behavior in mice

Repetitive behavior is one of the core symptoms of autism. To measure whether early postnatal NMDA activation affects this behavior, self-grooming test was performed. It was found that NMDA treatment at 5 mg/kg showed an increasing tendency of grooming time in males [F (2, 27)=0.9467, p=0.4005] (Fig. 3A) and increased grooming behavior of female mice [F (2, 25)=15.37, p=0.0110] (Fig. 3B). This indicates that the NMDA injection may augment the repetitive behavior of the injected mice at adolescent period.

Impaired locomotor and motor coordination in NMDA injected male mice

The locomotor activity of mice was measured using the open field test. Male mice injected with 5 mg/kg NMDA displayed a significant increased distance moved [F (2, 55)=6.332, p=0.0034] (Fig. 4A) while the females did not [F (2, 53)=0.6508, p=0.5257] (Fig. 4B). To test the anxiety-like behavior of the subject mice, the time spent in the center of the open field arena was also measured and both the male [F (2, 26)=0.9855, p=0.3868] (Fig. 4C), and female [F (2, 31)=1.24, p=0.3032] (Fig. 4D). And to test the motor coordination, rotarod test was performed and only the male mice induced with 5 mg/kg NMDA showed decreased latency in falling from the rotating rod [F (2, 26)=7.134, p=0.0034] (Fig. 5A). The females did not show any statistical difference [F (2, 31)=0.02503, p=0.9753] (Fig. 5B).

To confirm this, elevated plus maze test was performed, which is a standardized method to determine anxiety-like behaviors. In male mice, both the percentage of time spent in the open arms [F (2, 27)=0.04102, p=0.9599] (Fig. 6A) and the percentage of frequency in open arms [F (2, 27)=0.02258, p=0.9777] are not different in NMDA injected mice as compared with control (Fig. 6B). This is also true in female's percentage of time spent in the open arms [F (2, 27)=0.4315, p=0.6539] (Fig. 6C) and the percentage of frequency in open arms [F (2, 27)=0.4857, p=0.6206] (Fig. 6D). These data suggest that NMDA injection in both male and female mice did not induce anxiety-related behaviors but induced locomotor and motor coordination impairment in male mice.

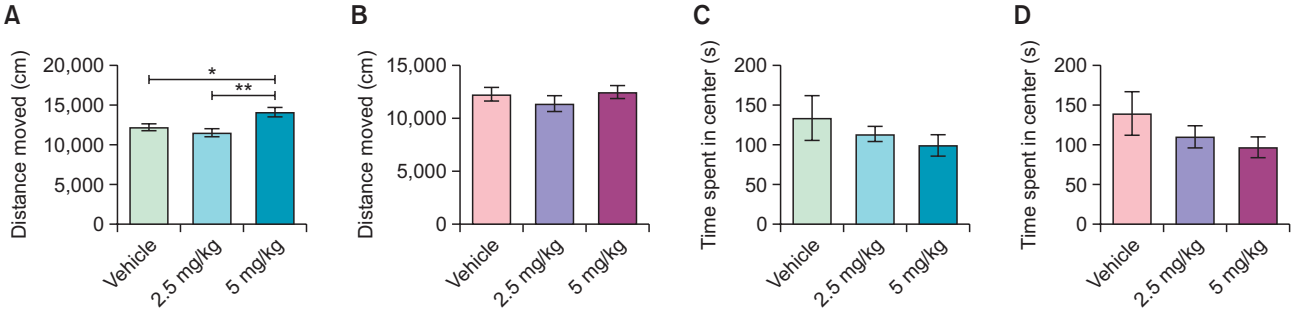

Fig. 4. Open field test of male and female NMDA injected mice. Distance Moved of male (A) and female (B), and time spent in the center of arena in male (C) and female (D) mice injected with saline, 2.5 mg/kg and 5 mg/kg of NMDA at PND 7-P 11. Bars expresses the mean ± SEM. **p*<0.05, ***p*<0.01. N=10-22 per group.

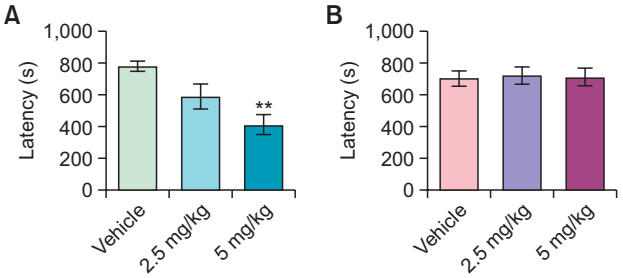

Fig. 5. Rotarod test of male and female NMDA injected mice. Latency to fall in male (A) and female (B) mice injected with saline, 2.5 mg/kg and 5 mg/kg of NMDA at PND 7-PND 11. Bars expresses the mean ± SEM. ***p*<0.01. N=8-16 per group.

