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Research paper

Effect of intrahippocampal microinjection of VU0155041, a positive allosteric modulator of mGluR4, on long term potentiation in a valproic acid-induced autistic male rat model

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ABSTRACT

The precise cause of autism spectrum disorder (ASD) is not fully understood. Despite the involvement of glutamatergic dysregulation in autism, the specific contribution of mGlu4 receptors to synaptic plasticity remains unclear. Using the positive allosteric modulator VU0155041, we aimed to restore long-term potentiation (LTP) in the perforant path-dentate gyrus (PP-DG) pathway in VPA-induced autistic rat model. High-frequency stimulation was applied to the PP-DG synapse to induce LTP, while the VU0155041 was administered into the DG. Unexpectedly, VU0155041 failed to alleviate the observed LTP reduction in VPA-exposed rats, further resulting in a significant decrease in population spike LTP. This unexpected outcome prompts discussion on the complex nature of mGlu4 receptor modulation, highlighting potential interference with physiological processes underlying synaptic plasticity.

1. Introduction

Autism spectrum disorder (ASD) is a complex and heterogeneous neurodevelopmental condition characterized by a range of social, communicative, and behavioral impairments (Ousley and Cermak, 2014; Kientz and Dunn, 1997). While the etiology of ASD remains multifaceted, growing evidence suggests that imbalances in glutamatergic neurotransmission (Shinohe et al., 2006; Nisar et al., 2022) and synaptic plasticity (Hansel, 2019; Mohammadkhani et al., 2022), may play a pivotal role in the pathophysiology of this disorder. Among the various receptors within the glutamatergic system, metabotropic glutamate (mGlu) receptors have garnered attention due to their ability to modulate synaptic transmission and influence neuronal connectivity (Gubellini et al., 2004).

The mGlu receptor family consists of eight subtypes, including mGlu1 to mGlu8 (Huang et al., 2024). Each subtype has different functions and is found in different brain regions, contributing to various aspects of synaptic and neuronal regulation (Gubellini et al., 2004). mGlu4 receptors has emerged as a potential target for understanding the neurobiological underpinnings of autism (Becker et al., 2014). mGlu4 is a G-protein-coupled receptor known to regulate glutamatesignaling and synaptic plasticity in various brain regions (Xiang et al., 2021). Although studies have explored mGlu4 receptor in the context of various neurological and neuropsychiatric disorders, including autism (Becker et al., 2014), its specific role in the modulation of synaptic plasticity in ASD is still under investigation. Moreover, mGlu4 receptor has high density in the dentate molecular layer of hippocampus (Corti et al., 2002). Immunoreactivity for mGlu4 receptors is prominently localized on granule cell bodies within the dentate gyrus (DG), indicating significant expression in this cell population (Bradley et al., 1996). Additionally, a subset of interneurons in the hilus region exhibits immunopositivity for mGlu4, reflecting its diverse distribution within hippocampal circuitry (Corti et al., 2002; Bradley et al., 1996). Furthermore, mGlu4 labeling extends to the terminal zones of the perforant path (PP) within the DG (Corti et al., 2002). These observations suggest mGlu4's involvement in regulating synaptic transmission and excitability within specific DG circuits.

Synaptic plasticity encompasses phenomena such as long-term

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potentiation (LTP) or long-term depression (LTD) (Huganir and Nicoll, 2013; Turrigiano, 2008). Prior studies have demonstrated a reduction in hippocampal LTP, considered a biological model for learning and memory, at the synapse between the medial perforant path and dentate gyrus (PP-DG) in an animal model of ASD (Mohammadkhani et al., 2022; Yun and Trommer, 2011; Takeuchi et al., 2013). Nevertheless, the exact underlying mechanism(s) for this phenomenon has yet to be determined.

This research paper endeavors to shed light on the involvement of hippocampal mGlu4 receptors in PP-DG LTP in the context of autism, using a valproic acid (VPA)-induced autistic rat model. VPA, an antiepileptic drug, has been shown to induce autism-like behaviors and alterations in synaptic plasticity in animal models (Ghahremani et al., 2022), making it a valuable tool for studying the disorder. Clinical research has reported that the maternal usage of VPA, a commonly prescribed antiepileptic drug and mood stabilizer (Löscher, 2002), can impact fetal brain development during pregnancy, leading to various abnormalities in the offspring (Christensen et al., 2013). Prenatal exposure to VPA is recognized as an environmental risk factor in the development of ASD in humans, and rats exposed to VPA during pregnancy serve as a well-established experimental model for autism (Nicolini and Fahnestock, 2018).

