

Supporting Information

Zhang et al. 10.1073/pnas.1212971110

SI Results

Mosaic Cre Expression in Thalamus of Serotonin Transporter-Cre Mice. Serotonin transporter (SERT)-Cre mice were bred to the Ai14 reporter mice to produce Ai14;SERT-Cre⁺ (SERT-Ai14) mice. Cre-mediated recombination was observed as early as postnatal day (P) 2 in the cortex and thalamus (Fig. S1A). The percentage of ventral posteromedial nucleus (VPM) neurons with recombination was examined at P7 and P14. Neurons were identified with anti-NeuN (SI Materials and Methods), and Cre-positive cells were identified with tdTomato signal. At P7 and P14, all tdTomato-expressing cells in the VPM were also NeuN-positive (Fig. 1 A and B), indicating that Cre expression was confined to neurons in the VPM. Percentages of VPM neurons with Cre expression were 49 ± 3% (*n* = 3 mice) at P7 and 55 ± 9% (*n* = 3 mice) at P14. The reason for the mosaic Cre expression in the SERT-Cre strain is unknown. The VPM of the mouse is thought to contain a homogenous population of sensory relay neurons. Progeny tests indicate that the transgene is not X-linked.

Cre Expression in Principal Trigeminal Nucleus (Pr5) of SERT-Cre Mice and Excitatory Transmission in Pr5 Neurons of SERT-Grin1^{-/-} Mice. Coronal sections of the brainstem of SERT-Ai14 mice were immunostained for NeuN. The Pr5 can be easily identified in coronal sections using the motor trigeminal nucleus (Mo5) and sensory root of the trigeminal nerve (s5) as landmarks (Fig. S2A). Few Pr5 neurons were Cre-positive as indicated by the lack of tdTomato signal in the majority of Pr5 neurons (Fig. S2B). The percentage of Pr5 neurons with Cre expression was 6.1 ± 1.0% (*n* = 5 mice) at P12 to P14.

We examined excitatory synaptic transmission in Pr5 neurons in horizontal slices. Bipolar stimulation electrodes were placed in s5, and whole-cell recording was obtained from Pr5 neurons (Fig. S2C). Inhibitory transmission was blocked by picrotoxin (100 μM) and strychnine (1 μM). In total, we recorded 19 cells from two SERT-Grin1^{-/-} mutant mice and 15 cells from two control mice at P13 to P14. All 19 Pr5 neurons in mutant mice showed NMDA receptor (NMDAR) excitatory synaptic currents (EPSCs). The amplitudes of AMPA receptor (AMPA)- and NMDAR-EPSCs were not different from those of Pr5 neurons in control mice (Fig. S2 D and E).

Deletion of NMDARs Reduced Quantal Excitatory Transmission in VPM Neurons. Spontaneous quantal EPSCs [i.e., miniature EPSCs (mEPSCs)] were recorded from VPM neurons of SERT-Grin1^{-/-} mice in the presence of tetrodotoxin (0.4 μM in the bath). The presence or absence of NMDARs was determined by applications of NMDA (50 μM, 300-ms puff) to recorded cells. In total, 23 VPM neurons were recorded from two SERT-Grin1^{-/-} mice aged P12 to P13; 11 cells showed NMDA responses and 12 cells did not show any response to NMDA application. Cells without NMDARs had fewer mEPSC events than those with NMDARs (Fig. S5A). Mean frequencies of mEPSCs were 5.5 ± 0.7 Hz (*n* = 12) for neurons without NMDARs and 14.8 ± 2.7 Hz (*n* = 11) for neurons with NMDARs (Fig. S5B; *P* = 0.014). Consistent with the results of Sr-mEPSCs, mEPSCs of neurons without NMDARs showed faster decay and smaller peak amplitude than those of neurons with NMDARs (Fig. S5 C–E). The reduction in the frequency of mEPSCs supports the idea that many excitatory synapses in VPM neurons without NMDARs have few or no AMPARs. A caveat, however, is that lemniscal and corticothalamic synapses contribute to mEPSCs recorded in these neurons.