DISCUSSION

The prototypic excitatory amino acid N-methyl-D-aspartate, have been used in previous studies to elicit seizures in mice as an experimental animal model of epilepsy or neurotoxicity (Wang *et al*., 2012; Yum *et al*., 2015; Rodríguez-Muñoz *et al*., 2018; Edwards and Zup, 2021). This is because hyperexcitability caused by a disrupted balance in the excitation and inhibition of the brain's neurotransmission is one of the main features of epilepsy (Lemmens *et al*., 2005; Shao *et al*., 2019). Similarly, neurodevelopmental disorders such as ASD have been linked to being affected by excitatory/inhibitory imbalance in the brain (Foss-Feig *et al*., 2017; Gonçalves *et al*., 2017). According to Clancy's report, the postnatal day 7 to 10 period corresponds to human third trimester in pregnancy when major brain growth occurs (Clancy *et al*., 2007). Du Bois and Huang (2007) also states that postnatal days 7 to 14 is when substantial development synaptogenesis in the brain occurs in rodents. Thus, we injected NMDA to mice during significant synaptic development periods in the brain, in this case at PND 7-11 to test whether overactivation of the NMDA receptors will result in behavioral changes similar to that of ASD.

Despite being described as a specific theory explaining the underlying mechanism of ASD, E/I imbalance is complex in itself since the brain involves myriads of neuronal connections, pathways, and regions with different or common functions with one another. Identifying specific areas, root causes, and classifications of these imbalances are steps that should be taken much further. The goal to stratify ASD individuals should be taken with efforts to recognize biomarkers for subtypes of the E/I imbalance if there are some. By pinpointing specific mechanism that caused the E/I imbalance, scientists and medical practitioners can help stratify ASD individuals into subgroups and introduce appropriate treatment modalities. A target-based approach is needed in drug development and it is difficult to apply one class of a drug to a complex disorder like ASD.

Several reports in clinical studies support the E/I imbalance theory of ASD showing either increased glutamatergic signaling (Shinohe *et al*., 2006) or reduced GABA transmission (Robertson *et al*., 2016). These results suggest that any disruption in both excitatory and inhibitory neurotransmission may trigger the development and drive the pathophysiology of ASD (Blatt *et al*., 2001; Oblak *et al*., 2011; Robertson *et al*., 2016). One important aspect of E/I imbalance is the increased excitatory neurotransmission coupled with either decreased or unchanged inhibitory neurotransmission. For example, in the valproic acid model of autism, perturbation of E/I balance has been reported which includes increases in glutamatergic neuronal differentiation markers, decreased GABAergic markers, increased brain plasticity, and connectivity, increased NMDA/AMPA ratio, increased glutamate kinetic profiles, and increased seizure susceptibility (Rinaldi *et al*., 2007, 2008; Fukuchi *et al*., 2009; Kim *et al*., 2014b, 2016). These reports suggest a hyper-excitation type of autistic model (Schneider and Przewlocki, 2005; Kim *et al*., 2011, 2014a, 2017). Interestingly, treatments with NMDA receptor antagonists such as memantine or MK-801 ameliorated the social and repetitive behavior defects (Kim *et al*., 2014b; Kang and Kim, 2015). *IRSp53*, a gene involved in excitatory postsynaptic scaffolding, regulates the F-actin in dendritic spines. Mice lacking this gene show impaired social interaction and increased ratio of NMDA/AMPA leading to an enhanced NMDA receptor function in the hippocampus (Chung *et al*., 2015). Accordingly, treatment with memantine or MPEP (mGluR5 antagonist) rescued the social deficits and restored the hippocampal NMDA function and plasticity as well as the mPFC neuronal firing in the *IRSp53* knockout mice (Chung *et al*., 2015). Clearer evidence of the direct role of E/I balance in the medial prefrontal cortex on the modulation of ASD-like behaviors was studied previously using optogenetic tools (Yizhar *et al*., 2011). Activation of mPFC and hence the increased E/I ratio by light stimulation techniques immediately induced social deficits in freely

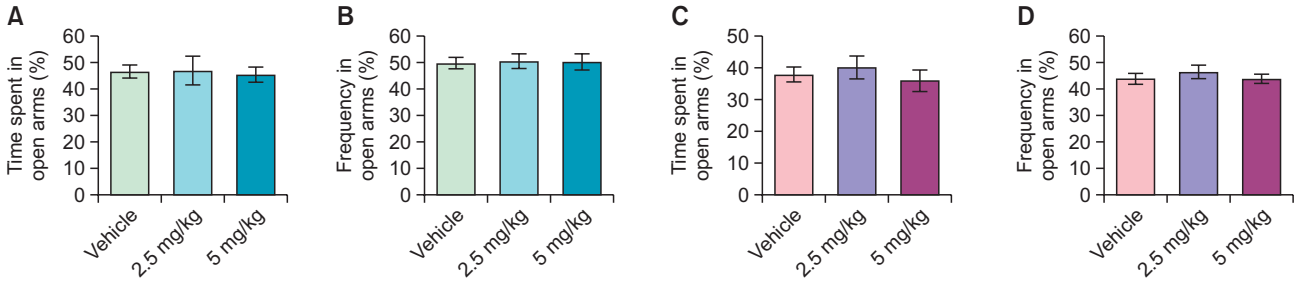

Fig. 6. Elevated plus maze test of male and female NMDA injected mice. %Time spent in open arms (A, C) and %frequency to enter to open arm (B, D) in male and female mice injected with saline, 2.5 mg/kg and 5 mg/kg of NMDA at PND 7-PND 11. Bars expresses the mean ± SEM. N=10 per group.

moving mice (Yizhar *et al*., 2011). Interestingly, the abnormal response by excitatory stimulation was partially rescued by increasing the inhibitory tone and restoring the E/I balance.

