Supporting Information

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SI Materials and Methods

Generation of HTB Transgenic Mice and Animals. The $Rosa26^{Lox\text{-}stop\text{-}LoxHTB}$ (HTB) was generated by cloning a tricistronic open reading frame containing Histone-2B-GFP, TVA and B19 glycoprotein separated by 2A cleavage sequences (HTB: H, histone-tag GFP; T, TVA; B, rabies glycoprotein B19 G) into a Rosa26 targeting vector containing a CAG promoter and a stop signal flanked by LoxP sites. Embryonic stem (ES) cells were electroporated with this targeting construct and positive clones were used to generate knock-in mice.

All experimental procedures followed protocols approved by the Animal Care and Use Committee at The Salk Institute for Biological Studies. For circuit mapping experiments with rabies virus, we used both female and male HTB mice. For electrophysiological recording experiments, we used female C57BL/6 mice. All C57BL/6 mice were purchased from Harlan and housed in standard cages (five mice per cage) under a constant 12-h light/dark cycle. All animals were anesthetized with a ketamine/xylazine (100 mg/kg, 10 mg/kg) mixture.

Virus Preparations. All viral production followed the biosafety guidelines approved by the Salk Institute. Moloney murine leukemia retroviral vector was used to express enhanced GFP or Cre under the promoter CAG. The retrovirus was prepared by transfecting three separated plasmids containing the viral proteins (CMV-gap/pol), capsid (CMV-vs. vg), and viral vector (CAG-GFP or CAG-Cre) into the HEK293T cells and by concentrating the supernatant (1). The rabies virus was produced as previously described (2).

Slice Preparation and Recording. Brains were transferred to an icy cold cutting solution containing (in mM): 110 choline-Cl^-, 2.5 KCl, 2.0 NaH_2PO_4, 25 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, 1.3 Na^+-ascorbate, 3.1 Na^+-pyruvate, 20 dextrose, and 4 kynurenic acid (bubbled with 95% O_2 and 5% CO_2). Four hundred micrometer-thick horizontal brain slices were cut in a Vibratome (VT 1000S, Leica) and recovered for 30–60 min in artificial CSF containing (32 °C, in mM): 125 NaCl, 2.5 KCl, 2.0 NaH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$, 1 $MgCl_2$, 1.3 Na^+-ascorbate, 3.1 Na^+-pyruvate, and 10 dextrose; they were then stored at room temperature. Patch clamp recordings were performed at room temperature with Axopach-200B amplifier (Molecular Device). Patch pipettes were pulled from filamented glass tubes (Flaming-Brown P-97 micropipette puller, Sutter Instrument). Pipettes for whole-cell recording (pipette resistance, 5–7 MΩ) were filled with internal solution containing the following (in mM): 120 K-gluconate, 15 KCl, 4 $MgCl_2$, 0.1 EGTA, 10 Hepes, 4 MgATP, 0.3 Na_2GTP, and 7 phosphocreatine (pH, 7.2). Biocytin (2 mg/mL) was added to the internal solution for morphological analysis. Signals were filtered at 2 kHz and sampled at 5 kHz using a Digidata 1320A analog-digital interface (Molecular Devices). Input resistance and series resistance were monitored continuously during recordings. Series resistance was ~20 MΩ. Resting membrane potentials were determined in $I = 0$ mode. A bipolar tungsten electrode was placed in the middle third of the ML at >300 μm from the recorded cell for extracellular stimulation (0.5 Hz). All antagonists were purchased from Sigma.

Photostimulation. For the photostimulation experiment, cells were held at a potential of 0 mV to record outward inhibitory current, and potassium in the internal solution was replaced with cesium. 4-Methoxy-7-nitroindolinyl-caged-L-glutamate (MNI-caged glutamate, 100 μM, Tocris) was added to recirculating ACSF. UV light (50 mW at 355 nm and 10 mW under the specimen) from an argon-ion laser (Series 3500, DPSS Lasers) with 10-ms duration was delivered to brain slices through a 40× microscope objective. More than 800 sites were scanned in a grid with 25 μm of space between the adjacent horizontal and vertical rows. To avoid glutamate receptor desensitization and local caged glutamate depletion, the sequence pattern of laser scanning was designed by computer, which prevented the adjacent site being stimulated within 10 s.

Differential interference contrast (DIC) images of brain slices were taken after scanning, and then tissue was fixed in 4% paraformaldehyde (PFA) overnight. Cell morphology was detected by biocytin staining. Overlying the DAPI image with the DIC image was used to establish the position of recording cells and laminar borders of the DG. The border and cells were reconstructed with Neurolucida software (MBF Bioscience). Stimulation sites were aligned to DG subregions by a custom program written in MATLAB (MathWorks).