SI Materials and Methods

Generation of Conditional Grin1 Allele. ES cell lines carrying the conditional-ready mutation of *Grin1* (*Grin1*^{tm1a(EUCOMM)Wtsi}; Fig. S3) were obtained from European Mouse Mutant Cell Repository Helmholtz Zentrum Munchen. The ES cells were injected into blastocysts of C57BL6 (B6) mice of white coat color [B6(Cg)-Tyr^{c-2f}/J; stock no. 0058; Jackson Laboratory]. Chimeric males were crossed to B6(Cg)-Tyr^{c-2f}/J females to generate offspring heterozygous for the mutation. Crossing mice carrying the mutation *Grin1*^{tm1a(EUCOMM)Wtsi} to the germ-line FLP strain ACT-FLPe [B6.Cg-Tg(ACTFLPe)9205Dym/J; stock no. 005703; Jackson Laboratory] removed the promoter-driven cassette and resulted in a conditional allele (*Grin1*^{2lox}) wherein the exon 4 (ENSMUSE00000164820) of *Grin1* is flanked by loxP sites. The distance between the loxP sites is 879 bp.

Generation of Grin1-Null Allele. We crossed another conditional strain of *Grin1* B6.129S4-Grin1^{tm2Stu}/J (stock no. 005246; Jackson Laboratory) (1) to the germ-line Cre strain EIIa-Cre [B6.FVB-Tg(EIIa-Cre)C5379Lmgd/J; stock no. 003724; Jackson Laboratory]. This resulted in a 12-kb deletion in *Grin1* that removed coding sequences for all transmembrane domains of GluN1. Mice carrying the null mutation were crossed to B6 to remove the EIIa-Cre transgene.

Characterization of Cre Expression of SERT-Cre Strain. The SERT-Cre strain *Tg(Slc6a4-Cre)ETI27Gsat* generated by GENSAT (2) was obtained from the Mutant Mouse Regional Resource Center at University of California, Davis (stock no. 017261-UCD). Originally on a mix FVB/B6/129/Swiss background, this strain was backcrossed to B6 for five generations before the experiments. Cre expression was analyzed by crossing SERT-Cre⁺ mice to the fluorescence reporter strain B6.Cg-Gt(ROSA)26Sor^{tm14(CAG-tdTomato)Hze}/J (Ai14; stock no. 007914; Jackson Laboratory) (3). Immunostaining was performed as described in a subsequent section. Confocal images were taken with a 63× objective, and analyzed by using ImageJ and NeuroLucida; NeuN- or tdTomato-positive neurons were counted manually. For each mouse, six sections were randomly chosen for quantification; the results from the six sections were averaged.

Mosaic Deletion of NMDARs. Mice with mosaic deletion of NMDARs (*Grin1*^{2lox/null};SERT-Cre⁺, or *Grin1*^{2lox/2lox};SERT-Cre⁺) were generated by crossing *Grin1*^{null/wt};SERT-Cre⁺ or *Grin1*^{2lox/wt};SERT-Cre⁺ to *Grin1*^{2lox/2lox} mice. Genotypes were determined by PCR with the following primers: 5'-CTACAAGGCAAAGATA-CAAGAC-3', 5'-GCATCAGATCTCATTACAGATGG-3', and 5'-ACTTCCATTTGTCACGTCCTGCAC-3' (which amplify a 580-bp fragment from the *Grin1*-null allele and a 158-bp fragment for the WT allele); 5'-CTATAGGAGCAAGAGGAATCCG-3' and 5'-GGACGGAGACTAGAAAGAGAC-3' [which, respectively, amplify 110-bp and 302-bp fragments from the *Grin1* WT and conditional (2lox) alleles]; and 5'-GCATTACCGTTCGA-TGCAACGAGTGATGAG-3' and 5'-GAGTGAACGAACCTG-GTCGAAATCAGTGCG-3' (which amplify a 410-bp fragment from SERT-Cre).