On the other side of the coin is the reduced NMDA receptor function that also leads to ASD phenotypes. *Neuroligin-1*, a cell-adhesion molecule, directly interacts with and promotes the synaptic localization of NMDA receptors (Budreck *et al*., 2013). In mice that lack neuroligin-1, a reduced NMDA/AMPA ratio in the corticostriatal synapses was found (Blundell *et al*., 2010). This finding was associated with increased repetitive grooming behavior relevant to ASD, which was rescued by treatment of an NDMAR partial co-agonist D-cycloserine (DCS). Another gene called *Shank2* involved in the excitatory synaptic structure creates a scaffolding important for synaptic assembly and signaling. If *Shank2* is knocked out in mice, it causes a dysregulated postsynaptic development resulting in a reduced NMDAR function as observed in the hippocampus (Won *et al*., 2012). The *Shank2* knockout mice have social behavior deficits, which were also ameliorated by treatment of DCS. These models are among the various ones, which show the involvement of NMDA receptor regulation and function in the manifestation of autistic symptoms. Given the involvement of NMDA receptor mechanisms in the development and pathophysiology of ASD, it is interesting that in this study, similar to these animal models, inducing E/I imbalance through subconvulsant and subtoxic dose of systemic NMDA injection affects the social and repetitive behaviors which are core symptoms of ASD.

ASD is a neurodevelopmental disorder defined by marked social communication deficits and repetitive behavior (American Psychiatric Association, 2013). Three chamber sociability test was performed to check the sociability and it was found that compared to the control group, the sociability of the NMDA-treated mice was significantly decreased, especially the ones treated with 5 mg/kg of NMDA (Fig. 2A). A study we reported previously in mice with acute treatment of NMDA during the adolescent stage yielded a similar result using the home cage sociability test (Adil *et al*., 2021). This is consistent with previous reports stating that an increased E/I ratio can yield behavioral changes and decreased sociability (Yizhar *et al*., 2011). The repetitive or stereotypic behavior tested through the self-grooming test, showed an increasing tendency in males injected with 5 mg/kg of NMDA while there was a significant increase in the females injected with the same dose (Fig. 3). This increased grooming behavior might be explained by the binding of NMDA to peripheral NMDA receptors which has been reported to cause hypersensitivity and play a role in nociception (Jang *et al*., 2015). Intraperitoneal administration of NMDA has been reported to increase grooming behavior especially on juvenile mice (Kim *et al*., 2019b), and our results demonstrate that, if injected during the neonatal stage, these effects can be observed up to the adolescent stages suggesting current experimental condition induces long-lasting changes in neurobehavioral circuits related to repetitive behaviors, which cannot be explained by the direct effect of NMDA on peripheral tissues. It is interesting to note that the NMDA affects the behavior of the injected mice in a dose-dependent manner. Based on these results, the higher dose (5 mg/kg) could be the optimum condition to induce and validate the possibility of using hyperactivation of glutamatergic neurotransmission through NMDA injection as an easy and versatile pharmacological model of ASD.

The higher male prevalence (4:1) in ASD remains a current issue in need of explanations (Maenner *et al*., 2020). Many theories have been introduced to explain these differences and a few of those notable ones include the empathizing-systemizing theory (Baron-Cohen, 2009) and the female protective theory (Jacquemont *et al*., 2014). Several animal models also show somewhat mild autistic-like symptoms in females as compared to their male counterparts (Baron-Cohen *et al*., 2005; Cox and Rissman, 2011; Kim *et al*., 2016). The sex differences in the current study could be related to many factors such as physical, genetic, and hormonal differences throughout development. Therefore, the response of each sex against the NMDA during the early period of development might be dependent on the complex interplay of factors that resulted in sexual dimorphism in the behavioral levels. Although we did not explore further the mechanism of these differences such as the critical period of NMDA introduction, changes in NMDA receptor expression during the developmental period, dose and frequency of NMDA treatment, we believe that it is worthy to delve into moving forward to help uncover some mechanistic links between the unequal prevalence, degree and/or variety of symptoms (behavioral domains) of the female population.

Previous reports have associated hyperactivity with ASD and it has been suggested that there is comorbidity between ASD and attention-deficit hyperactivity disorder (Leyfer *et al*., 2006; Meidenbauer *et al*., 2011). Results in the open field test revealed an increase in the distance moved of the NMDA-induced male mice but not the female mice (Fig. 4A, 4B). This is also true in the measurement of the motor co-