It has been shown that positive mGlu4 receptor allosteric modulator VU0155041 alleviates autistic symptoms in mice lacking the mu opioid receptor gene ($Oprm^{-/-}$) (Becker et al., 2014). Oprm−/− animals replicate core and several associated behavioral symptoms observed in autism. Additionally, they exhibit anatomical, neurochemical, and genetic features characteristic of the ASD. Furthermore, qRT-PCR analysis revealed a decrease in the transcription of Grm4, which encodes mGlu4 receptors, in certain brain areas of $Oprm^{-/-}$ animals.

Given the pivotal role of mGlu receptors in modulating synaptic transmission, we reasoned that increasing mGlu4 receptor activity in VPA-exposed animals using a positive allosteric modulator (PAM) could potentially restore the deficit in LTP. In this study, we tested whether intra-DG injection of VU0155041, a PAM and partial agonist of mGlu4 receptor can alleviate the impairment of hippocampal LTP in VPA-induced autistic rat model.

2. Methods

2.1. The VPA rat model of autism

The experimental protocols were granted approval by the Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1398.527) and were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All measures were taken to ensure minimal stress during the handling of animals. To induce pregnancy, two female Wistar rats were paired overnight with a sexually mature male rat (6 weeks old) of the same strain. Confirmation of successful mating on embryonic day 1 was determined by the presence of a vaginal plug or sperm in the vaginal smear the next morning. For the establishment of a rat model of autism, sodium valproate (NaVPA, Sigma, UK) was dissolved in normal saline to create a solution with a concentration of 150 mg/ml (pH 7.3). On embryonic day 12.5, VPA-dams received a single intraperitoneal (i.p.) injection of NaVPA (500 mg/kg, 3.3 ml/kg), while control groups received a single injection of saline as a vehicle (i.p., 3.3 ml/kg). The animals were housed in conditions with a room temperature of 23 ± 3 °C, a 12:12 h light/dark cycle, and had ad libitum access to food and tap water. Dams were individually housed and allowed to rear their own litters. The offspring were utilized for recording long-term potentiation (LTP).

2.2. Surgical and microinjection procedures

Between postnatal days 55 and 65, male rats were anesthetized using urethane and positioned in a stereotaxic apparatus for surgery, electrode implantation, and LTP recording. The procedures employed here were akin to those outlined in previous studies conducted by our laboratory (Mohammadkhani et al., 2022; Ghahremani et al., 2022; Safari et al., 2021). In essence, under urethane-induced anesthesia through intraperitoneal injection (1.5 g/kg), the rat's head was secured in the stereotaxic apparatus for both surgery and recording. A heating pad was employed to maintain the animals' temperature at 36.5±0.5∘C. Skull holes were made, and subsequently, two bipolar electrodes, composed of stainless steel with a Teflon cover (125 μm diameter, Advent Co., UK), were positioned in the right hippocampus. The stimulating electrode was situated in the perforant path (PP) [AP: −8.1 mm from bregma; ml: +4.3 mm from midline; DV: 3.2 mm from the skull surface],], while the recording electrode was positioned in the dentate gyrus (DG) granular cell layer [AP:−3.8 mm from bregma; ml: +2.3 mm from midline; DV: 2.7–3.2 mm from the skull surface], following the Paxinos and Watson atlas of the rat brain (Paxinos and Watson, 2005; Karimi et al., 2013). The electrodes were meticulously lowered at a rate of 0.2 mm/min from the cortex to the hippocampus to minimize trauma to the brain tissue. Cis-2-[[(3,5-Dichlorophenyl)amino]carbonyl]cyclohexanecarboxylic acid (VU0155041) (Tocris, UK), was dissolved in normal saline (0.9 % NaCl). VU0155041 was microinjected through an injection needle connected to a 5-μL Hamilton microsyringe with a polyethylene tube. VU0155041 was administered into the DG over a 5-min period at concentrations of 50 μg/0.5 μl saline during the baseline recording, midway through the baseline recording session (Ebrahimi et al., 2021). Control groups were administered saline.

