Experimental Neurology 305 (2018) 1–12

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Impaired social behaviors and minimized oxytocin signaling of the adult mice deficient in the *N*-methyl-D-aspartate receptor GluN3A subunit

Jin Hwan Lee[a,1], James Ya Zhang[a], Zheng Zachory Wei[a,b], Shan Ping Yu[a,b,*]

[a] Department of Anesthesiology, Emory University School of Medicine, Atlanta, GA 30322, USA
[b] Center for Visual and Neurocognitive Rehabilitation, VA Medical Center, Atlanta, GA 30033, USA

ARTICLE INFO

Keywords:
GluN3A
NR3A
Social behaviors
Oxytocin signaling

ABSTRACT

The *N*-methyl-D-aspartate receptor (NMDAR) has been implicated in the pathophysiology of neurological diseases, such as schizophrenia, autism spectrum disorders (ASD), and Alzheimer's disease (AD), whose unique clinical hallmark is a constellation of impaired social and/or cognitive behaviors. GluN3A (NR3A) is a unique inhibitory subunit in the NMDAR complex. The role of GluN3A in social behavioral activities is obscure. In this study, we sought to evaluate altered social activities in adult GluN3A knockout (KO) mice. GluN3A KO mice spent less time in reciprocal social interaction in the social interaction test compared to wild-type (WT) mice. A social approach test using a three-chamber system confirmed that mice lacking GluN3A had lower sociability and did not exhibit a preference for social novelty. GluN3A KO mice displayed abnormal food preference in the social transmission of food preference task and low social interaction activity in the five-trial social memory test, but without social memory deficits. Using a home cage monitoring system, we observed reduced social grooming behavior in GluN3A KO mice. Signaling genes that might mediate the altered social behaviors were examined in the prefrontal cortex, hippocampus, and thalamus. Among nine genes examined, the expression of the oxytocin receptor was significantly lower in the prefrontal cortex of GluN3A KO mice than that in WT mice. Oxytocin treatment rescued social activity deficits in GluN3A KO mice. These findings support a novel idea that a chronic state of moderate increases in NMDAR activities may lead to downregulation of the oxytocin signaling and impaired behavioral activities that are seen in psychiatric/neurodegenerative disorders.

1. Introduction

Glutamate mediates the majority of excitatory synaptic transmission in the central nervous system (CNS) (Meldrum, 2000). It activates three distinct classes of ionotropic receptors: *N*-methyl-D-aspartate (NMDA) receptors (NMDARs), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPARs), and kainic acid receptors (Hollmann and Heinemann, 1994; Karlsson et al., 2002; Lee et al., 1998). Many studies have focused on the NMDARs because of their widespread distribution in the CNS and broad functions involving brain development, neural plasticity, cognitive functions, excitotoxicity, and neurodegenerative diseases (Bettler and Mulle, 1995; Choi, 1992; Dingledine et al., 1999; Mota et al., 2014; Neill et al., 2014). NMDARs are hetero-multimers (with a tetrameric or pentameric structure) that are composed of at least two GluN1 subunits, and two or three GluN2A-D subunits (Nakanishi and Masu, 1994; Seeburg, 1993). GluN1 (NR1) is required for the fundamental formation of NMDARs, and the GluN2 (NR2) subunit is important in different functional properties, such as learning and memory (Lee et al., 1998; Seeburg, 1993). On the other hand, GluN3A (NR3A) and GluN3B (NR3B) were discovered later and its expression is relatively lower in the adult brain (Al-Hallaq et al., 2002; Ciabarra et al., 1995; Das et al., 1998). The GluN3 subunits are unique inhibitory NMDAR subunits; when a GluN3 subunit is assembled with GluN1 and GluN2, it reduces the Ca^{2+} permeability, affecting NMDAR-mediated synaptic currents and the overall NMDA responses (Nishi et al., 2001; Sucher et al., 1995; Tong et al., 2008). Demonstrated in patch clamp recordings, the expression of the GluN3 subunit substantially suppressed NMDA currents (Cavara and Hollmann, 2008; Ciabarra et al., 1995; Das et al., 1998; Sucher et al., 1995).