ordination of mice through the rotarod test (Fig. 5) where only the male mice showed a decrease in the latency to fall which indicates a decrease in motor coordination. Similar results have been reported on other types of ASD animal models. In genetic shank3(e4-9) homozygous mutant mice, male mice display more severe impairments than females in motor coordination as well as abnormal social behaviors (Wang *et al*., 2011). Also it was reported that only male mice from the polyinosinic:polycytidylic acid (Poly I:C) MIA animal model of ASD showed reduced social interactions as well as motor development and coordination deficits (Haida *et al*., 2019). These reports suggest that females are better protected against developmental insults leading to ASD-like symptoms in mice. This gender difference in motor coordination as well as ASD-like symptoms could possibly be explained by the hormonal influences of estrogen on female mice. Estrogen is a hormone that plays a role in regulating emotions, learning and memory and other body functions such as motor coordination (Kalkbrenner and Standley, 2003; Brann *et al*., 2007; Varshney *et al*., 2021). It is also widely known for its neuroprotective effects in several neurological disorders and evidence have emerged supporting this throughout the years (Liu and Zhao, 2013). Experimental studies revealed that estrogen and progestin administration improved the outcome after traumatic brain injury and ischemia (Roof and Hall, 2000). An NMDA-induced seizures study on mice revealed that there was a considerable neuronal loss due to decreased endogenous estrogen following NMDA-induced seizure and estrogen was indicated to have a neuroprotective effect on female mice (Kalkbrenner and Standley, 2003). Another study on revealed that excitotoxicity in the basal forebrain cholinergic neurons was prevented by estrogen after NMDA infusions (Horvath *et al*., 2002). This neuroprotective action has been attributed to its antioxidant abilities (Xia *et al*., 2009) and capability to suppress neuronal hyperexcitability (Roof and Hall, 2000). Interestingly, it was reported that higher glutamate binding is observed in males compared to the female counterparts and this observation is more sensitive during the perinatal period (postnatal day 3 to 10) than in adulthood (McCarthy *et al*., 1997). Thus, this early period of neuroprotective action (or lesser vulnerability) to female mice observed in the motor coordination of the NMDA-injected mice, possibly via higher level of estrogen, could also be at play here in this study. Anxiety behavior has also been reported to be associated with autism in previous reports (Kim *et al*., 2000; Ozsivadjian and Knott, 2011). Anxiety behavior was initially assessed in the open field test by my measuring the time spent in the center of the arena. Anxious mice tend to take more time staying in the side of the arena. We found a decreasing tendency to stay in the center in mice treated with NMDA. To confirm this, an elevated plus-maze test was conducted. Mice that are anxious stay inside the covered arm of the EPM apparatus more than their time spent in the open arms. However, there were no implications of anxiety behavior found in both male and female subject mice.

Systemic administration of NMDA in mice shows increased tolerance along with increasing age with younger mice showing increased nociceptive and repetitive behaviors, suggesting that younger and developing mice are more sensitive to the effects of a similar dosage of NMDA than older mice (Kim *et al*., 2019b). One plausible explanation is the penetration of NMDA through the blood-brain barrier (BBB). NMDA is known to have poor penetration in the BBB especially in adult mice (PND 60), but younger and developing mice (PND 12 and PND 18) show increased response to NMDA, to which their BBB did not yet fully mature (Kábová *et al*., 1999). Thus, pharmacological induction of excitatory neurotransmission during the early stage of life more likely will cause a long-term impairment in behavior.

Seizure disorders and ASD are often associated to co-occur (Jeste and Tuchman, 2015) suggesting that these disorders have something in common in terms of their pathogenesis. Animal models and human studies indicate that this epilepsy-ASD comorbidity arises from hyperexcitability or E/I imbalance in several brain regions (Bozzi *et al*., 2018). Animal models made from inducing imbalance by injecting NMDA during the early developmental stage may give rise to a deeper understanding of this comorbidity and help in the development of future therapeutic strategies. In this study, experiments performed were limited to behavioral experiments. Further experiments on the cellular and molecular aspect, as well as other behavioral experiments on different domains, would give additional information which would be helpful in the understanding of NMDA receptors and the brain in general.

ACKNOWLEDGMENTS

This work was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) & funded by the Korean Government (MSIT) (NRF-2017M3A9G2077568) and the NRF (2016R1A5A2012284).

REFERENCES

Adil, K. J., Gonzales, E. L., Remonde, C. G., Boo, K. J., Jeon, S. J. and Shin, C. Y. (2021) Autism-like behavioral phenotypes in mice treated with systemic N-methyl-D-aspartate. *Biomol. Ther. (Seoul)* doi: 10.4062/biomolther.2021.133 [Online ahead of print].

American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders: DSM-5. Arlington, VA.

Baron-Cohen, S., Knickmeyer, R. C. and Belmonte, M. K. (2005) Sex differences in the brain: implications for explaining autism. *Science* **310**, 819-823.

Baron-Cohen, S. (2009) Autism: the empathizing–systemizing (E-S) theory. *Ann. N. Y. Acad. Sci.* **1156**, 68-80.

Bejjani, A., O'Neill, J., Kim, J. A., Frew, A. J., Yee, V. W., Ly, R., Kitchen, C., Salamon, N., McCracken, J. T., Toga, A. W., Alger, J. R. and Levitt, J. G. (2012) Elevated glutamatergic compounds in pregenual anterior cingulate in pediatric autism spectrum disorder demonstrated by 1H MRS and 1H MRSI. *PLoS ONE* **7**, e38786.