2.3. Electrophysiological recordings and LTP induction

Single 0.1 ms biphasic square wave pulses were administered through the stimulation of the perforant path (PP) to acquire input–output current profiles, determining the appropriate stimulus intensity for each animal.

Field potential recordings were conducted in the granular cells of the DG subsequent to the stimulation of the PP. Test stimuli were administered to the PP at 10-s intervals, with electrodes strategically placed to elicit the maximum amplitude of the population spike (PS) and field excitatory postsynaptic potentials (fEPSP). After determining the appropriate stimulus intensity for each animal, the baseline stimulus intensity was set at 50 % of the maximum response. Once a steady-state baseline response was established for 40 min, LTP was induced using a high frequency stimulation (HFS) protocol of 400 Hz (10 bursts of 20 stimuli, 0.2 ms stimulus duration, 10 s interburst interval). Following the HFS protocol (with roughly 80 % of the maximum response), the intensity of stimulation was adjusted back to 50 % of the maximum amplitude. Both fEPSP and PS were recorded 5, 30, and 60 min after the HFS in order to determine any changes in the synaptic response of DG neurons. At each designated time point, 10 consecutive evoked responses were averaged at a 10-s stimulus interval. This process allowed for the comprehensive analysis of synaptic changes over time in the DG neurons (Taube and Schwartzkroin, 1988; Karimi et al., 2015; Salehi et al., 2018). For the stimulations, the parameters of the stimuli were defined using custom software and were transmitted through a data acquisition board connected to a constant current isolator unit (A365 WPI, USA) before being delivered to the PP via the stimulus electrodes. The resulting field potential response from the DG underwent a series of processes: it was first passed through a preamplifier, then amplified (1000×) using a Differential amplifier DAM 80 WPI (USA), and finally, filtered (band pass 1 Hz to 3 kHz). This response was then digitized at a sampling rate of 10 kHz, made visible on a computer, and saved in a file for subsequent offline analysis.

2.4. Measurement of evoked potentials

The elicited field potential in the DG exhibits two distinct

components: the PS and the fEPSP. Throughout electrophysiological recordings, alterations in both PS amplitude and fEPSP slope were quantified (Karimi et al., 2013).

The calculations for fEPSP slope and PS amplitude were determined using Eqs. (1) and (2), respectively (refer to Fig. 1 for details).

$$EPSP = \frac{\Delta V}{\Delta T} \quad (1)$$

$$PS = \frac{\Delta V_1 + \Delta V_2}{2} \quad (2)$$

Eq. 1 expresses the calculation for the EPSP, defined as the ratio of the potential difference (ΔV) between points a1 and a2 to the time difference (ΔT) between points a3 and a5.

Eq. 2 describes the computation for the population spike (PS), which is equal to the average of the potential differences (ΔV_1 and ΔV_2) between points a4 and a6, and points a6 and a7, respectively (as illustrated in Fig. 1).

2.5. Statistical analysis

The presentation of data involved expressing values as the mean ± standard error of the mean (SEM), with analysis conducted using GraphPad Prism® 8.0.2 software. To assess normality, the Shapiro-Wilk test was applied. The ensuing statistical examination comprised a two-way repeated measures ANOVA, followed by the Tukey's multiple comparisons post-test. LTP data underwent normalization, referencing the mean of fEPSP slopes and PS amplitude recorded before LTP induction, as described by Eq. 3 (Scott-McKean et al., 2018). Significance was established at a probability threshold of 0.05.

$$\text{LTP} = \frac{\text{the EPSP or PS value after HFS induction} \times \quad 100\%}{\textit{the average EPSP or PS at baseline}} \quad (3)$$

3. Results

3.1. Effects of VU on field EPSP LTP in PP-DG pathway

LTP, evoked by HFS stimulation of the PP-DG pathway, was reduced by VPA exposure. Representative examples of evoked field potential in the DG recorded prior to and 60 min after high-frequency stimulation are shown in Fig. 2. The pre- and post-HFS EPSP slopes were as follows (mV/ms): Control (pre: 0.58 ± 0.06, post: 0.91 ± 0.08), VPA-exposed (pre: 0.48 ± 0.03, post: 0.62 ± 0.04), Control + VU (pre: 0.6 ± 0.06, post: 1.01 ± 0.09), and VPA-exposed + VU (pre: 0.51 ± 0.03, post: 0.79 ± 0.02). The results showed that prenatal exposure to VPA resulted in decreased fEPSP potentiation in DG granular neurons (Fig. 3). Two-way repeated-measures ANOVA revealed significant effect of time- points [F (1.208, 48.30) = 16.83, P<0.0001], group effect [F (3, 40) = 1.351, P=0.2717], and interaction [F (9, 120) = 1.456, P=0.1721] in slope of fEPSP potentiation in the granular cell of DG (Fig. 3). Post-hoc comparisons indicated that VPA-exposed rats exhibited significantly less fEPSP slope potentiation than control animals (P= 0.0435). But intrahippocampal injection of VU0155041, has no effects on slope of EPSP potentiation in control and VPA-exposed rats.

