Supporting Information

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SI Materials and Methods

Mice. All mouse lines used in these experiments were backcrossed to C57/B6 mice for >10 generations. Both male and female mice were used in all experiments, and all mice were between 8 and 10 wk of age at the start of experiments. All control animals were heterozygous at the *Grin1* locus in a fraction of cells. Heterozygosity at this locus does not alter protein expression in knockout mice (1). Mice with a conditional allele for *Grin1* (2) and $Slc6a3^{Cre/+}$ mice (3) were crossed to generate $Slc6a3^{Cre/+}$;$Grin1^{\Delta/lox}$ knockout mice and $Slc6a3^{Cre/+}$;$Grin1^{lox/+}$ controls as described (4).

$Drd1a^{Cre/+}$ mice were generated as described (5). To generate $Drd1a^{Cre/+}$;$Grin1^{\Delta/lox}$ animals, one copy of the *Grin1* locus was globally inactivated by crossing $Grin1^{lox/lox}$ animals with $Mox2^{Cre/+}$ mice to generate $Grin1^{\Delta/+}$ mice. $Drd1a^{Cre/+}$;$Grin1^{\Delta/+}$ males were crossed to $Drd1a^{+/+}$;$Grin1^{lox/lox}$ females to generate $Drd1a^{Cre/+}$;$Grin1^{\Delta/lox}$ knockouts and $Drd1a^{Cre/+}$;$Grin1^{lox/+}$controls. These knockout mice are slightly smaller than littermate controls, but are otherwise grossly normal.

$Gpr88^{Cre/+}$ mice were generated by targeting Cre-GFP to the first coding exon of *Grp88* locus and then removing the frt-flanked neomycin-resistance gene that was used for positive selection by crossing them with *Gt(ROSA)26Sor* mice expressing Flp recombinase.

$Grin1^{lox/lox}$ and $Grin1^{lox/+}$ animals used in viral experiments were generated by crossing $Grin1^{lox/+}$ and $Grin1^{lox/lox}$ animals. Following viral injection and recovery, these knockout mice were indistinguishable from littermate controls.

For electrophysiology experiments, $Drd1a^{Cre/+}$;$Grin1^{\Delta/+}$ males were crossed to $Grin1^{lox/lox}$;$Rosa26^{TdTomato/TdTomato}$ females, in which Cre-mediated recombination leads to expression of the fluorescent reporter TdTomato under the control of the cytomegalovirus chicken β-actin (CBA) promoter, as described previously (mouse line Ai14) (6).

All animal protocols were approved by the University of Washington Institutional Animal Care and Use Committee.

AMPH Sensitization. On days 1–3, animals were placed in activity chambers (Columbus Instruments) for 90 min before receiving i.p. saline (0.01 mL/g), after which their locomotion was monitored for an additional 90 min. On days 4–8, day 12 (3 d DW), and day 30 (21 DW), animals were placed in locomotion chambers for 90 min before receiving i.p. AMPH (2.5 mg/kg; Sigma), after which their locomotion was monitored for an additional 90 min.

AMPH CPP. Baseline compartment preference was measured for 15 min in a three-chamber CPP apparatus, and mice were paired using an unbiased paradigm, such that on average the mice had no baseline preference for the AMPH-paired side (7). The following day, mice received two 15-min pairing sessions, one with saline and one with AMPH (1.5 mg/kg), in the contextually distinct compartments of the apparatus. On day 3, compartment preference was measured again in a 15-min session. Preference is shown as difference in time spent in AMPH- and saline-paired sides of the chamber at baseline and on the test day.

MK-801–Induced Locomotion. Animals were placed in locomotion chambers for 90 min before receiving i.p. MK-801 (0.5 mg/kg; Sigma), after which their locomotion was monitored for an additional 90 min. Total distance traveled in 90 min after injection is reported.

Overnight Locomotion. Animals were placed in locomotion chambers with ad libitum access to food and water for 48 h. Distance traveled in the first hour is reported as a novelty response. Distance traveled during the second light and dark cycles are reported.