Blatt, G. J., Fitzgerald, C. M., Guptill, J. T., Booker, A. B., Kemper, T. L. and Bauman, M. L. (2001) Density and distribution of hippocampal neurotransmitter receptors in autism: an autoradiographic study. *J. Autism Dev. Disord.* **31**, 537-543.

Blundell, J., Blaiss, C. A., Etherton, M. R., Espinosa, F., Tabuchi, K., Walz, C., Bolliger, M. F., Sudhof, T. C. and Powell, C. M. (2010) Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. *J. Neurosci.* **30**, 2115-2129.

Bozzi, Y., Provenzano, G. and Casarosa, S. (2018) Neurobiological bases of autism-epilepsy comorbidity: a focus on excitation/inhibition imbalance. *Eur. J. Neurosci.* **47**, 534-548.

Brann, D. W., Dhandapani, K., Wakade, C., Mahesh, V. B. and Khan, M. M. (2007) Neurotrophic and neuroprotective actions of estrogen: basic mechanisms and clinical implications. *Steroids* **72**, 381-405.

Budreck, E. C., Kwon, O. B., Jung, J. H., Baudouin, S., Thommen, A., Kim, H. S., Fukazawa, Y., Harada, H., Tabuchi, K., Shigemoto, R., Scheiffele, P. and Kim, J. H. (2013) Neuroligin-1 controls synaptic