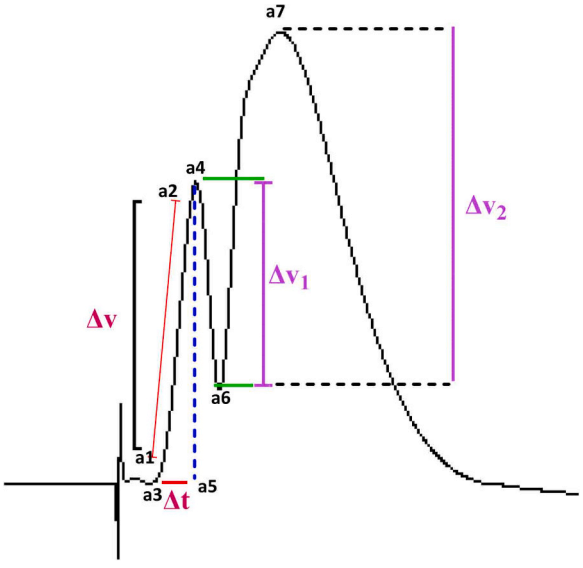

Fig. 1. Assessment of Evoked Potentials. Calculations for EPSP slope and PS amplitude were performed as per Eqs. 1 and 2, respectively (see details in the text). ΔV indicates the potential difference, and ΔT signifies the time difference.

3.2. Effects of VU on field PS LTP in PP-DG pathway

As shown in Fig. 4, differences in population spike LTP were evident in the magnitude of PS amplitude. The pre- and post-HFS PS amplitude were as follows (mV): Control (pre: 0.81 ± 0.09, post: 2.3 ± 0.1), VPA-exposed (pre: 0.64 ± 0.05, post: 1.65 ± 0.08), Control + VU (pre: 0.65 ± 0.09, post: 2.4 ± 0.24), and VPA-exposed + VU (pre: 0.75 ± 0.04, post: 1.12 ± 0.09). Two-way repeated-measures ANOVA revealed significant effect of time- points [F (1.203, 43.31) = 35.65, P<0.0001], group effect [F (3, 36) = 2.161, P=0.1096], and a significant interaction [F (9, 108) = 2.172, P=0.0294] in PS amplitude of the granular cell of DG (Fig. 4). Post-hoc comparisons indicated that VPA-exposed rats exhibited significantly less PS LTP than control animals. Moreover, VU0155041 significantly decreased PS LTP at PP-DG pathway in VPA-exposed rats.

4. Discussion

This study tested whether intra-DG injection of VU0155041, a PAM and partial agonist of mGlu4 receptor can alleviate the impairment of hippocampal LTP in VPA-induced autistic rat model. Employing HFS in the PP-DG pathway, the study revealed a reduction in LTP manifested by a decline in fEPSP slope potentiation and PS amplitude in DG granular neurons following VPA exposure. Interestingly, the administration of VU0155041, through intrahippocampal microinjection did not alter fEPSP slope potentiation in either control or VPA-exposed rats. Remarkably, the administration of VU0155041 not only fails to alleviate this reduction but also resulted in a significant decrease in PS LTP in the PP-DG pathway in VPA-exposed animals. Although we lack specific data regarding the stability and migration speed of this compound in the brain, drawing from our prior experience with VU0155041, we can affirm that its effects endure for more than 3 h within the brain.

The observed reduction in hippocampal LTP following VPA exposure suggests an imbalance in excitatory and inhibitory signaling, which aligns with the notion that disruptions in the excitation/inhibition (E/I) balance are associated with neurodevelopmental disorders such as autism (Manyukhina et al., 2022; Qi et al., 2022). Furthermore, it has been shown that the detrimental effects of VPA exposure extend not only to excitatory synapses but also to inhibitory synapses (Qi et al., 2022).

