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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.

Keywords

GABAergic synapse; synapse development; mIPSC; NMDA receptor; dopamine neuron; cortical pyramidal neuron; in utero electroporation

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

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Author Contributions

XG and WL designed the research; XG performed all experiments. WL wrote the paper, and all authors read and commented on the manuscript, and approved the final version of the manuscript.

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Conflict of Interest None

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 up-regulates AMPA receptor (AMPA)-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 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.

Material and Methods

Mouse genetics

Animal housing and handling was conducted according to the ACUC guidelines at NINDS, NIH. *Grin1^{fl/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.

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.

In utero electroporation.—*In Utero* electroporation was performed as described previously [24]. Briefly, a timed-pregnant *Grin1^{fl/f}* mouse at 14.5 day of gestation (E14.5) was anesthetized with isoflurane. The abdominal cavity was opened and 6-8 embryos in the uterine horns were gently exposed. Plasmid DNA (approximately 1 to 2 μ l) were manually injected into the lateral ventricles of each embryo at the concentration of 2 μ g/ μ l mixed with 0.05% fast green (Sigma 68724). The injection glass pipettes were beveled with the BV-10 micropipette Beveler (Sutter) before injection. After each injection, voltage steps via tweezer electrodes (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.

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 μ m 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 minutes. 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.

Electrophysiology.—For whole-cell recording in acute slices, coronal cortical slices (300 μ m) or horizontal midbrain slices (for recording in VTA dopamine neurons, 200 μ m) were cut from dissected brain on a DTK Microslicer vibratome (Ted Pella) in chilled high sucrose cutting solution, containing (in mM): KCl 2.5, CaCl₂ 0.5, MgCl₂ 7, NaH₂PO₄ 1.25, NaHCO₃ 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, NaHCO₃ 26, Na₂PO₄ 1, glucose 11, CaCl₂ 2.5, MgCl₂ 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 μ M) and picrotoxin (100 μ M) were added to ACSF. For recording mIPSCs at -70 mV, NBQX (10 μ M) and TTX (0.5 μ M) 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 epifluorescence microscopy. The intracellular solution for NMDA EPSC recording contained (in mM) CsMeSO₄ 135, NaCl 8, HEPES 10, Na₃GTP 0.3, MgATP₄, EGTA 0.3, QX-314 5, and spermine 0.1. The intracellular solution for GABA mIPSC recording contained (in mM) CsMeSO₄ 70, CsCl 70, NaCl 8, EGTA 0.3, HEPES 20, MgATP 4 and Na₃GTP 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- M Ω borosilicate glass pipettes. For recording evoked responses in VTA dopamine neurons, the tip of the monopolar glass electrode was placed 50-100 μ m 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 μ m 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.

Statistics

Statistical analysis was performed using GraphPad Prism 6. All data were presented as Mean $\pm$ 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.

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 dopamine neurons in which the GluN1 subunit of the NMDAR was genetically deleted.

We first performed *in utero* electroporation to express Cre fused to mCherry (Cre-mCherry) in layer 2-3 primary motor cortical progenitor neurons of E14.5 *Grin1^{f/f}* mice (Fig. 1A). About three weeks after *in utero* electroporation, we performed a histological assay and found that Cre-mCherry were specifically expressed in layer 2-3 pyramidal neurons in the primary motor cortex (Fig. 1B). We then prepared acute coronal cortical slices and performed simultaneous dual whole-cell recordings to measure NMDAR-mediated excitatory postsynaptic currents (EPSCs) at +40 mV between a transfected Cre-mCherry-positive layer 2/3 pyramidal neurons and a nearby control pyramidal cell in the primary motor cortex in the presence of NBQX and picrotoxin to block AMPAR- and GABA_A-mediated synaptic transmission, respectively. We found that NMDAR-mediated EPSCs were lost in Cre-positive neurons, demonstrating that functional NMDARs are genetically eliminated in these cells (Fig. 1C).