abundance of NMDA-type glutamate receptors through extracellular coupling. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 725-730.
Castillo-Gomez, E., Perez-Rando, M., Belles, M., Gilabert-Juan, J., Llorens, J. V., Carceller, H., Bueno-Fernandez, C., Garcia-Mompo, C., Ripoll-Martinez, B., Curto, Y., Sebastia-Ortega, N., Molto, M. D., Sanjuan, J. and Nacher, J. (2017) Early social isolation stress and perinatal NMDA receptor antagonist treatment induce changes in the structure and neurochemistry of inhibitory neurons of the adult amygdala and prefrontal cortex. *eNeuro* **4**, ENEURO.0034-17.2017.
Chung, W., Choi, S. Y., Lee, E., Park, H., Kang, J., Park, H., Choi, Y., Lee, D., Park, S. G., Kim, R., Cho, Y. S., Choi, J., Kim, M. H., Lee, J. W., Lee, S., Rhim, I., Jung, M. W., Kim, D., Bae, Y. C. and Kim, E. (2015) Social deficits in IRSp53 mutant mice improved by NMDAR and mGluR5 suppression. *Nat. Neurosci.* **18**, 435-443.
Clancy, B., Finlay, B. L., Darlington, R. B. and Anand, K. J. (2007) Extrapolating brain development from experimental species to humans. *Neurotoxicology* **28**, 931-937.
Coppola, A. and Moshé, S. L. (2012) Animal models. *Hand. Clin. Neurol.* **107**, 63-98.
Cox, K. H. and Rissman, E. F. (2011) Sex differences in juvenile mouse social behavior are influenced by sex chromosomes and social context. *Genes Brain Behav.* **10**, 465-472.
Du Bois, T. M. and Huang, X. F. (2007) Early brain development disruption from NMDA receptor hypofunction: relevance to schizophrenia. *Brain Res. Rev.* **53**, 260-270.
Edwards, K. A. and Zup, S. L. (2021) Serotonin pretreatment abolishes sex-specific NMDA-induced seizure behavior in developing rats. *Neuroscience* **463**, 184-196.
Ey, E., Leblond, C. S. and Bourgeron, T. (2011) Behavioral profiles of mouse models for autism spectrum disorders. *Autism Res.* **4**, 5-16.
Foss-Feig, J. H., Adkinson, B. D., Ji, J. L., Yang, G., Srihari, V. H., McPartland, J. C., Krystal, J. H., Murray, J. D. and Anticevic, A. (2017) Searching for cross-diagnostic convergence: neural mechanisms governing excitation and inhibition balance in schizophrenia and autism spectrum disorders. *Biol. Psychiatry* **81**, 848-861.
Fukuchi, M., Nii, T., Ishimaru, N., Minamino, A., Hara, D., Takasaki, I., Tabuchi, A. and Tsuda, M. (2009) Valproic acid induces up- or down-regulation of gene expression responsible for the neuronal excitation and inhibition in rat cortical neurons through its epigenetic actions. *Neurosci. Res.* **65**, 35-43.
Golitabari, N., Mohammadian, F., Salari, A. A. and Amani, M. (2021) Neonatal NMDA blockade alters the LTP, LTD and cognitive functions in male and female Wistar rats. *Neuropharmacology* **205**, 108896.
Gonçalves, J., Violante, I. R., Sereno, J., Leitão, R. A., Cai, Y., Abrunhosa, A., Silva, A. P., Silva, A. J. and Castelo-Branco, M. (2017) Testing the excitation/inhibition imbalance hypothesis in a mouse model of the autism spectrum disorder: *in vivo* neurospectroscopy and molecular evidence for regional phenotypes. *Mol. Autism* **8**, 47.
Haida, O., Al Sagheer, T., Balbous, A., Francheteau, M., Matas, E., Soria, F., Fernagut, P. O. and Jaber, M. (2019) Sex-dependent behavioral deficits and neuropathology in a maternal immune activation model of autism. *Transl. Psychiatry* **9**, 124.
Horvath, K. M., Hårtig, W., Van der Veen, R., Keijser, J. N., Mulder, J., Ziegert, M., Van der Zee, E. A., Harkany, T. and Luiten, P. G. (2002) 17β-Estradiol enhances cortical cholinergic innervation and preserves synaptic density following excitotoxic lesions to the rat nucleus basalis magnocellularis. *Neuroscience* **110**, 489-504.
Jacquemont, S., Coe, B. P., Hersch, M., Duyzend, M. H., Krumm, N., Bergmann, S., Beckmann, J. S., Rosenfeld, J. A. and Eichler, E. E. (2014) A higher mutational burden in females supports a "female protective model" in neurodevelopmental disorders. *Am. J. Hum. Genet.* **94**, 415-425.
Jang, J. H., Nam, T. S., Jun, J., Jung, S. J., Kim, D. W. and Leem, J. W. (2015) Peripheral NMDA receptors mediate antidromic nerve stimulation-induced tactile hypersensitivity in the rat. *Mediators Inflamm.* **2015**, 793624.
Jeste, S. S. and Tuchman, R. (2015) Autism spectrum disorder and epilepsy: two sides of the same coin? *J. Child Neurol.* **30**, 1963-1971.
Kábová, R., Liptakova, S., Šlamberová, R., Pometlová, M. and Velíšek, L. (1999) Age-specific N-methyl-D-aspartate—induced seizures: perspectives for the west syndrome model. *Epilepsia* **40**, 1357-1369.
Kalkbrenner, K. A. and Standley, C. A. (2003) Estrogen modulation of NMDA-induced seizures in ovariectomized and non-ovariectomized rats. *Brain Res.* **964**, 244-249.
Kalueff, A. V., Stewart, A. M., Song, C., Berridge, K. C., Graybiel, A. M. and Fentress, J. C. (2016) Neurobiology of rodent self-grooming and its value for translational neuroscience. *Nat. Rev. Neurosci.* **17**, 45-59.
Kang, J. and 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.
Kim, J. A., Szatmari, P., Bryson, S. E., Streiner, D. L. and Wilson, F. J. (2000) The prevalence of anxiety and mood problems among children with autism and Asperger syndrome. *Autism* **4**, 117-132.
Kim, J. W., Park, K., Kang, R. J., Gonzales, E. L. T., Kim, D. G., Oh, H. A., Seung, H., Ko, M. J., Kwon, K. J., Kim, K. C., Lee, S. H., Chung, C. and Shin, C. Y. (2019a) Pharmacological modulation of AMPA receptor rescues social impairments in animal models of autism. *Neuropsychopharmacology* **44**, 314-323.
Kim, J. W., Seung, H., Kim, K. C., Gonzales, E. L. T., Oh, H. A., Yang, S. M., Ko, M. J., Han, S. H., Banerjee, S. and Shin, C. Y. (2017) Agmatine rescues autistic behaviors in the valproic acid-induced animal model of autism. *Neuropharmacology* **113**, 71-81.
Kim, J. W., Seung, H., Kwon, K. J., Ko, M. J., Lee, E. J., Oh, H. A., Choi, C. S., Kim, K. C., Gonzales, E. L., You, J. S., Choi, D. H., Lee, J., Han, S. H., Yang, S. M., Cheong, J. H., Shin, C. Y. and Bahn, G. H. (2014a) Subchronic treatment of donepezil rescues impaired social, hyperactive, and stereotypic behavior in valproic acid-induced animal model of autism. *PLoS ONE* **9**, e104927.
Kim, K. C., Choi, C. S., Kim, J. W., Han, S. H., Cheong, J. H., Ryu, J. H. and Shin, C. Y. (2016) MeCP2 modulates sex differences in the postsynaptic development of the valproate animal model of autism. *Mol. Neurobiol.* **53**, 40-56.
Kim, K. C., Kim, P., Go, H. S., Choi, C. S., Yang, S. I., Cheong, J. H., Shin, C. Y. and Ko, K. H. (2011) The critical period of valproate exposure to induce autistic symptoms in Sprague-Dawley rats. *Toxicol. Lett.* **201**, 137-142.
Kim, K. C., Lee, D. K., Go, H. S., Kim, P., Choi, C. S., Kim, J. W., Jeon, S. J., Song, M. R. and Shin, C. Y. (2014b) Pax6-dependent cortical glutamatergic neuronal differentiation regulates autism-like behavior in prenatally valproic acid-exposed rat offspring. *Mol. Neurobiol.* **49**, 512-528.
Kim, S., Kim, D. G., Gonzales, E. L., Mabunga, D. F. N., Shin, D., Jeon, S. J., Shin, C. Y., Ahn, T. and Kwon, K. J. (2019b) Effects of intraperitoneal N-methyl-D-aspartate (NMDA) administration on nociceptive/repetitive behaviors in juvenile mice. *Biomol. Ther. (Seoul)* **27**, 168-177.
Kim, Y. S. and Leventhal, B. L. (2015) Genetic epidemiology and insights into interactive genetic and environmental effects in autism spectrum disorders. *Biol. Psychiatry* **77**, 66-74.
Kishikawa, Y., Kawahara, Y., Yamada, M., Kaneko, F., Kawahara, H. and Nishi, A. (2014) The spontaneously hypertensive rat/lzm (SHR/lzm) shows attention deficit/hyperactivity disorder-like behaviors but without impulsive behavior: therapeutic implications of low-dose methylphenidate. *Behav. Brain Res.* **274**, 235-242.
LaSalle, J. M. (2013) Epigenomic strategies at the interface of genetic and environmental risk factors for autism. *J. Hum. Genet.* **58**, 396-401.
Latusz, J. and Mackowiak, M. (2020) Early-life blockade of NMDA receptors induces epigenetic abnormalities in the adult medial prefrontal cortex: possible involvement in memory impairment in trace fear conditioning. *Psychopharmacology* **237**, 231-248.
Leblond, C. S., Nava, C., Polge, A., Gauthier, J., Huguet, G., Lumbroso, S., Giuliano, F., Stordeur, C., Depienne, C., Mouzat, K., Pinto, D., Howe, J., Lemière, N., Durand, C. M., Guibert, J., Ey, E., Toro, R., Peyre, H., Mathieu, A., Amsellem, F., Rastam, M., Gillberg, I. C., Rappold, G. A., Holt, R., Monaco, A. P., Maestrini, E., Galan, P., Heron, D., Jacquette, A., Afenjar, A., Rastetter, A., Brice, A., Devillard, F., Assouline, B., Laffargue, F., Lespinasse, J., Chiesa, J.,