Perhaps the most intriguing and unexpected result is that the administration of VU0155041 not only failed to alleviate the reduction in LTP induced by VPA but also resulted in a significant decrease in PS LTP in the PP-DG pathway in VPA-exposed animals. This implies a potential adverse effect (at concentrations of 50 μg) or interference with the other physiological processes underlying synaptic plasticity in the hippocampus of VPA-induced autistic rats.

Research suggests that mGlu4 receptor plays a role in maintaining the excitatory and inhibitory signaling within neural circuits. mGlu4 receptor is typically located on presynaptic terminals. When activated by glutamate, it can inhibit the release of further glutamate (Ramos et al., 2012). This presynaptic inhibition helps regulate the overall excitatory input to the neuron, preventing excessive glutamate release.

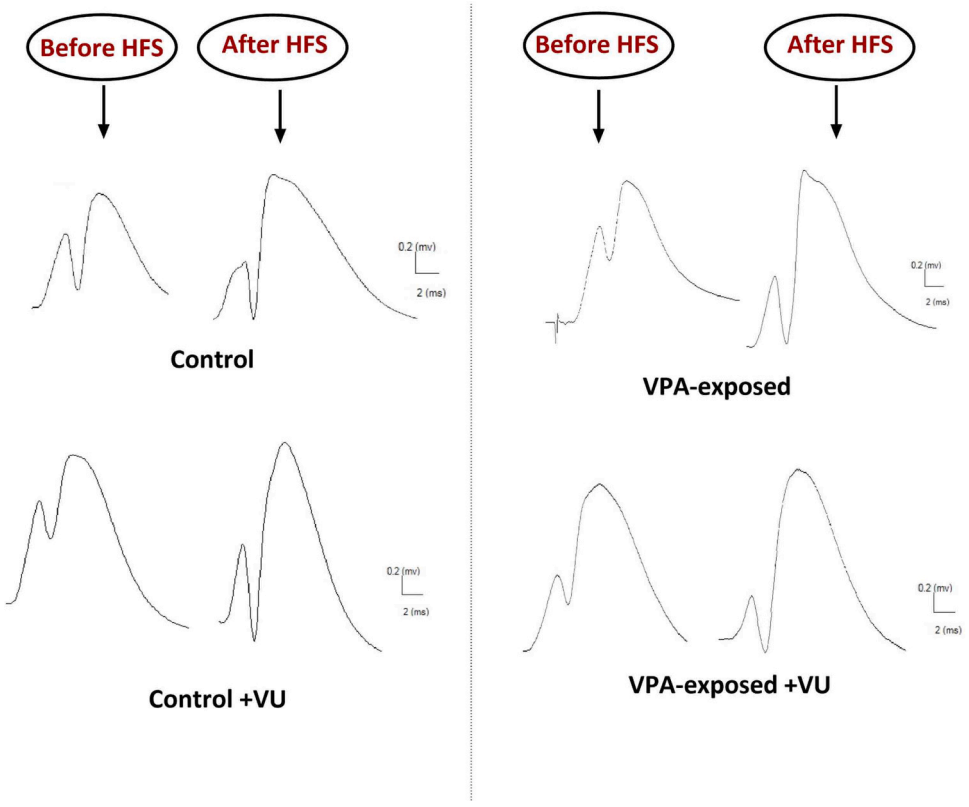

Fig. 2. Exemplar traces of evoked field potential in the DG recorded before and after high-frequency stimulation across all groups.

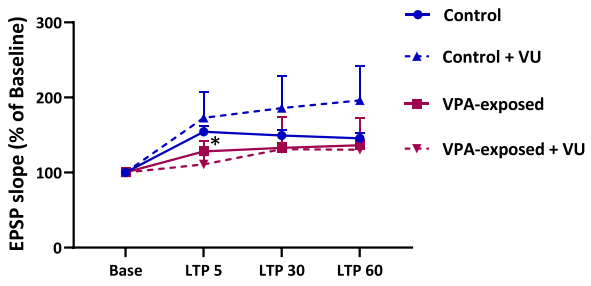

Fig. 3. The selective mGlu4 allosteric agonist, VU0155041, did not impact the EPSP slope potentiation in both control and VPA-exposed rats. *P <0.05.