The GluN3A expression is widely observed in brain regions, such as

Abbreviations: GluN3A, glutamate NMDA receptor subunit 3A or NR3A; NMDA, *N*-methyl-D-aspartate; NMDAR, *N*-methyl-D-aspartate receptor; KO, knockout; WT, wild-type; CNS, central nervous system; ASD, autism spectrum disorders; AVP, vasopressin; 5-HTR, serotonin receptor; 5-HTT, serotonin transporter; BDNF, brain-derived neurotrophic factor; CD38, cluster of differentiation 38; CD73, cluster of differentiation 73; TNFR1, tumor necrosis factor receptor 1

* Corresponding author at: 101 Woodruff Circle, WMB suite 620, Emory University School of Medicine, Atlanta, GA 30322, USA.
E-mail address: spyu@emory.edu (S.P. Yu).

[1] Authors made the equal contribution to this paper.

https://doi.org/10.1016/j.expneurol.2018.02.015
Received 12 October 2017; Received in revised form 5 February 2018; Accepted 26 February 2018

the cortex, hippocampus, thalamus, and olfactory bulb, whereas GluN3B expression is identified in the brain stem, spinal cord, cortex, hippocampus, amygdala, cerebellum, and olfactory bulb (Bendel et al., 2005; Fukaya et al., 2005; Sucher et al., 1995). The GluN3A expression is identified in the brain of rodents, monkeys, and humans (Henson et al., 2010). GluN3A expression peaks during early postnatal life, and diminishes to lower levels in adulthood (Ciabarra et al., 1995; Perez-Otano et al., 2001). Therefore, most functional studies on the role of GluN3A/3B have focused on the developing stage; the functional role of these subunits in the adult brain remains incompletely defined. We recently demonstrated that even though the GluN3A expression level undergoes a downregulation after the neonatal period, GluN3A in the adult brain has profound impacts on locomotor activity, pain sensation, neuroprotection, olfactory, and cognitive functions (Lee et al., 2016a; Lee et al., 2015; Mohamad et al., 2013). Specifically, deletion or reduced level of GluN3 generates a chronic state of moderately increased activities of NMDAR in the brain. The consequence of this chronic regulation of NMDAR on behavior activities has rarely been investigated before.

NMDAR function has been implicated in the pathophysiology of neuropsychiatric and neurodegenerative diseases, such as schizophrenia, autism spectrum disorders (ASD), and Alzheimer's disease (AD), whose unique hallmark is impaired social behaviors and/or cognitive function with impaired synaptic activities (Gladding and Raymond, 2011; Kocahan and Dogan, 2017; Neill et al., 2014; Rosenthal-Simons et al., 2013). For example, the glycine binding site in NMDARs can regulate social behavior in schizophrenia and ASD (Ghanizadeh, 2011; Labrie et al., 2008). GluN1-deficient mice have behavioral abnormalities, including reduced social interactions, hypolocomotor activity, and deficits in cognitive inflexibility (Duncan et al., 2004; Mohn et al., 1999). NMDAR antagonists, such as ketamine and MK-801, have been shown to have inhibitory or regulatory effects in adult rodent sociability (Silvestre et al., 1997; Siviy et al., 1995). Basic and clinical evidence demonstrate antidepressant and anxiolytic effects of ketamine and other NMDAR antagonists (Murrough et al., 2017). Furthermore, GluN3B knockout (KO) mice showed markedly increased social interactions with their familiar cage mates in their home cage, but moderately increased anxiety-like behavior and decreased social interaction in a novel environment (Niemann et al., 2007). Recent studies have also linked sustained moderate activations of NMDA receptors to neurodegenerative changes in AD (Kocahan and Dogan, 2017). In the present investigation, we tested the hypothesis that chronic deletion of the GluN3A subunit might have a significant impact on the social behavior in adulthood. We also explored the alteration of social genes, such as oxytocin in the GluN3A knockout brain, as an underlying mechanism in the behavior changes.