Rivier, F., Bonneau, D., Regnault, B., Zelenika, D., Delepine, M., Lathrop, M., Sanlaville, D., Schluth-Bolard, C., Edery, P., Perrin, L., Tabet, A. C., Schmeisser, M. J., Boeckers, T. M., Coleman, M., Sato, D., Szatmari, P., Scherer, S. W., Rouleau, G. A., Betancur, C., Leboyer, M., Gillberg, C., Delorme, R. and Bourgeron, T. (2014) Meta-analysis of SHANK mutations in autism spectrum disorders: a gradient of severity in cognitive impairments. *PLoS Genet.* **10**, e1004580.

Lemmens, E. M., Lubbers, T., Schijns, O. E., Beuls, E. A. and Hoogland, G. (2005) Gender differences in febrile seizure-induced proliferation and survival in the rat dentate gyrus. *Epilepsia* **46**, 1603-1612.

Leyfer, O. T., Folstein, S. E., Bacalman, S., Davis, N. O., Dinh, E., Morgan, J., Tager-Flusberg, H. and Lainhart, J. E. (2006) Comorbid psychiatric disorders in children with autism: Interview development and rates of disorders. *J. Autism Dev. Disord.* **36**, 849-861.

Liu, S. b. and Zhao, M. g. (2013) Neuroprotective effect of estrogen: role of nonsynaptic NR2B-containing NMDA receptors. *Brain Res. Bull.* **93**, 27-31.

Maenner, M. J., Shaw, K. A., Baio, J., Washington, A., Patrick, M., DiRienzo, M., Christensen, D. L., Wiggins, L. D., Pettygrove, S., Andrews, J. G., Lopez, M., Hudson, A., Baroud, T., Schwenk, Y., White, T., Rosenberg, C. R., Lee, L. C., Harrington, R. A., Huston, M., Hewitt, A., Esler, A., Hall-Lande, J., Poynter, J. N., Hallas-Muchow, L., Constantino, J. N., Fitzgerald, R. T., Zahorodny, W., Shenouda, J., Daniels, J. L., Warren, Z., Vehorn, A., Salinas, A., Durkin, M. S. and Dietz, P. M. (2020) Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2016. *MMWR Surveill. Summ.* **69**, 1-12.

McCarthy, M. M., Davis, A. M. and Mong, J. A. (1997) Excitatory neurotransmission and sexual differentiation of the brain. *Brain Res. Bull.* **44**, 487-495.

Meidenbauer, J. J., Mantis, J. G. and Seyfried, T. N. (2011) The EL mouse: a natural model of autism and epilepsy. *Epilepsia* **52**, 347-357.

Moy, S., Nadler, J., Perez, A., Barbaro, R., Johns, J., Magnuson, T., Piven, J. and Crawley, J. (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.

Oblak, A. L., Gibbs, T. T. and Blatt, G. J. (2011) Reduced GABAA receptors and benzodiazepine binding sites in the posterior cingulate cortex and fusiform gyrus in autism. *Brain Res.* **1380**, 218-228.

Ozsivadjian, A. and Knott, F. (2011) Anxiety problems in young people with autism spectrum disorder: a case series. *Clin. Child Psychol. Psychiatry* **16**, 203-214.

Rinaldi, T., Kulangara, K., Antoniello, K. and Markram, H. (2007) Elevated NMDA receptor levels and enhanced postsynaptic long-term potentiation induced by prenatal exposure to valproic acid. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 13501-13506.

Rinaldi, T., Perrodin, C. and Markram, H. (2008) Hyper-connectivity and hyper-plasticity in the medial prefrontal cortex in the valproic acid animal model of autism. *Front. Neural Circuits* **2**, 4.