By modulating glutamate release, mGlu4 receptor contributes to maintaining an appropriate level of excitatory signaling (Ramos et al., 2012). In addition to affecting glutamatergic neurotransmission, mGlu4 receptor can also influence the activity of gamma-aminobutyric acid (GABA)ergic neurons. GABA is the main inhibitory neurotransmitter in the brain. Activation of mGlu4 receptor on GABAergic neurons may reduces GABA release (Hopkins et al., 2009). This contributes to the overall balance between excitatory and inhibitory neurotransmission. Our findings suggest that mGlu4 receptor modulation with VU0155041 does not simply restore the balance in synaptic plasticity disrupted by VPA exposure. Instead, it appears to have an unexpected impact, potentially exacerbating the imbalance. This underscores the complexity of mGlu4 receptor function in the context of neurodevelopmental disorders and highlights the need for a more nuanced understanding of its role in maintaining the E/I balance.

Moreover, despite previous reports suggesting a high-density expression of mGlu4 receptors in the dentate molecular layer of the hippocampus (Corti et al., 2002), there is an ongoing debate regarding the precise localization of mGlu4 receptors. Some arguments propose that mGlu4 receptors is exclusively situated in the CA2 region of the

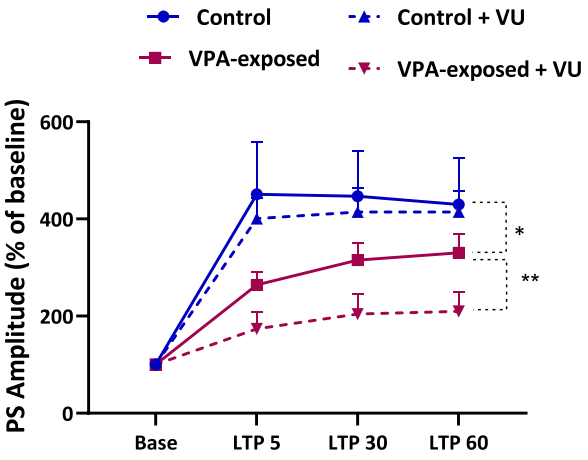

Fig. 4. The Selective mGlu4 receptor allosteric agonist, VU0155041, resulted in a reduction of PS LTP in the PP-DG pathway in VPA-exposed animals. *P <0.05, **P <0.01.

hippocampus. Given this uncertainty, it is noteworthy that our injections were targeted specifically into the DG. This spatial specificity in our experimental approach could potentially account for the observed results, as the effectiveness of interventions may depend on the accurate localization of the targeted receptors. The discrepancy in receptor distribution emphasizes the importance of considering regional variations in mGlu4 receptor expression when interpreting experimental outcomes and underscores the need for further investigations into the precise anatomical localization of mGlu4 receptors within the hippocampus.

The unexpected reduction in PS-LTP observed in the PP-DG pathway following VU0155041 administration in VPA-exposed rats prompts a pertinent discussion in the context of the heterodimerization of mGluRs,

specifically mGlu2 and mGlu4 receptors (Xiang et al., 2021). While our study did not directly investigate mGlu2/mGlu4 heterodimers, Xiang et al., underscored the importance of synaptic specificity in heterodimeric regulation (Xiang et al., 2021). Heterodimerization can lead to nuanced regulatory outcomes, influencing excitatory transmission in a manner distinct to specific synaptic pathways (Xiang et al., 2021). These findings underscore the intricacies of mGlu receptor modulation and heterodimerization, prompting further exploration into the molecular and cellular mechanisms underlying the observed effects and their potential implications for targeted therapeutic strategies in neurological and neuropsychiatric disorders.

In addition, the selective inhibition of PS increase by VU in the VPA-treated animals suggests a decrease in firing probability of dentate granule cells, without the change in excitatory synaptic drive. One possibility is that VU0155041 may modulate intrinsic excitability within dentate granule cells. Rather than directly altering excitatory synaptic transmission, VU0155041 might influence the membrane properties of these cells, affecting their firing threshold and propensity to generate action potentials. This modulation of intrinsic excitability could occur through various mechanisms, such as the regulation of ion channel function or intracellular signaling pathways. For example, activation of mGlu4 increases two-pore domain K^+ (K_2P) channels activity, and inhibition of PKA is involved in the observed channel activation by mGlu4 (Cain et al., 2008). K_2P channels contributes to the intrinsic excitability of dentate granule cells in hippocampus (Yarishkin et al., 2014).

