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Mice lacking *GRIP1/2* show increased social interactions and enhanced phosphorylation at GluA2-S880

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HIGHLIGHTS

- Neuron-specific *Grip1/2*-knockout mice show increased social interactions.
- Increased GluA2-pS880 in frontal cortex and decreased GABAβ3 expression in striatum were identified.
- Results support a role of Grip1/2-mediated AMPA signaling in regulating social behavior.
- Disturbances of AMPA- and GABA-signaling are implicated in autism social deficits.

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ABSTRACT

Glutamate receptor interacting proteins 1 and 2 (GRIP1/2) play an important role in regulating synaptic trafficking of AMPA receptor 2/3 (GluA2/3) and synaptic strength. Gain-of-function *GRIP1* mutations are implicated in social behavioral deficits in autism. To study mechanisms of Grip1/2-mediated AMPA signaling in the regulation of social behaviors, we performed social behavioral testing on neuron-specific *Grip1/2*-double knockout (DKO) and wild type (WT) mice that are matched for age, sex, and strain background. We determined the expression profile of key signaling proteins in AMPAR, mGluR, mTOR, and GABA pathways in frontal cortex, striatum, and cerebellum of DKO mice. Compared to WT mice, DKO mice show increased sociability in a modified three-chamber social behavioral test [mean ± sem for interaction time in seconds; WT: 44.0 ± 5.0; n = 10; DKO: 81.0 ± 9.0; n = 9; two factor repeated measures ANOVA: $F(1,37) = 14.45$; $p < 0.01$ and planned *t*-test; $p < 0.01$] and in a dyadic male–male social interaction test (mean ± sem for total time in seconds: sniffing, WT-WT, 18.9 ± 1.1; WT-DKO, 42.5 ± 2.1; *t*-test: $p < 0.001$; following, WT-WT, 7.7 ± 0.72; WT-DKO,14.4 ± 1.8; *t*-test: $p < 0.001$). Immunoblot studies identified an increase in phosphorylation at GluA2-Serine 880 (GluA2-pS880) in frontal cortex (mean ± sem; WT: 0.69 ± 0.06, n = 5; DKO: 0.96 ± 0.06, n = 6; *t*-test; $p < 0.05$) and reduced GABAβ3 expression in striatum (mean ± sem; WT: 1.16 ± 0.04, n = 4; DKO: 0.95 ± 0.06, n = 4; *t*-test; $p < 0.05$) in DKO mice. GluA2-S880 phosphorylation is known to regulate GluA2synaptic recycling, AMPA signaling strength and plasticity. GABAβ3 has been implicated in the etiology and pathogenesis in autism. These data support an important role of Grip1/2-mediated AMPA signaling in regulating social behaviors and disturbance of glutamate- and GABA-signaling in specialized brain regions in autism-related social behavioral deficits.

1. Introduction

Severe deficits in reciprocal social interactions are core features of several neuropsychiatric disorders including Autism spectrum disorders (ASDs). Both genetic and environmental risk factors are known to contribute to behavioral phenotypes in ASDs [1,2]. Despite extensive studies in recent years, neural mechanisms of social behavioral regulation and pathogenesis of social deficits in autism remain poorly understood.

Abbreviations: GRIP1/2, glutamate receptor interacting proteins 1 and 2; AMPA, 2-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; GluA2, AMPA glutamate receptor 2; GABAβ3, gamma-aminobutyric acid receptor beta 3 subunit.

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Glutamate mediates the majority of excitatory neurotransmission in central nerve system through its families of receptors including ionotropic (AMPA, NMDA, Kainate) and metabotropic glutamate (mGluR) receptors [3]. AMPA receptors cycle between postsynaptic membrane and endosomal vesicles at basal state and in response to synaptic activities [4]. These dynamic changes in receptor availability at postsynaptic membrane play an important role in determining synaptic strength and plasticity [4]. Synaptic trafficking of AMPA receptors is tightly regulated by neural scaffolding proteins such as glutamate receptor interacting proteins 1 and 2 (GRIP1/2) [5–7] and PICK1 [8]. GRIP1/2 are neuron-enriched scaffolding proteins with 7 PDZ domains [5,6], bind to the c-terminal domains of AMPA receptors 2 and 3 (GluA2/3) via its PDZ4-6, and play an important role in GluA2/3 trafficking, synaptic organization and transmission [5]. GRIP1/2 are abundantly expressed in both glutamatergic and GABAergic synapses [9], suggesting a role in regulating both excitatory and inhibitory synaptic functions. Loss of GRIP1/2 expression in neurons results in decelerated recycling of GluA2 to synaptic membrane in hippocampal neurons [10] and lack of LTD expression in cerebellum [7].