Slice Preparation, Patch-Clamp Recording, and Analysis. Sagittal slices containing the VPM were prepared as we described previously (4). Briefly, mice were anesthetized with tribromoethanol and decapitated. Brains were dissected quickly and chilled in ice-cold solution containing (in mM): 210 sucrose, 3 KCl, 1 CaCl₂, 3 MgCl₂, 20 glucose, and 26 NaHCO₃, saturated with 95% O₂ and

5% CO₂. Sagittal sections 300 μm thick were cut by using a Vibratome (VT1200; Leica), and sections were allowed to recover at room temperature in artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 1.5 CaCl₂, 1.3 MgCl₂, 1.0 NaH₂PO₄, 26 NaHCO₃, and 20 glucose, saturated with 95% O₂ and 5% CO₂. All procedures are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and have been approved by the Jackson Laboratory Animal Care and Use Committee.

For recording, a slice was transferred to a submersion-type chamber, where it was bathed in ACSF saturated with 95% O₂ and 5% CO₂ flowing at the rate of 2.0 to 2.2 mL/min and heated to 32 to 33 °C. Picrotoxin (100 μM) was included in the ACSF to block GABAergic transmission. Slices were first viewed with a 4× objective, and a concentric bipolar electrode (FHC) was placed in the medial lemniscus just before it enters the VPm. Patch pipettes were pulled from borosilicate glass using puller (P97; Sutter Instruments). Patch electrodes had resistance of 2 to 4 MΩ when filled with pipette solution containing (in mM): 110 cesium methylsulfate, 15 CsCl, 20 TEA-Cl, 10 Hepes, 4.0 ATP magnesium, 0.3 GTP sodium, 4.0 QX-314, 0.5 spermine, and 0.5 EGTA (pH 7.2; 270–280 mmol/kg with sucrose). Individual neurons in VPm were identified by using Nomarski optics and infrared video microscopy. Whole-cell recordings were made at the soma of VPm neurons with a MultiClamp 700B amplifier (Molecular Devices). The series resistance (R_s), usually between 10 and 14 MΩ, was not compensated. Data were discarded when R_s was >16 MΩ. Data were filtered at 4 kHz and recorded at 16 kHz using AxoGraph X (AxoGraph Scientific).

Data were analyzed by using AxoGraph X and IgorPro (WaveMetrics). Amplitudes of AMPAR-EPSCs were measured at the peak of EPSCs at –70 mV. NMDAR-EPSCs recorded at +40 mV were measured by averaging a 1-ms segment starting at 10 ms after the onset of EPSCs. For each cell, the NMDAR/AMPA ratio was calculated by using the amplitudes of NMDAR-EPSCs and AMPAR-EPSCs evoked at the same intensity. A cell is considered to be without NMDARs when it meets two criteria: (i) the ratio of NMDAR/AMPA is <0.1 and (ii) the amplitude of the maximal NMDAR-EPSC is <50 pA. The number of inputs for each VPm neuron was estimated by analyzing EPSCs at –70 mV over a wide range of stimulus intensity (100 μs, 20–900 μA, 0.1 Hz). For each cell, the intensity of stimulus was increased with

steps of 10 or 20 μA, and, typically, two responses were recorded at each intensity. An increment was determined as a sudden increase in EPSC amplitude at a given intensity that is maintained at higher intensities.

For quantal analysis, data were filtered at 1 kHz, and events were detected by using a variable-amplitude template with the rise time set at 0.5 ms and decay time at 1.8 ms. For each neuron, 100 to 500 events were analyzed and averaged.