We next measured miniature inhibitory postsynaptic currents (mIPSCs) at the -70 mV to examine GABAergic synaptic transmission in Cre-mCherry positive layer 2-3 neurons in the primary motor cortex in the presence of NBQX and AP-5 to block glutamatergic synaptic transmission. In developing neurons from P9-10 cortical slices, GABAergic synaptic transmission was strongly reduced in Cre-mCherry positive, layer 2-3 pyramidal neurons as there was a nearly 60% reduction of mIPSC frequency (Fig. 2A-B). In addition, although there was a trend for lower mIPSC amplitude in Cre-mCherry positive neurons, it was not significant (Fig. 2A-B). Similarly, in layer 2-3 neurons at P17-18 mice, genetic deletion of NMDARs also caused a substantial reduction of mIPSC frequency, but not amplitude (Fig. 2C-D), suggesting that genetic deletion of NMDARs during development has a lasting effect on GABAergic synaptic transmission in these neurons. Taken together, these data indicate that loss of NMDARs in cortical pyramidal neurons strongly impairs the establishment of GABAergic synaptic transmission.

It is well-known that pyramidal neurons in hippocampus and cortex share many common morphological and functional properties[26]. We thus sought to test whether NMDARs are important for the establishment of GABAergic synaptic transmission in a distinct type of neurons, such as midbrain dopamine neurons. To this end, we crossed a well-characterized knockin mouse line in which the expression of Cre is under the control of the DAT (dopamine transporter) promoter[25] that expresses Cre specifically in midbrain dopamine neurons at around E15 with the conditional GluN1 knockout (*Grin1^{f/f}*) mice. In addition, we crossed a Cre-dependent tdTomato reporter mouse line (Ai14, tdTomato^{Cre}) with *Grin1^{f/f}*. We then crossed *Grin1^{f/+}*/DAT-Cre with *Grin1^{f/f}*/tdTomato^{Cre} to generate *Grin1^{f/f}*/DAT-Cre/tdTomato^{Cre} mice or *Grin1^{f/+}*/DAT-Cre/tdTomato^{Cre} mice that served as the control (Fig. 3A). In this three-gene modified mouse line, Cre expression led to genetic deletion of the conditional GluN1 alleles and simultaneously the expression of tdTomato, a red fluorescent protein, in dopamine neurons, to facilitate electrophysiological recordings (Fig. 3B). We further confirmed the loss of NMDARs in dopamine neurons with a dual whole-cell recording approach in horizontal brain slices containing the ventral tegmental area (VTA) as described before[27]. In these experiments, we performed paired whole-cell recordings of NMDAR-mediated evoked EPSCs at +40 mV from a tdTomato positive neuron (dopamine neuron) and a neighboring tdTomato negative neuron (non-dopamine neuron) in *Grin1^{f/f}*/DAT-Cre/tdTomato^{Cre} mice or *Grin1^{f/+}*/DAT-Cre/tdTomato^{Cre} mice. The stimulation

electrode was placed at the rostral part of the VTA area to evoke a measurable, monosynaptic EPSC in both cells. We found that NMDAR-mediated EPSCs were eliminated in dopamine neurons in *Grin1^{f/f}/DAT-Cre/tdTomato^{Cre}* mice, but not in *Grin1^{f/+}/DAT-Cre/tdTomato^{Cre}* mice (Fig. 3C-E), demonstrating the loss of NMDARs in dopamine neurons from *Grin1^{f/f}/DAT-Cre/tdTomato^{Cre}* mice.

We next measured mIPSCs in dopamine neurons to examine GABAergic synaptic transmission. We found that mIPSC frequency was strongly reduced in dopamine neurons in *Grin1^{f/f}/DAT-Cre/tdTomato^{Cre}* mice (Fig. 4A-B). However, there was no significant difference of mIPSC amplitudes in dopamine neurons from *Grin1^{f/f}/DAT-Cre/tdTomato^{Cre}* and *Grin1^{f/+}/DAT-Cre/tdTomato^{Cre}* mice (Fig. 4A-B). These data indicate that NMDARs are also important for proper establishment of inhibitory synaptic transmission in midbrain dopamine neurons.

Discussion

Neural circuit function relies on rapid communication through both excitatory and inhibitory chemical synapses, and synaptic dysfunctions can lead to devastating neurological and psychiatric disorders [3-6, 28-30]. Thus, it is important to understand the regulatory mechanisms underlying the formation and maturation of synapses. Hippocampal neurons have been widely used to study the molecular and cellular mechanisms for synapse development in the central nervous system. However, as enormous diversity of neuronal types and function exist in the brain, it is important to examine whether the mechanisms discovered in hippocampal neurons also apply in other neuronal types. We have previously demonstrated that NMDARs play a critical role in controlling the establishment of GABAergic synaptic transmission in developing hippocampal neurons[24]. Here we examined the role of NMDARs in development of GABAergic synaptic transmission in two distinct types of neurons.