Robertson, C. E., Ratai, E. M. and Kanwisher, N. (2016) Reduced GABAergic action in the autistic brain. *Curr. Biol.* **26**, 80-85.

Rodríguez-Muñoz, M., Onetti, Y., Cortés-Montero, E., Garzón, J. and Sánchez-Blázquez, P. (2018) Cannabidiol enhances morphine antinociception, diminishes NMDA-mediated seizures and reduces stroke damage via the sigma 1 receptor. *Mol. Brain* **11**, 51.

Roof, R. L. and Hall, E. D. (2000) Gender differences in acute CNS trauma and stroke: neuroprotective effects of estrogen and progesterone. *J. Neurotrauma* **17**, 367-388.

Rosenberg, R. E., Law, J. K., Yenokyan, G., McGready, J., Kaufmann, W. E. and Law, P. A. (2009) Characteristics and concordance of autism spectrum disorders among 277 twin pairs. *Arch. Pediatr. Adolesc. Med.* **163**, 907-914.

Rubenstein, J. and Merzenich, M. M. (2003) Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav.* **2**, 255-267.

Schneider, T. and Przewlocki, R. (2005) Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. *Neuropsychopharmacology* **30**, 80-89.

Sealey, L., Hughes, B., Sriskanda, A., Guest, J., Gibson, A., Johnson-Williams, L., Pace, D. and Bagasra, O. (2016) Environmental factors in the development of autism spectrum disorders. *Environ. Int.* **88**, 288-298.

Shao, L. R., Habela, C. W. and Stafstrom, C. E. (2019) Pediatric epilepsy mechanisms: expanding the paradigm of excitation/inhibition imbalance. *Children* **6**, 23.

Shinohe, A., Hashimoto, K., Nakamura, K., Tsujii, M., Iwata, Y., Tsuchiya, K. J., Sekine, Y., Suda, S., Suzuki, K., Sugihara, G., Matsuzaki, H., Minabe, Y., Sugiyama, T., Kawai, M., Iyo, M., Takei, N. and Mori, N. (2006) Increased serum levels of glutamate in adult patients with autism. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **30**, 1472-1477.

Uzunova, G., Pallanti, S. and Hollander, E. (2016) Excitatory/inhibitory imbalance in autism spectrum disorders: implications for interventions and therapeutics. *World J. Biol. Psychiatry* **17**, 174-186.

Varshney, M. K., Yu, N. Y. L., Katayama, S., Li, X., Liu, T., Wu, W. F., Töhönen, V., Krjutškov, K., Kere, J., Fan, X., Inzunza, J., Gustafsson, J. Å. and Nalvarte, I. (2021) Motor function deficits in the estrogen receptor beta knockout mouse: role on excitatory neurotransmission and myelination in the motor cortex. *Neuroendocrinology* **111**, 27-44.

Wang, X., McCoy, P. A., Rodriguiz, R. M., Pan, Y., Je, H. S., Roberts, A. C., Kim, C. J., Berrios, J., Colvin, J. S., Bousquet-Moore, D., Lorenzo, I., Wu, G., Weinberg, R. J., Ehlers, M. D., Philpot, B. D., Beaudet, A. L., Wetsel, W. C. and Jiang, Y. H. (2011) Synaptic dysfunction and abnormal behaviors in mice lacking major isoforms of Shank3. *Hum. Mol. Genet.* **20**, 3093-3108.

Wang, Y. J., Zhang, Y., Liang, X. H., Yang, G. and Zou, L. P. (2012) Effects of adrenal dysfunction and high-dose adrenocorticotropic hormone on NMDA-induced spasm seizures in young Wistar rats. *Epilepsy Res.* **100**, 125-131.

Won, H., Lee, H. R., Gee, H. Y., Mah, W., Kim, J. I., Lee, J., Ha, S., Chung, C., Jung, E. S., Cho, Y. S., Park, S. G., Lee, J. S., Lee, K., Kim, D., Bae, Y. C., Kaang, B. K., Lee, M. G. and Kim, E. (2012) Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. *Nature* **486**, 261-265.

Xia, Y., Xing, J. and Krukoff, T. (2009) Neuroprotective effects of R,R-tetrahydrochrysene against glutamate-induced cell death through anti-excitotoxic and antioxidant actions involving estrogen receptor–dependent and –independent pathways. *Neuroscience* **162**, 292-306.

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'Shea, D. J., Sohal, V. S., Goshen, I., Finkelstein, J., Paz, J. T., Stehfest, K., Fudim, R., Ramakrishnan, C., Huguenard, J. R., Hegemann, P. and Deisseroth, K. (2011) Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* **477**, 171-178.

Yum, M. S., Lee, M., Woo, D. C., Kim, D. W., Ko, T. S. and Velíšek, L. (2015) β-Hydroxybutyrate attenuates NMDA-induced spasms in rats with evidence of neuronal stabilization on MR spectroscopy. *Epilepsy Res.* **117**, 125-132.

Zerbo, O., Qian, Y., Yoshida, C., Grether, J. K., Van de Water, J. and Croen, L. A. (2015) Maternal infection during pregnancy and autism spectrum disorders. *J. Autism Dev. Disord.* **45**, 4015-4025.