Immunostaining. Mice were perfused with 4% (wt/vol) paraformaldehyde and postfixed overnight at 4 °C. Sagittal sections 50 or 60 μm thick were cut with a Vibratome (VT1000s; Leica). For SERT-Cre expression analysis, immunostaining was performed on free-floating sections; an antibody of NeuN (mouse; MAB377; 1:1,000; Millipore) was used to label all neurons. For analysis of synaptic terminals, an antibody of vesicular glutamate transporter 2 (guinea pig; AB2251; 1:4,000; Millipore) was used to label synaptic terminals of Pr5 axons; an antibody of RFP (rabbit, no. 600-401-379, 1:2,000; Rockland) was used to enhance tdTomato signal. Sections were incubated with primary antibodies at 4 °C for 48 h. Secondary antibodies, conjugated with Alexa dyes (1:300; Life Technologies), were incubated for 1 h at room temperatures. Sections were coverslipped in ProLong Gold antifade medium (Life Technologies).

Image Acquisition and Analysis. Confocal images were taken with a 63× objective (NA 1.3, glycerol) and 4× optical zoom on a Leica SP5 laser-scanning microscope. The same laser and microscope settings were used for mutant and control neurons. Z-stacks were taken with 250-nm steps.

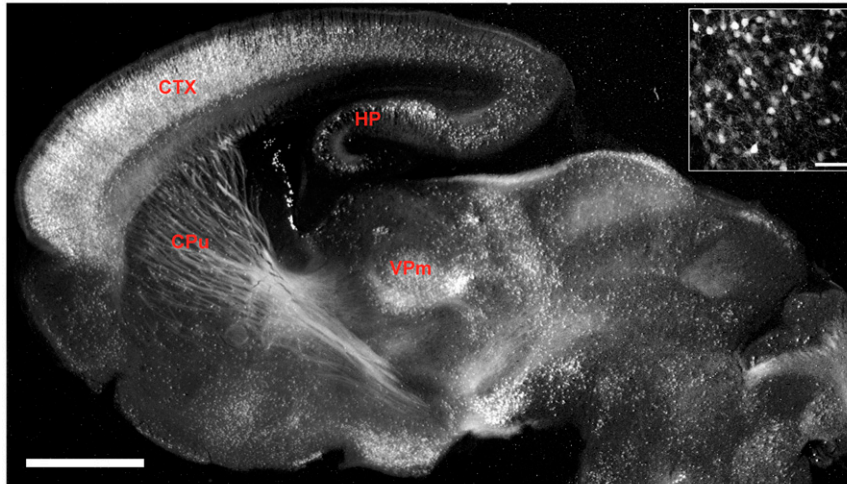
Confocal images were analyzed blind by using ImageJ and Neurolucida. To quantify somatic innervation, five consecutive optical sections around the middle section of the cell body were used for z-projection at the maximal intensity. Perimeters of cell bodies and the portion in contact with vesicular glutamate transporter 2-positive terminals were measured by using Neurolucida.

Statistics. Statistics was analyzed by using IgorPro and InStat (GraphPad). Throughout, means are given ± SEM. Means were compared by using two-tailed Mann–Whitney tests or non-parametric ANOVA. Distributions of cells receiving different number of inputs were compared by using an χ^2 test.

1. Tsien JZ, et al. (1996) Subregion- and cell type-restricted gene knockout in mouse brain. *Cell* 87(7):1317–1326.
2. Gong S, et al. (2007) Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci* 27(37):9817–9823.

3. Madisen L, et al. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133–140.
4. Arsenault D, Zhang ZW (2006) Developmental remodelling of the lemniscal synapse in the ventral basal thalamus of the mouse. *J Physiol* 573(pt 1):121–132.