Disturbances in glutamate signaling and glutamatergic synaptic functions have been implicated in ASDs. Studies have identified an increase in transcript levels of AMPA glutamate receptor 1, glial glutamate transporter 1, and GRIP1 in postmortem tissues of cerebellum from autism patients [11]. Rare point mutations and/or *de novo* microdeletions or duplications were found in numerous genes including *NLGN3/4, SHANK3, NRXN1, and CNTNAP2* that are known to involve in glutamate receptor clustering, as well as synaptogenesis, dendritic development and maturation in glutamatergic synapsis [12–16]. At the brain circuit levels, abnormal functional connectivity between cortical–cortical and cortical-striatal areas have been identified in autism patients [17,18]. For example, abnormal functional connectivity in frontal lobe circuits was detected in patients with *CNTNAP2* mutations [19]. An altered balance of excitatory and inhibitory synaptic functions was found in well-established autism mouse models including Fragile X syndrome [20], Rett syndrome [21], and Neuroligin 3 mutant mice [22]. Furthermore, growing evidence has linked dysfunction in GABAergic signaling pathways to ASDs. For instance, studies have identified a reduced expression of GABAergic signaling proteins and altered GABAergic neurons in brain tissues from autism patients [23,24]. These pathological changes in the GABA-signaling pathway could be a direct effect or a consequence of mutations in other signaling or scaffolding proteins that affect GABA synapses. Together, these results support that dysregulation of both excitatory and inhibitory synaptic function, brain circuits, and network synchrony contributes to behavioral deficits in certain forms of ASDs [25–27].

Recent studies from our laboratories implicated AMPA glutamate signaling disturbance in autism's social deficits [28]. We identified multiple missense mutations clustered at PDZ4-6 of GRIP1 in a cohort of patients with autism [28,29]. These autism-associated mutations showed a gain-of-function effect manifesting an increase in binding with GluA2 in yeast-two-hybrid assay and an accelerated recycling of GluA2 in hippocampal neurons [28]. Correspondingly, loss of *Grip1/2* expression causes a decelerated recycling of GluA2 in neurons [10]. Genotype-phenotype correlation studies of affected sib-pairs in proband families supported that presence of these GRIP1 mutations contribute to an increase in the severity of autism-related social behaviors as measured by cumulative ADI-R scores in reciprocal social interactions [28].

To understand the roles of GRIP1/2 in modulating social behaviors and pathogenesis of social deficits in autism, we systematically studied social behaviors in neuron-specific *Grip1/2* double knockout (DKO) mice and examined key AMPA- and GABA-signaling proteins in brain regions that are implicated in autism [30–32]. We found that DKO mice with a complete loss of Grip1/2 neuronal expression exhibit an increase in social interactions, which is consistent with our prior finding that GRIP1 gain-of-function mutations contribute to social deficits in autism [28]. Further, our studies showed that GluA2-S880 phosphorylation (GluA2-pS880) is increased in frontal cortex and cerebellum while GABAβ3 expression is reduced in striatum of DKO mice. These results implicate that GRIP1/2-mediated GluA2/3 trafficking play a role in the modulation of social behaviors and that dysregulation of glutamatergic and GABAergic signaling contribute to the pathogenesis of social behavioral deficits in autism.

2. Methods

2.1. Animals

Grip1/2 double knockout (DKO) mice were generated by crossing *Grip2* conventional KO mice with conditional *Grip1* KO mice (*Grip1* flox/flox) to circumvent embryonic lethality of conventional *Grip1* KO mice [7,33]. Neuron-specific *Grip1* deletion was achieved by crossing with Nestin-Cre transgenic mice. The cre-dependent *Grip1* deletion in neurons was verified in lysates in multiple brain regions by immunoblot (Supplementary Fig. S1). Adult male DKO mice and WT controls (WT) were generated by breeding littermates heterozygous for *Grip1/2* deletions and were matched for age, sex, and strain background. WT and DKO mice were genotyped using PCR of tail DNA following a published protocol [28]. All mice were housed in temperature-controlled rooms with 12-h light/dark cycle (9:00 and 21:00) and had free access to water and standard mouse chow. Animal breeding and procedures were conducted in strict accordance with the NIH Guide for Care and Use of Laboratory Animals. The Animal Care and Use Committee (ACUC) at the Johns Hopkins University approved this study protocol.

2.2. Mouse behavioral testing

Mouse behavioral tests were conducted at the Animal Behavioral Core of the Johns Hopkins University School of Medicine following standard protocols from Animal Behavioral Core User Manual (http://www.brainscienceinstitute.org/index.php/cores) [34,35] and published protocols from our laboratories [28,36]. The test order and age of the study cohorts of mice for individual test (in parenthesis) are as follow: Open Field (2 month), elevated plus maze (2 month), rotarod (3 month), sociability and preference for social novelty (3 month), dyadic male–male interaction (4 month), resident-intruder test (4 month), general olfaction (5 month). For individual test, WT and DKO mice were always tested together to minimize variations. Test animals have at least one week break in between these tests. The average ambient lighting (lux) for each tests: open field (318), elevated plus maze (492), dyadic male–male interaction, sociability, preference for social novelty, and resident-intruder test (595) as described in previous published studies [28,36].

2.2.1. Open-field test

Each individual test mouse was placed in a photo-beam (n = 16 at equal spacing of 2.5 cm) equipped clear plastic chamber (45 × 45 cm) and was allowed to explore free from interference for 30 min. The peripheral area (425 sq cm) was defined by the two side-photo beams, #1-2 and #15-16 while the central area (1600 sq cm) was defined by photo beams #3-14 at each direction. Movements in the chamber were tracked using a SDI Photobeam Activity System (San Diego Instruments). The patterns of ambulatory movement, fine movement, and rearing behavior at central and peripheral areas were automatically recorded and analyzed.