An inhibitory current map was made by measuring and detecting outward currents of each stimulation site in MiniAnalysis software (Synaptosoft). Events occurring 10–150 ms after stimulation were counted as synaptic responses. Direct responses evoked by activating glutamate receptors on recorded neurons, which usually occurred within 10 ms after stimulation, were excluded from the analysis. IPSC amplitude and numbers of event at each site were measured by MiniAnalysis. The value at each site was aligned to its layer, and laminar summaries were calculated as the mean response across all stimulation sites within each layer. This method permits identification of the locations of cell bodies of the neurons that provide synaptic input but cannot determine the location of synapses on the dendritic arbor.

Immunohistochemistry and Microscopy. Mice were transcardially perfused by 4% PFA and postfixed in 4% PFA overnight, then equilibrated in 30% sucrose. Forty-micrometer coronal serial brain sections were prepared by sliding microtome. Brain slices were incubated overnight at 4 °C with goat anti-ChAT (1:100, Chemico) and rabbit anti-tyrosine hydroxylase (TH) (1:200, Protos Biotech), rabbit anti-parvalbumin (1:1,000, Swant), rabbit anti-calretinin (1:500, Swant), and rabbit anti-somatostatin (1:500, Swant). Cyanine 3- and 5-conjugated secondary antibodies were used against primary antibodies (1:250, Jackson ImmunoResearch). Biocytin signals were detected by cyanine 3-conjugated streptavidin (1:1,000, Jackson ImmunoResearch). DAPI was used to reveal nuclei. Image stacks were collected with a 20× or 40× objective lens of laser scanning confocal microscope (Radiance 2100, Bio-Rad; LSM 710, Zeiss).

Statistics and Data Presentation. Data were analyzed with t test (unpaired or paired), one or two-way ANOVA with Bonferroni's post hoc comparison. Results associated with $P < 0.05$ were considered to be statistically significant and all data were presented as mean ± SEM.

1. Tashiro A, Zhao C, Gage FH (2006) Retrovirus-mediated single-cell gene knockout technique in adult newborn neurons in vivo. *Nat Protoc* 1(6):3049–3055.

2. Osakada F, et al. (2011) New rabies virus variants for monitoring and manipulating activity and gene expression in defined neural circuits. *Neuron* 71(4):617–631.

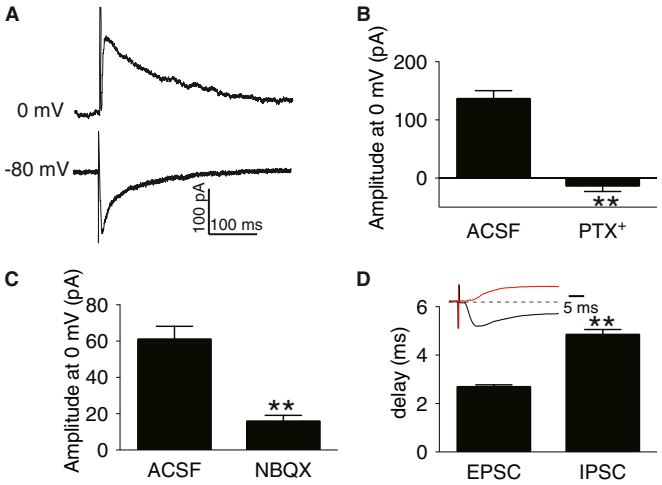

Fig. S1. Disynaptic inhibition received by dentate granule cells (DGCs) via perforant path (PP) stimulation. (*A*) Average of five traces recorded from the same DGC at different holding potentials via PP stimulation. (*B*) PP stimulation (0.9 mA) induced outward currents from DGCs in ACSF ($n = 6$) and in cells perfused with picrotoxin (PTX) ($n = 10$). Evoked IPSCs was blocked by PTX, confirming their $GABA_A$ receptor-mediated nature. Bars represented mean IPSC amplitude ± SEM. $^{**}P < 0.001$ (unpaired t-test). (*C*) Outward currents recorded from DGCs via PP stimulation (0.5 mA) were decreased by 1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium salt hydrate (NBQX, 10 μM, $n = 6$, ACSF, $n = 6$). Values represented mean IPCS amplitude ± SEM. $^{**}P < 0.001$ (unpaired t-test). (*D*) Traces represent whole-cell recording from the same cell at different holding potentials. Black represented EPSC recorded in voltage clamp at -80 mV (average of five traces) and red represented IPSC at 0 mV (average of five traces). Bottom of graph represented the onset of EPSCs and IPSCs evoked by PP stimulation ($n = 10$). $^{**}P < 0.001$ (paired t-test).