A Ai14;SERT-Cre, P2



B Ai14;SERT-Cre, P14

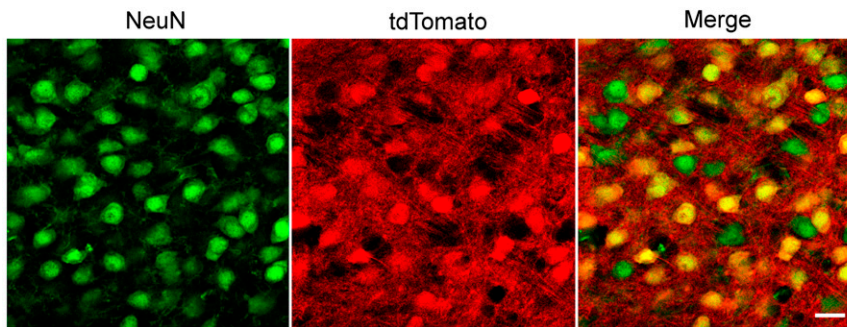


Fig. S1. Mosaic Cre recombinase expression in the thalamus of SERT-Cre mice. (A) Pattern of Cre expression in the brain of a SERT-Ai14 mouse at P2. Four images taken from a 70- μ m-thick sagittal section were used to build a montage using Fiji. (Scale bar: 1 mm.) CPU, caudate putamen; CTX, neocortex; HP, hippocampus. *Inset:* Expression pattern in VPM. (Scale bar: 50 μ m.) (B) Confocal images of the VPM in a section of a SERT-Ai14 mouse brain immunostained for NeuN. Neurons that express Cre recombinase were localized with tdTomato signal. (Scale bar: 10 μ m.)