In contrast to our findings, the study on Fragile X Syndrome (FXS) demonstrated that the activation of mGlu4 receptor has a rescuing effect on various aspects of synaptic function and behavior in a mouse model of FXS. Specifically, the activation of mGlu4 receptor in the FXS model is associated with the restoration of parallel fiber synaptic transmission, LTP, motor learning, and social behavior. This suggests that mGlu4 receptor modulation may play a beneficial role in ameliorating the deficits observed in FXS, presenting a potential therapeutic avenue for addressing cognitive and behavioral impairments associated with this genetic disorder (Martín et al., 2023). While the outcomes in the FXS model showcase a positive impact of mGlu4 receptor activation, the contrast with our results, highlights the complexity and context-specific nature of mGlu receptor function in different neurological conditions. Factors such as the specific model, underlying genetic or environmental factors, and the regional distribution of mGlu4 within the brain may contribute to the divergent outcomes observed between the two studies.

Adding to the complex landscape of mGlu4 receptor modulation in neurodevelopmental disorders, the study by Becker et al. reveals that VU0155041 has a therapeutic effect in alleviating autistic symptoms in Oprm $^{-/-}$ mice (Becker et al., 2014). The reported improvement in autistic symptoms following VU0155041 administration suggests a potential role for mGlu4 receptor modulation not only in FXS but also in other genetic models of autism, expanding the scope of its therapeutic relevance. The contrasting outcomes between this study and our own results in a VPA-induced rat model further underscore the intricate interplay of genetic and environmental factors in determining the effectiveness of mGlu4 receptor modulation across different models of neurodevelopmental disorders. These diverse findings emphasize the need for a nuanced understanding of mGlu4 receptor function and its potential as a therapeutic target within the complex spectrum of autism-related conditions.

Considering this contrast, further investigation is warranted to elucidate the nuanced mechanisms underlying mGlu4 receptor function in different models of autism and to refine our understanding of how modulation of this receptor may vary across diverse models of neurodevelopmental disorders.

Finally, while our study sheds light on the role of mGlu4 modulation in synaptic plasticity within an ASD context, several limitations should be noted. We focused exclusively on the PP-DG pathway and the effects of VU0155041 in a VPA-induced rat model, potentially overlooking interactions with other brain regions or neurotransmitter systems relevant to ASD. Additionally, the VPA-induced rat model may not fully reflect the complexity of human ASD. Notably, our study did not assess the expression levels of mGlu4 in the hippocampus of VPA-treated rats, nor did we investigate potential changes in spontaneous excitatory postsynaptic currents (EPSCs) or intrinsic excitability in DG granule cells of VPA-treated rats, which could provide further insights into the synaptic alterations associated with ASD. Furthermore, we did not examine the effects of VU0155041 on spontaneous inhibitory postsynaptic currents (IPSCs) of DG granule cells in VPA-treated rats, limiting our understanding of its impact on inhibitory neurotransmission in this model. These additional considerations underscore the complexity of ASD pathophysiology and highlight avenues for future research.

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CRediT authorship contribution statement

Zahra Ebrahimi: Data curation, Investigation, Methodology, Software. **Reza Ghahremani:** Investigation, Methodology, Software, Writing – review & editing. **Abdolrahman Sarihi:** Conceptualization, Data curation, Funding acquisition, Validation. **Parsa Gholipour:** Data curation, Investigation, Methodology, Software, Writing – review & editing. **Reihaneh Mohammadkhani:** Data curation, Investigation, Methodology, Writing – review & editing. **Seyed Asaad Karimi:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Resources, Software, Supervision, Writing – original draft, Writing – review & editing. **Alireza Komaki:** Conceptualization, Data curation, Funding acquisition, Validation. **Iraj Salehi:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Writing – review & editing.

Author contribution

SAK designed the project, wrote the manuscript, performed the statistical analysis, revised the manuscript, and supervised the project. **PG**, **RM**, **ZE**, and **RG** conducted the experiment and analyzed the data. **AS**, **AK**, and **IS** collaborated on designing the experiments, analyzing the data, and securing funding. All authors thoroughly reviewed and approved the final results.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT in order to check grammar and syntax. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of Competing Interest

Authors declare that they have no conflict of interest.

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