Viral Injections. For virus-mediated rescue of *Grin1* in the NAc, an AAV1-fsNR1 virus was used. This virus contains a floxed SV40 late polyA-addition site and seven in-frame termination codons followed by three HA-tag sequences inserted after amino acid 31 of the rat NMDAR1-3a splice variant (8). After Cre-mediated recombination, expression of *Grin1* is driven by the CBA promoter. The ORF is followed by the bovine growth hormone (bGH) polyadenylation sequence. For sensitization experiments, $Drd1a^{Cre/+}$;$Grin1^{\Delta/lox}$ and $Drd1a^{Cre/+}$;$Grin1^{\Delta/+}$ animals were stereotactically injected bilaterally at the coordinates ($x = \pm 1.35$ mm; $y = 1.70$ mm; $z = -3.75$ mm) from bregma, and allowed 1 wk to recover before behavioral testing began. To verify that AAV1-fsNR1 expresses full-length NR1 in a Cre-dependent manner $Drd1a^{Cre/+}$and $Drd1a^{+/+}$ animals were injected at the same coordinates and killed for Western blotting 1 wk later. For all experiments, each hemisphere was injected with 0.5 μL of virus titered at 6.0×10^9 viral genomes per microliter.

For virus-mediated *Grin1* knockout experiments, an AAV1-Cre-GFP virus was used. This virus contains a myc-tagged Cre-GFP fusion gene with a 5′ nuclear localization signal. Expression of Cre-GFP is driven by the CBA promoter, and the gene is followed by WPRE and bGH polyadenylation sequences. $Drd1a^{Cre/+}$;$Grin1^{\Delta/lox}$, $Drd1a^{Cre/+}$;$Grin1^{lox/+}$, $Grin1^{lox/lox}$, and $Grin1^{lox/+}$ animals were stereotactically injected with 1.0 μL of virus titered at 1.2×10^8 viral genomes per microliter. Virus was injected bilaterally at the coordinates given above, and animals were allowed 2 wk to recover before behavioral testing to ensure more complete turnover of NR1 protein.

Viruses were prepared in HEK cells with AAV1 coat serotype, purified by sucrose and CsCI gradient centrifugation steps, and then resuspended in HBSS at the indicated titers.

Immunohistochemistry. After behavioral experiments, all stereotactically injected animals were euthanized and perfused as described (9). Briefly, animals were given a lethal dose of pentobarbital and transcardially perfused with PBS followed by 4% paraformaldehyde (PFA) in PBS. Brains were removed, postfixed in PFA overnight, cryoprotected in 30% sucrose, and frozen. Free-floating coronal sections (30 μm) were immunostained.

For $Drd1a^{+/Cre}$;$Grin1^{\Delta/lox}$ animals rescued with AAV1-fs-*Grin1*, viral targeting and expression was confirmed by immunostaining with an anti-HA antibody (1:1,200; Applied Biological Materials) and Cy3-conjugated secondary (1:200; Jackson ImmunoResearch). For $Grin1^{lox/lox}$ and $Drd1a^{Cre/+}$;$Grin1^{\Delta/lox}$ animals injected with AAV1-Cre-GFP, viral targeting and expression was confirmed by direct observation of GFP using a Nikon Eclipse E600 microscope.

Electrophysiology. Mice were anesthetized with pentobarbital (200 mg/kg i.p.) and coronal brain slices (300 μm) were prepared in a chilled solution containing (in mM) 87 NaCI, 2.5 KCI, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 0.5 $CaCI_2$, 7 $MgCI_2$, and 75 sucrose. Slices recovered >30 min at 32 °C in artificial cerebral spinal fluid (ACSF) that contained (in mM) 124 NaCI, 5 KCI, 1.25 NaH_2PO_4, 2 $MgCI_2$, 2 $CaCI_2$, 10 D-glucose, and 26 $NaHCO_3$ (295–305 mOsm). Whole-cell patch-clamp recordings in voltage-clamp mode were obtained from MSNs visualized in slices on