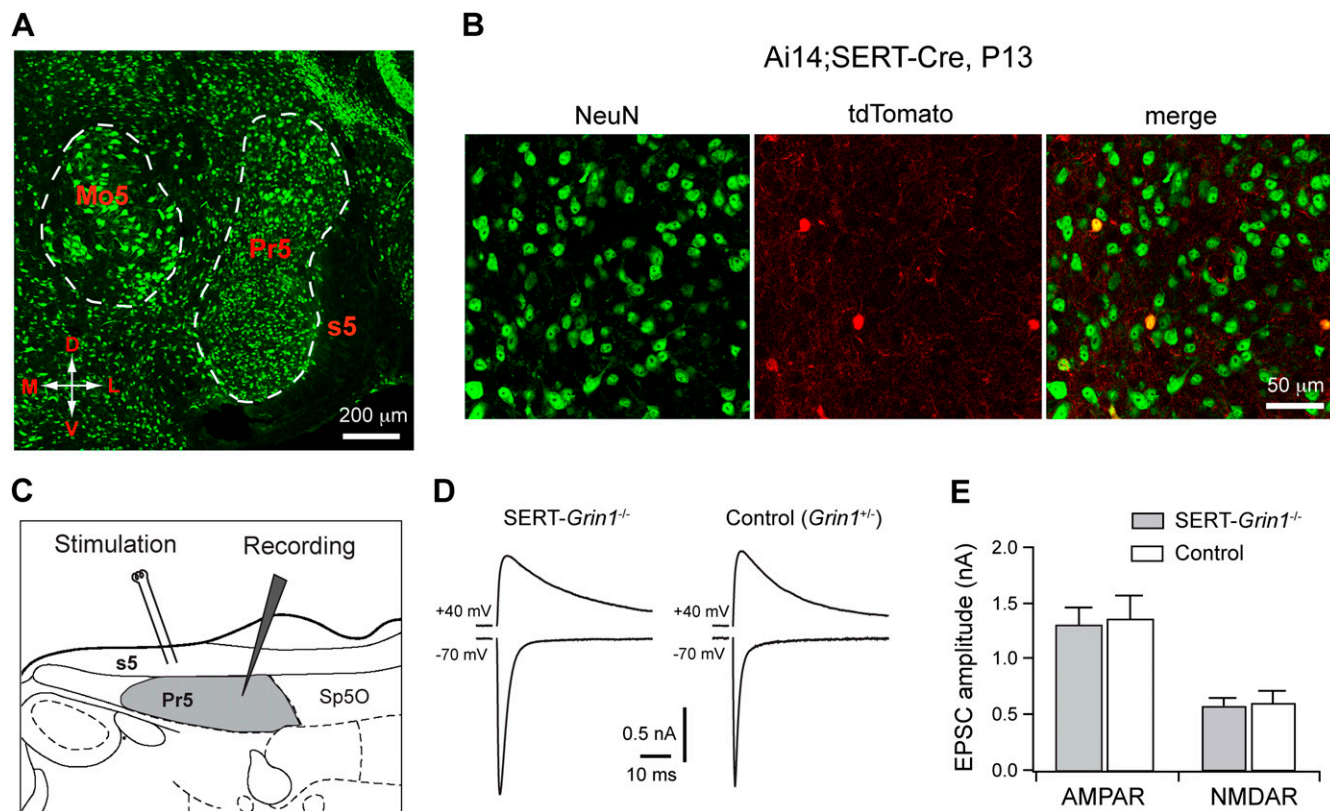


Fig. S2. Excitatory transmission in the Pr5 was not significantly altered in SERT-*Grin1*^{-/-} mice. (A) Confocal image of the Pr5 in a coronal section immunostained for NeuN. Mo5, motor trigeminal nucleus. (B) Confocal images of Pr5 neurons in a coronal section from a SERT-Ai14 mouse at P13. Few Pr5 neurons were Cre-positive as indicated by tdTomato signal. (C) Schematic view of the horizontal slice used for recording synaptic transmission in Pr5 neurons. EPSCs were evoked in Pr5 neurons by the stimulation of s5. Inhibitory transmission was blocked by picrotoxin and strychnine. (D) EPSCs recorded from two Pr5 neurons of a SERT-*Grin1*^{-/-} and control (*Grin1*^{+/+}) mouse, respectively. (E) Amplitudes of EPSCs recorded from Pr5 neurons of SERT-*Grin1*^{-/-} (gray bars) and control (empty bars) mice at P13 to P14. Mean amplitudes of AMPAR-EPSCs were 1.30 ± 0.14 nA ($n = 19$) for mutant mice and 1.36 ± 0.21 nA ($n = 15$) for control mice ($P = 0.73$); mean amplitudes of NMDAR-EPSCs were 0.57 ± 0.06 nA for mutant and 0.60 ± 0.11 nA for control ($P = 0.49$).

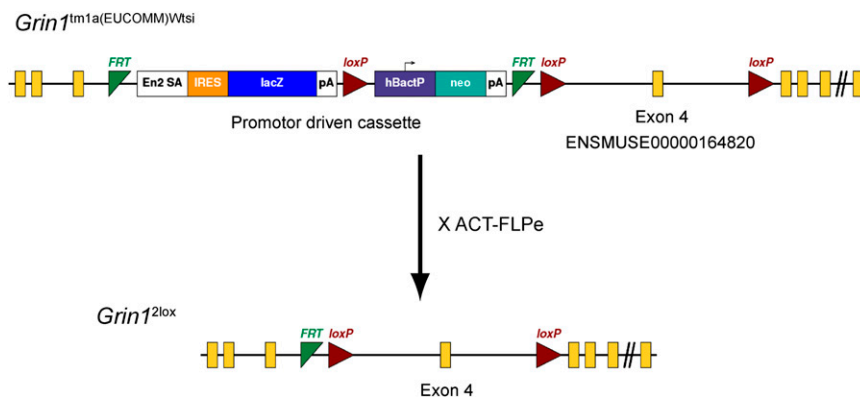


Fig. S3. Generation of a conditional allele of *Grin1* (*Grin1*^{2lox}). ES cells carrying the conditional-ready mutation of *Grin1* [*Grin1*^{tm1a(EUCOMM)Wtsi}] were injected into blastocysts of B6-Tyr^{c-2j} mice. Chimeric males were crossed to B6-Tyr^{c-2j} females to generate offspring heterozygous for the mutation. The promoter-driven cassette was removed by using the germ-line FLP strain ACT-FLPe. The resulting conditional allele (*Grin1*^{2lox}) has loxP sites flanking the exon 4 of *Grin1*. The distance between the loxP sites is 879 bp.

