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Research article

Genetic deletion of NMDA receptors suppresses GABAergic synaptic transmission in two distinct types of central neurons

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ABSTRACT

NMDA-type ionotropic glutamate receptors (NMDARs) play an important role in the regulation of synapse development and function in the brain. Recently we have shown that NMDARs are critical for GABAergic synapse development in developing hippocampal neurons. However, it remains unclear whether NMDARs are important for establishment of GABAergic synaptic transmission in other types of neurons in the brain. Here we report that in both cortical pyramidal neurons and midbrain dopamine neurons in ventral tegmental area (VTA), genetic deletion of the GluN1 subunit, which is required for assembly of functional NMDARs, leads to a strong reduction of GABAergic synaptic transmission. These data demonstrate that NMDARs play an important role in the development of GABAergic synaptic transmission in two types of neurons with distinct developmental origins, and suggest that NMDARs are commonly involved in development of GABAergic synaptic transmission in different types of neurons in the brain.

1. Introduction

In the central nervous system, neurotransmitter-mediated synaptic communication between neurons is essential for proper neural circuit function. The formation and functional maturation of synapses are highly regulated processes and subject to activity-dependent refinement [1,2]. When synapse development goes awry, devastating neurological and psychiatric diseases may occur [3–6]. Thus, it is important to understand the molecular and cellular mechanisms underlying the regulation of synapse development.

NMDA-type ionotropic glutamate receptors (NMDARs) are a subclass of glutamate-gated ion channels [7]. In mature neurons, NMDARs are highly concentrated at glutamatergic synapses, whereby they contribute to excitatory synaptic transmission and are implicated in several forms of synaptic plasticity [8]. Interestingly, NMDARs are expressed on neuronal surface long before glutamatergic synaptogenesis. For example, functional NMDARs are detectable in embryonic day 14–16 (E14-16) cortical neurons [9–12]. In developing neurons, it has been shown that NMDARs negatively regulate functional maturation of glutamatergic synapses. Indeed, genetic deletion of NMDARs in developing hippocampal, cortical and midbrain dopamine neurons strongly upregulates AMPA receptor (AMPARs)-mediated synaptic transmission [13–21]. As AMPARs are the primary mediators of fast excitatory synaptic transmission, these data indicate that NMDARs in developing neurons prevent AMPARs from trafficking to synapses, and thus keep glutamatergic synapses in the silent state [13,15,16]. In addition, there is evidence showing that NMDARs are not required for glutamatergic synaptogenesis in hippocampal pyramidal neurons [15,16,22], but are important in other types of neurons [13,14,21].

In addition to regulating glutamatergic synapses, NMDARs have been implicated in inhibitory GABAergic synapse development. Early work has shown that NMDAR activity promotes GABAergic synapse development [23]. Recently we have demonstrated that NMDARs in immature hippocampal neurons are necessary for GABAergic synapse development [24]. Specifically, single-cell genetic deletion of the NMDAR GluN1 subunit in hippocampal progenitor cells at E14 strongly impaired GABAergic synapse formation and reintroduction of NMDARs into the GluN1-deficient neurons rescued the GABAergic synapse deficit [24]. These data demonstrate an important role for NMDARs in the development of GABAergic synaptic transmission in developing hippocampal neurons. However, it remains unclear whether NMDARs are also important for GABAergic synapse development in other neuronal

Abbreviations: NMDA, N-methyl-D-aspartate; GABA, γ-aminobutyric acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; VTA, ventral tegmental area; TH, tyrosine hydroxylase; DAT, dopamine transporter; mIPSC, miniature inhibitory postsynaptic currents; EPSC, excitatory postsynaptic currents; PBS, phosphate-buffered saline; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzoquinoxaline-2,3-dione

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types. The answer to this question will be valuable as it will determine whether NMDAR-dependent GABAergic synapse development is a mechanism widely employed by different types of neurons in the brain. Here we employed a gene inactivation approach to remove NMDARs in developing cortical pyramidal neurons and midbrain dopamine neurons, and examined GABAergic synaptic transmission. We found that GABAergic synaptic transmission is strongly reduced in both types of developing neurons lacking NMDARs, indicating that NMDARs are important for the establishment of GABAergic synaptic transmission in a variety of neuronal types in the brain.

2. Material and methods

2.1. Mouse genetics

Animal housing and handling was conducted according to the ACUC guidelines at NINDS, NIH. *Grin1*$^{f/f}$ (#005246) and Ai14 tdTomato (tdTomato^{Cre}) (#007908) mice were purchased from Jackson laboratory. DAT-Cre mice [25] were provided by Dr. Thomas Hnasko at UCSD.

2.2. Plasmids

Lentiviral vector pFUGW-Cre-mCherry (Cre fused to mCherry, Cre-mCherry) plasmid was used in the current study. In this plasmid, Cre-mCherry expression was driven by a ubiquitin promoter.

2.2.1. In utero electroporation

In Utero electroporation was performed as described previously [24]. Briefly, a timed-pregnant *Grin1*$^{f/f}$ mouse at 14.5 day of gestation (E14.5) was anesthetized with isofluorane. The abdominal cavity was opened and 6–8 embryos in the uterine horns were gently exposed. Plasmid DNA (approximately 1–2 μl) were manually injected into the lateral ventricles of each embryo at the concentration of 2 μg/μl mixed with 0.05% fast green (Sigma 68724). The injection glass pipettes were beveled with the BV-10 micropipette Beveller (Sutter) before injection. After each injection, voltage steps via tweezertrodes (5 mm round, platinum electrodes and BTX electroporator, BTX, ECM830) positioned on either side of the head were applied across the uterus to target hippocampal or cortical neural progenitors. Voltage was 45 V for 5 pulses at 1 Hz, each pulse lasting 50 ms. The embryos were moistened with warmed PBS and returned to the abdominal cavity. Buprenex (0.1 mg/kg) was put into to the abdominal cavity before the wound was sutured. Ketoprofen (5 mg/kg) was administered daily for three days after surgery.