2. Experimental procedures

2.1. Wild-type and GluN3A KO mice

Young adult (2–3-month-old) male GluN3A KO and littermate WT mice were tested in this investigation. All animal handling and experimental protocols were approved by the Emory University Institutional Animal Care and Use Committee (IACUC), in compliance with National Institutes of Health (NIH) guidelines. The GluN3A KO mice and WT counterparts were originally provided by Nobuki Nakanishi and Stuart A. Lipton at Sanford-Burnham Medical Research Institute (La Jolla, California, USA). Das and colleagues have described detailed information on the background of these mice (Das et al., 1998). Briefly, embryonic stem cells derived from 129/SvJ were electroporated with DNA carrying disrupted GluN3A gene, and then injected into blastocysts from C57BL/6 mice. The resulting chimeric males were crossed with BlackSwiss or 129SvEv females to produce F1 heterozygotes. GluN3A KO homozygote mice were then produced by cross-breeding F1 mice. In our lab, homozygote colonies of either WT or GluN3A KO mice were maintained under the same conditions with identical room temperature (22 °C), same food and water supply, and animal care environment.

2.2. Genotyping of DNA sequence

The genotyping method used in this study was performed as described previously (Mohamad et al., 2013). DNA for genotyping was extracted from tail snips (approximately 2–4 mm). Two separate sets of primers were used for the GluN3A KO and WT mice, respectively. For the WT reaction, forward primer: 5′–CCACGGTGAGCTTGGGGAAG-3′ and reverse primer: 5′-TTGGGGAGCGCCCTGCATGG-3′. For the KO reaction, forward primer: 5′-CCACGGTGAGCTTGGGGAAG-3′ and reverse primer: 5′-GCCTGAAGAACGAGATCAGG-3′. DNA (2 μl) was amplified on a thermal cycler (MJ minim, Personal Thermal Cycler, Bio-Rad, CA, USA) for 40 cycles (95 °C for 60 s, 58 °C for 30 s, 72 °C for 60 s). Afterwards, the samples were incubated for an additional 10 min at 72 °C before being held at 4 °C. PCR products were subjected to electrophoresis in 2% agarose gel with ethidium bromide. The relative intensity of PCR bands was analyzed using InGenius3 manual gel documentation system (Syngene, Frederick, MD, USA).

2.3. Oxytocin treatment

WT and GluN3A KO mice were intraperitoneally (i.p.) administered 10 mg/kg oxytocin (Sigma-Aldrich, St. Louis, MO, USA) or saline vehicle treatment once daily for 7 days. This dosage was selected based on previous reports (Mooney et al., 2014).

2.4. Behavioral tests

In behavioral assays, all animals were tested at the same time of the day (early or late evening). An individual mouse was tested no more than twice a day.

2.4.1. Reciprocal social interaction test

This test was conducted with untreated, unfamiliar, weight-matched, age-matched, and sex-matched mice partners. Subject and stranger mice were put together in a clean empty home cage and recorded by the TopScan System (Clever Sys Inc., Reston, VA, USA). We scored time spent in social interaction of the animals for 10 min between 1 and 5 PM. The following social interaction categories were all blindly scored: social sniffing (both nose-to-nose sniffing and nose-to-anogenital sniffing), following, grooming, biting, and wrestling. Cages were washed with 70% ethanol solution and water before we performed the next test in order to prevent possible inter-subject cross-contamination.

2.4.2. Social approach test using the three-chamber system

The social approach test was utilized to test general sociability and response to social novelty. The test was performed in a three-chambered box with openings between the chambers. Glass panels were used to cover the openings during phase changes. First, the test subject was placed into the empty box and allowed to explore all chambers freely for 10 min. After the habituation period, a stranger (non-littermate) mouse contained in a wire cage was placed into the left chamber. The mouse was then allowed to explore all three chambers. Both the time spent with the stranger mouse (stranger #1) and the time in the empty chamber was recorded over a 10 min session. The test mouse was then returned to the center chamber and the openings were blocked. In the social novelty test, a second stranger mouse (stranger #2) was placed in the empty chamber. The central chamber door was opened, and the test mouse was again allowed to freely explore the chambers and interact with strangers #1 and #2. Since the test mouse already had contact with stranger #1 but not #2, the time spent with stranger #2 vs #1 tested the subject's preference for novel social interaction.