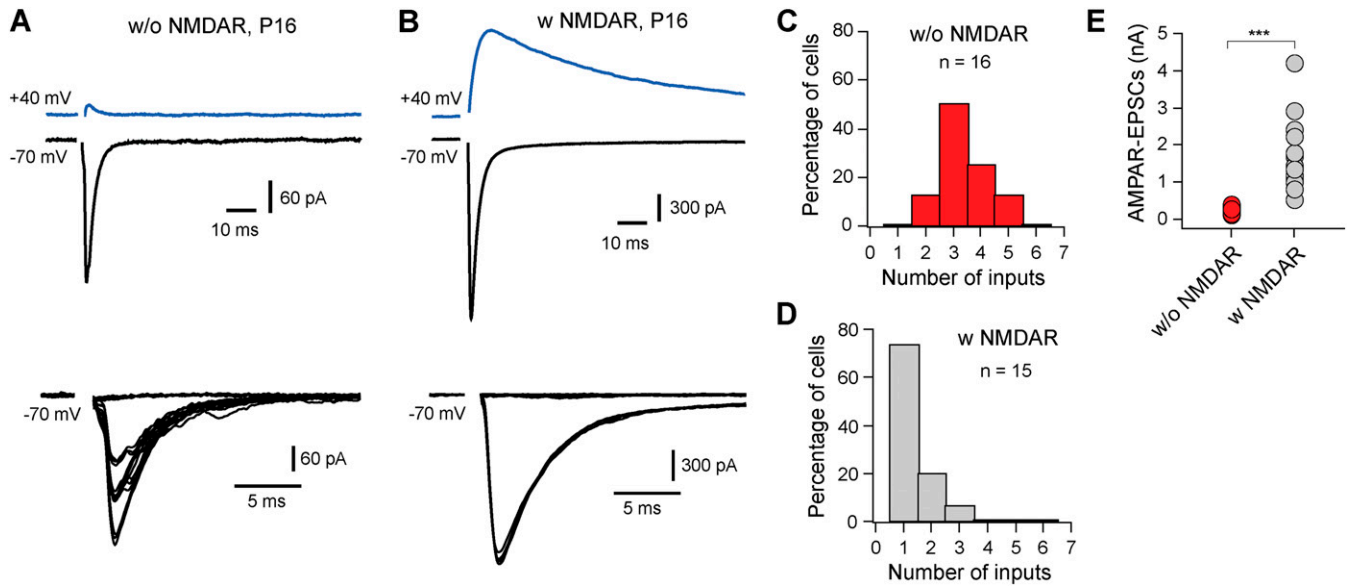


Fig. 54. Deletion of NMDARs altered input number and synaptic strength in VPM neurons at P16 to P17. (**A**) EPSCs from a VPM neuron without NMDARs at P16. This neuron showed three increments in response to a range of stimulation intensities, indicating that it received three inputs. (**B**) Equivalent results from a VPM neuron at P16 with NMDARs. (**C** and **D**) Distributions of neurons at P16 to P17 receiving different numbers of inputs. Mean numbers of inputs per neuron were 1.3 ± 0.2 for neurons with NMDARs and 3.4 ± 0.2 for those without NMDARs ($P < 0.0001$). (**E**) Peak amplitudes of AMPAR-EPSCs from VPM neurons with (gray) or without (red) NMDARs. Mean amplitudes were 1.70 ± 0.24 nA ($n = 15$) for neurons with NMDARs and 0.24 ± 0.02 nA ($n = 16$) for neurons without NMDARs ($P < 0.0001$).