2.2.2. Immunohistochemistry

Mice were perfused via cardiac infusion with 4% paraformaldehyde in cold PBS. To obtain frozen sections, brain tissues were removed and submerged in 30% sucrose for 24 h and sectioned at 40 μm thickness with a cryostat (Leica CM1850). Three to five brain slices containing the ventral tegmental area (VTA) or the Cre-mCherry targeted area of primary motor cortex in in utero electroporation were permeabilized in 0.3% Triton X-100 for 15 min. Slices were incubated in primary antibodies (with 3% normal goat serum and 0.3% Triton X-100) overnight at 4 °C after blocking in 10% normal goat serum for 1 h at room temperature. After extensively washed in PBS, slices were incubated with Alexa 488, Alexa 594 or Alex 633-conjugated secondary antibodies (1:500). Slices were mounted with DAPI Fluoromount-G (SouthernBitotech 0100-01) and imaged by LSM510 confocal microscope with 20X objective for overview images of primary motor cortex and VTA, and 40X objective for detailed images of dopaminergic neuron in VTA. Antibodies specific to tyrosine hydroxylase (TH) (Millipore AB152, 1:500) and NeuN (Millipore MAB377, 1:1000) were used to stain dopaminergic neurons and all neurons in VTA, respectively.

2.2.3. Electrophysiology

For whole-cell recording in acute slices, coronal cortical slices (300 μm) or horizontal midbrain slices (for recording in VTA dopamine neurons, 200 μm) were cut from dissected brain on a DTK Microslicer vibratome (Ted Pella) in chilled high sucrose cutting solution, containing (in mM): KCl 2.5, $CaCl_2$ 0.5, $MgCl_2$ 7, NaH_2PO_4 1.25, $NaHCO_3$ 25, glucose 7, sucrose 210 and ascorbic acid 1.3. Freshly cut slices were placed in an incubating chamber containing carbogenated artificial cerebrospinal fluid (ACSF), containing (in mM) NaCl 119, KCl 2.5, $NaHCO_3$ 26, Na_2PO_4 1, glucose 11, $CaCl_2$ 2.5, $MgCl_2$ 1.3, and recovered at 32 °C for ~30–60 min. Slices were then maintained in ACSF at room temperature prior to recording. After 0.5–1 h incubation at room temperature, slices were transferred to a submersion chamber on an upright Olympus microscope, perfused in ACSF. For recording NMDAR-mediated EPSCs at +40 mV, NBQX (10 μM) and picrotoxin (100 μM) were added to ACSF. For recording mIPSCs at −70 mV, NBQX (10 μM) and TTX (0.5 μM) were added to ACSF. All the pharmacological reagents were purchased from Abcam.

Neurons were visualized by infrared differential interference contrast microscopy. mCherry positive neurons were identified by epi-fluorescence microscopy. The intracellular solution for NMDA EPSC recording contained (in mM) $CsMeSO_4$ 135, NaCl 8, HEPEs 10, Na_3GTP 0.3, MgATP 4, EGTA 0.3, QX-314 5, and spermine 0.1. The intracellular solution for GABA mIPSC recording contained (in mM) $CsMeSO_4$ 70, CsCl 70, NaCl 8, EGTA 0.3, HEPES 20, MgATP 4 and Na_3GTP 0.3. Osmolality was adjusted to 285–290 mOsm and pH was buffered at 7.25–7.35. Neurons were recorded with 3- to 5- MΩ borosilicate glass pipettes. For recording evoked responses in VTA dopamine neurons, the tip of the monopolar glass electrode was placed 50–100 μm from the recorded neurons. For recording evoked responses in layer 2/3 neurons in primary motor cortical region, the monopolar glass electrodes were placed ~50 μm lateral to layer 2/3 pyramidal neurons. All evoked responses in acute slices were paired recordings involved simultaneous whole-cell recordings from one fluorescence-positive neuron and a neighboring fluorescence-negative neuron. The stimulus was adjusted to evoke a measurable, monosynaptic EPSC in both cells. Most mIPSC recordings in acute brain slices were also paired recordings involved simultaneous whole-cell recordings from one fluorescence-positive neuron and one neighboring fluorescence-negative neuron. Series resistance was monitored and not compensated, and cells in which series resistance varied by 25% during a recording session were discarded. Synaptic responses were collected with a Multiclamp 700B-amplifier (Axon Instruments, Foster City, CA), filtered at 2 kHz, digitized at 10 Hz. All recordings were performed at room temperature.

3. Statistics

Statistical analysis was performed using GraphPad Prism 6. All data were presented as Mean ± SEM (standard error of mean). Paired whole-cell recordings were compared with paired *t*-test. Other comparisons between two groups were made using two-tailed, unpaired Student's *t*-test (Mann-Whitney test). Cumulative distributions were compared by the Kolmogorov-Smirnov test. Statistical significance was defined as $p < 0.05$, 0.01 or 0.001 (indicated as *, ** or ***, respectively). p values ≥ 0.05 were considered not significant.

4. Results

We have previously shown that NMDARs are important for the development of GABAergic synaptic transmission in developing hippocampal neurons [24]. However, it remained unclear whether NMDAR-dependent development of GABAergic synaptic transmission is a hippocampal neuron-specific phenomenon, or, NMDARs are also important for establishing GABAergic synaptic transmission in other types of neurons in the brain. To study this question, we examined GABAergic synaptic transmission in cortical neurons or midbrain