Our data show that single-cell genetic deletion of the GluN1 subunit leads to a strong reduction of GABAergic synaptic transmission in cortical layer 2-3 pyramidal neurons. Similarly, in midbrain dopamine neurons loss of NMDARs causes a significant decrease of inhibitory synaptic transmission. Taken together with our previous findings of an important role of NMDARs in GABAergic synapse development in hippocampal neurons[24], these data collectively demonstrate that neurons with distinct developmental origins commonly rely on NMDAR-dependent mechanisms for establishing GABAergic synaptic transmission during development. It is also important to note that it is a partial loss of GABAergic synaptic transmission in neurons lacking NMDARs. This suggests that there are other mechanisms existing in neurons important for GABAergic synapse development. Currently, the molecular mechanisms for either NMDAR-dependent or independent processes for GABAergic synapse development remain largely unclear. Recent studies have shown the importance of several postsynaptic cell surface proteins in GABAergic synapse development [31-42]. Thus, it will be critical to investigate the functional interactions between NMDARs and above-mentioned molecules in GABAergic synaptogenesis in the future.

Previous work has shown that NMDARs negatively regulate AMPAR-mediated excitatory synaptic transmission in pyramidal neurons and dopamine neurons. In both hippocampal CA1 and cortical pyramidal neurons, genetic deletion of NMDARs strongly up-regulate AMPA EPSCs [13-17]. Similarly, loss of NMDARs in midbrain dopamine neurons significantly increases AMPAR-mediated synaptic transmission [18, 19]. Furthermore, it has been shown that NMDARs negatively regulates AMPA EPSCs in hippocampal neurogliaform interneurons and adult-born granule cells [20, 21]. Taken together, these data indicate that NMDARs suppress functional maturation of AMPAR-mediated fast excitatory synaptic transmission and maintain the excitatory synapses in a silent state during development [15]. Thus, NMDARs in developing neurons promote GABAergic synapse development as demonstrated here and before [24], and at the same time suppress the maturation of glutamatergic synaptic transmission [13-21]. One potential advantage for such dual regulation of synapse development by NMDARs may be to prevent AMPAR-mediated over-excitation of neurons before proper development and maturation of GABAergic inhibitory transmission. Therefore, it is tempting to speculate that the NMDAR is a critical signaling molecule for controlling the establishment of excitation/inhibition balance during development.

Our findings that GABAergic synaptic transmission is strongly reduced in different types of neurons lacking NMDARs may have important implications. Genetic deletion of NMDARs in different brain regions has been widely used to probe the function of NMDAR-dependent synaptic transmission and/or plasticity in animal behavior [18, 19, 27, 43-50]. The reduction of inhibitory synaptic transmission in several types of neurons lacking of NMDARs as shown by us indicates that the behavioral outcomes induced by NMDAR knockout could be generated by a combined effect of loss of NMDARs, increased AMPAR-mediated synaptic transmission and/or reduced synaptic inhibition. Thus, innovative approaches to precisely manipulate NMDAR function, instead of simply genetically deleting the receptors, may be the key to determine the role of NMDAR activity in neural circuit function and behavior.

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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-benzoquinoline-2,3-dione.

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Highlight

- GluN1 knockout in developing cortical neurons suppresses GABAergic transmission
- Loss of NMDARs in developing dopamine neurons reduces synaptic inhibition
- NMDARs are commonly involved in development of GABAergic synaptic transmission

Figure 1. Genetic deletion of NMDARs in layer 2-3 pyramidal neurons in motor cortex
(A) Schematic of *in utero* electroporation of Cre-mCherry performed at E14.5 $GRIN1^{ff}$ embryos.

(B) Expression of Cre-mCherry in layer 2/3 pyramidal neurons in $Grin1^{ff}$ primary motor cortex.

(C) Dual recordings showed that Cre expression led to loss of NMDA EPSCs in layer 2/3 pyramidal neurons in cortical slices made from P13-P17 mice (Control (Cnt), 110.9 ± 20.5 pA, $n = 6$; Cre, 0.80 ± 0.32 pA, $n = 6$; $p < 0.01$; paired t-test). Scale bar: 50 pA and 50 ms.