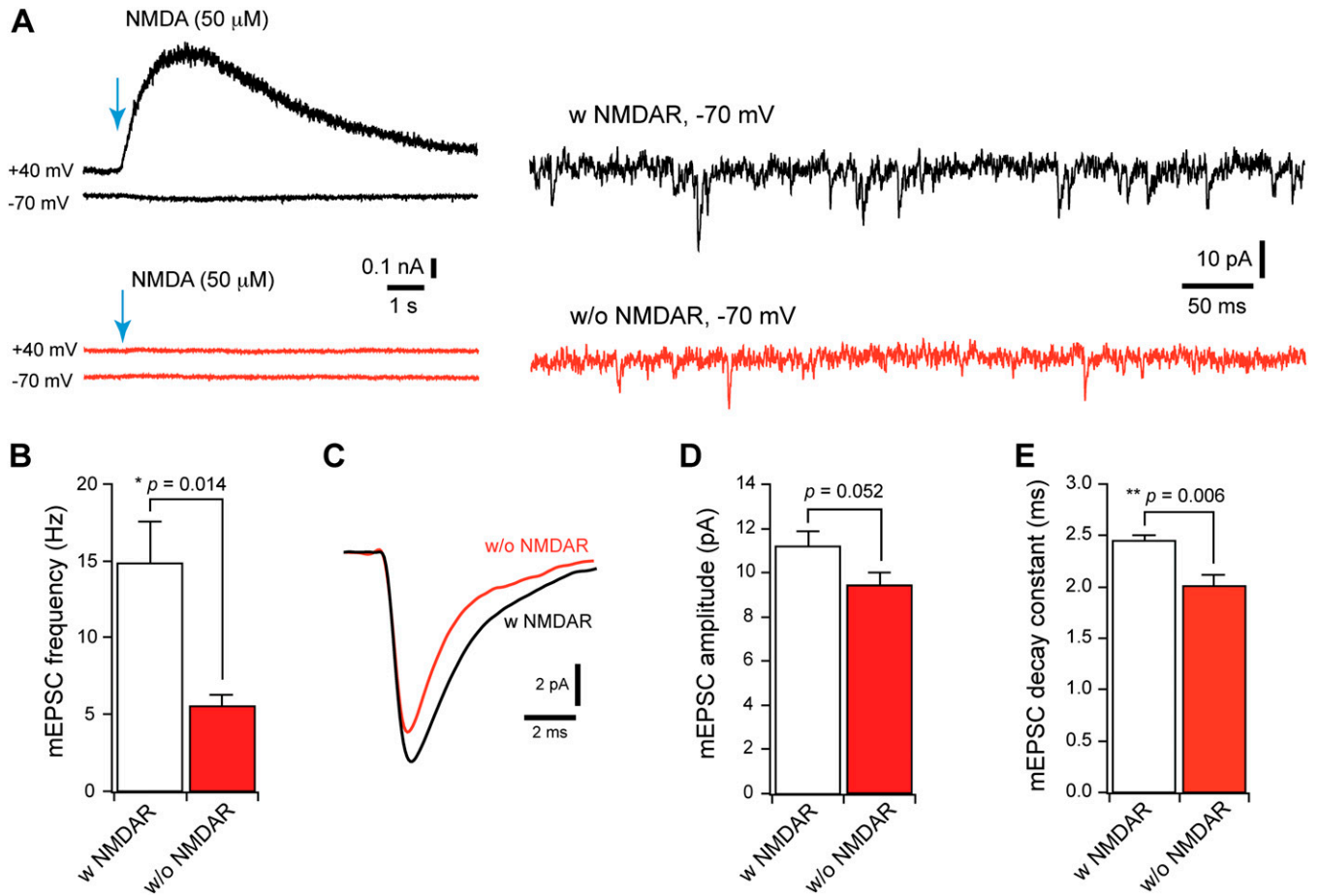


Fig. 55. Deletion of NMDARs reduced quantal excitatory transmission in VPM neurons. (A) Two VPM neurons recorded from a SERT-*Grin1*^{-/-} mouse at P13; traces in black were from a cell with NMDARs, and traces in red were from a cell without NMDARs. *Left:* Traces show responses (or lack thereof) to NMDA application (50 μ M, 300-ms puff). Each cell was tested at +40 mV and -70 mV. *Right:* Traces show spontaneous mEPSC events. (B) The mean frequency of mEPSCs of neurons with or without NMDARs. (C) Averaged mEPSCs from neurons with (black) or without NMDARs (red). (D and E) Peak amplitude and decay constant of mEPSCs from neurons with or without NMDARs.