a Zeiss Axioskop FS microscope with the aid of infrared videomicroscopy coupled with differential interference contrast optics and fluorescent filters optimized to detect EGFP fluorescence and infrared light. Recordings were made with an Axopatch 700A amplifier with filtering at 1 KHz, using 2–5 MΩ electrodes filled with an internal solution containing (in mM) 44 cesium methanesulfonate, 9 glycol-bis(2-aminoethylether)-*N*,*N*,*N′*,*N′*-tetraacetic acid, 5 *N*-2-hydroxyethylpiperazine-*N′*-2-ethane sulfonic acid, 4 NaCI, 3 KCI, 1 $MgCI_2$, 5 Mg-ATP, 1 Mg-GTP (pH = 7.2–7.4, 260–280 mOsm). ACSF at room temperature was continuously perfused at a rate of ∼2.0 mL/min and included bicuculline (10 μM) to block $GABA_A$ receptor currents. Clampex was used for data acquisition. Neurons were held at +40 mV, and a bipolar stimulating electrode was placed locally to elicit EPSCs at 0.1 Hz. d-APV (100 μM) and CNQX (10 μM) were used to block NMDA and AMPA receptor currents, respectively. The effect of glutamate receptor antagonists on EPSCs was determined by comparing 30 s of stimulations during baseline, after d-APV, and after d-APV with CNQX.

Western Blots. Mice were cervically dislocated and their striata were rapidly dissected on ice and frozen in liquid nitrogen. Tissue was sonicated in RIPA buffer containing protease and phosphatase inhibitors (2.5% wt/vol), and spun at 10,000 × *g* at 4 °C for 10 min. Supernatant was collected and protein concentration assayed using a BCA assay (Thermo Scientific). Loading buffer was added to 25 μg total protein, and samples were heated to 65 °C for 15 min and run on a precast 4–20% gradient polyacrylamide gel (Bio-Rad). Protein was transferred onto nitrocellulose membranes, blocked for 1 h, probed with anti-HA (1:1,000; Covance), anti-NR1 (1:1,000; Millipore), and anti-β-actin (1:50,000; Sigma) antibody, washed, incubated with HRP-conjugated secondary antibodies, and visualized (ECL; Amersham).

Statistics. Sensitization and CPP data were analyzed using two-way, repeated-measures ANOVA. Fisher LSD post hoc tests were performed. Acute MK-801and APV responses were compared using unpaired *t* tests. All data are reported as means ± SEM.

1. Forrest D, et al. (1994) Targeted disruption of NMDA receptor 1 gene abolishes NMDA response and results in neonatal death. *Neuron* 13:325–338.
2. Tsien JZ, Huerta PT, Tonegawa S (1996) The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell* 87:1327–1338.
3. Zhuang X, Masson J, Gingrich JA, Rayport S, Hen R (2005) Targeted gene expression in dopamine and serotonin neurons of the mouse brain. *J Neurosci Methods* 143:27–32.
4. Zweifel LS, Argilli E, Bonci A, Palmiter RD (2008) Role of NMDA receptors in dopamine neurons for plasticity and addictive behaviors. *Neuron* 59:486–496.
5. Heusner CL, Beutler LR, Houser CR, Palmiter RD (2008) Deletion of GAD67 in dopamine receptor-1 expressing cells causes specific motor deficits. *Genesis* 46:357–367.
6. Madisen L, et al. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13:133–140.
7. Heusner CL, Palmiter RD (2005) Expression of mutant NMDA receptors in dopamine D1 receptor-containing cells prevents cocaine sensitization and decreases cocaine preference. *J Neurosci* 25:6651–6657.
8. Zukin RS, Bennett MV (1995) Alternatively spliced isoforms of the NMDARI receptor subunit. *Trends Neurosci* 18:306–313.
9. Hnasko TS, et al. (2006) Cre recombinase-mediated restoration of nigrostriatal dopamine in dopamine-deficient mice reverses hypophagia and bradykinesia. *Proc Natl Acad Sci USA* 103:8858–8863.