Figure 2. Loss of NMDARs in layer 2-3 pyramidal neurons in motor cortex decreases GABAergic synaptic transmission

(A-B) Cre expression caused a strong reduction of mIPSC frequency in layer 2/3 pyramidal neurons from primary motor cortex from P9-10 *Grin1^{f/f}* mice (Frequency: Cnt (0.77 ± 0.14 Hz, $n = 10$), Cre (0.34 ± 0.06 Hz, $n = 11$), $p < 0.01$, t-test; Amplitude: Cnt (-25.5 ± 2.1 pA, $n = 10$), Cre (-21.8 ± 1.6 pA, $n = 11$), $p = 0.67$, t-test). Cumulative distributions were compared by the Kolmogorov-Smirnov test ($p = 0.05$ for amplitude and $p < 0.001$ for interevent interval). Scale bar: 20 pA and 1s.

(C-D) Cre expression caused a significant reduction of mIPSC frequency in layer 2/3 pyramidal neurons from primary motor cortex from P17-18 *Grin1^{f/f}* mice (Frequency: Cnt (5.1 ± 0.5 Hz, $n = 9$), Cre (3.3 ± 0.3 Hz, $n = 8$), $p < 0.01$, t-test; Amplitude: Cnt (-22.4 ± 1.6 pA, $n = 9$), Cre (-21.0 ± 1.5 pA, $n = 8$), $p = 0.74$, t-test). Cumulative distributions were compared by the Kolmogorov-Smirnov test ($p = 0.05$ for amplitude and $p < 0.001$ for interevent interval). Scale bar: 20 pA and 1s.

Figure 3. Genetic deletion of NMDARs in VTA dopamine neurons

(A) Breeding scheme to generate $Grin1^{f/f}/DAT-Cre/tdTomato^{Cre}$ mice.

(B) Immunohistochemical assays in P13 $Grin1^{f/+}/DAT-Cre/tdTomato^{Cre}$ mice confirmed Cre expression selectively in dopaminergic neurons as indicated by complete colocalization of tdTomato, the product of Cre-mediated recombination and tyrosine hydroxylase (TH, green), a cellular marker for dopamine neurons. Note that there were both tdTomato-positive dopamine neurons (solid arrow) and tdTomato-negative nondopamine neurons (open arrow) in the VTA region. NeuN is a neuronal marker (purple). SNc, Substantia nigra pars compacta.

(C-D) Scatter plots and sample traces of NMDA EPSCs in tdTomato-expressing VTA dopamine neurons and nearby tdTomato-negative non-dopamine control neurons from the same slices showed the loss of NMDA EPSCs in dopamine neurons from *Grin1^{f/f}/DAT-Cre/tdTomato^{Cre}* (C), but not in *Grin1^{f/+}/DAT-Cre/tdTomato^{Cre}* mice (D).

(E) Bar graph showed normalized NMDA EPSC ratio in dopamine neurons vs nondopamine neurons from *Cre⁺/Cre⁻* in *Grin1^{f/+}/DAT-Cre/tdTomato^{Cre}* mice ($66.36 \pm 24.02\%$, $n = 6$) and in *Grin1^{f/f}/DAT-Cre/tdTomato^{Cre}* mice ($1.35 \pm 1.01\%$, $n = 9$), $p < 0.001$, t-test. Deletion of the GluN1 subunit abolished NMDA EPSCs in VTA DA neurons from *Grin1^{f/f}/DAT-Cre/tdTomato^{Cre}* mice. Scale bar: 50 pA and 50 ms.

Figure 4. GABAergic synapse development in cortical neurons and in VTA dopamine neurons requires NMDARs.

(A-B) mIPSC frequency was strongly reduced in Cre-positive VTA dopamine neurons from *Grin1^{f/f}/DAT-Cre/tdTomato^{Cre}* mice (Frequency: *Grin1^{f/+}* (1.59 ± 0.20 Hz, $n = 21$), *Grin1^{f/f}* (0.61 ± 0.08 Hz, $n = 22$), $p < 0.001$, t-test; Amplitude: *Grin1^{f/+}* (-20.84 ± 0.79 pA, $n = 21$), *Grin1^{f/f}* (-21.62 ± 1.26 pA, $n = 22$), $p = 0.61$, t-test). Cumulative distributions were compared by the Kolmogorov-Smirnov test ($p = 0.05$ for amplitude and $p < 0.001$ for interevent interval). Scale bar: 20 pA and 1s.